

Editorial

NewGeneris: A European Study on Maternal Diet during Pregnancy and Child Health

Domenico F. Merlo,¹ Christopher P. Wild,² Manolis Kogevinas,^{3,4,5,6} Soterios Kyrtopoulos,⁷ and Jos Kleinjans⁸ on behalf of the NewGeneris Consortium

¹National Cancer Research Institute, Genoa, Italy; ²Molecular Epidemiology Unit, Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, Leeds, United Kingdom; ³Centre for Research in Environmental Epidemiology, ⁴Municipal Institute of Medical Research, and ⁵CIBER Epidemiología y Salud Pública, Barcelona, Spain; ⁶Medical School, University of Crete, Heraklion, Greece; ⁷Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, Athens, Greece; and ⁸Department of Health Risk Analysis and Toxicology, Maastricht University, Maastricht, the Netherlands

Introduction

The effect of *in utero* exposures on health in childhood and adulthood is a growing area of research interest with major public health implications (1). Evidence from observational studies on exposure-disease associations is being complemented by mechanistic data demonstrating that gene expression in the fetus can be modulated by maternal exposures, reflecting developmental plasticity. It is suggested that an adaptive response in the fetus to *in utero* exposures could result in persistent changes that influence health later in life (1). Relevant exposures are likely to include maternal nutrition, the main focus in the article by Gluckman and colleagues (1), as well as environmental chemicals.

Among the important health effects consequent to prenatal and early-life exposures are childhood cancers and immune disorders (2). Significant increases in the incidence of childhood cancer in general and specifically leukemia, in Europe, and of the prevalence of immune diseases worldwide have been recently reported (3, 4). In fact, there may be relationships between the development of childhood cancer and immunologic diseases in cases in which genotoxic carcinogens also have immunotoxic activity (5). Infant leukemia is one example in which *in utero* exposures are suggested to affect cancer risk, with chromosomal abnormalities occurring during fetal development often involving rearrangements of the *MLL* gene. Epidemiologic and experimental studies provide some support for the hypothesis that topoisomerase II inhibitors, including dietary flavonoids, may affect risk by inducing such translocations (6-8). Recognition of the important role that epigenetic processes, including DNA methylation and histone modification, play in gene expression (9) provides alternative

pathways by which early-life exposures may influence disease risk (10). This is of particular interest because of the potential to reverse epigenetic changes through interventions.

In addition to the importance of early-life exposures, a substantial body of evidence suggests that in comparison with adults, children may exhibit different vulnerabilities with respect to both exposure and acute and chronic adverse health outcomes (11). The importance of understanding the dynamic changes in susceptibility of the child to environmental exposures due to age-related differences in toxicokinetics has been highlighted in relation to risk assessment (11). There may be "critical windows" of exposure that will differ for different exposures depending on their pattern of exposure and chemical properties (12, 13). Makri and colleagues (13) also stressed the importance of considering not only maternal exposure during gestation but also the possibility of mobilization of chemicals, such as polychlorinated biphenyls from maternal fat deposits.

Given the above background, it is of scientific interest to investigate the role played by exposure in the very earliest stages of life (e.g., *in utero*) to selected classes of agents with genotoxic and/or immunotoxic properties, in the development of immune disorders and cancer during childhood as well as in other disorders.

Study Design

The Newborns and Genotoxic exposure risks project (NewGeneris;⁹ ref. 14) is a multidisciplinary research project conducted within the European Union Food Quality and Safety area of the 6th Framework Programme (15). Its main objectives are to investigate associations between fetal exposure to dietary contaminants, including polycyclic aromatic hydrocarbons, heterocyclic amines, nitrosamines, acrylamide, the mycotoxin deoxynivalenol, dioxin and polychlorinated biphenyls, alcohol and DNA-reactive aldehydes, and the occurrence of early health effects (Table 1). The hypothesis to be tested is that maternal exposure to these dietary compounds results in

