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Original article

Transgenerational Influences

Does childhood trauma influence offspring's birth characteristics?

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Abstract

Background: A recent epigenetic hypothesis postulates that 'a sex-specific male-line transgenerational effect exists in humans', which can be triggered by childhood trauma during 'the slow growth period' just before puberty. The evidence is based on a few rather small epidemiological studies. We examine what response childhood trauma predicts, if any, in the birth size and prematurity risk of almost 800 000 offspring.

Methods: Children of parity 1, 2 or 3, born 1976-2002 in Sweden, for whom we could trace both parents and all four grandparents, constituted generation 3 (G3, $n = 764\ 569$). Around 5% of their parents, G2, suffered parental (G1) death during their own childhood. The association of such trauma in G2 with G3 prematurity and birthweight was analysed, while controlling for confounders in G1 and G2. We examined whether the slow growth period was extra sensitive to parental loss.

Results: Parental (G1) death during (G2) childhood predicts premature birth and lower birthweight in the offspring generation (G3). This response is dependent on G2 gender, G2 age at exposure and G3 parity, but not G3 gender.

Conclusions: The results are compatible with the Pembrey-Bygren hypothesis that trauma exposure during boys' slow growth period may trigger a transgenerational response; age at trauma exposure among girls seems less important, suggesting a different set of pathways for any transgenerational response. Finally, parental death during childhood was not important for the reproduction of social inequalities in birthweight and premature birth.

Key words: Childhood trauma, parental loss, transgenerational response, birthweight, prematurity, slow growth period, inequalities, Sweden

Key Messages

- A recent epigenetic hypothesis suggests that childhood trauma may trigger a sex-specific male-line transgenerational effect in humans.
- We studied whether death of a parent during childhood was associated with prematurity risk and birthweight deficit in the next generation, using all Swedish births 1976-2002, for whom both parents and all four grandparents could be traced, $n = 764\,000$.
- Parental (G1) death during (G2) childhood predicts prematurity and lower birthweight in the offspring generation (G3). This response is dependent on G2 gender, G2 age at exposure and G3 parity, but not on G3 gender.
- The results are compatible with the hypothesis that trauma exposure during boys' slow growth period may trigger a response in their offspring.
- Age at trauma exposure among girls seems less important, suggesting a different set of pathways for any transgenerational response.

Introduction

A human being's early experience is known to be formative for development, physical growth, health and cognitive ability. A suggested mechanism is 'biological programming' *in utero* or infancy. The focus on fetal and infant growth, however, is somewhat narrow, as early social and family circumstances may be equally important and modify early biological programming.

There is considerable continuity in birthweight, school achievement and health across generations.[1–3] One tradition has typically stressed its genetic basis. A different tradition, in the humanities and the social sciences, has identified learning from parents and the transmission of knowledge, material resources and social opportunity across generations as a driver of such continuity.

The concept of 'transgenerational response', however, does not focus primarily on phenotypic continuity but on determinant-outcome associations across generations. Here also there are two principal, but not mutually exclusive, types of pathway. Learning from parents and opportunity created by parental circumstances represent cultural or social pathways.[4] Alternatively, exposures in previous generations may modify gene expression in later generations. Although of great theoretical interest, there is at best scarce evidence that any transgenerational response among humans is driven by modifications of gene expression. However, if it were a common phenomenon it would open up new ways of thinking about disease risk, including its prevention.[5,6] Our aim in this study is to add to the knowledge about transgenerational response among humans by studying the association between parental loss in childhood, and birth outcomes in the next generation. A further question is whether or not such response may contribute to the reproduction of health inequalities across generations.[7]

We look at one particular childhood experience: the loss of a parent through death. Such loss has consequences later in life, psychologically, for morbidity and mortality.[8–13] Carey[14] suggests that childhood trauma contributes to later neuropsychiatric disorders via epigenetic mechanisms. In mammals and plants, early exposure can modify gene expression, not only in the soma line but in the germ line too, without changing the DNA sequence. Such modifications (through methylation, histone acetylation or microRNAs) represent a specific biological mechanism for transgenerational response. In mice, early trauma caused changes in sperm microRNA content, consistent with a specific male-line response.[15]

Few, if any, studies on humans demonstrate such a mechanism. Bygren *et al.* identified starvation during 'the slow growth period' as a trigger of transgenerational response along the paternal line.[16,17] Pembrey, following Bygren, found that boys who took up smoking before age 11 tended to have offspring with a different metabolism from that of other boys.[18] We note two observations in these studies: (i) 'the slow growth period', just before puberty, is suggested as sensitive for triggering a transgenerational response; (ii) this response, observed in successive generations, differs by whether it is paternally or maternally transmitted. Although Pembrey and Bygren did not have access to biological samples, their results are generally interpreted as support for an epigenetic mechanism. The authors themselves write[18] that their studies '…provide proof of principle that a sex-specific male line transgenerational effect exists in humans', a point which was reinforced by the editor, Emma Whitelaw, in the same issue.[19] Behind this claim is the idea that spermatogenesis is environmentally programmed during boys' slow growth period, which is also the period when the testis starts developing. If trauma influenced such programming and if this had an effect on offspring's birth characteristics we should be able

