Challenges and Novel Approaches in the Epidemiological Study of Early Life Influences on Later Disease

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Abstract The influence of factors acting during early life on health outcomes of offspring is of considerable research and public health interest. There are, however, methodological challenges in establishing robust causal links, since exposures often act many decades before outcomes of interest, and may also be strongly related to other factors, generating considerable degrees of potential confounding. With respect to pre-natal factors, the degree of counfounding can sometimes be estimated by comparing the association between exposures experienced by the mother during pregnancy and outcomes among the offspring with the association of the same exposures experienced by the father during the pregnancy period and offspring outcomes. If the effects are due to an intra-uterine exposure, then maternal exposure during pregnancy should have a clearly greater influence than paternal exposure. If confounding by socio-economic, behavioural or genetic factors generates the association then maternal and paternal pregnancy exposures will be related in the same way with the outcome. For early life exposures it is also possible to compare outcomes in siblings who are concordant or discordant for the exposure, which will reduce the influence of family-level confounding factors. A different approach is that of Mendelian randomization, which utilises genetic variants of known effect that can proxy for modifiable exposures and are also not in general related to potential confounding factors, or influenced by disease. In other settings the use of non-genetic instrumental variables is possible. A series of examples of the application of these approaches are presented and their potentials and limitations discussed. Other epidemiological strategies are briefly reviewed.

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It is concluded that the naïve acceptance of findings utilising conventional epidemiological methods in this setting is misplaced.

Keywords Causal inference • developmental origins • family studies • Mendelian randomization

Abbreviations ALSPAC: Avon Longitudinal Study of Parents and their Children; BMI: body mass index; IV: instrumental variable; MTHFR: methyltetrahydrofolate reductase; NTD: neural tube defects; RCT: randomised controlled trials

1 Introduction

There is considerable interest in the proposition that exposures acting in early life have long-term consequences for health in adulthood. The early life factors include those acting during (or before) the period of fetal development – such as maternal diet, smoking or alcohol use; those acting in infancy – such as breast or bottle feeding; and those acting in childhood – such as passive exposure to tobacco smoke. Nearly all domains of later health experience – including cardiovascular disease, various cancers, respiratory disease and cognitive decline – have been associated with early-life exposures of one kind or another. To formulate effective public health policy it is crucial to be able to separate out causal associations, which offer the possibility of intervention and disease prevention, from non-causal associations. If the non-causal associations are mistaken for causal associations this could lead to misguided strategies that at best waste resources and divert attention from effective approaches, and at worst could have health-damaging consequences.

2 Challenges Facing Epidemiological Studies of Early Life Influences

There are several issues that render the epidemiological study of the influence of early life exposures on later health outcomes problematic. These relate to the long time-gap between exposure and outcome. The assessment of exposure may be difficult, since retrospective approaches may be required to obtain information regarding exposures acting many decades before the health outcome is observed. There are no truly prospective large-scale studies with detailed and continuous data – including biological measures – from before birth through to late adulthood. Such problematic exposure assessment can lead to random errors, with the expectation that these errors would attenuate effect estimates and make it more

difficult to establish robust associations. This will lead to studies being underpowered, but not to spurious associations being observed. Perhaps more seriously retrospective assessment may be biased by knowledge of later health outcomes. Biased exposure reporting can, in this situation, generate spurious associations when none actually exist.

A further consequence of the long time gap between exposure and outcome is that even when associations are observed from well-conducted prospective studies, and therefore likely to be robust, their relevance to contemporary pregnant women, infants and children is unclear. Thus findings in lifecourse epidemiology may be context dependent, and long time gaps between exposure and outcome render it more likely that they will not be applicable to current exposure patterns.