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Requests for reprints: Christopher P. Wild, Centre for Epidemiology and Biostatistics, Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, Leeds LS2 9JT, United Kingdom. Phone: 44-113-343-6602; Fax: 44-113-343-6603. E-mail: c.p.wild@leeds.ac.uk

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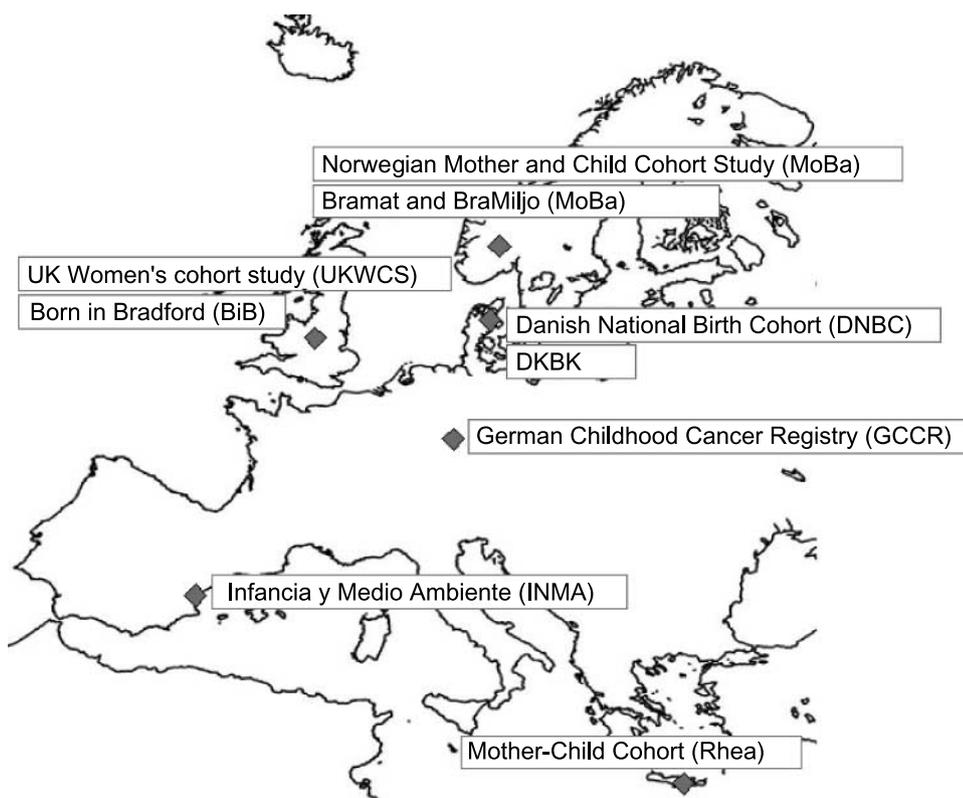
⁹ <http://www.newgeneris.org/>

Table 1. Exposure to specific agents investigated in mother-child pairs, their source of exposure, and class of toxicity

Model compound	Chemical class	Source of exposure	Class of toxicity
Benzo(a)pyrene	Polycyclic aromatic hydrocarbons	Environmental contamination of the food chain; formation during baking and frying; smoking and exposure to environmental tobacco smoke	Genotoxic carcinogenesis, immunotoxicity
2-Amino-3-methylimidazo[4,5]quinoline 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine	Heterocyclic amines	Formation during baking and broiling	Genotoxic carcinogenesis
Monoacrylamide	Acrylamides	Formation during baking and frying	Genotoxic carcinogenesis
Dimethylnitrosamine	Nitrosamines	Environmental nitrate contamination of the food chain and subsequent endogenous formation	Genotoxic carcinogenesis
Deoxynivalenol	Mycotoxins	Environmental contamination of the food chain	Immunotoxicity
Dioxin (TCDD) PCB	Organochlorines	Environmental contamination of the food chain	Cocarcinogenesis, immunotoxicity, endocrine disruption
4-Hydroxynonenal malondialdehyde	Aldehydes (DNA-reactive)	Macronutrients	Genotoxicity via lipid peroxidation immunotoxicity
Ethanol	Alcohols	Lifestyle factor	Cocarcinogenesis immunotoxicity

