Maternal and Gestational Factors and Micronucleus Frequencies in Umbilical Blood: The NewGeneris Rhea Cohort in Crete

Kim Vande Loock,1 Eleni Fthenou,2 Ilse Decordier,1 Georgia Chalkiadaki,2 Maria Keramarou,2 Gina Plas,1 Mathieu Roelants,3 Jos Kleinjans,5 Leda Chatzi,5 Franco Merlo,6 Manolis Kogevinas,7,8,9,10 and Micheline Kirsch-Volders1

1Laboratory of Cell Genetics, Faculty of Science and Bio-engineering, Vrije Universiteit Brussel, Brussels, Belgium; 2Department of Histology, School of Medicine, University of Crete, Heraklion, Greece; 3Laboratory of Anthropogenetics, Faculty of Science and Bio-engineering, Vrije Universiteit Brussel, Brussels, Belgium; 4Department of Health Risk Analysis and Toxicology, University of Maastricht, Maastricht, the Netherlands; 5Department of Social Medicine, Medical School, University of Crete, Heraklion, Greece; 6Environmental Epidemiology and Biostatistics, National Cancer Research Institute, Genoa, Italy; 7Centre for Research in Environmental Epidemiology (CIBERESP), Spain; 8Municipal Institute of Medical Research, Barcelona, Spain; 9CIBER Epidemiología y Salud Pública (CIBERESP), Spain; 10National School of Public Health, Athens, Greece

BACKGROUND: The use of cancer-related biomarkers in newborns has been very limited.于是我们研究了母体的形成于微核（MN）在全期和早产新生儿及其父母的Rhea队列（Crete），由于在满月时的脐血中验证了这个系统，以及在全期的单核和双核T淋巴细胞。

OBJECTIVE: We investigated the formation of micronuclei (MN) in full-term and preterm newborns and their mothers from the Rhea cohort (Crete), applying for the first time in cord blood a validated semiautomated analysis system, in both mono- and binucleated T lymphocytes.

METHODS: We assessed MN frequencies in peripheral blood samples from the mothers and in umbilical cord blood samples. We calculated MN in mononucleated (MNMONO) and binucleated (MNBN) T lymphocytes and the cytokinesis block proliferation index (CBPI) in 251 newborns (224 full term) and 223 mothers, including 182 mother–child pairs. Demographic and lifestyle characteristics were collected.

RESULTS: We observed significantly higher MNBN and CBPI levels in mothers than in newborns. In newborns, MMN and MBN were correlated (r = 0.35, p < 0.001), and we found a moderate correlation between MMNMONO in mothers and newborns (r = 0.26, p < 0.001). MNMONO frequencies in newborns were positively associated with the mother’s body mass index and inversely associated with gestational age and mother’s age, but we found no significant predictors of MNBN or CBPI in newborns.

CONCLUSIONS: Although confirmation is needed by a larger study population, the results indicate the importance of taking into account both mononucleated and binucleated T lymphocytes for biomonitoring of newborns, because the first reflects damage expressed during in vivo cell division and accumulated in vivo, and the latter includes additional damage expressed as MN during the in vitro culture step.

KEY WORDS: folate, gestational age, micronuclei, mononucleated cells, newborns, vitamin B12.

Environ Health Perspect 119:1460–1465 (2011). http://dx.doi.org/10.1289/ehp.1003246 [Online 27 May 2011]
in both MONO and BN T lymphocytes and to derive the CBPI in 251 newborns and 223 mothers, including 182 mother–child pairs. We hypothesized that gestational factors and delivery type would influence MN levels in newborns, in addition to maternal smoking and the child’s age and sex.

Materials and Methods

Subject recruitment. The recruitment of the mother–child pairs and preparation of the MN slides were performed at the University of Crete. The Rhea mother–child cohort is a study of pregnant women (Greek and immigrants) who are residents of the prefecture of Heraklion in Crete, Greece (Chatzi et al. 2009). Female residents (Greek and immigrants) who were pregnant during a 12-month period starting in February 2007 were contacted and asked to participate in the study. The first contact was made at the time of the first major ultrasound examination, around the 12th week of gestation. The inclusion criteria for study participants were as follows: residents in the study areas, pregnant women > 16 years of age, first visits to hospitals or private clinics at the time of the first major ultrasound examination at the 10th–13th week of gestation, and no communication handicap. The study was approved by the ethical committee of the University Hospital in Heraklion, Crete, Greece, and all participants provided written, informed consent after complete description of the study. Structured questionnaires and medical records were used to obtain information on several factors, including maternal age, prepregnancy body mass index (BMI), lifestyle (tobacco smoking, alcohol consumption, and fruit intake), delivery type, type of anesthesia, supplement intake, birth weight, child sex, gestational age (GA), and singleton versus twin status. GA was based on the interval between the last menstrual period and the date of delivery of the baby for 84% of the subjects. When the menstrual estimate of GA was inconsistent by ≥ 7 days with the ultrasound measurement taken in the first trimester of pregnancy, a quadratic regression formula describing the relationship between crown–rump length and GA was used instead (Westerveld et al. 2000).

