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MiniReview

Characterization of the Exposure–Disease Continuum in Neonates of Mothers Exposed to Carcinogens during Pregnancy

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Abstract: The impact of maternal exposure to carcinogens during pregnancy on childhood cancer risk may be especially relevant for genetically susceptible infants. A molecular epidemiological approach, which has the potential to characterize processes between exposure and subsequent health effects in newborns by using biomarkers, is expected to provide valuable information for actually identifying such vulnerable neonates. Therefore, biomarkers of exposure (e.g. levels of cotinine and metals in cord blood), biomarkers of the biologically relevant dose (e.g. DNA and protein adducts) and biomarkers of early effects (e.g. the occurrence of somatic mutations in cord blood) have been studied in relation to birth outcomes. In this *MiniReview*, the most important data concerning these biomarker studies in relation to potential adverse health effects in neonates will be summarized and will be compared to the outcome of a small study population (59 mother–child pairs) in which all these biomarkers were assessed simultaneously. Overall, it can be concluded that plasma cotinine levels, macromolecule–carcinogen adduct levels and hypoxanthine phosphoribosyltransferase mutant frequencies are increased in cord blood of neonates of mothers who were exposed during pregnancy and their levels correlated with proxies of health effects, such as reduced birth weight. Moreover, DNA damage was found to be the highest in those neonates that carried risk alleles in genes that code for biotransformation enzymes. These results were confirmed in our study, which indicates that it is possible to identify a susceptible subgroup of newborns. In summary, there is a reason for profound concern of genotoxic effects in newborns of exposed mothers.

It is well known that the foetus is exposed via the placenta to potentially genotoxic xenobiotics, which are present in the peripheral circulation of the mother. For instance, the demonstration of tobacco smoke-related compounds and their metabolites (e.g. nicotine and cotinine), metals (e.g. lead and cadmium) and DNA damage induced in umbilical cord blood, placenta and foetal tissues [1–4] has led to concerns about diseases in childhood or in later life (adolescence or adulthood) possibly induced by maternal exposures during pregnancy. Epidemiological studies have shown that exposure to environmental carcinogens, tobacco smoke and environmental tobacco smoke during pregnancy increases the risk for severe health impairments in the newborn children [5,6]. The risk for most of these conditions has been found to increase with the level of exposure, for instance the number of cigarettes smoked during pregnancy, while newborns of women who stopped smoking during early pregnancy were at lower risk for most of those pathologies [7]. In exposed adults, it is now generally accepted that DNA damage by genotoxic compounds is an important initial step in the process of carcinogenesis [8,9]. However, assessing the level of genotoxic damage in the foetus can be very challenging, because (i) the placenta forms a relevant barrier, (ii) compounds that eventually reach the foetus can be partially metabolized by the maternal system and the placenta before they reach the foetal circulation, and (iii) compounds may accumulate in the foetal compartment due to urinary excretion of water-soluble derivatives by the foetus and subsequent foetal swallowing of amniotic fluid. The dose of exogenous genotoxins that actually reaches the developing foetus is therefore unknown, and foetal exposure is thus assessed with substantial error. As a result, statistical relations between exposure and disease occurrence will be greatly attenuated. In other words, the ability to estimate exposures accurately can be the difference between establishing a causal link and an inconclusive study.

Although epidemiologists are very interested in the biological basis of their observed associations, the process between exposure and disease is mostly treated as a 'black box'. Molecular epidemiology is a relatively new field of research that tries to further characterize the series of events between exposure and disease, in order to break open this 'black box' [10]. To this end, molecular epidemiology integrates molecular biology into epidemiological research

