Summary

This chapter first discusses the urgent need for prevention of childhood diseases that impose a huge and growing burden on families and society. It provides a review of recent research in this area to illustrate both the strengths and limitations of molecular epidemiology in drawing needed links between environmental exposures and illness in children. For illustration, three of the major diseases in children are discussed: asthma, cancer and developmental disorders. All three impose significant difficulties, have increased in recent decades, and are thought to be caused in substantial part by environmental factors, such as toxic exposures due to lifestyle choices (i.e. smoking and diet), pollutants in the workplace, ambient air, water and the food supply. These exogenous exposures can interact with “host” factors, such as genetic susceptibility and nutritional deficits, to cause disease. Molecular epidemiology has provided valuable new insights into the magnitude and diversity of exposures beginning in utero, the unique susceptibility of the young, and the adverse preclinical and clinical effects resulting from the interactions between these factors. However, molecular epidemiology also faces certain constraints and challenges that are specific to studies of the very young, including ethical issues, technical issues due to the limited amount of biological specimens that can be obtained, and communication of results to parents and communities. These challenges are particularly apparent when incorporating the newer epigenetic and “omic” techniques and biomarkers into studies of children’s diseases.

Introduction

Molecular epidemiology, which combines epidemiologic methods and molecular/genetic techniques to measure biomarkers, has been a valuable tool in the study of environmental causes of diseases and disorders in children. Over the past 25 years, the field has made many notable contributions to intervention and prevention efforts that have significantly improved the health of children. These
contributions include the phase-out of lead in gas in the 1970s, which was a result of studies on the negative effects of low-levels of lead on child neurodevelopment, and documentation of the benefits to fetal growth of a 2001 regulation which restricted the use of the pesticide chlorpyrifos (CPF). These positive changes provided the impetus for US federal policies that require agencies to explicitly address risks to children (e.g. the US Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)) and the revised US Environmental Protection Agency’s (EPA) Cancer Guidelines (1990s–2000s)).

In the context of studies of disease in children, molecular epidemiology has enhanced the power and capabilities of researchers to better delineate mechanisms and causal pathways involved in the exposure-outcome pathway. It has also improved estimates of dose-biologic dosimetry; reduced misclassification of exposure and disease status; augmented the understanding of the variability in individual responses and risk, especially interactions between environment and genes or other susceptibility factors; identified preclinical cases for intervention, thereby enabling earlier interventions; and improved quantitative risk assessment and public policy.

However, molecular epidemiology also faces certain generic constraints and challenges, as well as others that are specific to studies of the very young, including ethical issues, technical issues due to the limited amount of biological specimens that can be obtained, and communication of results to parents and communities. These challenges are apparent in thinking about future directions, such as the incorporation of newer epigenetic and “omics” into studies of children’s diseases.

Context and public health significance: The need for prevention

Between 1980 and 1995, the percentage of children with asthma has doubled in the USA (from 3.6% in 1980 to 7.5% in 1995), and has also increased in other countries (1). An estimated 9 million (12.5%) children aged <18 years in the USA have had asthma diagnosed at some time in their lives (2); an estimated 8.7% (6.3 million) of children had asthma in 2001 alone (1). Rates vary by geographic area and ethnic group; a recent study found that over 25% of elementary schoolchildren in Harlem, New York had asthma (3).

The overall cancer incidence rate increased from the mid-1970s in the USA, but rates in the past decade have been fairly stable (4). Leukaemia is the most common diagnosis for those < 15 years of age, but the relative proportion of it decreases with age: from 36% for those < 5 years of age, to 22% for 10–14-year-olds, and 12% for adolescents 15–19 years of age. Incidence rates in Europe have shown an increase over time since the middle of the last century: the yearly increase averages 1.1% for the 1978–1997 period and ranges from 0.6% for the leukemias to 1.8% for soft-tissue sarcomas (5). According to the databases of population-based cancer registries, which have been created and registered for commercial use with the US Environmental Protection Agency (EPA) (8), and 2000–3000 new chemicals are submitted for review by the EPA every year (9). Nearly 3000 registered substances are produced in quantities of almost 500 000 kg every year, yet no basic toxicity information is publicly available for 43% of the high volume chemicals manufactured in the USA; a full set of basic toxicity information is publicly available for 43% of the high volume chemicals manufactured in the USA.

Environmental factors, such as toxic exposures due to lifestyle choices (i.e. smoking and diet), pollutants in the workplace, ambient air, water, and the food supply, can interact with ‘host’ factors, such as genetic susceptibility and nutritional deficits, to cause disease. Therefore, there is a need to understand the role of both environmental and susceptibility factors in childhood disease and neurodevelopmental disorders, and to identify the primary environmental toxins affecting them so that preventive measures can be taken.

A focus of this research must be early exposure to toxic chemicals, which has risen exponentially in the past 50 years. Over 80 000 synthetic chemical compounds have been created and registered for commercial use with the US Environmental Protection Agency (EPA) (8), and 2000–3000 new chemicals are submitted for review by the EPA every year (9). Nearly 3000 registered substances are produced in quantities of almost 500 000 kg every year, yet no basic toxicity information is publicly available for 43% of the high volume chemicals manufactured in the USA; a full set of basic toxicity

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information is available for only 7% of these chemicals, and there is no information about developmental or paediatric toxicity for 80% (8).

The exposures of concern include genotoxic and non-genotoxic chemicals, as well as chemicals that exert both types of effects. Recent research suggests that endocrine-related cancers, or susceptibility to cancer, may be a result of developmental exposures (10). There is differential exposure of the young to diverse toxic chemicals. During pregnancy and lactation, certain toxicants stored in the bodies of mothers can become bioavailable, exposing the fetus and child. In addition, the fetus and child clearly absorb less readily than an adult (11–15). Young children breathe air closer to the ground, exposing them to particles and vapours present in carpets and soil. While playing and crawling on the floor, children can inhale or dermally absorb toxicants, which are subsequently absorbed more efficiently in children than in adults (16). Compounding the effects of these behaviours is the fact that infants have twice the breathing rate of the average adult. Hand-to-mouth behaviour and thumb sucking habits can also increase exposure.