Blood collection. Peripheral blood samples from the mothers and umbilical cord blood samples from the children were collected in heparinized tubes (BD Vacutainer, Plymouth, UK) immediately after the delivery. To prevent clotting, 0.5 mL extra heparin (Leo Pharmaceutical Products, Ballerup, Denmark) was added to the tubes used to collect umbilical cord blood. After collection, the samples were kept at 4°C and processed within 24 hr (Doddier et al. 2007).

Folate and vitamin B_{12}. Plasma vitamin B_{12} and erythrocyte folate concentrations were measured in subgroups of 88 and 42 mothers, respectively, and 79 and 33 children, respectively (including 76 and 30 mother–child pairs, respectively) at the National Cancer Institute (Genoa, Italy).

In vitro CBMN assay. The CBMN assay was carried out according to the standardized protocol developed by us for the semi-automated image analysis system (Decordier et al. 2009). Sampling and making of cultures occurred in Crete. The protocol included preparation of whole-blood cultures (5 mL) within 24 hr after collection and cultivation at 37°C. Umbilical cord blood was diluted (1:3) with phosphate-buffered saline before culture preparation. After 44 hr of phytohemagglutinin A 16 (Remel, Kent, UK) stimulation, cytochalasin B (Sigma, Steinheim, Germany) was added at a final concentration of 6 µg/mL. At 72 hr, the whole-blood cultures were harvested and subjected to a cold hypotonic treatment, using 90 mM KCl (Fisher Bioreagents, Pittsburgh, PA, USA) for umbilical cord blood and 110 mM KCl for venous blood. After fixation according to the protocol, cell suspensions were dropped onto clean slides. Duplicate cultures and two slides per culture were prepared per donor.

Staining. After slide preparation, slides were sent to Vrije Universiteit Brussel, where staining and MN analysis occurred. Slides were stained for 20 min with freshly prepared 5% Giemsa in Sorensen buffer (pH 6.8; Prosan, Metelbeke, Belgium), which was filtered twice through Whatman 41 filters (Whatman International Ltd., Maidstone, UK).

Slides scoring and interactive validation. The automated scoring procedure followed by visual validation of selected MN cells was carried out by the same researcher (K.V.L.), using the PathFinder platform installed by Imstar (version 6; Paris, France) at the Laboratory of Cell Genetics from Vrije Universiteit Brussel, consisting of a PathFinder CELLCAN capture station and two PathFinder MN analysis workstations. At the end of the processing step, the cells containing detected MN are presented one by one on the computer screen for visual confirmation or rejection by the scorer, according to Human MicroNucleus project (HUMN) scoring criteria (Fenech et al. 2003). After validation, the total numbers of mono- and binucleated T lymphocytes with MN (MNMONO and MNBN, respectively) and without MN, and the CBPI, were recorded in a data file. CBPI was calculated as (number mononucleate cells + 2x number binucleate cells + 3x number polynucleate cells) + total number of cells.

Statistics. Consistent with Organisation for Economic Co-operation and Development (OECD) guideline T487 (OECD 2010), data were included only for subjects with at least 1,000 BN lymphocytes counted (except for two mothers and two children, where 985, 986, 995, and 997 binucleates were counted). Normality was evaluated by the Kolmogorov–Smirnov goodness-of-fit test, and because not all data were normally distributed, differences between groups were tested using the nonparametric Mann–Whitney U-test. Associations between genotoxicity parameters and demographic characteristics were tested using Spearman’s rho correlation analysis. Multivariable linear regression analysis with backward selection was used to identify maternal and child factors that were significant predictors of the different genotoxicity parameters (MNB, MN, MNMONO, and CBPI). The backward selection was done by eliminating variables that were not significantly associated with the outcome (p < 0.05). Each initial model included maternal age, birth weight, sex of the child (0 = boy, 1 = girl), prepregnancy BMI, GA, delivery type (0 = vaginal, 1 = cesarean delivery), smoking at the 30th week of pregnancy (0 = no, 1 = yes), and the use of antioxidant supplements during pregnancy (0 = no, 1 = yes). The models were restricted to observations with complete data for all variables. For the analysis of all newborns, perterm status (0 = term birth, 1 = GA < 37 weeks) was added as an independent variable. Additional models of newborn data from mother–child pairs included maternal genotoxicity parameters as initial predictors. Twin births (four pairs, eight children in total) were modeled as individual observations, without accounting for correlated observations. To reduce the number of independent variables and limit multiple testing, highly intercorrelated (Spearman’s rho correlation) independent variables (vitamin B_{12} and folate concentrations) were excluded from the regression model. The level of significance was set at p < 0.05 for all statistical analyses. We used SPSS (version 16.0; SPSS Inc., Chicago, IL, USA) to analyze the data.