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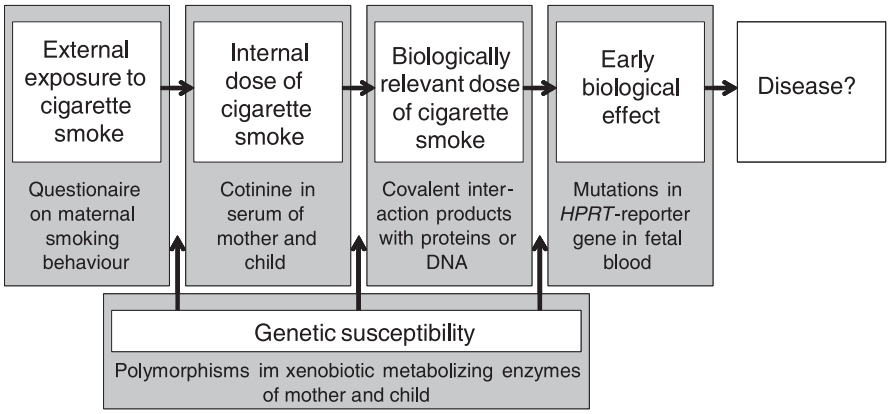

Fig. 1. Overview of the exposure disease continuum and potential biomarkers in neonates. To further characterize possible health effects of maternal exposure to genotoxins during pregnancy, a molecular epidemiological approach can be applied as inventory of early biological effects. Health effects may become apparent at older ages (adulthood), and may thus remain undiscovered by epidemiological studies, which predominantly focused on young age groups.

and focuses on the contribution of potential genetic and environmental risk factors, as identified at the molecular level, to the aetiology of diseases. These so-called biomarkers comprise all measurable cellular or molecular indicators of exposure (internal dose or biologically effective dose), effect or susceptibility to disease (fig. 1). It is one of the major goals of the 6th Framework Programme European Union-integrated project 'NEWGENERIS' (www.newgeneris.org) to apply these types of biomarkers to cohorts of mothers and their newborn children to obtain further insights into the link between exposure to genotoxins during pregnancy and potential health effects in their children. Such insights are important in the light of the increasing incidence of childhood cancer [11].

This *MiniReview* will summarize the most important findings with regard to such biomarker studies in relation to potential adverse health effects in neonates that were *in utero* exposed to carcinogenic compounds (through the mother via cigarette smoking, or exposure to environmental tobacco smoke or environmental carcinogens in heavily polluted areas). The biomarkers chosen for this *MiniReview* are considered to be the ones that have (at least partly) been validated in studies with adults, but may still need validation for neonatal blood. The data reported here will be compared to a small study population in which all these biomarkers were assessed simultaneously. This study population consisted of 59 mother–child pairs from the southern part of the country of which 15 smoked throughout pregnancy (maternal age: 28 ± 6 years, smoking 12 ± 8 cigarettes per day), 14 stopped smoking in the first trimester of pregnancy (maternal age: 31 ± 3 years, used to smoke 13 ± 8 cigarettes per day) and 30 were never-smokers (maternal age: 30 ± 4 years). This study was approved by the medical ethical committee of the Maastricht University, Maastricht, The Netherlands. Informed consent was obtained from all mothers. Results obtained from a subset of these subjects were published previously [12].

In utero exposures and foetal sensitivity

There is ample evidence that the foetus/newborn is more vulnerable for exposure to certain toxic compounds, which may not only be due to the complex exposure during *in utero* life, but also to the physiological immaturity of most tissues, leading to different metabolic or repair capacities as compared to adults [13]. Especially, the nervous system seems to be a relevant target tissue, leading to deficits in cognitive functioning, lower intelligence and subsequent poorer school performances at older ages [5]. An obvious example of maternal exposure to hazardous chemicals with easily identifiable health effects is cigarette smoking (for instance intra-uterine growth retardation and impaired mental development [5,6]). However, with regard to childhood cancer, the data are much less clear; a recent meta-analysis, including more than 30 studies on maternal tobacco use during pregnancy and childhood cancer, indicated that there is only a small increase in the risk of all neoplasms, but not for specific types of tumours [14]. Tobacco smoke contains numerous compounds that have been classified as potential human carcinogens. Most of these compounds are thought to elicit their carcinogenic properties via the formation of DNA damage. Cell division is thought to be a prerequisite to fix this DNA damage as a mutation; an initial and irreversible step in the process of carcinogenesis. Because cell turnover is very high during foetal development, it is surprising that the impact of maternal smoking on cancer development in the offspring is so little.