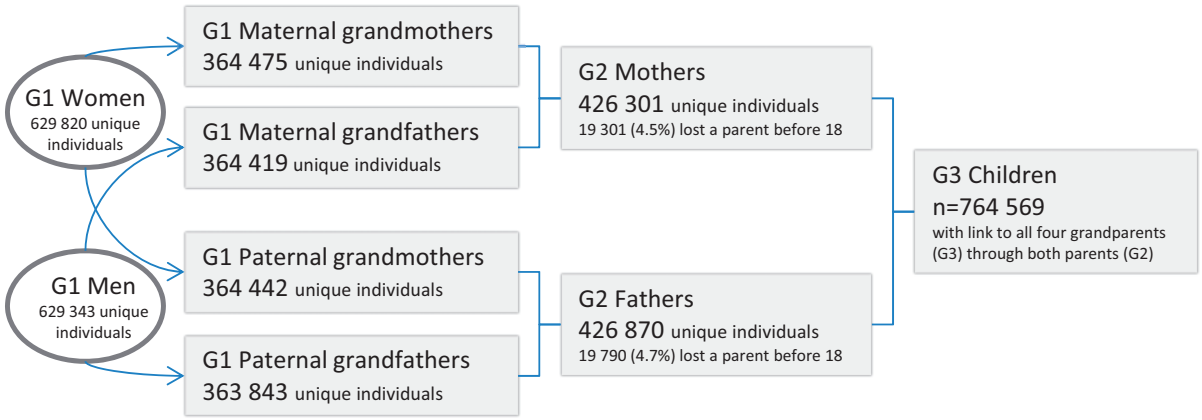

Figure 1. Description of the three generations included in the analysis.

to detect this in population studies, with data on age at trauma exposure and birth outcomes, even without genetic or epigenetic data.

We make no a priori assumptions about the mechanisms that cause transgenerational response. We hypothesize that (i) experiencing severe childhood trauma such as parental death may trigger a cascade of events impacting on development across several generations, detectable in their children's birth characteristics. We examine (ii) whether the slow growth period is a sensitive 'window of susceptibility', allowing such a transgenerational response to evolve, and (iii) whether its impact is similar along paternal and maternal lines of transmission.

Methods

Population under study

The *social mobility database* is a large, temporary and anonymized database covering all individuals resident in Sweden at any point after 1960 and born 1932-2002, irrespective of country of birth. Additionally, there is information on their biological parents from the Swedish Multigeneration Register even when born before 1932. In total, the database consists of 13.6 million individuals, linked to information from several Swedish national registers. We used the following: the Population and Housing Census 1960, Cause of Death Register 1961-2002, the Swedish Register of Education 1990 and the Swedish Medical Birth Register (MBR) 1973-2002. Stockholm Regional Ethics Committee gave ethical permission [2009/1115-32].

We extracted all births from the MBR as generation 3 (G3) and established ancestral lineages by tracing their parents (G2) and grandparents (G1). Our study population was restricted to single births (97.7% of all births) and to births of parity 1, 2 or 3 (96.3%) in G3; to families where both parents (G2) were born from 1961 onwards (i.e. it was possible to trace G1 deaths in the Cause of Death Register; 33.0%); and families with complete links between G1, G2 and G3 (83.6% of all G1-G2-G3 families). This gave 764 569 individuals in G3 with complete linkages to G2 and G1, in total 2 876 903 unique individuals across the generations (Figure 1). Figure 2 describes birth year distributions.

Variables

Exposure

Parental (G1) death during the childhood of G2, 0-17 years, was experienced by 4.6%; in over 70% of these cases this was the father's death, in 2% the death of both parents. If both parents died, G2 age at the first death was considered. G2 age at parental loss was calculated as calendar year when the loss occurred minus birth year; then classified as 0-2, 3-7, 8-12 or 13-17. Age 8-12 is considered the slow growth period.

Outcomes

Birthweight was treated as a continuous variable. Implausible values (< 0.01% of records) were removed. Births were classified as premature (< 37 completed weeks of gestation) or not premature.

Covariates

Education of G2 was defined as short (compulsory, 8-9 years), medium (secondary, usually 12 years), long (post-secondary/university) or missing. Missing constituted a separate category in order not to systematically exclude the youngest part of G2. Social class of G1 was categorized as non-manual, self-employed, manual, other or missing. Missing was treated as a separate category.

Method of analysis

We compared children (G3) of individuals (G2) who experienced parental (G1) death during their own childhood