Results

Study population. Demographic data were available for most of the subjects (Table 1). The mean age of the mothers was 29 years and ranged between 20 and 41 years. Most of the women were of Greek origin (86.2%), and their prepregnancy BMI ranged between 14 and 47. Almost all of the women took diet supplements during pregnancy (94.4%). Before becoming pregnant, 43.8% of the mothers smoked; 37.3% were still smoking at the first week of the pregnancy, and 20.1% at the 30th week. Concerning alcohol consumption, 22.6% declared to have consumed alcohol during pregnancy, resulting in a mean ± SD of 8.25 ± 31.96 g/day. Half of the deliveries (50.9%) occurred naturally, and 49.5% were without any anesthesia. Most of the deliveries with anesthesia occurred using general (39%) or spinal (42%) anesthesia. Only 19% were with epidural anesthesia. As far as the newborns are concerned, GA ranged between 33 and
When restricted to data from participants in mother–child pairs (Table 2). Median CBPI values in maternal samples (1.70) were statistically significantly higher than those in cord blood (1.58; p < 0.001). Median MNMONO frequencies were not significantly different between maternal and child samples (total population: 0.42 and 0.44 per 1,000, respectively; paired samples: 0.50 and 0.40 per 1,000, respectively). Genotoxicity parameters did not differ significantly between preterm and full-term newborns (data not shown). In newborns, MNMONO and MNBN frequencies were positively correlated with MNBN (r = 0.346) (data not shown). Within the pairs (data not shown), we found a significant positive correlation between the number of MNMONO/1,000 MONO from newborns and mothers (r = 0.263).

### Multivariable analysis
None of the variables included in the initial model (maternal age, birth weight, child sex, maternal BMI, GA, delivery type, preterm status, smoking, and supplement intake) were significant predictors of MNBN and CBPI among the 173 children with complete data for all potential predictors (Table 3). However, MNMONO frequency was significantly inversely associated with maternal age, GA and preterm status and positively associated with maternal prepregnancy BMI.

To reduce potential bias due to pregnancy complications associated with preterm delivery, we also conducted multivariable linear regression analysis using data from full-term births only (n = 156). As for all births combined, no variables significantly predicted MNBN frequency or CBPI (Table 3). GA was associated with significantly decreased MNMONO frequency, whereas maternal BMI was associated with increased MNMONO frequency. In addition, MNMONO frequency was significantly higher in female than in male infants. We confirmed the results in a paired sample subset (data not shown).

Next, we performed a multivariable regression analysis for the full-term infants with paired samples from their mothers to estimate associations with maternal genotoxicity parameters (MNBN, MNMONO, and CBPI) in addition to the variables evaluated previously (data not shown). As for previous models, we found no significant predictors of MNBN, including maternal genotoxicity parameters. In addition, maternal genotoxicity parameters did not predict child’s MNMONO frequency. However, we found a significant positive association between maternal CBPI and child’s CBPI (data not shown).

We also evaluated predictors for maternal genotoxicity parameters (Table 3). In the total population (n = 163), mothers delivering a girl had significantly fewer MBNN than those delivering a boy. MNMONO frequencies increased significantly with maternal age. Mothers who delivered by cesarean section had significantly higher CBPI values compared with those who delivered naturally, and higher prepregnancy BMI also was associated with higher CBPI values.