However, no relevant human studies have tried to relate foetal exposure to early onset of cancer at older ages (i.e. after childhood), to evaluate the hypothesis that the lag time between cancer initiation and clinical manifestation can be significantly reduced due to prenatal exposure. Moreover, although the impact of maternal smoking during pregnancy on childhood cancer risk was found to be small for neonates

in general, adverse effects of maternal cigarette smoking may be more relevant in subsets of susceptible newborns [5,12]. In determining this susceptibility, it might also be necessary to take the susceptibility of the mother into account, because the metabolism of pre-carcinogens in the mother may determine the actual dose that reaches the foetus.

It is very difficult to identify those compounds responsible for potentially adverse health effects in newborn children after exposure to complex mixtures of genotoxic agents. Suspected environmental genotoxins that are also present in cigarette smoke include polycyclic aromatic hydrocarbons (PAH), aromatic amines, cadmium (Cd) and lead (Pb). It should be noted that also other genotoxins, such as nitrosamines, benzene, alcohol or acrylamide may have consequences for the developing foetus, but these will not be discussed in this *MiniReview*.

For some PAH and aromatic amines, there is sufficient evidence from animal studies for health effects in the offspring after transplacental exposure [15–17]. Increased frequencies of stillbirths, malformations and re-absorptions were observed, and foetal weight, number of live pups and uterine weight were significantly reduced. There is also some evidence from studies in human beings, linking maternal PAH exposure during pregnancy to low birth weights, premature births and decreased head circumference [5].

Accumulation of Cd already starts at a young age, because excretion is limited. Although the placenta functions as an efficient barrier for Cd, teratogenic and developmental effects were observed in experimental animals and human beings after exposure to Cd during pregnancy [18]. The placenta is not an efficient barrier for Pb that is present in the maternal circulation. Interestingly, Pb can be released from maternal body storages (predominantly resorption from bone) at the last stages of pregnancy and can reach the developing foetus [19]. Some reported non-carcinogenic effects of Pb on the foetus are spontaneous abortion [20], low birth weight [21] and cognitive outcomes in the child [22,23]. Fortunately, nowadays the effects of Cd and Pb are rarely observed in Western societies due to the low levels of exposure in the general population. Nonetheless, exposure to these compounds remains a matter of concern [22].

Biomarkers of internal dose

Definition. The concentration of a certain compound or its metabolites in body fluids (urine, blood/plasma, saliva, etc.) that is indicative for exposure and uptake of these compounds or class of compounds [10].

A biomarker of internal dose in newborns is considered to be superior to measuring external exposure levels of a compound, because it takes into account the maternal uptake and distribution and subsequent passing of the placental barrier. Biomarkers of internal dose in maternal blood or urine can only be used to predict the exposure of the foetus, if the levels in maternal and foetal body fluids correlate. Maternal exposure to potential genotoxic agents during pregnancy is probably best characterized in studies that focused on cigarette smoking, which is usually assessed by questionnaires but can also efficiently be monitored by the assessment of cigarette smoke specific compounds in the umbilical cord blood (e.g. plasma cotinine levels) [1]. Cotinine is a metabolite of nicotine, and the data of a relationship between cotinine levels in blood or urine and recent smoking behaviour is overwhelming. It is, however, still unclear whether cotinine concentrations are higher in the foetal or maternal system [1,24–26]. Nonetheless, cotinine levels in newborns showed a strong relationship with maternal smoking behaviour during pregnancy [1,24]. In our study population, cotinine levels were indeed higher in the umbilical cord blood of those mothers who smoked throughout pregnancy (64.7 ± 15.5 ng/ml) as compared to those who did not smoke (6.5 ± 0.3, $P < 0.05$) and those who stopped smoking in the first trimester of pregnancy (11.7 ± 6.2, $P < 0.05$). Levels in maternal blood correlated with those in umbilical cord blood ($r = 0.59$, $P < 0.001$). The collection of maternal blood was less than 24 hr before delivery, which is necessary due to the relatively short half-life of cotinine in blood.

For assessing general exposure to PAH in adults, hydroxylated metabolites in urine have been widely applied [27]. However, no studies were found in which this biomarker was used to assess exposure of neonates, probably because it is practically difficult to obtain urine of newborns. There are, however, studies available that measured 1-hydroxypyrene in young children or adolescents, and a correlation with asthmatic symptoms was reported [28].