in utero exposure and in molecular events in the unborn child, leading to increased risks of cancer and immune disorders in childhood. The study therefore focuses on assessing the relationship between (a) dietary questionnaire data and biomarkers of exposure and (b) exposure and biomarkers of effect, relevant to both carcinogenicity and immune status. At a later time point, when disease data are available, direct correlations with risk will be possible.

To address these aims, questionnaires and a wide range of biomarkers are being used as a way of measuring exposure, individual susceptibility to toxic agents, and early effects. The biomarkers are measured in blood samples collected from mother-child pairs recruited in three existing and three newly established European mother and child cohorts (Fig. 1). Different categories of biomarkers of exposure are being applied, including chemical metabolites and both DNA and protein

**Figure 1.** European mother-child cohorts contributing to NewGeneris.

adducts. Validated biomarkers of genotoxic (micronuclei frequencies) and immunotoxic (cytokines) effects are being measured simultaneously with novel effect biomarkers based on transcriptomics and proteomics to study how dietary exposure can affect patterns of gene expression and protein production. In addition, inter-individual differences in genotoxic responses will be evaluated genotypically and phenotypically, in relation to the level of DNA repair. Moreover, polymorphisms encoding for susceptibility will be investigated and promising candidate genotypes identified using DNA samples of brain tumor, lymphoma, and leukemia patients were selected from the German Childhood Cancer Registry (16). The overall work plan is presented in Fig. 2.

Information on dietary and environmental exposures in pregnancy is obtained through questionnaires administered to the mothers during pregnancy and/or at birth. A detailed food frequency questionnaire (FFQ) is administered, which, in addition to questions on the consumption of specific food items, provides information on the method and degree of cooking for meats and fish, which influence the content of chemicals of interest such as polycyclic aromatic hydrocarbons and heterocyclic amines. Information on environmental (e.g., active smoking, environmental tobacco smoke) and occupational exposures is collected through short questionnaires. FFQ-based exposure assessment for chemicals of interest (Table 1), as well as to other food components (e.g. lipids, micronutrients, etc.), is conducted through standardized methodology utilizing food contamination or food composition tables constructed using national or European data. Questionnaire-based exposure assessment will eventually include preconception environmental exposures of the mothers and, for those cohorts

in which corresponding information is available, of the fathers.

Additional “workpackages” (Fig. 2) address the fate of foodborne genotoxins and immunotoxins during trans-placental transport, thereby contributing to a more reliable exposure risk assessment for the fetus, as well as investigating the effect on childhood cancer risk via exposure of the father’s germ cells to these compounds.

The project obviously raises ethical considerations. These refer to seeking approval from local ethics committees with regard to sampling of biological specimens from mothers, fathers, and their newborn children, as well as from ethics committees in other countries to obtain approval for performing biomarker analyses on samples collected elsewhere. Questions also have to be addressed with respect to the transboundary exchange of human samples and data.

Study Size

Each mother-child cohort contributing to NewGeneris (Fig. 1) will provide maternal and umbilical cord blood. The number of subjects in each cohort, together with a basic description of the population, is presented in Table 2. All the biological measurements (biomarkers) will be gathered and centralized to allow a pooled statistical analysis. Pooling is required to provide the necessary variation in levels of dietary contaminant exposures to permit the study of dose-response relationships.

As an example, the estimated sample size required to test the hypothesis that maternal exposure measured during pregnancy increases by 20% the frequency of micronucleated cells measured in circulating lymphocytes is 1,140 and 1,490 with a statistical power of 80% and 90%, respectively.