### Table 1. Clinical and lifestyle characteristics of the total mother–newborn population.

| Characteristic | n       | Percent | Mean ± SD | Range |
|---------------|---------|---------|-----------|-------|
| Mothers       |         |         |           |       |
| Age (years)   | 201     | 29.26 ± 4.85 | 20–41     |
| BMI before pregnancy | 188 | 24.79 ± 9.51 | 14.68–47.46 |
| Origin (Greece) | 217 | 86.2 |         |       |
| Smoking       |         |         |           |       |
| During last 3 months before pregnancy (yes) | 208 | 43.8 |         |       |
| During first weeks of pregnancy (yes) | 209 | 37.3 |         |       |
| At 12th week of pregnancy (yes) | 209 | 19.6 |         |       |
| At 30th week of pregnancy (yes) | 214 | 20.1 |         |       |
| Delivery type (vaginal) | 222 | 50.9 |         |       |
| Anesthesia (yes) | 212 | 50.5 |         |       |
| Type of anesthesia (spinal/epidural/general) | 105 | 42.0/19.0/39.0 |         |
| Supplements during pregnancy (yes) | 196 | 94.4 |         |       |
| Total fruit intake (g/day) | 165 | 410.40 ± 264.17 | 0.00–1,142 |
| Alcohol (yes) | 164 | 22.6 |         |       |
| Total alcohol intake (g/day) | 164 | 8.25 ± 31.96 | 0.00–330.00 |
| Wine (g/day) | 164 | 3.09 ± 11.97 | 0.00–106.00 |
| Beer (g/day) | 164 | 5.13 ± 29.28 | 0.00–330.00 |
| Newborns      |         |         |           |       |
| GA (weeks)    | 241     | 38.33 ± 3.13 | 33–41     |
| Birth weight (g) | 238 | 3194.89 ± 439.31 | 1,860–4,300 |
| Sex (male)    | 241     | 51.9 |         |       |
| Twin (yes)    | 241     | 4.1  |         |       |
| GA < 37 weeks (yes) | 246 | 8.9 |         |       |

### Table 2. Biomarker distribution measured in umbilical cord blood and maternal blood from the total mother–newborn population.

| Biomarker | Mothers | Newborns |
|-----------|---------|----------|
|           | n       | Mean ± SD (25th–75th percentile) | Median | Mean ± SD (25th–75th percentile) | p-Value (mothers vs. newborns) |
| MNBN/1,000 BN | 223 | 2.85 ± 2.18 | 2.36 (1.40–3.85) | 251 | 1.86 ± 1.59 | 1.53 (0.77–2.47) | <0.001 |
| MNMONO/1,000 MONO | 223 | 0.72 ± 0.14 | 0.42 (0.00–1.01) | 251 | 0.62 ± 0.71 | 0.44 (0.00–0.97) | 0.770 |
| CBPI | 223 | 1.64 ± 0.25 | 1.70 (1.47–1.83) | 251 | 1.56 ± 0.22 | 1.58 (1.44–1.71) | <0.001 |
| Folate (ng/mL) | 42 | 851.40 ± 365.32 | 794.20 (525.25–1119.53) | 33 | 766.65 ± 272.63 | 714.20 (580.35–917.95) | 0.445 |
| Vitamin B12 (pg/mL) | 88 | 234.67 ± 115.97 | 204.70 (152.28–300.28) | 79 | 275.50 ± 156.33 | 256.60 (163.80–321.30) | 0.130 |

Differences between mothers and newborns were analyzed with a Mann–Whitney U-test.
Discussion

The present study investigated factors that predict the frequency of MN in blood samples from newborns (full term and preterm) and mothers in the Rhea cohort (Crete), conducted within the European Union project NewGen. Detailed questionnaires provided information concerning gestational factors, demographic data, medical status, smoking, alcohol consumption, and other factors.

The CBMN assay is a well-validated reference biomarker for early genetic effects, and the present study is, to the best of our knowledge, the first to analyze MN frequencies in peripheral and umbilical cord blood from mothers and newborns using a semiautomated image analysis system. This system has several advantages, including a well-defined detection algorithm; applicability to human T lymphocytes; discrimination among MONO, BN, and polynucleated cells; scoring MN according to HUMN scoring criteria; thorough validation with false-positive and false-negative rates as low as possible; and determination of cell proliferation (for review, see Decordier et al. 2011). Unique to this study is the evaluation of MNBN, MNMONO, CBPI, and GA of the newborns, whereas a weakness of the study was the limited data on folate concentration in erythrocytes and plasma.

MN in newborns. In literature, all studies focusing on MN levels in umbilical cord blood reported low frequencies in BN T lymphocytes from newborns, consistent with our results of 1.53 per 1,000 cells (median) in the total population (Das and Karuppasamy 2009; Decordier et al. 2007; Hando et al. 1994; Levario-Carrillo et al. 2005; Lope et al. 2010, Milošević-Djordjević et al. 2005, 2007; Nath et al. 1995; Neri et al. 2005; Pedersen et al. 2009, 2010; Stankovic et al. 2004; Wyatt et al. 2007; Zalacain et al. 2006). We detected lower levels than previously reported levels in literature (Grujicic 1994; Levario-Carrillo et al. 2005; Lope et al. 2010, Milošević-Djordjević et al. 2005, 2007; Stankovic et al. 2004). Although we detected lower levels than previously reported, relative differences in frequencies between newborns and their mothers were comparable with previous reports.