Dietary habits of children also cause increased exposure to foodborne toxicants. In the USA, children under five years of age eat 3–4 times more food per unit of body weight than the average adult; the average one-year-old drinks 10–20 times more juice than the average adult (17). Dermal exposures may also be higher, as a typical newborn has more than double the surface area of skin per unit of body weight than an adult (18).

**Susceptibility of the young**

The biological susceptibility of the young is another important research area. Experimental and human data indicate that the fetus and young child are especially vulnerable to the toxic effects of environmental tobacco smoke (ETS), polycyclic aromatic hydrocarbons (PAHs), particulate matter, nitrosamines, pesticides, polychlorinated biphenyls (PCBs), metals and radiation (11). There is mounting evidence, much of it from molecular epidemiologic studies, that the fetus, infant and child are biologically more sensitive to a variety of environmental toxicants than adults (7,11,19). Specifically, the *in utero* and childhood periods are characterized by rapid physical and mental growth, and gradual maturation of major organ systems. In fact, typical newborns double their weight within six months of birth, while integral parts of the nervous and immune systems are formed during the first six years of life (20). Additionally, sex organ development, myelination, and alveoli formation begin late in pregnancy and continue until adolescence (16). Since cells are proliferating rapidly and organ systems are immature, they are sensitive to the potentially harmful effects of environmental toxins. Absorption, metabolism and excretion pathways in infants and children differ from those in adults. These pathways dictate the amount of a toxicant, in its various forms, that is present in the body. Epidemiological studies with biomarkers have demonstrated placental transfer of toxicants, and in some cases slower fetal clearance of chemicals such as PAHs, PCBs and mercury (21–23). An infant’s kidney filtration rate is lower than an adult’s, thus increasing potential susceptibility (16). DNA repair systems are also immature in the fetus and young child, leading to higher levels of genetic damage per unit of exposure in cord blood leukocytes compared to maternal blood leukocytes (24). Studies have also shown a 65-fold range of variability in sensitivity to the pesticide chlorpyrifos between the most sensitive newborn and the least-sensitive mother (based on paraoxonase 1 (*PON1*) status) (25).

Finally, infants and children have more years of future life than most adults. Thus, there is more time for early exposures to trigger diseases that have long latency periods. For example, early exposure to carcinogens will more likely lead to cancer than the same exposure experienced later in life. In addition, it has been hypothesized that fetal growth restriction due to nutritional deprivation in early life is an important cause of some of the most common, costly and disabling medical disorders of adult life, including coronary heart disease and the related disorders hypertension, stroke and type 2 diabetes (26). It has been proposed that individuals with a ‘thrifty phenotype’ will have “…a smaller body size, a lowered metabolic rate and a reduced level of behavioural activity… adaptations to an environment that is chronically short of food” (27). This hypothesis, now widely (though not universally) accepted, is known as the Barker Early Origins Hypothesis or thrifty phenotype hypothesis; it is a source of concern for societies undergoing a transition from poor to better nutrition (28). All in all, there is increasing evidence that exposures in early life strongly influence risk of chronic diseases in adulthood, including heart disease and cancer (29).
Additional susceptibility factors

The differential susceptibility of the fetus, infant and child can be influenced by nutritional deficits, genetic predispositions, and psychosocial stressors that can modify (i.e. reduce or increase) the toxic effect of exposures.

Nutritional factors

Micronutrients are known to have major effects on child health and development, and there is evidence that they interact with environmental exposures. Certain micronutrient deficiencies have been associated with childhood asthma, adverse birth outcomes, child development and childhood cancer. For example, essential fatty acid deficiencies are associated with low birth weight, smaller head circumference, and reduced cognitive and motor function (30–32). Antioxidants modulate inflammatory response to air pollution and its effects on childhood asthma (33–35). By removing free radicals and oxidant intermediates, antioxidants protect DNA from the genotoxic, procarcinogenic effects of chemicals that bind to DNA (36,37). Nutritional deficiencies are often closely related to lower socioeconomic status, although variations exist within each socioeconomic bracket.

Genetics

Genetic susceptibility can take the form of common polymorphisms or haplotypes that modulate the individual response to a toxic exposure. For example, two genes have been identified that can increase an individual’s vulnerability to organophosphates (OPs), such as chlorpyrifos (CPF), by reducing the reservoir of functioning protective enzymes (38). The first gene has a prevalence of 4% and results in a poorly functioning form of the enzyme acetylcholinesterase. The second gene results in a relatively inactive form of the enzyme paraoxonase (PON1) (prevalence of 30–38%), an enzyme that detoxifies CPF before the toxin can inhibit acetylcholinesterase (39–41). The effect of CPF on head circumference at birth was significant only among women with low PON1 activity, which could be evidence of an interaction between the PON1 genotype and OP pesticides (42). However, there are still limited data relating PON1 to clinical outcomes in individuals exposed to OPs. Other examples of gene–environment interactions of interest include the gene coding for the d-ALA enzyme that affects lead metabolism and storage (39,43), and a genetic polymorphism in the dopamine transporter that is associated with increased behavioural problems in children prenatally exposed to tobacco smoke (44). Other research has found that the P450 and glutathione-S-transferase gene families play a role in the activation and detoxification of various xenobiotics. They are involved in the metabolism of PAHs and can influence the level of PAH–DNA damage. PAH-DNA adduct levels in human placenta were significantly higher in infants with the CYP1A1 MspI restriction site, a genetic marker associated with lung cancer risk, than in infants without the restriction site (45). The GSTT1 genotype was also shown to be a susceptibility marker for lower birth weight and pre-term birth among babies of pregnant women who actively used tobacco (37,46). Children with the GSTM1 genotype who were exposed in utero to tobacco smoke had increased risk for persistent asthma and wheezing (47,48). The GSTM1 genotype in asthmatic children is also associated with increased susceptibility to the harmful effects of ozone, such as reduced forced expiratory flow (49).