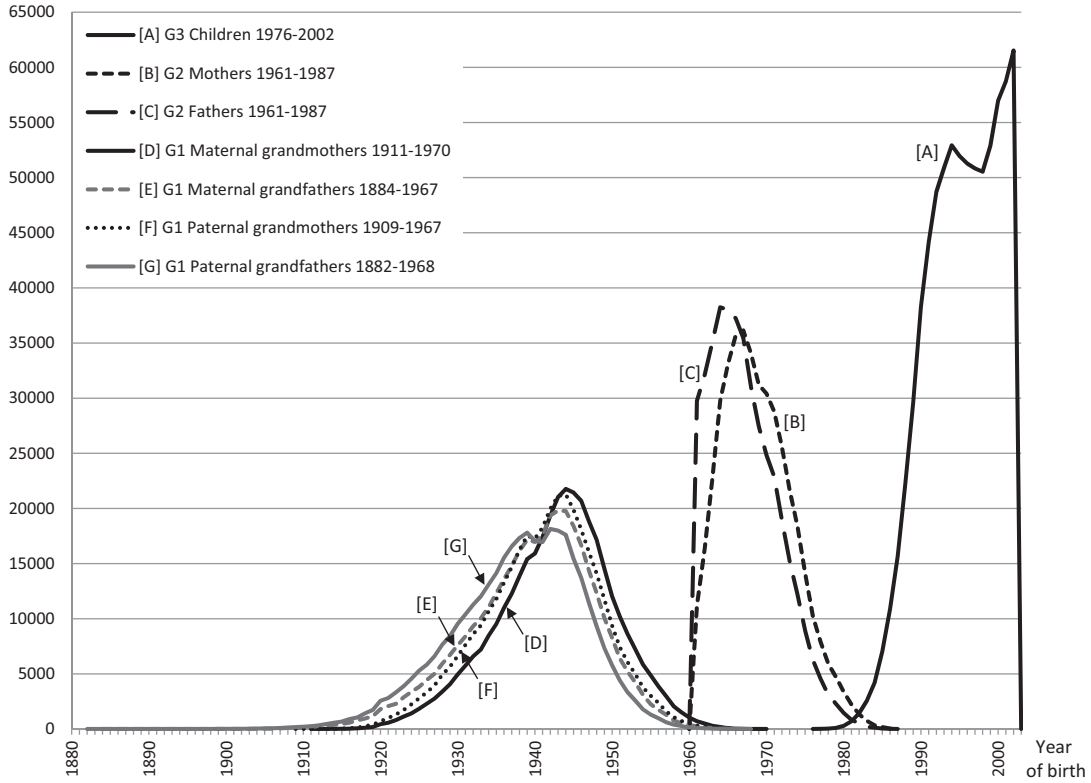

Figure 2. Birth year distribution in G1 (left), G2 (middle) and G3 (right) The two lines in G2 represent mothers and fathers, respectively. The four lines in G1 represent the four kinds of grandparental ancestor.

with children of other G2 individuals. G3 birthweight and prematurity were analysed by G2 age at parental death and G2 gender, while adjusting for potential confounders/mediators using linear or logistic regression. To take into account the interdependence between siblings, we clustered the standard errors at the family level throughout analyses. Parity-specific analyses were undertaken, since previous research suggests that parity may modify transgenerational response.[20] Initially, analyses were run separately for male and female offspring (G3). As the result did not differ (not shown), G3 genders were combined. Variables were categorized as in Table 1.

Confounders

Mortality falls over time. Birthweight increases.[21] To account for temporal trends, the birth year of G2 individuals was treated as a potential confounder behind any association between parental loss and offspring birth outcomes. G1 age and social class at G2 birth predict G2 parental loss. They may also predict both G2 birthweight[22] and education, being potential confounders. We addressed confounding by controlling for G2 birth year, age of G1 (mother) when G2 was born and social class of G1 (father) in 1960.

Mediating factors

These help us to understand pathways between parental loss and offspring birth characteristics. Achieved education and age at first childbirth of G2 individuals may be influenced by a parent's death during childhood and predict their offspring's birth characteristics; these are therefore possible mediating factors. Additionally, achieved education can be viewed as a marker of earlier social circumstances and may thus at the same time confound and mediate the association between parental death and offspring birth outcomes.

Consequently, in analyses of prematurity and birthweight, three models were applied: (1) crude differences; (2) model 1, plus adjustment for confounding factors; and (3) model 2, plus additional adjustments for hypothesized mediators. In birthweight analyses we considered, but avoided, adjustments for prematurity as this may introduce 'collider bias' in the estimated association.[23] Examining whether any period of boys' childhood is particularly sensitive to parental loss allowed us to test a recent epigenetic hypothesis.

Results

Table 1 describes the population stratified by G2 gender and parental loss in childhood.