MNMONO, GA, and smoking in newborns. Multivariable linear regression analysis revealed a significant inverse association between GA and MNMONO in the newborn population, with significantly lower MNMONO frequencies in preterm newborns (GA < 37 weeks) compared with term births at ≥ 37 weeks. To reduce bias due to pregnancy complications, we performed subsequent multivariate regression analysis using data from full-term infants only. We identified no significant predictors of MNBN levels in full-term newborns, but MNMONO frequency was inversely correlated with GA. Zalacain et al. (2006) and Pedersen et al. (2009) observed elevated MNBN levels in newborns of smoking versus nonsmoking mothers, whereas Milošević-Djordjević et al. (2007) reported nonsignificantly higher mean MNBN frequencies in infants of nonsmoking mothers. We know of only one report focusing on MN levels in umbilical cord blood reported low frequencies in BN T lymphocytes, whereas Milošević-Djordjević et al. (2007) reported nonsignificantly higher mean MNBN frequencies in infants of nonsmoking mothers. We know of only one report focusing on MN levels in umbilical cord blood reported low frequencies in BN T lymphocytes, whereas Milošević-Djordjević et al. (2007) reported nonsignificantly higher mean MNBN frequencies in infants of nonsmoking mothers. We know of only one report focusing on MN levels in umbilical cord blood reported low frequencies in BN T lymphocytes, whereas Milošević-Djordjević et al. (2007) reported nonsignificantly higher mean MNBN frequencies in infants of nonsmoking mothers. We know of only one report focusing on MN levels in umbilical cord blood reported low frequencies in BN T lymphocytes, whereas Milošević-Djordjević et al. (2007) reported nonsignificantly higher mean MNBN frequencies in infants of nonsmoking mothers. We know of only one report focusing on MN levels in umbilical cord blood reported low frequencies in BN T lymphocytes, whereas Milošević-Djordjević et al. (2007) reported nonsignificantly higher mean MNBN frequencies in infants of nonsmoking mothers. We know of only one report focusing on MN levels in umbilical cord blood reported low frequencies in BN T lymphocytes, whereas Milošević-Djordjević et al. (2007) reported nonsignificantly higher mean MNBN frequencies in infants of nonsmoking mothers. We know of only one report focusing on MN levels in umbilical cord blood reported low frequencies in BN T lymphocytes, whereas Milošević-Djordjević et al. (2007) reported nonsignificantly higher mean MNBN frequencies in infants of nonsmoking mothers.

We proposed by Bonassi et al. (2003), tobacco smoking may induce damage to the lymphocytes, and damaged cells may not survive the culture period in the CBMN assay or may not divide. If they do not divide, they will not form a BN and will thus be scored as a MONO T lymphocyte. Moreover, some compounds of tobacco smoke can cross the placenta (Jauniaux et al. 1999; Lackmann et al. 1999) and can induce DNA damage such as DNA adducts, chromosomal instability, and oxidative damage (de la Chica et al. 2005; Finette et al. 1998; Perera et al. 1999; Pluth et al. 2000; Ramsey et al. 1995). Full-term newborns are well equipped to cope with the stress of oxidative damage (Decordier et al. 2007). Presence of MN within a MONO lymphocyte indicates chromosome breakage/loss before the blood was sampled and thus reflects damage accumulated during pregnancy (in utero exposure only). In contrast, MN in BN cells may originate from preexisting MN plus DNA or protein lesions that are expressed as chromosome breaks/loss during in vitro replication. MNBN levels may therefore reflect genetic damage induced by stress during delivery. The different (although not significant) response we detected in MNBN levels between preterm and full-term children can result from differences in DNA repair or apoptotic capacity. A possible explanation for the higher MNBN frequencies in preterm children might be the matura-

Table 3. Significant predictors from multiple regression analysis with backward selection of preterm status (total newborns only), maternal age, birth weight, child sex, mother BMI, GA, delivery type, smoking, and supplement intake on genotoxicity biomarkers in newborns and mothers.