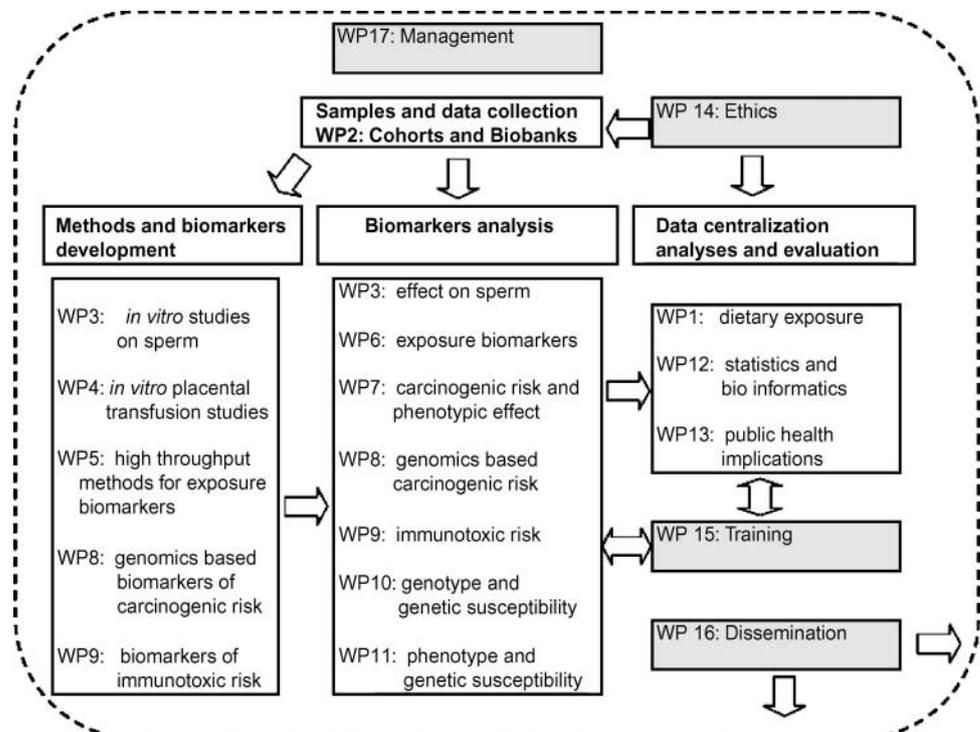


Figure 2. NewGeneris logical framework.

Blood Sample Collection, Processing, Storage, and Distribution

Data pooling requires a standardization of the procedures relating to the collection, processing, and storage of samples by each NewGeneris cohort to permit the same biomarkers to be measured in a comparable way across all cohorts. In this way, there is a greater likelihood that biomarker levels will reflect true differences in exposure rather than differences that arise as an artifact due to the lack of a common protocol for blood collection and processing.

Detailed procedures for blood collection, processing, storage, and distribution have been developed during a pilot phase of the study. They include the type and number of vials to be used for blood collection and their identification with ID codes, the optimal handling of samples on the site of sampling, the separation or pooling of samples according to the biomarkers to be measured, the centrifugation speed and time, the processing and storage temperatures, and the instruction for shipping processed samples to the identified laboratories. These procedures are central to the written research protocol enabling any mother-child cohort to rigorously follow the steps indicated to guarantee biomarker comparability across all cohorts. As an example, the required steps for the processing of blood to be used for effect biomarkers, based on transcriptomics (e.g., gene expression), is shown in Fig. 3.

Future Perspectives

There is increasing scientific interest in the role of critical exposures at the earliest stages of life and how these factors influence disease risk later in life (1, 17). Critical to understanding the importance of these exposures will be investigation of the associated cellular and physiologic effects to provide biological plausibility to the reported exposure-disease associations. A promising recent example in this regard comes from the work of Fry and colleagues (18), who showed that arsenic exposure in pregnant Thai women was related to differences in gene expression in the cord blood at the time of birth of their child. Among the affected biological pathways were those indicative of stress, inflammation, metal exposure, and apoptosis.

