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Increased *MLL* gene rearrangements in amniocytes from fetuses of mothers who smoke

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ABSTRACT

We assess the possible genotoxic effect of maternal smoking on amniotic fluid cells, based on the presence of an increasing of structural abnormality of the 11q23 band bearing the *MLL* gene rearrangements. In this observational and prospective study cultured amniocytes were obtained from 20 control and 20 women who smoke (>10 cigarettes/day for >10 years and during pregnancy). We performed fluorescence in situ hybridization (FISH) analysis in amniocytes. Comparison of FISH data between smoker and control groups showed statistical significance for the *MLL* gene rearrangements. Epidemiologic studies, including a large series of patients, will be needed to determine whether the offspring of parents who smoke have an increased lifetime risk of leukemia.

1. Introduction

Possible *in utero* effects of maternal smoking on hematopoietic cancer in the offspring have been addressed, although the results are inconclusive [1]. It is known that a high proportion of infants with acute leukemia have molecular rearrangements in chromosome band 11q23, where *MLL* (leukemia, myeloid/lymphoid or mixed lineage) gene is located [2]. According to some authors, there is strong evidence that 11q23 rearrangements occur *in utero* [3,4]. These findings show the importance of the involvement of 11q23 or *MLL* gene in events leading to leukaemogenesis in infants. A previous study by de la Chica et al. [5] in amniocytes from 25 control (healthy women) and 25 women who smoked (10 cigarettes/day for 10 years and during pregnancy) indicated an increased chromosomal instability and a special sensitivity of the11q23 band to genotoxic compounds contained in tobacco. We assess the possible genotoxic effect of maternal smoking on amniotic fluid cells, based on the presence of an increasing of structural abnormality of the 11q23 band bearing the *MLL* gene rearrangements (*MLL*+).

2. Materials and methods

2.1. Patients

In this observational and prospective study, cultured amniocytes were obtained through amniocentesis proceedings for routine cytogenetic prenatal diagnosis. The study group consisted of 20 smoker women (>10 cigarettes/day for >10 years and during pregnancy) and 20 nonsmoking women between the 14th and 20th gestational week. All patients were selected among consecutive pregnacies attending Cytogenetic Prenatal Amniocentesis in a Public Health Hospital (Hospital Vall d'Hebron, Barcelona). Women were first personally interviewed at length by one of the authors (C.M.). They were asked about their consumption of alcohol, coffee, tea, drugs, regular medication, her exposure to environmental contaminants and radiation, chronic diseases (diabetes, psiquiatric disorders, etc.) and infectious contact. Only if the answers were negative these women were asked to fill out the smoking questionnaire concerning their current and previous smoking habits, those of their husbands, and smoking in their occupational setting. Smoker ones had smoked 10 or more cigarettes per day usually over 16 years (range 4–27 years) and during pregnancy. Nonsmokers (controls) were not exposed to tobacco at home or at work (i.e., no passive smoking). Participants in both groups had similar characteristics regarding: age, paternal age, previous pregnancies, weeks of gestation and analyzed nuclei (Table 1), all were spontaneous gestations. Remarkably information about personal or family history of malignancies/medical problems are listed next: mother S6: Hemophilic Carrier. Family with three generations of hemophilia; mother S7: previous son, from a different couple, with hypersinsulinism; mother S12: Pregnant's father died of cirrhosis at age 33; mother S15: Pregnant's sister suffers sclerosis multiple (age of onset: 16); mother S19: Pregnant's niece with neuroblastoma; mother C 19: First pregnancy in a 43 old healthy woman. After amniocentesis results, ultrasound (US) scan performed at 21 weeks discovered radio agenesis, fetal blood was taken in order to rule out Fanconi anemia: fragility test was negative and a healthy boy was born. In other patients the indications for amniocentesis were anxiety, maternal age and positivist of triple screening. This study was approved by the ethic committee of Hospital del Mar and in accordance with

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Table 1
General characteristics of the mothers and the analyzed nuclei.

	Controls ($n = 20$)	Smokers ($n = 20$)	p Value
General characteristics of the mothers			
Mean maternal age in years (SD)	35.9 (3.7)	33.8 (6.6)	0.224
Mean paternal age in years (SD)	35.3 (4.1)	34.5 (6.1)	0.641
No. of previous pregnancies[a]	1 [0–2]	0.5 [0–2.8]	0.968
Mean years of previous maternal smoking (SD)	–	18.0 (7.0)	–
Mean weeks of gestation (SD)	16.4 (1.5)	15.8 (1.4)	0.190
Mean of analyzed nuclei (SD)	339.8 (104.7)	288.9 (101.6)	0.127
MLL gene rearranged cells[a]	0.60 [0.27–1.19]	0.30 [0.00–0.61]	0.108
No. of paternal smoking (%)	3 (15.0%)	9 (45.0%)	0.038
Cigarettes/day			
10	–	3 (15.0%)	–
11–20	–	11 (55.0%)	–
>20	–	6 (30.0%)	–
No. of mothers >16 weeks of gestation	8 (42.1%)	4 (20.0%)	0.135
No. of mothers with maternal age >37 years	8 (40.0%)	8 (40.0%)	1.000
Characteristics of the analyzed nuclei			
Weeks of gestation	>16 weeks ($n = 3879$)	≤16 weeks ($n = 8604$)	
MLL gene rearrangements	4 (0.11%)	27 (0.31%)	0.033
Maternal age	≤37 years ($n = 7561$)	>37 years ($n = 5012$)	
MLL gene rearrangements	12 (0.16%)	19 (0.38%)	0.015
Smoking	No smoker ($n = 5780$)	Smoker ($n = 6825$)	
MLL gene rearrangements	7 (0.12%)	24 (0.34%)	0.009