In addition, the blood or plasma concentrations of metals in umbilical cord blood can be considered as biomarkers of internal dose. In our [12] and other studies [18], the placenta was found to be an effective barrier for Cd. As a result, maternal and foetal blood correlations of Cd do not necessarily correlate and maternal levels can thus not be used as a *proxy* to estimate levels of Cd that reach the foetus. In fact, concentrations in neonatal blood are significantly lower than those in maternal blood, which was verified in our study population [12]. The barrier function of the placenta is much less effective for Pb, and good associations have been observed between Pb concentrations in maternal and foetal blood. Interestingly, whole-blood Pb levels have been observed to have a U-shaped curve during pregnancy [29], with a fall of blood Pb levels in the second trimester, and an increase during the final months of pregnancy. It has been suggested that the decrease in the second trimester may be due to haemodilution and/or possibly to deposition of Pb in bone [30]. The deposited Pb can subsequently be released in the third trimester. It is not yet clear whether the second-trimester fall in the total body load of Pb in the maternal circulation also represents a fall in foetal Pb levels. Nonetheless, the U-shaped curve indicates relatively stable foetal lead exposures and an increase during the final stages of pregnancy.

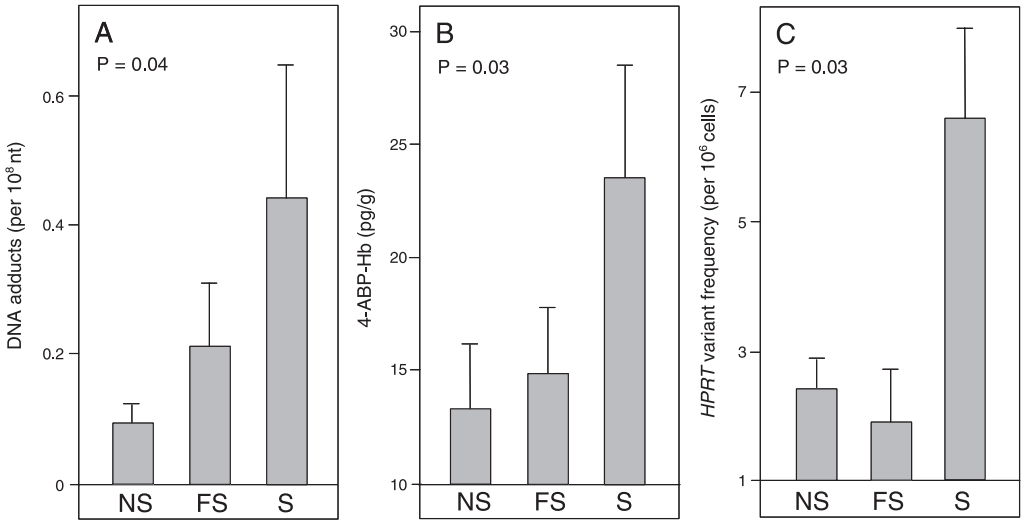

Fig. 2. Effects of the maternal smoking on biomarkers in umbilical cord blood of newborn children. (A) Aromatic DNA adduct levels in umbilical cord blood of non-smokers (NS), former smokers (FS, stopped smoking in the first trimester of pregnancy) and mothers who continued to smoke till several hours before delivery (S). (B) 4-Aminobiphenyl haemoglobin levels in umbilical cord blood of non-smokers, former smokers and smokers, respectively, and (C) the *HPRT* mutant frequency in umbilical cord blood of non-smoking, former smoking and smoking mothers.

Biomarkers of the biologically effective dose

Definition. The concentration of a certain compound or its reactive metabolites has interacted with cellular macromolecules such as DNA or proteins. The biologically effective dose is considered to be the net outcome of uptake and excretion, metabolism to reactive compounds and the ultimate interaction with relevant molecular targets [10].