Individual- and community-level psychosocial stressors

The notion that individual- and community-level conditions can produce profound effects on host susceptibility to disease is derived from the long-standing existence of strong social class gradients in health (50). Recent studies have shown that women who live in violent, crime-ridden, physically decayed neighbourhoods are more likely to experience pregnancy complications and adverse birth outcomes, after adjusting for a range of individual-level sociodemographic attributes and health behaviours (51,52). Other studies have suggested that the stresses of racism and community segregation are associated with lower birth weight (53). The effects of individual poverty on birth outcomes have been shown to be exacerbated by residence in a disadvantaged neighbourhood (54). In one of the few studies that has measured interactions between physical toxicants and individual psychosocial stressors, a prospective cohort study of Northern Manhattan (New York, USA) mothers and toddlers (by the Columbia Center for Children’s Environmental Health (CCCEH) cohort) found that the risk of developmental delay among children exposed prenatally to maternal ETS was significantly greater among those whose mothers experienced material hardship during pregnancy (55).
Examples/case studies: Molecular epidemiology and children's diseases related to environmental factors

The research reviewed in this article is based on the paradigm of a continuum of molecular/genetic alterations between exposure to an external agent and an adverse outcome that can be accessed using biomarkers to provide links in the chain of causality (Figure 26.1). The following is not an encyclopaedic review; rather, examples are provided for illustration of the methodology (Table 26.1).

Based on the growing understanding that children may be more susceptible to toxicants than adults, the last few decades of molecular epidemiologic research have provided a new perspective on studying environmental risks in paediatric populations. As such, the emphasis in conducting studies of disease in children has been placed on identifying less-invasive methods of biological specimen collection; specific approaches to interpretation and validation of biomarkers; methods for translating biomarker results into intervention strategies, and for integrating them with environmental monitoring and health data; optimal ways to obtain consent and provide information to children and/or their parents participating in the studies; and techniques for the effective communication with policy-makers and the public (56).

Respiratory disease/asthma

Air pollution and allergens are among the best-studied environmental risk factors, and have been established as important triggers for asthma and respiratory disease in childhood. There appears to be a critical window in both prenatal and postnatal development during which exposure to irritants and other toxicants can modify the formation and maturation of the lung, which occurs through years six to eight of life (57). Most of the research on air pollution has focused on the postnatal window of exposure and has not used biomarkers. For example, an association of poorer air quality with increased prevalence of respiratory symptoms in children has been documented in the Netherlands and in Indonesia (58,59). Moreover, increased levels of fine ambient particulate matter have been associated with decreased peak expiratory flow rates among inner-city children with asthma (60). These studies seem to implicate vehicle exhaust emissions and/or ambient particulate matter, especially diesel exhaust particles (DEP) and PAHs, in the exacerbation of asthma. Moreover, a community study of exposure to traffic evaluated 5–7 year old schoolchildren in southern California, USA, and found that residing near a major road was associated with asthma (61). Experimental studies have shown that DEP was associated with a greater risk of becoming sensitized to allergens (62,63). Importantly, a prospective study in southern California detected significant declines in lung function (FEV1) in association with exposure to nitrogen dioxide, acid vapour, PM$_{2.5}$, and elemental carbon (EC) among children ages 10–18 (64).