Table 1. Characteristics of the population under study

	G2 women		G2 men	
	Did not lose a parent	Lost a parent at < 18 years	Did not lose a parent	Lost a parent at < 18 years
Characteristics of G1	***n* = 407000**	***n* = 19301**	***n* = 407080**	***n* = 19790**
Age of G1 (grandmother) at childbirth				
< 21	59623 (14.7%)	2142 (11.1%)	60018 (14.7%)	2162 (10.9%)
21-25	152596 (37.5%)	5217 (27.0%)	148396 (36.5%)	5221 (26.4%)
26-34	163912 (40.3%)	8074 (41.8%)	164273 (40.4%)	8252 (41.7%)
≥ 35	30 869 (7.6%)	3868 (20.0%)	34 393 (8.5%)	4155 (21.0%)
Social class of G1 (grandfather) in 1960				
Non-manual	77444 (19.0%)	3577 (18.5%)	82367 (20.2%)	3907 (19.7%)
Self-employed	45735 (11.2%)	2286 (11.8%)	44747 (11.0%)	2428 (12.3%)
Manual	189757 (46.6%)	9477 (49.1%)	188307 (46.3%)	9618 (48.6%)
Other	59535 (14.6%)	2256 (11.7%)	56422 (13.9%)	2138 (10.8%)
Missing	34 529 (8.5%)	1705 (8.8%)	35 237 (8.7%)	1699 (8.6%)
Characteristics of G2	***n* = 407000**	***n* = 19301**	***n* = 407080**	***n* = 19790**
Birth year of G2				
1961-65	107444 (26.4%)	5732 (29.7%)	164457 (40.4%)	8818 (44.6%)
1966-70	160067 (39.3%)	7411 (38.4%)	149114 (36.6%)	6843 (34.6%)
1971-75	104200 (25.6%)	4380 (22.7%)	75027 (18.4%)	3179 (16.1%)
after 1975	35 289 (8.7%)	1778 (9.2%)	18 482 (4.5%)	950 (4.8%)
Age of G2 at parental death				
0-2	N.A.	1488 (7.7%)	N.A.	1486 (7.5%)
3-7		3860 (20.0%)		3882 (19.6%)
8-12		5623 (29.1%)		5903 (29.8%)
13-17		8330 (43.2%)		8519 (43.1%)
Achieved education of G2 in 1990				
Short	90931 (22.3%)	4974 (25.8%)	86635 (21.3%)	4923 (24.9%)
Medium	220119 (54.1%)	10266 (53.2%)	232607 (57.1%)	11342 (57.3%)
Long	45847 (11.3%)	1638 (8.5%)	58434 (14.4%)	2056 (10.4%)
Missing	50103 (12.3%)	2423 (12.6%)	29 404 (7.2%)	1469 (7.4%)
Mean age (SD) of G2 in years at the birth of the first child (G3)	26.2 (4.4)	25.7 (4.6)	27.9 (4.4)	27.5 (4.6)
Characteristics of G3	***n* = 729569**	***n* = 35000**	***n* = 728688**	***n* = 35881**
Birth order of children born to G2				
1	391709 (53.7%)	18326 (52.4%)	390994 (53.7%)	19041 (53.1%)
2	263108 (36.1%)	12642 (36.1%)	262870 (36.1%)	12880 (35.9%)
3	74752 (10.3%)	4032 (11.5%)	74824 (10.3%)	3960 (11.0%)
Prematurity (< 37 weeks of gestation) (*n*, %), by birth order				
1	23742 (6.1%)	1154 (6.3%)	23716 (6.1%)	1180 (6.2%)
2	9624 (3.7%)	504 (4.0%)	9655 (3.7%)	473 (3.7%)
3	2854 (3.8%)	197 (4.9%)	2885 (3.9%)	166 (4.2%)
Birthweight in grams (mean, SD), by birth order				
1	3481 (551)	3456 (559)	3480 (551)	3469 (557)
2	3645 (534)	3619 (542)	3644 (534)	3629 (550)
3	3680 (553)	3638 (561)	3678 (553)	3668 (561)

Compared with unexposed, the exposed G2 individuals had older mothers and more often fathers of manual class. They tended to have lower education and be younger when they had their first baby, within each period of birth of G2 men and women (not shown). Their children were premature more often and had lower birthweight, on average, than children of unexposed G2.

Table 2. Parental (G1) death during childhood of G2 and risk of offspring (G3) premature birth by G2 age at parental loss and G3 parity; OR (95% CI) estimated by logistic regression*