A further challenge to causal inference is the potential for substantial degrees of confounding. For example, a number of studies have investigated the effect of breast feeding on later health outcomes, such as obesity, blood pressure, cancer risk and cognitive function. However in many societies breast feeding is strongly related to higher socioeconomic circumstances and associated phenomena, such as maternal non-smoking, healthy diet, low toxic occupational exposures and a generally better quality of the physical and social environment. The links between breast feeding and these other factors would generate relationships between breast feeding and the many health outcomes that they influence. Thus it has been claimed that the association between breast feeding and IQ can be completely accounted for by such confounding (Der et al. 2006).

3 Examples of Mismatch Between Observational and Trial Evidence

Confounding and bias are capable of generating associations in observational epidemiological studies of adulthood risk factors and disease outcomes that are not causal. Consider cardiovascular disease, where observational studies suggesting that beta carotene, (Manson et al. 1991) vitamin E supplements, (Rimm et al. 1993; Stampfer et al. 1993) vitamin C supplements, (Osganian et al. 2003) and hormone replacement therapy (HRT) (Stampfer and Colditz 1991) were protective were followed by large randomised controlled trials (RCT) showing no such protection. (Omenn et al. 1996; Alpha-tocopherol 1994; Dietary supplementation 1999; Heart Protection Study 2002; Beral et al. 2002; Manson et al. 2003). In each case special pleading was advanced to explain the discrepancy, but it is likely that a general problem of confounding – by lifestyle and socioeconomic factors, or by baseline health status and prescription policies – is responsible (Davey Smith and Ebrahim 2001, 2006; Lawlor et al. 2004; Vandenbroucke 2004).

There is evidence that the intake or level of antioxidants is associated with known risk factors for coronary heart disease. In the British Women's Heart and Health study (BWHHS), for example, women with higher plasma vitamin C levels were less likely to be in a manual social class, have no car access, be a smoker or be

obese and more likely to exercise, be on a low fat diet, have a daily alcoholic drink, and be tall (Lawlor et al. 2004, 2005a). Furthermore for these women in their 60s and 70s, those with higher plasma vitamin C levels were less likely to have come from a home 50 years or more previously in which their father was in a manual job, or had no bathroom or hot water, or within which they had to share a bedroom. They were also less likely to have limited educational attainment. In short, a substantial amount of confounding by factors from across the lifecourse that predict elevated risk of coronary heart disease was seen.

There are similar instances of confounding and bias operating in studies of early-life factors and later health outcomes. Consider, for example, the influence of maternal diet on offspring health and development. In the Southampton Women's Study there are very strong associations between diet, socio-economic position and smoking. (Robinson et al. 2004). In observational studies vitamin C intake during pregnancy has been associated with higher birth weight of offspring (Matthews et al. 1999), however data such as those from Robinson et al. (2004) would suggest that mothers with higher vitamin C intake during pregnancy would have much lower rates of smoking and be of more privileged socio-economic background, generating substantial confounding. Figure 1 contrasts the results from the observational study with those from the largest RCT to date in which pregnant women were randomised to a supplement containing vitamin C and E (Poston et al. 2006). Findings from the two study designs are unlikely to be compatible.

Given the inherent difficulties in relating early life exposures to later health outcomes, in this chapter we will briefly discuss several methods that can be applied