The very early causal role of air pollutants in childhood asthma has been less well understood, but has recently benefited from prospective molecular epidemiologic studies that have enrolled pregnant women and assessed in utero exposures resulting from the maternal environment. Recent results from such a study in New York City (the CCCEH cohort study) highlight the importance of the prenatal period of development, showing that adaptive immune responses may begin in utero, as evidenced by the occurrence of cord blood T-cell proliferation in response to specific allergens, independent of maternal sensitization (65). Moreover, high prenatal exposure to certain airborne PAHs (e.g. pyrene) was found to increase the likelihood of children’s allergic response to cockroach, mouse and dust mite allergens as measured by elevated IgE (a biomarker of preclinical effect and a known asthma predictor) at two years of age (66).
<table>
<thead>
<tr>
<th>Type of marker</th>
<th>Biomarker</th>
<th>Sample</th>
<th>Indicator of exposure to</th>
<th>Outcome of concern</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marker of exposure</td>
<td>S-Phenylmercapturic acid (S-PMA)</td>
<td>Urine</td>
<td>Metabolite of benzene, thought to be derived from the condensation product of benzene oxide with glutathione</td>
<td>Cardiovascular risk (as determined by prevalence of arterial hypertension (AH) and pathologic changes in electrocardiography (ECG))</td>
<td>(128,129)</td>
</tr>
<tr>
<td>Internal dose</td>
<td>Levels of 2,2', 4,4', 5,5'-hexachloro-biphenyl (CB-153), as a proxy of the total PCB burden, and of p,p'-DDE</td>
<td>Serum</td>
<td>Persistent organochlorine pollutants, such as polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (p,p'-DDE)</td>
<td>Altered sperm DNA/chromatin integrity (i.e. male fertility)</td>
<td>(130)</td>
</tr>
<tr>
<td>Internal dose</td>
<td>Newborn cotinine levels (nanograms per milliliter)</td>
<td>Plasma</td>
<td>Active and passive cigarette smoke exposure of the mother</td>
<td>Birth weight and birth length</td>
<td>(131)</td>
</tr>
<tr>
<td>Biologically effective dose</td>
<td>PAH-DNA adducts</td>
<td>White blood cells (WBC)</td>
<td>Ambient air pollution (polycyclic aromatic hydrocarbons (PAH), particulate matter, and environmental tobacco smoke (ETS)), which is significantly associated with the amount of PAH bound to DNA</td>
<td>Birth weight, birth length, and head circumference (ultimately, reduction of head circumference at birth correlates with lower intelligence quotient, as well as poorer cognitive functioning and school performance in childhood)</td>
<td>(132)</td>
</tr>
<tr>
<td>Preclinical effect</td>
<td>CD4 count</td>
<td>Lymphocytes</td>
<td>HIV virus</td>
<td>HIV-related mortality and time to death, psychological resources (positive affect, positive expectancy regarding health outcomes, finding meaning in challenging circumstances) may be protective</td>
<td>(133)</td>
</tr>
<tr>
<td>Clinical disease</td>
<td>Soluble Nogo-A (a development-related molecule inhibiting axonal regeneration) is a major component of central nervous system (CNS) myelin</td>
<td>Cerebrospinal fluid (CSF) and central nervous system (CNS) tissue</td>
<td></td>
<td>Multiple sclerosis (MS) (the etiology of non-reversible neurologic dysfunctions is thought to have something to do with failure of damaged axons to regenerate)</td>
<td>(134)</td>
</tr>
<tr>
<td>Individual susceptibility</td>
<td>Polymorphism of enzymes paraoxonase (PON1) and glutathione S-transferase (GSTM1 and GSTT1), which are involved in the detoxification of pesticides</td>
<td>Erythrocytes</td>
<td>Pesticides, including organophosphates and paraquat (exposures to which were evaluated thanks to erythrocyte delta-amino-levulinic acid dehydratase (ALA-D), an important biological indicator of pesticide exposure)</td>
<td>PON1 and GSTT1 are relevant determinants of susceptibility to chronic pesticide poisoning and pesticide-related symptomatology</td>
<td>(135)</td>
</tr>
</tbody>
</table>
Laboratory studies have suggested possible mechanisms for the effects observed in children. In vitro studies have shown that nasal challenges with DEP, combined with ragweed allergen, heightened the production of the Th2 cytokine IL-4 and isotype class switching to IgE (67–69). In addition, DEP has been shown to upregulate the Th2 chemokines, including I-309 and PARC, even among non-atopic subjects (70,71). Combined, these studies suggest that DEP may promote asthma by upregulating IL-4-mediated IgE pathways in response to allergen exposure. DEP exposure has also been shown to increase procollagen gene expression and tissue hydroxyproline levels in explanted rat tracheas (72), which suggests that it may trigger airway remodeling, a pathological phenotype associated with severe asthma (73).

While it is clearly established that ETS exposure is associated with respiratory infections, reduced lung function, and asthma in children (74,75), recent studies suggest that ETS exposure may modulate the respiratory response to other toxic exposures, such as PAHs. There is evidence that cigarette smoke may delay pulmonary clearance of inhaled insoluble particles (76,77). ETS exposure has been shown to exacerbate the IgE-promoting effects of ragweed exposure (78), providing another mechanism whereby ETS can worsen airway disease. In the CCCEH cohort, more cough and wheeze were reported by 12 months of age among children exposed to prenatal PAH in concert with ETS postnataally. By 24 months, difficulty breathing and probable asthma were reported more frequently among children exposed to prenatal PAH and ETS postnatally (66). Most recently, a parallel cohort study in Poland has assessed PAH exposure during pregnancy by personal air monitors worn by women (n = 333) for 48 hours during the second or third trimester of pregnancy. After delivery, the mothers were interviewed every three months over the course of a year. Prenatal PAH exposure was associated independently with an increased risk for respiratory symptoms including wheezing without cold (RR = 3.8; 95% CI = 1.2–12.4) during the course of the infants first year of life (79). The data were adjusted for confounders including ETS, which was verified by cotinine, a biomarker of ETS exposure. These results suggest an independent effect of urban PAH exposure on respiratory outcomes in children (79).

**Cancer risk/genetic damage**

Environmental exposures are recognized as potentially important risk factors for childhood cancer (80), and again biomarkers are proving useful in assessing causal relationships. For example, carcinogen-DNA adducts are considered a biomarker of the biologically effective dose of PAHs and increased cancer risk (81). Experimental evidence shows that the amount of PAHs crossing the placenta and reaching the fetus is on the order of one-tenth of the dose to the mother (12,13), yet the levels of PAH–DNA adducts measured in rodent fetal tissue are higher than expected based on transplacental dose (14). Similarly, research in mothers and newborns has consistently shown that PAH–DNA adduct levels in the white blood cells (WBC) of newborns were similar to or exceeded those in paired maternal samples, despite the estimated 10-fold lower dose of the parent compound to the fetus (82,83). This research indicates that the differential effect of exposure to PAHs in the fetus is not limited to a particular ethnic or geographic group (84). Increased adducts in the fetus relative to the adult could result from lower levels of phase II (detoxification) enzymes and decreased DNA repair efficiency in the fetus (19,82,85,86). In addition, fetal plasma cotinine levels were higher than in paired maternal samples, suggesting reduced ability of the fetus to clear carcinogenic cigarette smoke constituents (82,83).

Chromosomal aberrations have been associated with increased risk of cancer in multiple studies, and are a well-validated biomarker of the preclinical effect of carcinogens (87,88). In New York City newborns, maternal exposure to airborne PAHs during pregnancy was associated with increased frequency of chromosomal aberrations in WBCs, suggesting that risk of cancer can be increased by exposure in utero (89). Studies have also linked maternal tobacco smoking to increased chromosomal aberrations in the WBCs of newborns (90). Other research has shown an approximately 10-fold higher risk of infant acute myeloid leukaemia (AML) with increasing maternal consumption of DNA topo 2 inhibitor-containing foods, raising concerns about benzene, a topo 2 inhibitor (91).