| Variable | Partial $r^2$ | Slope | $R^2$ | p-Value |
|----------|---------------|-------|-------|---------|
| Newborns (n = 173) | MNBN/1,000 BN | No significant predictors | | |
| | MNMONO/1,000 MONO | | | |
| | Mother’s age | 0.026 | −0.025 | 0.036 |
| | Mother’s BMI | 0.049 | 0.029 | 0.004 |
| | GA | 0.058 | −0.183 | 0.002 |
| | Preterm | 0.026 | −0.532 | 0.038 |
| | CBPI | 0.108 | 0.001* |
| Full-term newborns (n = 156) | MNBN/1,000 BN | No significant predictors | | |
| | MNMONO/1,000 MONO | | | |
| | Child’s sex | 0.026 | 0.228 | 0.048 |
| | Mother’s BMI | 0.069 | 0.033 | 0.001 |
| | GA | 0.074 | −0.199 | 0.001 |
| | CBPI | 0.120 | 0.000* |
| Mothers (n = 163) | MNBN/1,000 BN | No significant predictors | | |
| | MNMONO/1,000 MONO | | | |
| | Child’s sex | 0.026 | −0.677 | 0.026 |
| | Mother’s BMI | 0.027 | 0.037 | 0.036 |
| | Smoking | 0.018 | −0.293 | 0.091 |
| | CBPI | 0.144 | 0.008 | 0.022 |
| | Delivery type | 0.065 | 0.117 | 0.076 |
| | | 0.002* |

* $p < 0.05$
antioxidants such as vitamin B, vitamin C, and beta-carotene during the final days of gestation (Friel et al. 2004; Gerdin et al. 1985; Ripalda et al. 1989; Robles et al. 2001).

Predictivity of MNMONO in newborns. Up to now, MNMONO frequencies have been rarely taken into account in biomonitoring studies (Aka et al. 2004; Elhajouji et al. 1998; Godderis et al. 2004, 2006; Touli et al. 2002). The value of including both MNBN and MNMONO in biomonitoring studies has been emphasized by Kirsch-Volders and Fenech (2001), because of their different but complementary properties. MNMONO cells represent chromosomal damage induced and expressed in vivo before the start of the CBMN assay culture. MNBN cells, in addition to in vivo accumulated MN, reflect damage present on DNA or key proteins and expressed as MN during in vitro cell division.

Whether MN frequencies in newborns can be interpreted in the same way as in adults, and whether they are predictive for cancer, is not known at this time. Three major facts need to be considered regarding their biological significance in newborns compared with adults. In adults, circulating T lymphocytes may accumulate MN over several years, in contrast to newborns, where damage accumulates during a short in utero exposure time of up to 6 months (Pollin et al. 2004). Second, the response of T lymphocytes to phytohemagglutinin stimulation in umbilical cord blood is less efficient than the response of T lymphocytes in peripheral blood from adults (Eisenthal et al. 2003). Therefore, the significantly higher CRPI in mothers than in newborns was consistent with expectations. Third, despite the fact that MNMONO cells in mothers represent damage accumulated over several years, dependent on the life span of the lymphocytes (Tough and Sprent 1995), and in newborns only during in utero exposure, we observed a significantly positive correlation between MNMONO frequencies in mothers and newborns. This suggests that maternal exposure is reflected in their newborns, and one has to consider inherited similarities in DNA repair capacity.

Conclusion

Although confirmation in a larger study population is needed, multivariataly analysis revealed the importance of taking into account GA when studying MN frequencies in newborns. In addition, our results indicate the importance of assessing both MNMONO and MNBN for biomonitoring of newborns, because the first reflects damage expressed during in vivo cell division and accumulated in utero, and the latter includes additional damage expressed as MN during the in vitro culture step. Because of physiological differences and the age of circulating T lymphocytes, it is not yet clear whether MN frequencies in newborns can be interpreted in the same way as in adults—that is, whether they are predictive for cancer and childhood cancer in particular.

References

Aka P, Mateuca R, Buchet JP, Thiaren H, Kirsch-Volders M. 2004. Are genetic polymorphisms in DGGI, XRCC1 and XRCC3 genes predictive for the DNA strand break repair phenotype and genotoxicity in workers exposed to low dose ionising radiations? Mutat Res 556:101–181.

Behrmann RE, Kliegman RM, Arvin AM. 1995. Tables of references for laboratory tests. In: Nelson Textbook of Pediatrics. 15th ed. Philadelphia:W.B. Saunders, 2004–2006.

Bonni S, Pincus G, Lando C, Ceppi M, Lin Y, Chang WP, et al. 2003. Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human MicroNucleus project. Mutat Res 543:155–166.

Bominaar ED, Searle MP, Fenech M, London N, et al. 2007. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. Carcinogenesis 28(6):625–631.

Chatzi I, Piana E, Duranton D, Tastaniotis C, et al. 2009. Metabolic syndrome in early pregnancy and risk of preterm birth. Am J Epidemiol 170:829–836.

Das B, Karuppasamy CV. 2009. Spontaneous frequency of micronuclei among the Tamil high level natural radiation areas of Kerala in the southwest coast of India. Int J Radiat Biol 85(3):272–280.

Decordier I, De Bont K, De Bock K, Mateuca R, Roelants M, Ciardelli R, et al. 2007. Genetic susceptibility of newborn daughters for oxidative stress. Toxicol Lett 172:68–84.

