Environmental influences on childhood cancer risk: an umbrella review

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Abstract

Aim: To explore the validity and strength of evidence on the association between environmental factors and risk of developing childhood (0-14 years) cancer. **Methods:** An umbrella review was conducted including systematic reviews and meta-analyses of observational epidemiological studies that examined the association of any environmental exposure of either parent or child with any type of childhood cancer. PubMed and Scopus databases were searched until April 2020. Based on predefined criteria, the evidence was graded into strong, highly suggestive, suggestive or weak. **Results:** 509 meta-analyses explored environmental exposures and risk of developing 10 different types of childhood cancer. Only 2.4% of the associations were considered to have highly suggestive evidence inferred by strongly statistically significant results. These associations were confined to increased risk of overall leukemia, especially acute lymphoblastic leukemia (ALL), in relation to high birthweight, paternal smoking and exposure to pesticides, particularly insecticides. By contrast, maternal multivitamin supplementation during pregnancy (summary odds ratio [OR]: 0.64, 95% confidence intervals [CI]: 0.52, 0.80) and breastfeeding for more than 6 months (summary OR: 0.76, 95%CI: 0.68, 0.84) were supported by highly suggestive evidence for decreased risk of ALL. There was also highly suggestive evidence for increased risk of central nervous system (CNS) tumors, especially astrocytoma, in relation to high birthweight, as well as increased risk of testicular cancer in relation to testicular microlithiasis and isolated cryptorchidism. **Conclusions:** The present findings provide evidence that exposure to seven maternal/neonatal factors significantly affects the risk of childhood leukemia, CNS tumors and testicular cancer. Further evidence from sufficiently powered studies and large consortia with uniform reporting of analyses is needed to allow firmer conclusions to be drawn.

INTRODUCTION

Cancer is the leading cause of death in children and adolescents worldwide . Every year, just above 150,000 children are diagnosed with cancer . Since a large proportion of childhood cancers in low- and middle-income countries are never diagnosed, the more realistic annual number is estimated to be at least twice as high, i.e. above 360,000 children . Given these caveats, the age-standardized incidence (ASR) of the disease, estimated at 140.6 per million person-years in children aged 0-14 years, is increasing with leukemia being the most common cancer type (ASR: 46.4) followed by central nervous system (CNS) tumors (ASR: 28.2)

and lymphomas (ASR: 15.2) . Due to striking diagnostic and therapeutic advances, the number of deaths from childhood cancer has decreased by more than 50% between 1975 and 2017. Despite the high cure rates, current therapeutic modalities remain traumatic for young patients and their families with significant long-term complications. The question at stake is to what extent childhood cancer is a preventable disease that can be spearheaded as an utmost priority .

Current etiological speculations on childhood cancer involve a complex interplay between genetic factors, epigenetics and environmental influences , but there is so far little to no evidence of it. Among environmental factors, adaptation to westernized lifestyle patterns could, among others, explain the increasing prevalence of childhood cancer . There is a wealth of studies examining environmental and other non-genetic factors in relation to childhood cancer risk; yet, aside from the 10-15% of children with high-penetrance germline variation, the causes of the disease are not definitively known . Evidence is mainly compromised by specificity of exposure measurements, underpowered original research, selection bias in participation-based case-control studies, residual confounding and selective reporting of positive results, whereas systematic reviews and meta-analyses are often hampered by significant between-study heterogeneity . Thus, though some reported associations may be causal for some exposure measures, they are flawed owing to inherent biases that exaggerate their effect on cancer incidence.

Umbrella reviews have recently come to be conducted with the aim of systematic appraisal of the evidence on an entire topic across many meta-analyses of multiple putative risk factors on multiple outcomes . To our knowledge, no umbrella review has been published so far to summarize the existing evidence, appraise its quality and provide decision makers with the available, highest level of evidence relevant to the association of environmental influences on childhood cancer risk. Specifically, we performed an umbrella review of systematic reviews and meta-analyses that investigated the association between a series of environmental risk factors and the development of different types of childhood cancer aiming to explore the validity and strength of evidence, as well as potential biases and limitations of published literature.

METHODS

Literature search

The present umbrella review of systematic reviews and meta-analyses was based on a predefined research protocol. It was conducted in accordance with the preferred reporting items for overviews of systematic reviews (PRIO; Supplementary Table 1) .