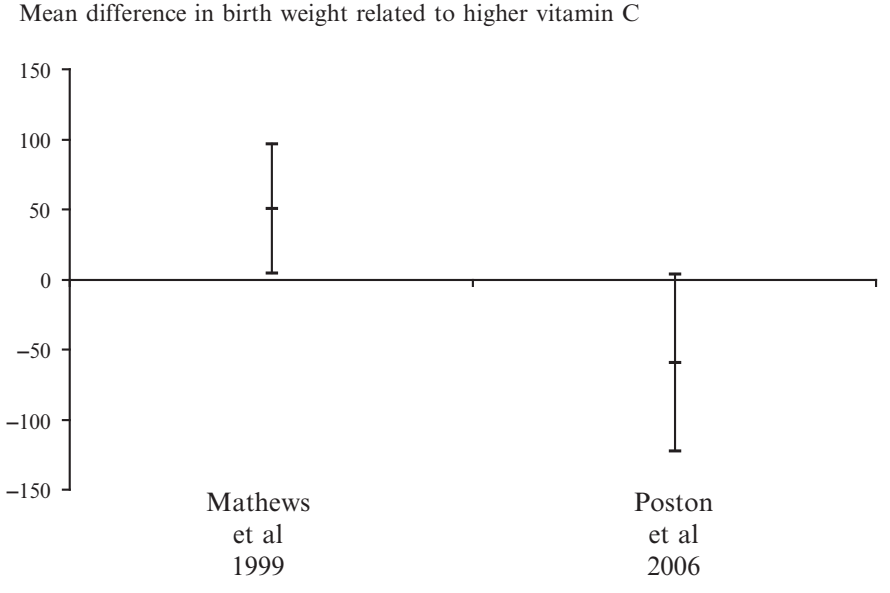

Fig. 1 Comparison of observational epidemiological evidence and randomised controlled trial evidence of the association between maternal vitamin C intake during pregnancy and birth weight

in epidemiological studies to increase the ability to draw causal inferences. These approaches include the use of maternal/paternal comparisons, studies of siblings, the identification of critical time periods and the use of genetic and non-genetic instrumental variable approaches. The approaches described here constitute a far from exhaustive list, but we hope illustrate methods that have some general utility.

4 Contrasting Maternal and Paternal Exposure Associations with Offspring Outcomes

We are often interested in the possibility that maternal exposures during pregnancy have a direct biological effect on offspring outcomes, through influencing the intrauterine environment in which the fetal development of the offspring occurs. Thus maternal smoking may influence offspring obesity, or maternal alcohol use may lead to impairments in various aspects of offspring functioning. However there are many confounding factors that could generate non-causal links between the smoking and drinking behaviours of mothers and the health of their children. One approach to this issue is to compare the strength of associations between an exposure among mothers and offspring outcomes with the association between the same exposure among fathers and the offspring outcomes (Davey Smith 2008). If there were a direct biological effect of intrauterine exposure on offspring health status, then the link with offspring health should be much stronger for exposure among mothers than for exposure among fathers. This can be illustrated with respect to an outcome where there is strong evidence of a causal influence of a maternal exposure – maternal smoking during pregnancy and on offspring health outcome, birthweight. Figure 2 demonstrates that in the Avon Longitudinal Study of Parents and their Children (ALSPAC) maternal smoking during pregnancy is associated with lower offspring birthweight, whereas smoking by the

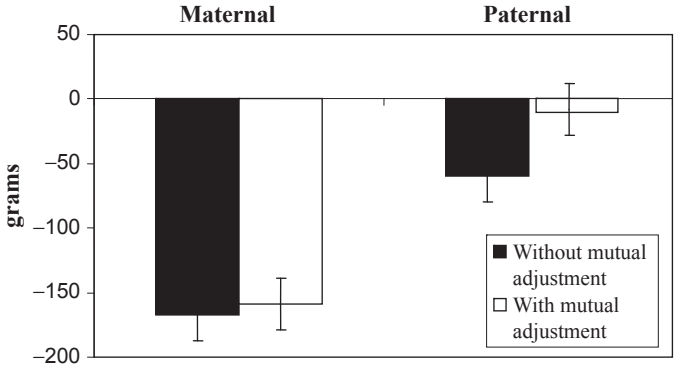

Fig. 2 Association between maternal/paternal smoking and offspring birthweight without and with mutual adjustment (difference in birthweight between offspring whose parents are smokers and non-smokers in grams)

father during pregnancy is only weakly associated (presumably because of some confounding with maternal smoking), and when both maternal and paternal smoking during pregnancy are taken into account the former shows a robust association that is little attenuated, whereas the latter association is essentially abolished.