Many genotoxic compounds, including PAH, have to be metabolized to reactive derivatives, before they can exert their genotoxic effects. The reactive metabolites can subsequently covalently bind to DNA (DNA adducts) or proteins (protein adducts). Both adduct types can be applied as biomarkers of the biologically effective dose in molecular epidemiological studies, but only DNA adducts are promutagenic structural modifications, which are thought to be involved in the onset of carcinogenesis [31]. Indeed, recent prospective studies showed that increased levels of DNA adducts represented an increased risk for lung cancer in adults [8,9]. It is yet unclear whether DNA adducts in newborns represent a similar enhanced risk for childhood cancer.

Several studies that have measured DNA adducts in umbilical cord blood of mothers that were exposed to PAH via contaminated air or cigarette smoking, showed that a higher maternal exposure coincides with higher levels of aromatic-DNA adducts in umbilical cord blood directly obtained after birth [32,33], but not all studies were able to find an effect of maternal exposures [34,35]. Therefore, more work is needed to clarify whether a link between maternal exposure and increased DNA adduct formation in the foetus exists. We observed significantly increased DNA adduct levels in neonates of mothers who reported to smoke (0.44 ± 0.20) as compared to those who reported to have quit smoking in the first trimester of pregnancy (0.27 ± 0.11) or who never smoked (0.10 ± 0.03, $P = 0.04$, fig. 2A). Furthermore, neonates that had relatively high levels of plasma cotinine (>10 ng/ml) levels also had increased adduct levels. However, due to large inter-individual variations, this relationship did not reach statistical significance. Literature is also not conclusive as to whether levels of DNA adducts are higher in maternal or neonatal blood. In our study population, DNA adduct levels were two to three times higher in maternal samples as compared to the paired umbilical cord blood samples, but others found contrasting results [5,35]. Nonetheless, both results can be interpreted as an indication for relatively high susceptibility of the human foetus for PAH, because animal studies indicated that only a small fraction (less than one tenth) of the maternal exposure actually reaches the foetus [36].

DNA adduct measurements represent a steady-state level between formation and repair, which both may vary between individuals. Protein adducts are not repaired and are therefore thought to have a better correlation with exposure. Indeed, studies that measured protein adducts in general showed a good association with recent maternal exposure, especially with cigarette smoking [37–40]. Although adducts on proteins (haemoglobin or albumin) do not reflect genetic risks, they indicate that genotoxins do pass the placenta and bind to foetal macromolecules after metabolic activation in the mother, the child or both. The literature on protein adducts thus adds to our understanding of the actual foetal genotoxic load after maternal exposure. We assessed 4-aminobiphenyl haemoglobin adducts in maternal and cord blood by gas chromatography-mass spectrometry [41] in our study population; the levels in neonates

were increased in newborns of smokers (fig. 2B) and correlated significantly with exposure as assessed by questionnaires (self-reported number of cigarettes smoked per day, $r = 0.55$, $P = 0.003$) and by the more objective assessment of exposure; cotinine in plasma ($r = 0.42$, $P = 0.003$). 4-Aminobiphenyl haemoglobin adduct levels were significantly lower in the child as compared to its mother. These data confirm previous findings [37].

Biomarkers of early effects

Definition. Measurable biochemical, physiological or other irreversible alteration within an organism that, depending on its magnitude, can be recognized as a consequence of the exposure and represents a process that is involved in disease aetiology [10].

DNA damage that is not repaired or not efficiently repaired may result in misincorporation of a nucleotide opposite the damaged site. As a consequence, the nucleotide sequence is altered, which is thought to be a critical initial step in carcinogenesis, because mutations in cell-cycle regulatory genes may disrupt normal cell growth. Somatic mutations in general are difficult to detect in ambient subjects. Mutations in so-called reported genes such as the gene for hypoxanthine phosphoribosyltransferase (*HPRT*) and glycophorin A (*GPA*) are easily detectable, but are not necessarily involved in the process of carcinogenesis. Still, these mutations are considered to reflect mutational events in genes relevant for cell-cycle control [42] and can be detected in cord blood from neonates [12]. All studies that investigated the formation of these types of mutations in newborns focused on cigarette smoke exposure (active and passive exposure). The literature on the induction of *HPRT*-mutations by maternal smoking shows conflicting results. Several studies reported increased levels of *HPRT* variants in cord blood [12,43,44], while others were unable to find an effect of cigarette smoking on *HPRT*-mutagenesis using the T-cell cloning assay [45,46]. This intriguing discrepancy between the results obtained by different methodologies deserves further attention before firm conclusions about the induction of *HPRT* mutations in cord blood by maternal smoking can be made. Overall, no statistically significant effects of maternal smoking were found regarding *GPA* mutations in newborns [44]. Although mutation frequencies were on average higher in smokers than in non-smokers, the difference did not reach statistical significance. It was suggested that increased mutation frequencies may originate from the pro-mutagenic lesions formed by aromatic genotoxic compounds from cigarette smoke [12]. A correlation between DNA adduct levels in umbilical cord blood and mutation frequencies was reported, which was thought to represent a molecular link between DNA damage and mutagenesis during foetal development [47].