Decordier I, Papine A, Plass G, Roesens S, Vande Loock K, Moreno-Palomao J, et al. 2009. Automated image analysis of cytokinesis-blocked micronuclei: an adapted protocol and a validated scoring procedure for biomonitoring. Mutagenesis 24(1):85–93.

Decordier I, Papine A, Vande Loock K, Plass G, Soussaline F, Kirsch-Volders M. 2011. Automated image analysis of micronuclei by IMSTAR for biomonitoring. Mutagenesis 26(1):163–168.

de la Chica RA, Ribas I, Giraldó J, Ejpezeu J, Fuster R. 2005. Chromosomal instability at fetuses from fetuses of mothers who smoke. JAMA 293(12):1722–1723.

Eisenthal A, Hassner A, Shenav M, Baron S, Lifschitz-Mercer B. 2011. Micronuclei in umbilical cord blood from newborns of mothers exposed to tobacco smoke. Nat Med 4(10):1144–1151.

Friel JK, Friesen RW, Harding SV, Roberts LJ. 2004. Evidence of oxidative stress in full term healthy infants. Pediatr Res 56(6):878–882.

Gerdin E, Tydén O, Eriksson UE. 1985. The development of antioxidant enzymatic defense in the perinatal rat lung: activities of superoxide dismutase, glutathione peroxidase and catalase. Pediatr Res 19(7):687–691.

Godderis L, Aka P, Mateuca R, Kirsch-Volders M, Lison D, Veulemans H. 2008. Dose-dependent influence of genetic polymorphisms on DNA damage induced by styrene oxide, ethylene oxide and gamma-radiation. Toxicolology 219:220–229.

Godderis L, De Boeck M, Hauvroid F, Emmyr M, Mateuca R, Gardinal S, et al. 2004. Influence of genetic polymorphisms on biomarkers of exposure to genotoxic effects in styrene-exposed workers. Environ Mol Mutagen 44:293–303.

Grujic D, Milošević-Djordjević O, Arsenijević S, Marinikovic D. 2007. Effect of orchiectomy and varicoceles on the frequency of micronuclei in peripheral blood lymphocytes of pregnant women. Clin Exp Med 7:11–15.

Hando JC, Nath J, Tucker JD. 1994. Sex chromosomes, micro -nuclei and aging in women. Chromosoma 103:186–196.

Huen K, Gunn L, Durdam P, Jeng M, Scaf R, Holland N. 2006. Application of a geographic information system to explore associations between air pollution and micronuclear frequencies in African American children and adults. Environ Mol Mutagen 47(4):236–246.

Jauniaux E, Gublis B, Achary G, Thiry P, Rodeck C. 1999. Maternal tobacco exposure and cotinine levels in fetal fluids in the first half of pregnancy. Obstet Gynecol 93(5):25–29.

Kaatsch P. 2010. Epidemiology of childhood cancer. Cancer Treat Rev 36:277–285.

Kirsch-Volders M, Elhajouji A, Coulander C, Van Hummelen P. 1997. The in vitro micronucleus test: a multi-endpoint assay to detect simultaneously mitotic delay, apoptosis, chromosome breakage, chromosome loss and non-disjunction. Mutat Res 382(21–19):90–100.

Kirsch-Volders M, Fenech M. 2001. Inclusion of micronuclei in non-divided mononuclear lymphocytes and necroptosis may provide a more comprehensive cytokinesis block micronucleus assay for biomonitoring purposes. Mutagenesis 16(1):51–58.

Kirsch-Volders M, Plas G, Elhajouji A, Lukamowicz M, Gonzalez L, Vande Loock K, et al. 2011. The in vitro MN assay in 2011: origin and fate, biological significance, protocols, high throughput methodologies and toxicological relevance. Arch Toxicol; doi:10.1007/s00204-011-0691-4 [Online 3 May 2011].

Lackmann GM, Salzberger U, Töllner U, Chen M, Carmella SG, Hecht SS. 1999. Metabolites of a tobacco-specific nitrosoamine carcinogen in urine from newborns. J Natl Cancer Inst 91:459–465.

Levario-Carrillo M, Sordo M, Rocha F, Gonzalez-Horta C, Atanio D, Ostrosky-Wegman P. 2005. Micronucleus frequency in human umbilical cord lymphocytes. Mutat Res 576(1):168–75.

Lope V, Pollan M, Fernandez M, de Leon A, Gonzalez MJ, Sanz J, et al. 2010. Cytogenetic status in newborns and their parents in Madrid: the BioMadrid study. Environ Mol Mutagen 51:267–277.