There is a growing body of evidence from studies of adults that polymorphisms of the DNA repair genes X-ray repair cross-complementing group 1 (XRCC1) and xeroderma pigmentosum group D (XPD) may constitute susceptibility factors to cancer. The results of a case–control study in a Chinese population suggested that XRCC1 194 Trp/Trp and XPD 751 Lys allele might be risk genotypes for lung cancer in this population.
While progress in this field is substantial, a basic understanding of the timing of the origin of the crucial molecular abnormalities, or the natural history of leukemic clones, is incomplete. The recognition of crucial temporal and developmental windows for the formation of leukemogenic genetic alterations will help to focus epidemiologic analysis as well as to prompt preventive strategies (103).

**Neurobehavioural disorders**

The exquisitely sensitive process of the development of the human central nervous system involves the production of 100 billion nerve cells and 1 trillion glial cells, which then must follow a precise stepwise choreography involving migration, synaptogenesis, selective cell loss, and myelination (104). A mistake at any point in the process can have permanent consequences. Experimental studies of prenatal and neonatal exposure to the organophosphate CPF have demonstrated, for example, neurochemical and behavioural effects, as well as selected brain cell loss (105–109). The behavioural and morphologic effects of developmental toxicants are highly dependent on the timing as well as on the dose and duration of exposure. This is illustrated by both rodent and human studies showing that the effect of irradiation on brain malformation is heightened during the window of susceptibility throughout fetal development (104). Adverse neurological development, including lowered intelligence, diminished school performance, and increased rates of behavioural problems have been associated with exposure to low-levels of several environmental toxicants and pollutants.

Cohort studies have demonstrated that low-level exposure to lead (even below 10ug/dL in blood) during early childhood is inversely associated with neuropsychological development through the first 10 years of life (110–114). Prenatal exposure to PCBs and methylmercury, predominantly from maternal seafood consumption, has been associated with neurocognitive deficits (115). In these studies, biomarkers (including levels of blood lead, tooth lead concentrations, blood mercury levels and cord tissue PCB) have been instrumental in quantifying the internal dose of the pollutants. Taking the recent example of pesticides, New York City children in the CCCEH cohort who were prenatally exposed to high levels of CPF, as measured by high cord plasma CPF levels, were significantly more likely than children with low cord levels to experience delay in both psychomotor and cognitive development at three years of age (116). In addition, the highly-exposed children were significantly more likely than less-exposed children to manifest symptoms of attention disorders, attention-deficit/hyperactivity disorder (ADHD) and pervasive personality disorder at age three. Although the EPA banned residential use of CPF in 2001, this pesticide is still widely used in agriculture. In addition, children with high prenatal exposure to airborne PAHs also had significantly lower test scores at age three on the Bayley test for cognitive development, after controlling for pesticide exposure (plasma CPF) (117), and at age 5 had significantly lower IQ (118). Moreover, in the same study, children prenatally exposed to ETS (cotinine-verified), especially children whose mothers experienced material hardship (unmet basic food, clothing and housing needs) had significantly
reduced scores on tests of cognitive development at two years of age (55). Finally, cohort children with high prenatal exposure to PAHs were more likely to experience developmental delays in childhood, after controlling for ETS and CPF (119).

Table 26.1 lists a few hand-picked examples from the literature of biomarkers that have been used to study relationships between exposure and disease in children.

**Strengths, limitations and lessons learned: Special considerations in mounting children’s studies**

Long-term studies that follow participants from the prenatal period into adolescence and early adulthood are considered essential to assess the full range of developmental consequences of exposure to environmental chemicals. (The advantages of prospective cohort studies and their logistical, ethical, and financial challenges are discussed in Chapter 17.)

To address some of the important lessons from prior research regarding the logistics, ethics, and the financing of these long-term studies in children, the platform in which many of these challenges were encountered, tackled and, for the most part, overcome is introduced.

**National centers for children’s environmental health**

In 1998, the US National Institute of Environmental Health Sciences (NIEHS) and the EPA collaborated to develop a research programme (the Children’s Centers) that would coordinate efforts to better understand toxic exposures to infants and young children, study the health effects of such exposures to clarify the mechanisms by which they work, and explore intervention strategies for reducing such exposures in a way that would provide evidence for practice. Each centre is designed around a central theme focusing on important questions regarding the role of exposures in one of the following health outcome areas: respiratory disease, childhood learning, and growth and development, including developmental disabilities. The purpose of the Children’s Centers programme was to create local research environments that promote multidisciplinary interactions among basic, clinical and behavioural scientists through university/community partnering, to accelerate translation of basic research findings into clinical prevention or intervention strategies. Additionally, it was designed to support a coordinated, nationwide network of scientists and community advocacy groups synergistically sharing their experiences to address relevant questions related to the role of environmental exposures in the health of children, to enhance community-level capacity to identify and address environmental threats and prevention opportunities (120). (A full description of the Children’s Centers can be found on the NIEHS web site (121), and a summary of the first eight centres has been more fully discussed by (122) and (123).)

The Children’s Centers have addressed and overcome many hurdles in their efforts to understand the link between environmental exposures and health outcomes, as well as interactions between exposures, and a variety of social and cultural factors. Out of their enterprise, several lessons have been learned on the practicalities of conducting longitudinal birth cohorts, such as the critical importance of long-term studies for assessing the full range of developmental consequences of environmental exposures, recognition of the unique challenges presented at different life stages for both outcome and exposure measurement, and the importance of ethical issues that must be dealt with in a changing medical and legal environment (120). In the following section, some of the more specific shared experiences are paraphrased as they pertain to the methodological, logistical, ethical and communication issues (124).

**Successes, challenges and lessons learned**

**Logistical issues in children’s studies**

**Barriers to recruitment.** The most common and important barriers to recruitment into prospective cohort studies, especially for working women, is the time required for each visit and the length of the follow-up period. Many members of a population are also distrustful of Western medicine and research. Solutions to these obstacles include hiring study staff familiar with or from the target population, recruitment by or at clinics known by the community to respect patient confidentiality, and allowing time for potential participants to discuss the study with their families before enrolment.