Age of G2 at parental death	Model 1	Model 2	Model 3
G2 women			
All G3 parities combined, $n = 760925$			
no parental death before 18	REF	REF	REF
at age 0-2	1.18 (0.99, 1.40)	**1.18 (1.00, 1.41)**	1.16 (0.97, 1.38)
at age 3-7	1.01 (0.90, 1.13)	1.02 (0.90, 1.14)	0.99 (0.88, 1.12)
at age 8-12	0.99 (0.90, 1.09)	1.00 (0.91, 1.10)	0.97 (0.88, 1.07)
at age 13-17	**1.12 (1.04, 1.21)**	**1.14 (1.05, 1.23)**	**1.11 (1.02, 1.20)**
Parity = 1, $n = 407678$			
no parental death before 18	REF	REF	REF
at age 0-2	1.10 (0.89, 1.36)	1.11 (0.90, 1.37)	1.10 (0.89, 1.36)
at age 3-7	0.93 (0.80, 1.07)	0.93 (0.81, 1.07)	0.92 (0.80, 1.06)
at age 8-12	1.01 (0.90, 1.13)	1.02 (0.91, 1.14)	1.01 (0.90, 1.13)
at age 13-17	**1.10 (1.00, 1.20)**	**1.11 (1.01, 1.21)**	**1.10 (1.00, 1.20)**
Parity = 2, $n = 274780$			
no parental death before 18	REF	REF	REF
at age 0-2	1.28 (0.95, 1.73)	1.28 (0.95, 1.74)	1.26 (0.93, 1.70)
at age 3-7	1.17 (0.96, 1.42)	1.18 (0.97, 1.43)	1.14 (0.94, 1.39)
at age 8-12	0.94 (0.78, 1.12)	0.95 (0.79, 1.13)	0.91 (0.76 1.08)
at age 13-17	1.13 (0.99, 1.29)	**1.14 (1.00, 1.31)**	1.10 (0.96, 1.26)
Parity = 3, $n = 78467$			
no parental death before 18	REF	REF	REF
at age 0-2	1.47 (0.89, 2.44)	1.47 (0.89, 2.44)	1.44 (0.87, 2.39)
at age 3-7	1.19 (0.86, 1.66)	1.20 (0.86, 1.68)	1.17 (0.84, 1.63)
at age 8-12	1.09 (0.82, 1.46)	1.11 (0.83, 1.48)	1.04 (0.78, 1.40)
at age 13-17	**1.38 (1.10, 1.71)**	**1.40 (1.13, 1.75)**	**1.34 (1.07, 1.67)**
G2 men			
All G3 parities combined, $n = 760925$			
no parental death before 18	REF	REF	REF
at age 0-2	0.90 (0.74, 1.09)	0.90 (0.74, 1.09)	0.88 (0.73, 1.07)
at age 3-7	0.92 (0.82, 1.04)	0.92 (0.82, 1.04)	0.91 (0.81, 1.02)
at age 8-12	**1.12 (1.02, 1.22)**	**1.12 (1.03, 1.23)**	**1.10 (1.00, 1.20)**
at age 13-17	1.03 (0.95, 1.11)	1.04 (0.96, 1.12)	1.02 (0.94, 1.10)
Parity = 1, $n = 407678$			
no parental death before 18	REF	REF	REF
at age 0-2	0.97 (0.78, 1.21)	0.97 (0.78, 1.21)	0.97 (0.78, 1.20)
at age 3-7	0.92 (0.80, 1.06)	0.92 (0.80, 1.06)	0.91 (0.79, 1.05)
at age 8-12	**1.11 (1.00, 1.23)**	**1.12 (1.00, 1.24)**	1.10 (0.99, 1.22)
at age 13-17	1.03 (0.94, 1.13)	1.04 (0.95, 1.14)	1.03 (0.94, 1.13)
Parity = 2, $n = 274780$			
no parental death before 18	REF	REF	REF
at age 0-2	0.76 (0.52, 1.11)	0.76 (0.52, 1.11)	0.74 (0.51, 1.09)
at age 3-7	0.94 (0.76, 1.16)	0.94 (0.75, 1.16)	0.92 (0.74, 1.14)
at age 8-12	1.11 (0.94, 1.31)	1.11 (0.94, 1.31)	1.09 (0.92, 1.28)
at age 13-17	0.98 (0.85, 1.13)	0.98 (0.85, 1.13)	0.96 (0.83, 1.11)
Parity = 3, $n = 78467$			
no parental death before 18	REF	REF	REF
at age 0-2	0.77 (0.40, 1.50)	0.78 (0.40, 1.51)	0.75 (0.39, 1.46)
at age 3-7	0.88 (0.59, 1.30)	0.88 (0.59, 1.31)	0.87 (0.59, 1.29)
at age 8-12	1.20 (0.91, 1.59)	1.22 (0.92, 1.61)	1.18 (0.89, 1.56)
at age 13-17	1.18 (0.93, 1.49)	1.19 (0.95, 1.51)	1.17 (0.92, 1.48)

Model 1: unadjusted.

Model 2: controlled for confounders: age of G1 grandmother at G2 birth, social class of G1 grandfather in 1960, birth year of G2.

Model 3: controlled as Model 2 + mediators: achieved education of G2 in 1990, age of G2 when G3 is born.

* Emboldened entries mark OR-s where the CI does not include unity.

Table 2 gives odds ratios (ORs) for premature birth of offspring (G3) of men and women (G2) exposed to parental (G1) death, compared with offspring of non-exposed G2. Offspring of women who lost their parent at the age of 0-2 or at the age of 13-17 had an increased risk for prematurity, after control for confounding (model 2). In all age groups, for births with parity 3 the point estimates were higher than for parity 1 and 2.

Combining all parities, offspring of men who lost a parent at ages 8-12 had an increased risk of prematurity [OR = 1.12 with 95% confidence interval (CI) 1.03-1.23 in model 2]. The point estimates were highest for this age group within each parity group but the parity-specific associations were less precise, with confidence intervals including unity.

Table 3 gives estimated differences in birthweight between offspring (G3) of individuals (G2) exposed to parental (G1) death during childhood and offspring of non-exposed G2. For women exposed to a parent's death at age 0-2, there was no significant deficit in their offspring's birthweight in any parity class. For women exposed at later ages we observed a deficit in birthweight, largest in children born as parity 3, and similar in magnitude across ages-at-exposure.

Among children whose fathers experienced parental loss, there was little variation between parity groups. Experiencing parental death at ages 8-12 in particular, or at ages 13-17, but not at ages 0-2 or 3-7, did predict having lighter offspring (model 2). The offspring of men exposed to parental loss at ages 8-12 were around 25-30 g lighter at birth than other children; the birthweight deficit among those exposed at 13-17 was around 15 g (model 2).

Adjustments for confounders (model 2 in Tables 2 and 3) had minor effects (mainly reductions) on the estimated ORs and birthweight differences, but among boys exposed during their slow growth period, the birthweight deficit appears to grow on adjustment for confounders. Adjusting for G2 education and age (model 3) tended to reduce the ORs and estimated birthweight deficit, more so in women than in men, indicating the importance of G2 education for G2 women's offspring.