Mateuca R, Lombaert N, Aka PV, Decordier I, Kirsch-Volders M. 2008. Chromosomal changes: induction, detection methods and applicability in human biomonitoring. Biochimie 88(11):1515–1531.

Merlo DF, Wild CP, Kogevinas M, Kleinjans J, Merlob P, et al. 2001. Environmental Health Perspectives

October 2011

Volume 119 | Number 10 | October 2011 | Environmental Health Perspectives

1464
Micronucleus frequencies in umbilical blood

Neri M, Ceppi M, Knudsen LE, Menlo DF, Barale R, Puntoni R, et al. 2005. Baseline micronuclei frequency in children: estimates from meta- and pooled analyses. Environ Health Perspect 113:1226–1229.

NewGeneris. 2010. NewGeneris Project: Newborns and Genotoxic Exposure Risks. Available: http://www.newgeneris.org [accessed 20 August 2011].

OECD (Organisation for Economic Co-operation and Development). 2010. OECD Guideline for the Testing of Chemicals: In Vitro Mammalian Cell Micronucleus Test. No. 487. Available: http://www.oecd-ilibrary.org/docserver/download/fulltext/9789264145916.pdf?expires=1314215653&id=id&accname=freeContent&checksum=5FCF86952C171F807A02C000A139BD04 [accessed 11 May 2010].

Pedersen M, Halldorsson TI, Mathiesen L, Mose T, Brouwer A, Hedegaard M, et al. 2010. Dioxin-like exposures and effects on estrogenic and androgenic exposures and micronuclei frequency in mother-newborn pairs. Environ Int 36:344–351.

Pedersen M, Wichmann J, Autrup H, Dang DA, Decordier I, Hvidberg M, et al. 2009. Increased micronuclei and bulky DNA adducts in cord blood after maternal exposures to traffic-related air pollution. Environ Res 109(6):1012–1020.

Perera FP, Jedrychowski W, Raush V, Whyatt RM. 1999. Molecular epidemiologic research on the effects of environmental pollutants on the fetus. Environ Health Perspect 107:451–460.

Pluth JM, Ramsey MJ, Tucker JD. 2000. Role of maternal exposures and newborn genotypes on newborn chromosome aberration frequencies. Mutat Res 465:101–111.

Pollin RA, Fox WW, Abman SH, eds. 2004. Fetal and Neonatal Physiology. 3rd ed. Philadelphia:W.B. Saunders.

Ramsey MJ, Moore DH, Briner JF, Lee DA, Olsen LA, Senft JR, et al. 1995. The effects of age and lifestyle factors on the accumulation of cytogenetic damage as measured by chromosome painting. Mutat Res 338:95–106.

Ripalda MJ, Rudoph N, Wong SL. 1989. Developmental patterns of antioxidant defense mechanisms in human erythrocytes. Pediatr Res 26(4):366–369.

Robles R, Palomino N, Robles A. 2001. Oxidative stress in the neonate. Early Hum Dev 65(suppl 1):S75–S81.

Stankovic M, Joksic D, Guc-Stecic M. 2004. Incidence of micronuclei in pregnant women and cord blood samples before and after the bombing of Serbia. Arch Oncol 12(4):200–202.

Tough DF, Sprent J. 1995. Lifespan of lymphocytes. Immunol Rev 141(1):1–12.

Touil N, Aka P, Buchet JP, Thierens H, Kirsch-Volders M. 2002. Assessment of genotoxic effects related to chronic low level exposure to ionizing radiation using biomarkers for DNA damage and repair. Mutagenesis 17(3):223–232.

Vineis P, Perera F. 2007. Molecular epidemiology and biomarkers in etiologic cancer research: the new in light of the old. Cancer Epidemiol Biomarkers Prev 16(10):1954–1965.

Westerway SC, Davison A, Cowell S. 2000. Ultrasonic fetal measurements: new Australian standards for the new millennium. Aust NZ J Obstet Gynaecol 40(3):297–302.

Wild CP. 2005. Complementing the genome with an “exposome”: the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev 14(8):1847–1850.

Wyatt NP, Falque-Gonzalez C, Farrar D, Tuffnell D, Whitelaw D, Knudsen L, et al. 2007. In vitro susceptibilities in lymphocytes from mothers and cord blood to the monofunctional alkylating agent EMS. Mutagenesis 22(2):123–127.

Zalacain M, Sierrasmemaga L, Larranaga C, Patimno-Garcia A. 2006. Effects of benzopyrene-7,8-diol-9,10-epoxide (BPDE) in vivo and of maternal smoking in vivo on micronuclei frequencies in fetal cord blood. Pediatr Res 60(2):180–184.