[a] This variable departed from normal distribution and is presented as median and 25–75 percentiles. SD: standard deviation.

the guidelines of the Declaration of Helsinki. Informed consent was given by all participants.

2.2. Fluorescence in situ hybridization analysis (FISH)

FISH studies were performed on fixed nuclei from amniotic fluid proceeding from conventional cytogenetics cultures. A commercial *MLL* dual color break apart probe (LSI *MLL*, Abbott Vysis, Abbott Park, IL, USA) was tested in order to detect numerical and structural abnormalities of this gene. It consisted of a 350-kb portion (5′ region) centromeric of the *MLL* gene breakpoint cluster region labeled in SpectrumGreen and a 190-kb portion (3′ region) largely telomeric of the bcr labelled in SpectrumOrange. Fluorescent signals were visualized under a epifluorescence microscope (Olympus BX51, Barcelona, Spain) equipped with a CCD camera and analyzed using image analysis software (Applied Imaging, Genetix, Hampshire, England). On the normal chromosome 11, FISH pattern is usually seen as a "fused" yellow signal rather than separate red and green signals, while rearrangements within *MLL* separate the two colors ("split signal") (Fig. 1). The study was blind and a median of 325 nuclei per exposed and not exposed groups were analyzed by consensus of two observers.

2.3. Statistical analysis

All continuous variables are presented as means (SD) or average value .The 25–75 percentiles are also presented. On the other hand, categorical variables are presented as percentages. Unpaired Student's t-test or non-parametric tests, and chi square test were performed when appropriate.

A multivariate logistic regression model with Generalized Estimating Equation (GEE) [6] was used to assess the differences in FISH patterns (*MLL* rearranged or not rearranged) between the smoker and the control groups. The GEE approach is an extension of Generalized Linear Models designed to explain repeated within-individual measurements. This technique is particularly indicated when the normality assumption is not reasonable as, for discrete data for instance. The GEE model was used instead of the classic Fisher exact test because the first takes into account the possible within-fetus correlation, whereas the latter assumes that all observations are independent. Since several nuclei were analyzed per fetus, the GEE model is more appropriate. In addition, this method allows the inclusion of additional explanatory variables as covariates in the model. The variable was defined as the number of total of *MLL* gene rearrangements/nuclei tested for each fetus. We

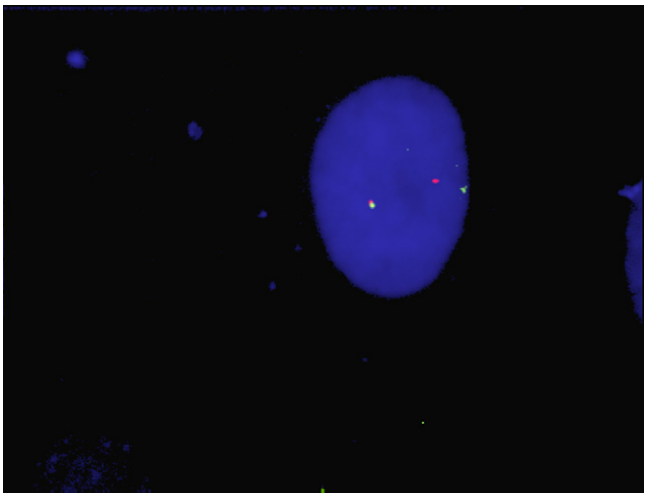

Fig. 1. Nucleus of amniocyte from fetuses carried by mothers who smoke showing *MLL* break. FISH pattern show a segregation of red (R) and green (G) signals and one single fusion (*MLL* germline). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 2
Odds ratio (OR) of *MLL* gene rearrangements observed in the variables in univariate analysis, and models from the saturated initial and final resulting multivariate analysis.

	Intercept	Smoker	Maternal age >37 years	Weeks of gestation ≤ 16
Univariate analysis[a]				
OR (95% CI)	–	1.409 (1.404–1.414)	1.171 (1.167–1.174)	1.409 (1.404–1.414)
p Value	–	<0.001	<0.001	<0.001
Saturated model				
OR (95% CI)	0.078	1.228 (1.027–1.468)	1.035 (0.887–1.207)	1.246 (1.002–1.549)
p Value	–	0.024	0.661	0.048
Final model				
OR (95% CI)	0.080	1.223 (1.037–1.442)	–	1.278 (1.036–1.577)
p Value	–	0.017	–	0.022

[a] OR values were obtained in a univariate analysis using Logistic Regression GEE model.