There has been considerable interest in the possibility that maternal smoking influences fetal development in a way that leads to higher body mass index (BMI) and risk of obesity in later life. In the case of offspring BMI at age 7 initial examination of the ALSPAC data show that the average BMI of children of smoking mothers is raised (Fig. 3; Leary et al. 2006a). However a similar sized association is seen with smoking by fathers, and including both maternal and paternal smoking behaviour in the same model leaves residual effects of similar magnitude (Fig. 3). These findings suggest, that in the case of offspring BMI, maternal smoking during pregnancy does not have a direct intrauterine effect, rather confounding factors associated with parental smoking and offspring BMI generate an association between both maternal and paternal smoking and offspring BMI. By contrast, for offspring leg length at age 7, maternal smoking shows stronger effects than paternal smoking (Leary et al. 2006b), suggesting a biological effect of maternal smoking on femur development, as supported by other studies of this issue (Jaddoe et al. 2007).

In our view, the strong implication of finding that maternal and paternal lifestyle-related factors during pregnancy are associated in similar ways with offspring outcomes suggests that such associations are generated by underlying socially-patterned environmental influences acting at the family level, and do not reflect direct biological influences of the exposures *per se*. However it is possible to interpret the findings differently. For example Pembrey et al. (2006) suggest that the association between paternal smoking and offspring BMI reflects epigenetic influences, and that such male-line transgenerational responses have important health implications. While it is possible that these male-line epigenetic factors exactly match the biological influence of maternal smoking on the intrauterine environment, to generate very similar associations, we feel this is unlikely. Informal or formal approaches to comparing explanatory models,

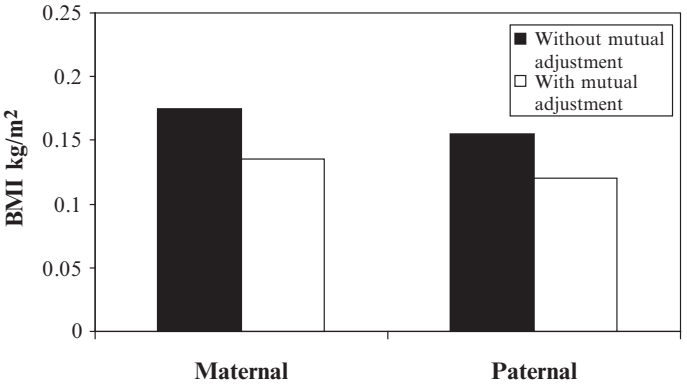

Fig. 3 Effect of maternal and paternal smoking during pregnancy on offspring body mass index without and with mutual adjustment

which adopt the parsimony principle of Occam's Razor (i.e. making the fewest possible assumptions) (Mackay 2003), would suggest that the likelihood of such perfectly matched effects, being produced by mechanistically distinct processes, is rather low.

5 Within and Between Sibship Comparisons

As we discuss above there are many factors that can confound associations between early life influences and later outcomes. One approach to this that has been widely utilised in the social sciences is to compare associations within sibling pairs and between all the people in a study, independent of their sibling group (Conley et al. 2003). Since familial socioeconomic background will be similar for siblings from the same family, comparing outcome differences in relation to discordant exposure levels within sibships is in effect 'matching' on fixed family characteristics (including socioeconomic position), whether these are evaluated or not in a study. As such this provides a stronger means of controlling for family socioeconomic position than multivariable adjustment, particularly where studies only have one or two indicators of socioeconomic position.

Consider, for example, the influence of breastfeeding on later growth. We know that in many situations breast-feeding is strongly socially patterned, and will be related to a whole series of factors that might lead to greater growth amongst people who are breast fed. In a prospective cohort study of 4,999 children from 1,352 families born in the 1920s and 1930s, breast feeding was related to later growth (Martin et al. 2002). When the analyses were undertaken in the whole cohort with no attention to within sibship associations, breast fed subjects were taller in childhood than never breast fed subjects. The association between breast feeding and childhood height and leg length persisted when the analysis was restricted to within sibship height/leg length differences in relation to within sibship differences in breast feeding. These findings were interpreted as demonstrating that breast feeding is causally related to greater skeletal growth (i.e. that the association is not confounded by fixed familial effects such as socioeconomic background). RCT evidence suggests that exclusive breast feeding results in accelerated growth in the first few months of life, but with no detectable difference by 12 months (effects on later childhood height have yet to be reported) (Kramer et al. 2002). However, the issue of context dependence discussed above may well apply here, as the food received by non-breast fed infants in the 1920s and 1930s in the U.K. will be very different to that received by the infants in the RCT, carried out in Belarus in the late 1990s.