In our study population, the *HPRT*-variant frequency appeared to be three times higher in cord blood of children from smokers (6.6 ± 1.4 per 10^6 lymphocytes, range: 0.4–12.2) than in blood of neonates with a non-smoking mother (2.3 ± 0.4 per 10^6, range: 0.4–5.2, $P = 0.03$, fig. 2C). A weak correlation was observed between the *HPRT*-variant frequency and DNA adduct levels ($r = 0.31$, $P = 0.16$), which was improved when the analysis was confined to never-smokers and active smokers only ($r = 0.75$, $P < 0.01$). This indicates that a difference in the half-life between adducts and mutations disturb the underlying relationship. Another explanation may be the confounding by co-exposures to metals such as Cd. Cd may be considered as a co-carcinogen, because it can decrease DNA repair capacities by competing efficiently with zinc for the incorperation into DNA repair enzymes that rely on zinc (e.g. XPA) [48]. Thus, simultaneous exposure to Cd and other DNA damaging agents could have synergistic effects with regard to the induction of DNA mutations. Indeed, a combined effect of DNA adducts and Cd on *HPRT* mutagenesis in newborns was reported earlier [12]. Furthermore, it should be noted that sometimes extremely high mutation frequencies were found in both *HPRT* and *GPA* assays. Such extreme variant frequencies may represent a subgroup of individuals with a high vulnerability to genotoxic exposures (see also next section); or these high values could have arisen by clonal expansion of haemopoietic precursor cells carrying mutations.

As mentioned above, the results of the *HPRT* variant analysis are influenced by the methodology used to assess variant frequencies, which makes the interpretation of the data difficult. Another analysis that is seriously influenced by the methodology is chromosomal aberrations (CA). In a prospective study, CAs were indicative for a higher cancer risk [49], and therefore CAs are considered to be a good biomarker for early effects, possibly also in neonates. However, increased levels of CA were only observed in neonates of exposed mothers when analysed by fluorescent *in situ* hybridization [50–52], while studies that used simple Giemsa staining were unable to find similar results [53,54]. In a recent study, it was, however, shown that Giemsa staining may be too insensitive to detect CA in newborns after *in utero* exposures [55].

Another biomarker of early effect that has received a lot of attention is the number of micronuclei (MN). Elevated MN frequencies have recently been linked to increased cancer risks and decreased cancer survival in adults [56]. Although increased MN frequencies have consistently been reported in blood of exposed children (age: 0–18 years) [3], more work is needed on MN in newborns and their implications for children's health. The amount of blood obtained in our smaller study population was unfortunately not sufficient to include CA and MN analysis.

Biomarkers of susceptibility

Definition. It is an indicator of inherent or acquired limitation of an organism's ability to respond to the exposure, or to respond to the effects of this exposure [10].

Although epidemiological studies showed that the impact of maternal exposures during pregnancy on childhood cancer risk is small in the general population of neonates [14], adverse effects of maternal exposures may be more relevant in genetically susceptible newborns. The genotypes of maternal biotransformation enzymes and environmental factors will affect the chemical properties and levels of xenobiotics reaching the placenta, where additional enzymatic reactions may occur. Biotransformation enzymes expressed by the foetus result in further activation or detoxification. Genetic polymorphisms in genes that are involved in the metabolism of carcinogens can be subdivided in so-called phase I enzymes that are generally thought to activate carcinogens to their reactive derivatives, and phase II enzymes that detoxify carcinogenic metabolites.