Contribution to inequalities in birthweight and prematurity

Table 4 displays differences in prematurity risk and birthweight in G3 by G1 social class and G2 educational achievement, after controlling for confounders (model 1). Controlling additionally for G2 exposure to parental loss had virtually no effect on these estimates (model 2). Thus, experiencing parental death during childhood does not seem important for the reproduction of social inequalities in birthweight and prematurity across generations. Family social background and parents' education mainly exercise their influence through other pathways.[24]

Discussion

Social, behavioural and natural scientists are puzzled by how the social world gets 'under the skin', or how social experience is 'embodied'.[25,26] The influence of previous generations can be conceptualized in many ways, traditionally either as social or cultural influences or as genetic ones. Genetic influences are not independent from those of the (social/cultural) environment; the latter may change gene expression.

Thus, the experiences of previous generations may influence later generations in ways that do not resemble Mendelian inheritance based on dominant and recessive genes. Gene expression is a more complex field than the study of polymorphisms and random mutations and their phenotypic correlates. The idea of transgenerational response tries to capture ancestral environmental influences, including those of the social environment, on human development, with[27,28] or without[4] reference to epigenetic modification of gene expression.

We were inspired by Pembrey and Bygren, who claimed to have 'proof of principle that a sex-specific male-line transgenerational effect exists in humans'.[18] We test their hypothesis in a much larger population, while at the same time trying to consider alternative, chiefly social, pathways.

We found that parental (G1) death during (G2) childhood predicts premature birth and lower birthweight in the offspring generation (G3). This response is dependent on G2 gender, G2 age at exposure and G3 parity, but not G3 gender. Parental death during childhood was not important for the reproduction of social inequalities in birthweight and premature birth.

Our analyses rely primarily on 'hard' exposure (parental death) and outcome (offspring prematurity; birthweight) data that were available in national registers. The Cause of Death Register and the Medical Birth Register are both virtually complete and of the highest quality. Data on social class and achieved education, derived from a census or the Swedish Register of Education, are routinely used in epidemiological studies. The Multigeneration Register allowed us to establish complete ancestral lineages. Its data structure, however, imposes a limitation. Any G2 death before 1991 (an unlikely event) will reduce the likelihood of tracing its G1 parents.[29] The loss of a G1-G2-G3 lineage will follow. This selection bias is likely to be small and conservative. A similar bias may be introduced by selecting only families with complete lineages G1-G2-G3.The large size of the G3 population should minimize random error in our results.

Table 3. Parental (G1) death during childhood of G2 and difference in offspring (G3) birthweight (in grams) by G2 age at parental loss and G3 parity; coefficients (95% CI) estimated by linear regression*

Age of G2 at parental death	Model 1	Model 2	Model 3
G2 women			
All G3 parities combined, $n = 760925$			
no parental death before 18	REF	REF	REF
at age 0-2	−8.0 (-34.1, 18.2)	−7.3 (-33.3, 18.8)	−0.0 (-25.9, 25.9)
at age 3-7	**-24.1 (-40.2, -8.0)**	**-23.2 (-39.3, -7.1)**	−15.6 (-31.7, 0.5)
at age 8-12	**-25.8 (-39.1, -12.4)**	**-24.9 (-38.2, -11.6)**	**-14.8 (-28.1, -1.5)**
at age 13-17	**-26.5 (-37.5, -15.5)**	**-26.0 (-37.0, -14.9)**	**-17.0 (-28.0, -6.0)**
Parity = 1, $n = 407678$			
no parental death before 18	REF	REF	REF
at age 0-2	−7.6 (-38.1, 22.8)	−6.9 (-37.3, 23.6)	−2.3 (-32.6, 28.0)
at age 3-7	−17.5 (-35.9, 1.0)	−16.8 (-35.3, 1.6)	−12.0 (-30.4, 6.4)
at age 8-12	**-27.5 (-42.6, -12.4)**	**-26.5 (-41.6, -11.5)**	**-20.9 (-35.9, -5.8)**
at age 13-17	**-28.7 (-41.2, -16.2)**	**-27.7 (-40.2, -15.1)**	**-22.2 (-34.7, -9.7)**
Parity = 2, $n = 274780$			
no parental death before 18	REF	REF	REF
at age 0-2	−2.6 (-37.7, 32.5)	−2.3 (-37.3, 32.7)	3.1 (-31.9, 38.0)
at age 3-7	**-31.3 (-52.8, -9.9)**	**-30.6 (-52.0, -9.2)**	**-23.6 (-44.9, -2.3)**
at age 8-12	**-32.4 (-49.9, -15.0)**	**-31.4 (-48.9, -14.0)**	**-21.3 (-38.7, -3.9)**
at age 13-17	**-23.0 (-37.7, -8.3)**	**-22.3 (-37.0, -7.6)**	−14.0 (-28.7, 0.6)
Parity = 3, $n = 78467$			
no parental death before 18	REF	REF	REF
at age 0-2	−38.5 (-102.4, 25.5)	−38.6 (-102.7, 25.5)	−33.7 (-97.0, 29.7)
at age 3-7	**-53.4 (-92.0, -14.9)**	**-53.3 (-92.0, -14.6)**	**-46.0 (-84.5, -7.4)**
at age 8-12	−23.7 (-56.0, 8.6)	−22.0 (-54.4, 10.4)	−7.1 (-39.4, 25.1)
at age 13-17	**-49.8 (-76.9, -22.7)**	**-50.2 (-77.3, -23.0)**	−38.1 (-65.0, -11.1)
G2 men			
All G3 parities combined, $n = 760925$			
no parental death before 18	REF	REF	REF
at age 0-2	10.3 (-14.4, 35.0)	7.7 (-17.0, 32.3)	13.4 (-11.2, 37.9)
at age 3-7	0.9 (-14.6, 16.4)	−1.2 (-16.7, 14.3)	4.7 (-10.7, 20.2)
at age 8-12	**-24.1 (-37.1, -11.1)**	**-26.4 (-39.4, -13.4)**	**-18.8 (-31.7, -5.9)**
at age 13-17	**-11.9 (-22.6, -1.2)**	**-15.1 (-25.8, -4.3)**	−8.2 (-18.9, 2.5)
Parity = 1, $n = 407678$			
no parental death before 18	REF	REF	REF
at age 0-2	8.9 (-18.6, 36.3)	7.6 (-19.9, 35.0)	10.8 (-16.5, 38.2)
at age 3-7	4.9 (-13.0, 22.8)	3.8 (-14.0, 21.7)	7.9 (-10.0, 25.7)
at age 8-12	**-23.7 (-38.6, -8.8)**	**-24.4 (-39.3, -9.6)**	**-19.2 (-34.0, -4.3)**
at age 13-17	**-12.6 (-24.9, -0.3)**	**-13.7 (-26.0, -1.4)**	−8.9 (-21.2, 3.5)
Parity = 2, $n = 274780$			
no parental death before 18	REF	REF	REF
at age 0-2	17.2 (-17.6, 52.1)	14.7 (-20.0, 49.5)	20.2 (-14.4, 54.8)
at age 3-7	−12.8 (-34.0, 8.4)	−14.1 (-35.3, 7.1)	−8.5 (-29.6, 12.6)
at age 8-12	**-27.7 (-45.6, -9.7)**	**-29.0 (-47.0, -11.1)**	**-22.9 (-40.8, -5.0)**
at age 13-17	**-15.0 (-29.6, -0.4)**	**-16.9 (-31.6, -2.2)**	−10.7 (-25.3, 4.0)
Parity = 3, $n = 78467$			
no parental death before 18	REF	REF	REF
at age 0-2	−15.6 (-75.5, 44.2)	−21.4 (-81.3, 38.5)	−13.3 (-73.1, 46.5)
at age 3-7	17.9 (-20.1, 55.9)	13.7 (-24.3, 51.7)	19.1 (-18.7, 57.0)
at age 8-12	−23.9 (-56.5, 8.7)	−29.3 (-61.8, 3.2)	−19.1 (-51.6, 13.3)
at age 13-17	−12.3 (-39.9, 15.2)	−18.0 (-45.6, 9.7)	−10.7 (-38.2, 16.7)