**Staffing issues.** Building trusting relationships with participants in the cohort is best accomplished by hiring bilingual, bicultural staff from the local community, who are assigned to follow particular families ideally from pregnancy through the child’s assessments. Although this is helpful, it can introduce systematic bias. Often, more in-depth training on data collection
techniques is needed than when hiring from within the academic community. In addition, the number of staff required to maintain a birth cohort, which includes conducting weekday, weekend and weekend assessments, as well as completing quality control tasks, is often much larger than projected. Gaps in funding are extremely detrimental.  

Retention. Besides the decrease in cohort size, one of the main issues with retention of participants is that loss to follow-up often differs from continuing participants with some demographic characteristics, such as age, marital status, medical insurance status, race and ethnicity. The most common reason for attrition is the inability to locate participants due to disconnected phones and/or frequent moves, regularly missed appointments leading to exclusion from the study, refusal to continue, or in a few cases, infant deaths. Different incentives that have been successful in improving retention rates include payments, often times incremental over the course of enrolment, gift certificates (e.g. to grocery stores), and bonus incentives for certain activities (e.g. calling study staff when in labour, returning on a separate day to finish an assessment or provide an additional sample, or providing new contact information upon moving). Incentives remain a major budget item to be considered in the planning stages of such a study. Maintaining contact every few months with birthday cards, brief telephone interviews about the child’s health, or simple check-ins with the family to remind them of the next phases of the study, is also critical.  

Environmental assessments. Home inspections to assess housing quality are time-consuming and require extensive training. It might be necessary to visit homes multiple times to reassess household exposures, which may vary by season or change when families relocate (125). Collecting environmental measurements often requires the purchase of expensive, specialized collection equipment (e.g. air monitors) and a delay between home assessments to allow for cleaning of equipment. In addition, standard practices for interpreting ambient measurements are not yet fully developed; for example, it is unclear for most contaminants whether house dust concentration (μg per gram of dust) or loading (μg of surface area) is a better predictor of children’s exposure or body burden.  

Delivery events. Shortened post-delivery hospital stays in the United States leave a limited window of opportunity to collect information and samples from mothers and neonates in the hospital. Because of the slow notification of participants’ admission for delivery, a large proportion of women fail to be tracked at the time of delivery. For efficient notification, researchers must rely on both participating women and delivery ward staff. Some of the solutions developed by researchers include: distributing cell phones to enable mothers without home phones to call the research team, or alternatively, distributing t-shirts or socks to wear to the hospital, which will alert the delivery staff; providing lists of participants approaching their due date to medical stations; and checking delivery logbooks daily. Cord blood samples are particularly difficult to obtain. Most missed collections occur when women’s delivery admissions are not reported to research staff; additional samples are missed from high-risk children with emergency deliveries. The greatest collection rate tends to be reported by the research teams that involve physicians in collecting the samples. Conducting neonatal assessments is also a difficult task. Few tests are available to assess newborn behaviour, and their predictive validity is not high. Many assessment tests require trained evaluators, who are not easily replaced when they leave projects, which can create gaps in cohort assessment. Within the context of shortened post-delivery hospital stays, post-delivery assessments have to be scheduled both after the effects of delivery medications wear off, and between the child’s sleeping and eating schedule. Finding a quiet assessment room in the hospital can also be a challenge. Due to these various impediments, assessments intended for the neonatal period are in many cases conducted several weeks after delivery. Early-morning assessments and assessment of the child both soon after delivery and again one month later tend to increase success with hospital assessments.  

Child assessments. Conducting assessments on small children in the home is nearly impossible, thus the provision of a standardized testing facility may be essential. Minimizing distractions to children during neurobehavioural assessment is particularly challenging. For children > 12 months of age, it is desirable to assess the child separately from the mother to reduce interference; this requires additional time for the tester to build a rapport with the child. Siblings can also be a source of distraction during assessments. On-site childcare, giving reimbursements for off-site childcare, and/or using videos or games to busy these children are some handy options; however, the ideal arrangement is on-site childcare with dedicated space and personnel.  

Problems in sample collection. Blood collection from children is a challenge. Collecting research
blood samples at the same time as clinical samples helps to avoid participant concerns about taking blood from children and pregnant women, especially in certain cultural groups. Consulting with community physicians to determine the amount of blood collection that is both clinically and culturally acceptable to the target population is also helpful. Collecting breast milk samples soon after delivery, although most convenient for the research team, can be daunting for mothers, as the milk supply may not yet be fully developed. Later collection of breast milk avoids some of these problems, but timing issues may arise for other sample types as well. Studies conducted in rural areas face additional barriers to successful collection and processing of samples, such as limited laboratory facilities that are not adequately equipped to process samples (e.g., to separate whole blood into blood products). In this case, it is necessary to transport samples over long distances, which increases costs. In locations where necessary goods and services (e.g., dry ice or courier services) are in short supply, it can also be difficult to ensure the prompt stabilization of samples. Finally, some rural areas may lack skilled paediatric phlebotomists.

**Participant fatigue.** Longitudinal studies are demanding for families. To minimize participant fatigue, researchers should aim to optimize contact frequency such that attrition is prevented, but participants are not overly burdened. It is important to design contact between researchers and study participants to be as brief and efficient as possible; respect for participants’ time may require that the focus of research be narrowed down. Strategies employed to minimize the impact of participant fatigue include using multiple workers to simultaneously collect information at each visit. This requires that each research worker is trained in multiple aspects of the study protocol (sometimes though, multiple short visits were preferred to one long visit, both for convenience and to prevent child fatigue). Other approaches were to provide snacks in case of lengthy and demanding assessments; develop qualitative assessments that allowed study staff to document participants’ level of fatigue, cooperation, and attention; and to record any changes made to the usual study protocol.