Genetic polymorphisms in the phase I enzymes cytochrome P450 1A1 and 1A2 (*CYP1A1* and *CYP1A2*) affect the metabolism of many aromatic compounds, including PAH and aromatic amines, and may thus modulate the biologically relevant dose in neonates. Indeed, there is evidence that a *CYP1A1* polymorphism, (involving an MspI restriction site thought to increase enzyme inducibility), is related to increased levels of DNA damage in newborns [57] and placenta [58]. Although the same polymorphism did not seem to affect *GPA* mutations [59], a significant association was observed with chromosome aberration frequencies ($P = 0.02$) in newborns [51]. *CYP1A2* is considered not to be expressed in high levels in the foetus until after delivery; nonetheless, this polymorphism may be relevant, because its presence in the mother may determine the dose of teratogenic compounds that reach the foetus [60].

Regarding phase II enzymes, so far most studies focused on glutathione S-transferases (*GSTM1* and *GSTP1*) and N-acetyltransferase 2 (*NAT2*). Genetic polymorphism has been described for these enzymes, which modulate an individual's ability to detoxify reactive metabolites and therefore may affect the formation of DNA adducts, possibly also in the foetus of a smoking mother. It has been reported that adults with a deletion of *GSTM1* have increased DNA adduct levels in lung tissue, and thus may have an increased risk for developing squamous cell carcinoma of the lung [31]. *N*-acetylation of aromatic amines by *NAT2* is also considered to be a detoxifying mechanism, but acetylation may as well be involved in the activation of xenobiotics, because intermediate metabolites (hydroxylamines and hydroxamic acids) can be further activated by *O*-acetylation. In addition, *NAT2* is genetically polymorphic and the most consistent findings show that fast acetylators have a lower risk for developing bladder cancer, but have an increased risk for colorectal cancer [61]. *NAT2* also affected the formation of DNA and protein adducts in peripheral blood of adult smokers [62]. Acetylation may be an important metabolic route in foetal tissue, because of the limited activity of glucuronidation enzymes [63].

In our study, no effect was observed for the *GSTM1* genotype on DNA adducts in neonates, which confirms earlier findings on cord blood PAH-albumin adduct levels [64] and is in line with the observation that *GSTM1* activity only develops after birth [65]. In our study population, also maternal *GSTM1* was not found to affect any biomarker for DNA damage in umbilical cord blood. A coding sequence polymorphism in *GSTP1* (i.e. an A→G transition in nucleotide 313 of *GSTP1*), has been identified that leads to an ile→val substitution. This polymorphism affects the hydrophobic binding site and catalytic efficiencies of the enzyme leading to greater activity towards PAH diol-epoxides. DNA adduct levels were found to be higher among *GSTP1* ile/val and ile/ile newborns compared to *GSTP1* val/val newborns ($P = 0.08$) [57]. Adduct levels were even 4-fold higher among *GSTP1* ile/ile newborns also carrying the *CYP1A1* restriction site compared to *GSTP1* val/val newborns who lacked the *CYP1A1* restriction site ($P = 0.04$) [57], which demonstrates a significant combined effect of phase I and phase II polymorphisms on DNA damage in foetal tissues.

Our own study population is too small to study effects of single polymorphisms. Therefore, we used an approach that was recently introduced by Matullo et al. [66] and Ketelslegers et al. [67], in which the total sum of risk alleles is studied rather than single polymorphisms. We observed an increasing trend of DNA adduct levels in both mother and child with increasing sums of risk alleles (involving polymorphisms in *CYP1A1* (3801T>C, 2455A>G and 2453C>A), *CYP1A2* (–164A>C), *GSTM1* (deletion), *GSTP1* (1404A>G and 2294C>T) and *NAT2* (341T>C, 590G>A and 857G>A) (fig. 3A). When the genetic polymorphisms in both mother and child were taken into account, the highest adduct levels were observed in those exposed neonates that carried a relatively high number of 'risk' alleles and who also had a mother with a relatively high number of 'risk' alleles (fig. 3B). Overall, it is concluded that genetic polymorphisms in both mother and child may affect the biologically effective dose of carcinogens that undergo metabolic activation/detoxification and subsequent health effects in newborns. Therefore, further studies on the role of maternal and neonatal genetic polymorphisms on genetic damage in neonates after maternal exposure to genotoxins are warranted.