Authors of studies that have compared within and between sibship associations point out that these studies provide insights into potential mechanisms between exposures and outcomes found in general population studies, beyond simply determining whether these are due to confounding or not (Lawlor et al. 2006b, 2007a). These comparisons require careful consideration of factors that are the same for siblings brought-up together. Thus, in a very large Swedish record linkage study

inverse associations of birth weight and gestational age with systolic blood pressure were found both within and between sibships (Lawlor et al. 2007). These findings suggest that the association between birth weight or gestational age and systolic blood pressure are not explained by factors, such as family socioeconomic position, that are the same or very similar for siblings. With additional intergenerational data included in the analyses the authors concluded that intrauterine factors, such as the effects of maternal metabolic or vascular health during pregnancy and/or placental function (characteristics that will vary from one pregnancy to the next in the same mother) were the most likely explanation for the association of birth weight/gestational age with blood pressure in the general population.

Several authors have used within and between sibling studies to explore whether the consistent positive association between birth weight and later intelligence (Lawlor et al. 2006a) is due to confounding or not (Record et al. 1969; Matte et al. 2001; Lawlor et al. 2005b, 2006c). These studies have produced discrepant findings, with some (e.g. Lawlor et al. 2005b; Matte et al. 2001) suggesting that birth weight discrepancies within sibships are related to differences in intelligence, and others (e.g. Lawlor et al. 2006c; Record et al. 1969) finding no such association. As two (Record et al. 1969; Lawlor et al. 2006c) of the largest studies found no within sibship association, and the third large study found a within sibship effect only for a sub-group (that was not clearly pre-specified) of males only (Matte et al. 2001) – it therefore seems reasonable to conclude that the association between birth weight and childhood intelligence seen in general populations of singletons is largely explained by factors, such as family socioeconomic background and parental education, that are shared by siblings. These studies demonstrate the need for large sample sizes if robust evidence is to be obtained from within and between sibship studies.

6 Relating an Exposure to a Critical Period

In some cases an exposure only influences a disease outcome if experienced during a critical exposure window. However confounding factors or biases would generally generate associations between exposure at any time and the outcome. Thus demonstrating the specific influence of an exposure at a particular critical window and a health outcome provides some evidence that the association is causal. For example, it has been suggested that radiotherapy for Hodgkin's lymphoma increases the later risk of breast cancer. However many factors could generate an association between Hodgkin's lymphoma and breast cancer. Such confounding factors would, however, apply to Hodgkin's lymphoma diagnosed at any stage of life. Thus the demonstration that exposure to radiotherapy for Hodgkin's lymphoma between menarche and first pregnancy – when the breast is particularly sensitive to mitogens – but not at other times is related to increased risk of breast cancer many years later provides some evidence that this is a causal association. That said, these analyses need to be pre-specified and those that are not should be clearly identified as post-hoc exploratory analyses that require confirmation.