**Quality control of assessments and interviews.** Proper staff training is critical. Adequate pilot testing is important, but often hindered by time, cost and the need for prior Institutional Review Board (IRB) approval. Insufficient time and resources are the main reasons why clear quality control protocols (e.g., direct observation, review of videotapes by the other evaluators and lead psychologists) fall short. Even after extensive staff training, inter-rater differences and reliability issues also remain a concern.

**Lack of transportation.** Transportation can be a barrier to successful completion of assessments. Paying for taxi services, reimbursing participants for alternate travel costs, transporting participants to the office for an assessment after completing a home visit, purchasing and outfitting an RV that could be driven to participants’ homes and used as a roving assessment room, or simply purchasing a car for the study to reduce mileage reimbursement costs and wear and tear on staff cars, are a few of the solutions to which Children’s Centers have turned to address this problem.

**Issues of literacy, language and culture.** The wording and phrasing of all study documents, including consent forms, must be simple; in addition, most study instruments, including those designed for self-administration, must be administered orally to attend to the issue of participants with limited education and low literacy. Potentially embarrassing topics that evade translation (e.g., specific birth control methods) may have to be described graphically. Other culturally sensitive issues to be attentive to include participants not knowing or not sharing their exact date of birth, being hesitant to provide biologic samples, and reporting pregnancy relatively late in gestation. Understanding these types of issues and planning the research accordingly relies heavily on focus groups with community members. Because young children cannot precisely answer questionnaires regarding behaviour or habits, researchers must often ask the child’s mother for a specific answer in a follow-up.

In terms of the logistics of a longitudinal birth cohort study, it is critical that funding be adequate for the start-up period, continuous without gaps, and extend for the long-term. Costs are often underestimated for labour-intensive activities, such as tracking and maintaining study participants.

**Ethical issues**

The ethical issues in a longitudinal birth cohort study are likely to become increasingly more complex in the changing medical and legal environment, and must be carefully considered in designing research protocols and following the cohort. It is particularly necessary to develop clear plans of referral when children with disease, developmental difficulties, or adverse social situations emerge.
Increasing ethical complexity. Since the implementation of the Health Insurance Portability and Accountability Act (HIPAA), it has become more time-consuming to obtain participants’ informed consent for studies in the USA. Concerns about potential lawsuits have increased, and conflicting ethical issues are routine (e.g. deciding when the health and safety of a child takes precedence over a promise of confidentiality).

Consent and assent. Because longitudinal studies demand lengthy and complex consent forms, ensuring that participants are well informed is challenging and requires the allocation of adequate time and resources. For studies using medical records in the USA, the completion of HIPAA subject authorization forms adds time to the consent process. It has been found to be important to inculcate in staff an understanding that consent is an ongoing process; instead of training staff to simply procure participant signatures, centres have trained staff to solicit and answer participants’ questions so that they can make informed decisions. Clearly, writing consent forms at a reading level understandable to all is critical. In cases where the level is suspected to be too high to assure comprehension, research workers have the option of reading consent forms aloud to participants to ensure that everyone, including participants who are embarrassed to admit their low literacy level, fully understands the information. Solicited feedback from community partners, community board members and community-based staff (in addition to the IRB) also helps ensure that appropriate language is used. Additional measures to enhance understanding of consent forms include providing study participants with timetables and schedules to communicate study procedures, or lists outlining the important items on the consent. Providing short checklists to verify that participants understand the key aspects of the study is also effective. All solutions that decrease the amount of complex information that participants have to digest at each visit and give them an opportunity to re-evaluate their participation at a midway point are helpful. While studies often operate with uncertainty about funding and the future direction in the long run, continuing requests for participation can be a source of frustration; full disclosure of the protocol up-front is thus preferable. Careful thought must be given to who must consent to participate at each stage of the research. In all cases, a pregnant woman or mother should be asked to consent to her own participation and that of her child. However, once children reach a certain age (generally 5–9 years), child assent is typically also required by the IRB, leading to new challenges.

Banked samples and informed consent. The process of banking samples for future studies requires special consideration, as participants must be informed about and consent to future uses of these samples. Consent forms may allow participants the option of either not having samples banked and/or not allowing future analysis of samples for unrelated studies. IRB re-approvals may additionally be required for each new analysis of banked samples.

Confidentiality and consideration of children at risk. Protecting the identity and personal information of all participants can be difficult in small or close-knit communities, especially when the research staff was hired from the local community. Confidentiality within computerized databases also requires particular attention. All computerized files should be password-protected with knowledge of passwords restricted to a small number of staff. The number of computer or paper files containing both the participant study number and identifying information (e.g. name) should be limited. In complex studies with multiple contacts, it can be necessary to work with both the IRB and the research staff to identify the types of linked information necessary for day-to-day operations, and to provide that information with the least possible risk to participants.

Protocols on intervening in cases of clear developmental delays or undiagnosed physical health problems are compulsory. Most protocols include timely screening of developmental assessments and questionnaires to ensure prompt referral or treatment. Reporting some exposure measures, such as lead results, to public health authorities when they exceed certain action levels is also essential. Lastly, certificates of confidentiality, which protect identifiable research information from forced disclosure, including in the case of legal action, are an important component in protecting participant confidentiality. However, an investigator might need to break the promise of confidentiality without participant consent, for example, in cases where child abuse, severe depression, drug use or traffic in the home, and other potentially dangerous conditions are observed. Disclosure of such requirements (e.g. the need to report child abuse) is typically incorporated into consent forms, despite concern that it would deter some participants.

Communication issues

Communicating study results is a key step in any research project. In addition to publishing results
in scientific journals, centres should seek to share findings with participants and community members. Researchers may benefit from soliciting the guidance of community collaborators to decide when and how to disseminate results, including how to clearly craft messages so they would be understood and of interest to the community. In some cases, communities expect interventions and actions that are outside the scope of the research; therefore it is important to concisely communicate the purposes and limitations of the research beforehand to prevent false expectations.