Model 1: unadjusted.

Model 2: controlled for confounders: age of G1 grandmother at G2 birth, social class of G1 grandfather in 1960, birth year of G2.

Model 3: controlled as Model 2 + mediators: achieved education of G2 in 1990, age of G2 when G3 is born.

* Emboldened entries mark coefficients where the CI does not include zero.

Table 4. Educational and social class differences in prematurity and birthweight in G3 ($n = 760325$) before (Model 1) and after (Model 2) accounting for G2 parental loss; OR (95% CI) from logistic regression, birthweight difference in grams (95% CI) from linear regression model

	OR for prematurity in G3		Birthweight difference in G3	
	Model 1	Model 2	Model 1	Model 2
Achieved education of G2				
G2 women				
Short	1.36 (1.30, 1.43)	1.36 (1.30, 1.42)	−89.2 (-95.3, -83.2)	−88.6 (-94.7, -82.5)
Medium	1.14 (1.10, 1.19)	1.14 (1.10, 1.19)	−36.7 (-41.5, -31.9)	−36.4 (-41.2, -31.6)
Long	REF	REF	REF	REF
Missing	1.40 (1.29, 1.51)	1.39 (1.29, 1.50)	−87.6 (-97.8, -77.3)	−87.1 (-97.3, -76.8)
G2 men				
Short	1.20 (1.15, 1.25)	1.20 (1.15, 1.25)	−66.7 (-72.2, -61.3)	−66.4 (-71.8, -61.0)
Medium	1.10 (1.06, 1.14)	1.10 (1.06, 1.14)	−30.5 (-34.9, -26.2)	−30.4 (-34.7, -26.0)
Long	REF	REF	REF	REF
Missing	1.24 (1.14, 1.34)	1.24 (1.14, 1.34)	−84.4 (-95.7, -73.2)	−84.1 (-95.3, -72.9)
Social class of G1				
G2 women				
Non-manual	REF	REF	REF	REF
Self-employed	1.01 (0.97, 1.06)	1.01 (0.97, 1.06)	0.3 (-5.4, 6.1)	0.5 (-5.2, 6.2)
Manual	1.08 (1.05, 1.12)	1.08 (1.05, 1.12)	−17.7 (-21.9, -13.6)	−17.5 (-21.7, -13.4)
Other	1.00 (0.96, 1.04)	1.00 (0.96, 1.04)	−0.8 (-6.1, 4.6)	−0.8 (-6.1, 4.6)
Missing	0.99 (0.94, 1.03)	0.99 (0.94, 1.03)	−41.2 (-47.6, -34.8)	−41.0 (-47.3, -34.6)
G2 men				
Non-manual	REF	REF	REF	REF
Self-employed	1.04 (1.00, 1.09)	1.04 (1.00, 1.09)	14.8 (9.2, 20.4)	14.9 (9.3, 20.5)
Manual	1.11 (1.08, 1.15)	1.11 (1.08, 1.15)	−13.0 (-16.9, -9.0)	−12.8 (-16.8, -8.9)
Other	1.05 (1.00, 1.09)	1.05 (1.00, 1.09)	−2.8 (-8.1, 2.5)	−2.8 (-8.0, 2.5)
Missing	1.07 (1.02, 1.12)	1.07 (1.02, 1.12)	−59.8 (-66.0, -53.6)	−59.6 (-65.8, -53.5)