7 Mendelian Randomisation

Mendelian randomization is the term that has been given to studies that use genetic variants in observational epidemiology to make causal inferences about modifiable (non-genetic) risk factors for disease and health related outcomes (Youngman et al. 2000; Davey Smith and Ebrahim 2003; Davey Smith 2007). Such studies exploit what is known as Mendel's second law or the law of independent assortment:

> *that the behavior of each pair of differentiating characters in hybrid union is independent of the other differences between the two original plants, and, further, that the hybrid produces just so many kinds of* egg *and pollen cells as there are possible constant combination forms*. (Mendel 1865)

In simple terms this means that the inheritance of one trait is independent of (i.e. randomised with respect to) the inheritance of other traits. The independent distribution of alleles (or blocks of alleles in linkage disequilibrium) from parents to their offspring means that a study relating health outcomes in the offspring to genetic variation transmitted from the parents will not suffer from confounding. This holds true for full-siblings who are not monozygotic twins. Despite the actual random allocation of groups of alleles being at the level of parent to offspring dyads, at a population level – when relating genetic variants to disease outcome – alleles are generally unrelated to those confounding factors (in particular socioeconomic position and lifestyle factors) that distort the interpretations of findings from observational epidemiology (Bhatti et al. 2005; Davey Smith et al. 2008). Furthermore, disease processes do not alter germline genotype and therefore associations between genotype and disease outcomes cannot be affected by reverse causality. Finally, for genetic variants that are related to a modifiable exposure this will generally be the case throughout life from birth to adulthood and therefore their use in causal inference can also avoid attenuation by errors (regression dilution bias) (Davey Smith and Ebrahim 2004), and provides an estimate of the effect of a modifiable exposure across the life course on disease outcome (Kivimaki et al. 2007). Mendelian randomization studies have been likened to a 'natural' RCT (Davey Smith and Ebrahim 2005; Hingorani and Humphries 2005). In addition to the major advantages with respect to confounding, unlike with conventional RCTs, Mendelian randomization studies can be conducted in a representative population sample without the need for exclusion criteria or for volunteers amenable to being randomly allocated to treatment.

Mendelian Randomization studies can provide unique insights into the causal nature of early life effects on later disease outcomes. For example, it is now widely accepted that neural tube defects (NTDs) can in part be prevented by periconceptual maternal folate supplementation (Scholl and Johnson 2000). RCTs of folate supplementation have provided the key evidence in this regard (MRC Vitamin Study 1991; Czeizel and Dudás 1992). But could we have reached the same conclusion before the RCTs were carried out, if we had access to evidence from genetic association studies? Studies have been carried out that have looked at the MTHFR 677C→T polymorphism (a genetic variant that is associated with methyltetrahydrofolate reductase activity and circulating folate and homocysteine

levels; the TT genotype being associated with lower circulating folate levels) in newborns with NTDs compared to controls, and have found an increased risk in TT versus CC newborns, with a relative risk of 1.75 (95% CI 1.41–2.18) in a meta-analysis of all such studies (Botto and Yang 2000). Studies have also looked at the association between this MTHFR variant in parents and the risk of NTD in their offspring. Mothers who have the TT genotype have an increased risk of 2.04 (95% CI 1.49–2.81) of having an offspring with a NTD compared to mothers who have the CC genotype (Botto and Yang 2000). For TT fathers, the equivalent relative risk is 1.18 (95% CI 0.65–2.12) (Botto and Yang 2000). This pattern of associations suggests that it is the intra-uterine environment – influenced by maternal TT genotype – rather than the genotype of offspring that is related to disease risk (Fig. 4). This is consistent with the hypothesis that maternal folate intake is the exposure of importance.

In this case the findings from observational studies, genetic associations studies and an RCT are similar. Had the technology been available, the genetic association studies, with the particular influence of maternal versus paternal genotype on NTD risk, would have provided evidence of the beneficial effect of folate supplementation before the results of any RCT had been completed. Certainly, the genetic association studies would have provided better evidence than that given by conventional epidemiological studies that had to cope with the problems of accurately assessing diet and also with the considerable confounding of maternal folate intake with a wide variety of lifestyle and socioeconomic factors that may also influence NTD risk. The association of genotype with NTD risk does not suggest that genetic screening is indicated – rather it demonstrates that an environmental intervention may benefit the whole population, independent of the genotype of individuals receiving intervention. There are an increasing number of examples in which Mendelian randomization can be utilized to understand the causal nature of intrauterine exposures, the major (but diminishing) limitations being the identification of genetic variants that are robustly associated with environmentally-modifiable