Biomarkers and neonatal health effects

The ultimate goal of biomarker studies is to evaluate early end-points that provide information on potential health effects in the offspring of exposed mothers, without having to wait for disease manifestation. To further validate these biomarkers, it is necessary to perform such prospective studies. In most studies, biomarkers have only been linked to endpoints at birth, such as birth weights, head circumference and intrauterine growth retardation, or end-points that occur relatively early in the offspring's life, such as growth retardation, intelligence, cognitive functioning and school performance in childhood [5]. Biomarkers indeed predicted some of these outcomes very well that thus underlines their potential for future studies. In addition, in our study population, birth weight were significantly about 500 g lower in children with

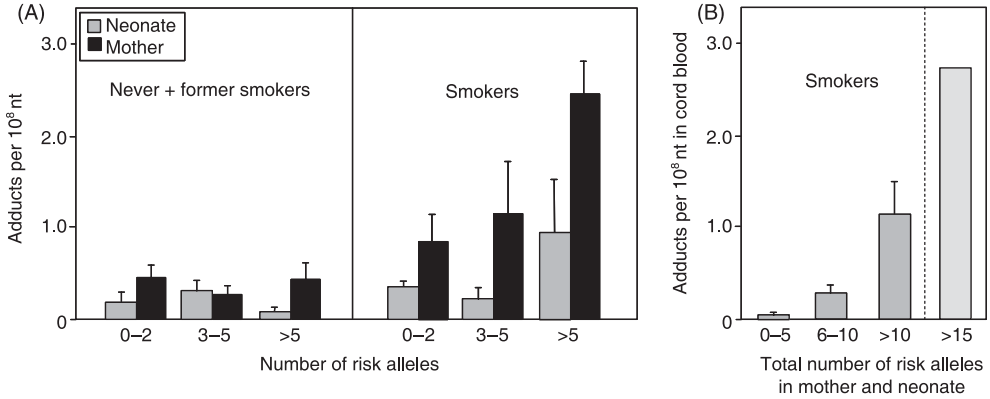

Fig. 3. Genetic polymorphisms in mother and/or child in relation to DNA adduct levels in cord blood. The total sum of 'at-risk' alleles from genetic polymorphisms in genes that are involved in the metabolism of genotoxins (*CYP1A1*, *CYP1A2*, *GSTM1*, *GSTP1* and *NAT2*, see text for more details) modulate DNA adduct levels in mother and child (3A) of smokers. When the sum of risk alleles from both mother and child are combined (3B), they predict increased DNA adduct formation in umbilical cord blood. Note: one neonate of a smoking mother had a total sum of risk alleles of >15 (see insert) and also had the highest DNA adduct level of this study population.

detectable DNA adduct levels in their cord blood. Future studies need to clarify whether these 'short-term' effects have consequences for the offspring in later life.

Relevance of biomarkers as indicators for health effects; need for further studies

Relatively few studies examined the genotoxic effects of maternal smoking on the foetus and its health implications, probably because it is problematic to obtain suitable and sufficient biological materials. Nonetheless, the development of new methods and end-points, especially genomic methods, and the establishment of biobanks (see www.newgeneris.org) offer hope for progress in this important area of public health. A definitive demonstration of transplacental mutagenesis by maternal exposure to carcinogens requires further investigation. Such studies will benefit from a molecular epidemiological approach, which can provide valuable information about processes that are relevant for mutagenesis during foetal development. Until now, there are sufficient indications to conclude that there is profound concern for genetic risks in neonates of mothers who were exposed to mutagens during pregnancy. Overall, it seems that some neonates may be more susceptible than others and an 'individualized' approach could identify these susceptible subgroups. This information can subsequently be used to improve experimental designs of future studies.

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