Model 1: controlled for confounders: age of G1 grandmother at G2 birth, social class of G1 grandfather in 1960, birth year of G2.
Model 2: controlled as Model 1 + age of G2 at parental death.

G3 births span nearly three decades; those of their grandparents span five. Birth years of generations of the same family, and birth years of married couples, are correlated. By controlling for birth year of the intermediate generation, we placed the whole G1-G2-G3 family on the time axis and controlled for temporal trends (such as in birthweight and mortality) influencing all generations, while avoiding over-controlling.

In interpreting the results, the principal problem was to differentiate between potential pathways, in particular between broader life-course explanations and epigenetic and genetic explanations, as we had no information about behaviours and biological markers or genes.

We considered genetic pathways first. Could G1 deaths during G2 childhood be a marker of G1 genetic characteristics which also predict G3 birthweight? It is known that a child's birthweight may predict its parents' subsequent all-cause and cardiovascular mortality, with weak associations for fathers and stronger ones for mothers.[30,31] Smith *et al.* discussed whether this could reflect common underlying genetic factors and concluded that, at most, this was a partial explanation. They saw no obvious reason why any genetic influence should be weaker along the paternal pathway than the maternal and concluded[31] that the stronger association between offspring birthweight and subsequent mortality of mothers was consistent with intergenerational effects on intrauterine growth: continuity in birthweight from mothers to their children plus fetal programming of cardiovascular profile. In our case, birthweights of G2 women, and of their G3 offspring, are very unlikely to be genetically linked to G2 parental loss in childhood, since most G1 deaths were fathers rather than mothers and since spouse correlations in birthweight are very low (< 0.02).[32] Separating the analyses by whether a G1 death was male or female gave no indication of a genetically transmitted risk (data not shown).

For the paternal line, it seems unlikely that any common genetic influence behind G2 parental loss and G3 birth outcomes should be particularly pronounced in those G2 men who were exposed at ages 8-12. Sensitivity during this

exposure period is, however, compatible with the epigenetic hypothesis that we wanted to test.

Parental loss during childhood may influence a person through absence of attachment to one or both parents. Two large studies[8,9] based on Swedish data found it to predict later morbidity, such as depression, and behavioural problems, such as drug and alcohol use/abuse and crime, as well as smoking-related cancer and HPV infection. It has been linked to later life obesity[33] and poor educational achievement.[34,35] Negative economic consequences for the child's family are also likely, in particular from the loss of a father. Thus, parental loss appears to trigger a cascade of events, psychological, behavioural, metabolic and social.

These influence, in a broad way, the life course of G2 women and may therefore also modify the intrauterine environment experienced by their babies. We may see this as a socially-mediated, female-line transgenerational response, consistent with our results for exposed G2 women. It is a general response in that, unlike for boys, trauma during girls' slow growth period appears to be no more detrimental than during other periods. Repeated childbirths among the exposed G2 women may represent a cumulative burden, resulting in the observed high birthweight deficits and prematurity ORs in parity three births. The elevated risk of G3 prematurity was observed for G2 women exposed at ages 0-2 and 13-17. In animal studies, maternal and grandmaternal stress in pregnancy, but not at later ages, has been linked to epigenetic programming of offspring prematurity.[36]

Parental loss is equally likely to influence the life course of G2 men but much less likely to directly influence the intrauterine environment of the children they father. In our data, the association of G2 men's parental loss with birthweight and prematurity in the next generation seems weaker, less general and not modified by parity. The finding that trauma during boys' slow growth period is linked to offspring birthweight deficit and prematurity in all models, therefore suggests a more specific pathway. Two recent studies of mice, one of which was inspired by the Pembrey-Bygren hypothesis, do indeed suggest that early trauma could modify sperm microRNA, with consequences for gene expression in subsequent generations, although the researchers could not separate the slow growth period from the rest of puberty.[14,37] Whether our finding is in fact epigenetic, carried via the male germ line as suggested by Pembrey-Bygren, we cannot say. However, it seems entirely possible that this could be the case.

Animal studies suggest that early trauma can influence gene expression in later generations, via the male germ line. There are so far very few human population studies addressing this issue. Our study fails to refute the hypothesis that a male-line epigenetic mechanism exists which may be triggered by trauma during boys' slow growth period. If ancestral trauma (psychological, nutritional or economic) can influence later generations' health and development in such a way, this will change the way we think of disease causation and prevention and will inspire new studies. It would highlight the long-term importance of historical events, such as wars and famines, and cast light on a new mechanism through which these may play a role.

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Conflict of interest

The authors have no conflict of interest.

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