At the same time, as the scientific community seeks to elucidate the effect of early-life exposures on the biology of the child, the attention of risk assessors, regulatory bodies, and governments has been drawn to the unique vulnerability of children to environmental risk factors and the need to pay specific attention to this group in risk assessment and risk management (13, 19). However, despite the acknowledged importance of this area, there is a paucity of data specifically linking exposure in young children with subsequent disease risk. One major challenge is the limited ability to accurately characterize exposure and provide a reliable evidence base for public health decision-making (19). This is particularly difficult in early life, including *in utero*. NewGeneris responds to

Table 2. Summary of the European mother-child cohorts contributing to NewGeneris

Cohort	Country	Recruitment (years)	Enrollment	Blood collection	Study population	Contribution to NewGeneris
Norwegian mother and child cohort study (22)	Norway	1999-2008	Prenatal	At delivery	National coverage, 52 hospitals; 100,000 pregnant women	FFQ data relevant to NewGeneris compound exposure
Bramat and BraMiljo-NewGeneris subcohorts	Norway	2007-2008	Prenatal	At delivery	Selected hospitals	400 maternal-cord blood samples; FFQ data
Infancia y Medio Ambiente (23)	Spain	2001-2005	Prenatal	At delivery	Selected hospitals; 3,500	200 maternal-cord blood samples; FFQ data
Infancia y Medio Ambiente-NewGeneris subcohort	Spain	2006-2008	Prenatal	At delivery	Selected hospitals	
United Kingdom women's cohort study (24)	United Kingdom	1995-1998	See*	See*	35,372 women across England, Wales, Scotland, and Northern Ireland	FFQ data relevant to NewGeneris compound exposure
Born in Bradford study (25)	United Kingdom	2007-2009	Prenatal	At delivery	Bradford Royal Infirmary; 10,000 planned	FFQ data relevant to NewGeneris compound exposure
BiB-NewGeneris subcohort	United Kingdom	2008-2009	Prenatal	At delivery	Bradford Royal Infirmary; planned elective caesarian sections	400 maternal-cord blood samples; mother's urine; FFQ data
Mother-child cohort (Rhea)	Greece	2007-2008	Prenatal	At delivery	Selected hospitals	350 maternal-cord blood samples; father's blood and sperm; FFQ data
The Danish Biobank (DKBK)	Denmark	2006-2007	Prenatal	At delivery	Copenhagen University Hospital	100 maternal-cord blood samples; FFQ data
Danish national birth cohort (26)	Denmark	1997-2003	Prenatal	At delivery	>100,000 pregnant women across Denmark	FFQ data and selected biomarkers

*Women in the United Kingdom women's cohort study were not recruited in relation to pregnancy, and biological samples were not systematically collected. However, the dietary questionnaires permit investigation of NewGeneris exposures of interest in relation to outcomes in the adult children of these women.