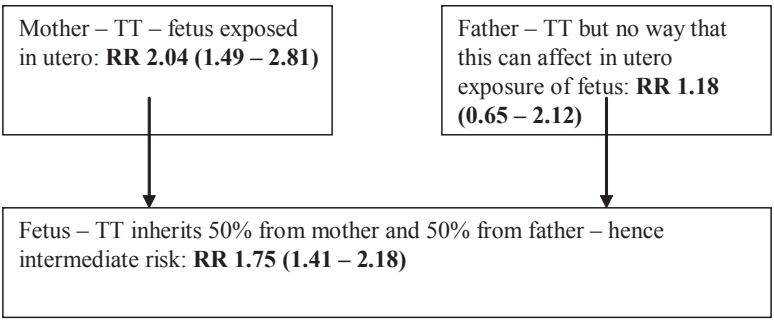

Fig. 4 Inheritance of MTHFR polymorphism, homocysteine and neural tube defects

risk processes and the size of the studies required to provide robust estimates of the association.

8 Non-genetic Instrumental Variables

The use of genotype, in Mendelian randomisation studies, to provide causal inference for the effect of a modifiable (non-genetic) exposure on disease outcome is an application of the general theory of *instrumental variables analysis* (Lawlor et al. 2008). An *instrumental variable* (IV) is a variable that is associated with the outcome only through its robust association with the exposure of interest. As such an instrumental variable will not be associated with factors that confound the association of exposure with outcome.

Dehydration in infancy has been associated with increased later blood pressure in one small study (Davey Smith et al. 2006). Furthermore a potential mechanism exists through a predictive adaptive response relating to the selective advantage of being able to respond to one episode of severe dehydration by sodium retention. However dehydration early in life will be strongly confounded with later-life exposures. One study interested in whether dehydration during early infancy was a risk factor for higher blood pressure later in life used climate conditions during infancy as an instrumental variable for the effect of dehydration (Lawlor et al. 2006d). Severe dehydration in early infancy might programme a taste for salty food in later life, which could result in greater blood pressure (Fessler 2003). Testing this hypothesis is problematic both because any assessment of dehydration in early life is difficult and the likelihood of an infant being dehydrated will be related to a number of socioeconomic and lifestyle confounding factors.

Rates of infant mortality and morbidity from diarrhoeal illnesses increased considerably during the summer months in the early decades of the twentieth century in Britain (Lawlor et al. 2006d). This summer diarrhoea and its associated infant mortality occurred in epidemic proportions during the hottest and driest (compared to cooler and wetter) summers. Thus, adults who were born in the early part of the last century and who experienced hot dry summers during the first year of their life are more likely than those who experienced cooler and wetter summers to have suffered infant diarrhoea and dehydration. Climate conditions in infancy for such a population would be a valid instrumental variable since there is no reason for it to be associated with socioeconomic and lifestyle confounding factors, as found amongst participants in the British Women's Heart and Health Study, a random sample of 3,964 British women born in the 1920s and 1930s. However, a one standard deviation (1.3°C) higher mean summer temperature in the first year of life was associated with a 1.12 mmHg (95% CI: 0.33, 1.91 mmHg) higher adult systolic blood pressure, and a one standard deviation higher mean summer rainfall (33.9 mm) with a lower systolic blood pressure (−1.65 [−2.44, −0.85] mmHg) (Lawlor et al. 2006d).

9 Conclusions

We have discussed various approaches to increasing the strength of causal inference in studies of early life exposure and later health outcomes. They have one characteristic in common, and that is that they generally require large sample sizes. This is because some of the methods require formal statistical tests between the strength of different associations (between maternal and paternal effects, for example), and others relate to situations of known small effect size (such as the association between common genetic variants and disease outcomes in the case of Mendelian randomization). However the price of large sample sizes is certainly one worth paying to get closer to reliable estimates of causal effects in epidemiological studies.

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