Timing of the results communication. It is advisable to disclose findings to participants and/or community advisory boards before their publication in journal or newspaper articles. This disclosure is an important step in building trust between researchers, participants, and communities. Community members resent hearing findings for the first time in the media.

Communication tools. Strategies to disseminate information, developed in collaboration with community advisory boards, have included newsletters, fact sheets, pamphlets, press releases in local papers, pay-stub inserts, radio programmes (particularly useful in rural areas), town hall meetings, and internet sites. Ideally, researchers concerned with children’s health would also like to communicate results to children themselves. Based on results from their study of pesticide exposure in children, the University of Washington’s Center for Children’s Environmental Health (Center for Child Environmental Health Risk Research) created colouring books and curricula to educate preschool and school-age children on how to prevent exposure to pesticides. Specialized tools are sometimes needed for studies that target low-literacy or non-majority-language-speaking communities. Publishing information in more than one language is essential, and successful attempts to develop pictorial rather than verbal messages have also been made (126) (http://www.checnet.org/healthhouse/education/articles-detail.asp?MainID=644).

Group- versus individual-level results. Perhaps the biggest communication issue has to do with whether to provide individual-level results, particularly for measures of exposure or internal dose. The argument in favour of providing such results is that participants have the right to know; the counterargument is that participants may be unnecessarily alarmed by results with no interpretable meaning. Generally, results with a clear clinical implication (e.g. blood lead levels) have been reported to participants, whereas results without clear clinical implications (e.g. urinary pesticide metabolite levels) have not been shared. One centre, however, on the basis of community advisory board input, decided to offer participants the option of requesting their individual pesticide levels. That centre is currently in the process of developing materials to provide these results and will work closely with community health care providers when clinical questions arise (124). Regardless of whether group- or individual-level results are returned, it is important to provide participants a context for these results. Providing a comparison, either to other study participants or nationwide data, has been particularly helpful. In communicating results, centres aim to clearly describe their implications for health and well-being; when these implications are not known (as in the case of pesticides), centres state this honestly (104).

Active and meaningful participation of the community is essential for determining the relevant research questions, enrolling and retaining the cohort in an intensive investigation over the long term, and contributing to translation of scientific principles and research results for communities and the public at large. This requires establishing trust and respecting differences in culture and knowledge of the community. Sufficient time and resources are necessary to develop community partnerships.

Future directions and challenges: Looking ahead

Summary

Recent molecular epidemiologic research has helped identify etiologic factors in childhood diseases. Exposures of particular concern for the fetus and young child are environmental tobacco smoke (ETS); polycyclic aromatic hydrocarbons (PAHs); particulate matter; nitrosamines; pesticides; polychlorinated biphenyls (PCBs); metals and radiation, which may be involved in respiratory disease and asthma; cancer risk and genetic damage; and neurobehavioural disorders. Molecular epidemiology has also been a useful tool in identifying the interactions between exposures and certain “host” factors, such as genetic susceptibility, nutritional deficits, and psychosocial stressors that can lead to disease. It has also provided compelling new evidence that early exposure to environmental factors is a likely contributor to disease in later years of life.

The powerful need for prevention, as directed by current trends in disease incidence, requires that strong, collaborative research be performed, especially
on populations most at risk. The logistics involved in designing and conducting such observational research, adequate communication of the research findings to populations enrolled in the study, as well as the ethics at stake, present real challenges. Strategies for conducting cutting-edge yet safe and responsible research must therefore be widely shared and understood.

**Future directions: Suggestions**

**New epigenetic and "omic" biomarkers**

The previous generation of biomarkers has contributed to our understanding of risk and susceptibility related to genotoxic carcinogens. As a result, interventions and policy changes have been mounted to reduce risk to children from several important environmental carcinogens. More recently developed biomarkers have considerable potential in molecular epidemiology, as they reflect another equally important mechanism of carcinogenicity: epigenetic alterations that affect the expression of genes and proteins. These can be measured by high-throughput methods, allowing large-scale studies that are ‘discovery-oriented.’ Research using these techniques is needed to study the effects of multiple exposures and their interactions both with each other and in combination with different types of susceptibility factors. Gene–environment interactions should be a major focus, but interactions with nutritional and psychosocial factors also deserve emphasis in future research. Studying the low-level exposure effects of global pollutants and their interactions with susceptibility factors in different geographic locations, exposure scenarios, and ethnic/racial groups will help in understanding etiology and confirm findings. However, most of these markers have not yet been validated, and their role in the causal paradigm is not clear. There is an urgent need for validation of these newer biomarkers so that they can be combined with the more traditional ones in hypothesis-testing studies. Large-scale, long-term prospective studies will be the key to achieving this.

**National Children’s Study**

In the USA, studies under way at the National Centers for Children’s Environmental Health were among the first in line to meet these needs. The National Children’s Study (NCS) (a programme that will follow 100 000 children across the US from before birth until age 21) will examine the effects of natural and man-made environment factors, biological and chemical factors, physical surroundings, social factors, behavioural influences and outcomes, genetics, cultural and family differences, and geographic location on the health and development of children in the cohort (127). (A summary of the participating cohorts and links to their web pages, as well as links to updates on the NCS, can be found at: http://www.nationalchildrensstudy.gov/studylocations/Pages/websites.aspx.)

**Conclusion**

Molecular epidemiology has contributed much to our knowledge of risk factors for environmental health-related diseases in children. The high rates of asthma, certain cancers and developmental disabilities, and the growing evidence that risk of certain adult diseases is associated with in utero and childhood exposures, indicate that maintaining an early focus in molecular epidemiology can have a significant impact on the overall burden of disease. When preventive measures have been enacted based on this knowledge in the past, children’s health has benefited. Incorporating strategic principles to translate existing and future data into public health policy will ensure benefits in children’s environmental health.

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