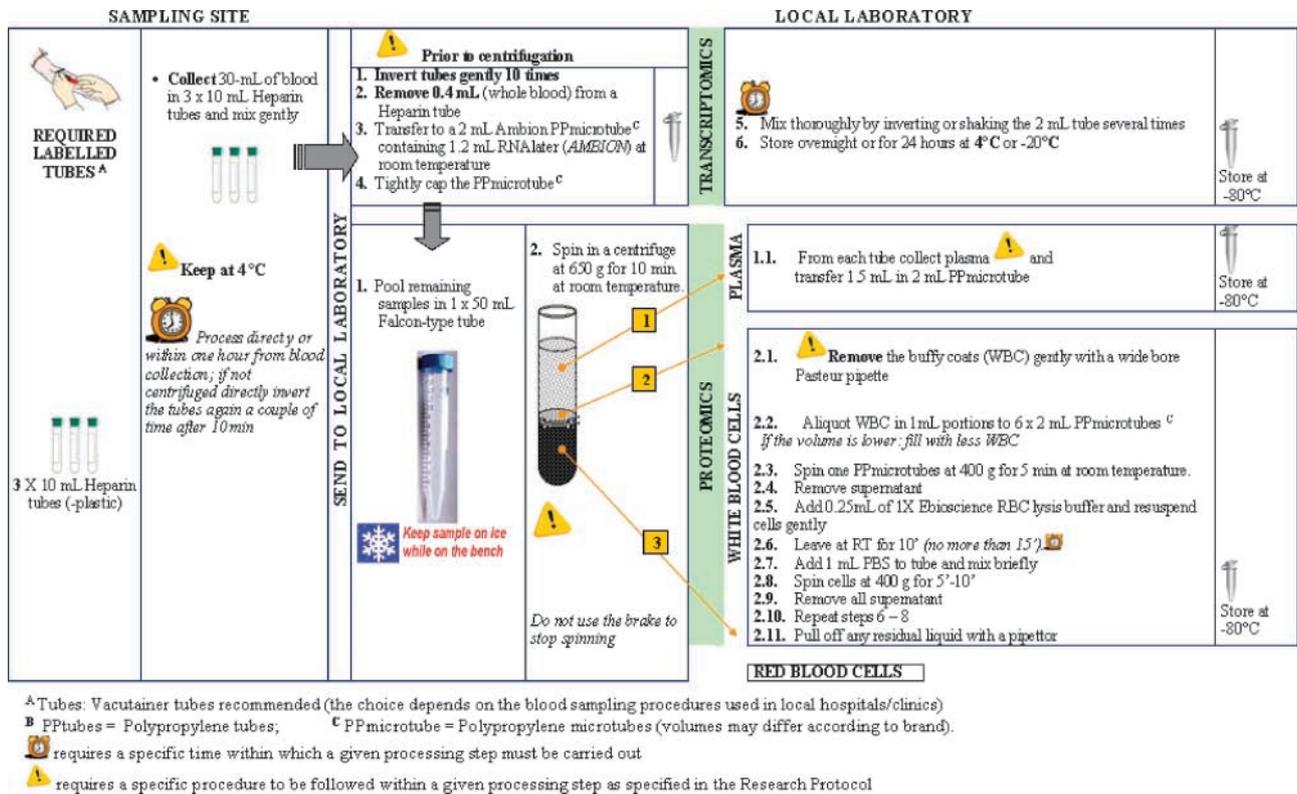


Figure 3. Maternal blood collection and processing steps for genomics biomarker analysis.

this challenge by complementing traditional, questionnaire-based exposure assessment with validated biomarkers of exposure measured in maternal as well as cord blood. The study of early-life exposure and childhood (and adult) diseases will benefit from prospective biological sample collection to enhance the understanding of etiology (20). Because cancer incidence is still low among young children, extremely large cohorts with long-term follow-up are required to assess exposure-disease relationships. The International Childhood Cancer Cohort Consortium is a complementary initiative to that of NewGeneris in this regard (21).

By focusing on subcohorts sampled from very large cohorts and by conducting biomarker-based analyses using umbilical and maternal blood, NewGeneris aims to make a difference in providing data on prenatal exposures and in relating this to dietary questionnaires during pregnancy. The project also seeks to establish biomarkers of effect that may provide information on whether a given exposure results in changes indicative of increased disease risk. These biomarkers of effect may also prove to be useful end points in intervention studies aimed at reducing exposure and disease risk.

Overall, the NewGeneris project should provide valuable information for a wide range of decision makers in the area of food safety in relation to young children. The study will also be a powerful platform for the evaluation of environmental exposures that are food-borne as well as other environmental agents, such as the association of air and water pollution, or environmental

tobacco smoke in relation to the measured biomarkers. The resource coming from these coordinated biobanks, therefore, should prove valuable for future scientific studies of putative health hazards to the very young.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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BLOOD CANCER DISCOVERY

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