We searched PubMed and Scopus databases until April 23 2020 for systematic reviews and meta-analyses on the association of any environmental risk factor and any type of childhood cancer employing a predefined search algorithm (Supplementary Material). No language or other restriction criteria were applied. Two independent investigators (MK and GM) examined the titles, abstracts and full texts of the shortlisted meta-analyses and discrepancies were resolved by consensus. We further hand searched the references of the retrieved systematic reviews and meta-analyses for systematic reviews/meta-analyses potentially missed by the initial electronic search and unpublished data.

Eligibility criteria

Eligible articles included systematic reviews and meta-analyses of observational epidemiological studies that examined the association of potential risk factors with childhood cancer types. Exposure variables included environmental exposures of either parent (mother and/or father) or child during preconception, pregnancy, delivery or childhood. Outcome variables included any type of cancer diagnosed in children aged 0-14 years. Studies on broader age groups which included sub-analyses on children 0-14 years were also eligible. Meta-analyses that solely examined the association of genetic or epigenetic factors with childhood cancer risk were excluded. Likewise, meta-analyses that investigated cancer survival or other outcomes among patients with cancer, as well as meta-analyses not reporting comprehensive study-specific information, such as effect sizes, 95% confidence intervals (CI) or sample sizes were also excluded. When one or more eligible systematic reviews and/or meta-analyses on the same research question were identified, the one with the largest number

of component studies, usually the most recently published study, was included. Each identified study was cross-checked for each quality and issues of overlap before including in the final set of eligible studies.

Data extraction

Data extraction was performed at a meta-analysis and individual study level. At a meta-analysis level, we extracted information on first author, year of publication, exposure, outcome, window of exposure (preconception, pregnancy, delivery or childhood), number of studies, effect size and 95% CI, level of adjustment and model of analysis (fixed effects or random effects). At an individual study level, we extracted information on first author, year of publication, epidemiological design, number of cases and total sample size in case-control studies or total population in cohort studies, maximally adjusted effect size and 95% CI.

The data extraction database included study quality indicators, in particular elements from the 11-point AMSTAR measurement tool to assess the methodological quality of systematic reviews. AMSTAR is considered a reliable and valid tool for quality assessment of systematic reviews and meta-analyses of both interventional and observational research . Based on the AMSTAR tool, the study quality was categorized into low (0-3 points), moderate (4-7 points), and high (8-11 points). Two investigators (CT, AK) independently performed the data extraction and quality assessment; disagreements were resolved by discussion with a third investigator (MK).

Data synthesis and analysis

1. Summary effect estimates and 95% CI for each association between environmental factors and childhood cancer risk were calculated through fixed and random effects models .

2. Heterogeneity was assessed with the Cochran's Q test and the I^2 statistic (ranging from 0% to 100%), defined as the ratio of between-study variance over the sum of the within-study and between-study variances . We further calculated the 95% CIs to assess the uncertainty around heterogeneity estimates .

3. Ninety-five percent prediction intervals for the summary random effect estimates were calculated to further assess heterogeneity and to estimate the effect that would be expected in future studies investigating the same association .

4. Small study effects, namely whether smaller studies tend to contribute higher effect estimates compared to larger studies were also examined; such differences between small and large studies may indicate publication bias or other reporting biases, genuine heterogeneity or chance . To account for small study effects, we used the Egger's regression asymmetry test (p [?]0.10) and we also assessed whether the random effects summary estimate was larger than the point estimate of the largest-most precise study, namely the study with the smallest standard error included in each meta-analysis.

5. Excess significance bias (set for individual meta-analyses at$p$ [?]0.10) were assessed exploring whether the observed number of studies with nominally statistically significant results ("positive" studies, p <0.05) within each meta-analysis was greater than the expected number of studies with statistically significant results. Specifically, we calculated the expected number of statistically significant studies in each meta-analysis from the sum of the statistical power estimates for each component study using an algorithm from a non-central t distribution . The power estimates of each component study depend on the plausible effect size for the tested association, which was assumed to be the smallest standard error, namely the effect of the largest study in each meta-analysis .

Grading the evidence

Based on the strength and validity of evidence, the associations between environmental risk factors and childhood cancer were classified into strong, highly suggestive, suggestive and weak. A strong association was indicated, when the p -value of the random-effects meta-analysis was below 10^{-6}, the number of cancer cases was greater than 1000 to significantly reduce false positive findings, the largest study in meta-analysis was nominally statistically significant (p<0.05), heterogeneity was low to moderate (I^2 <50%), there was no indication of small study effect or excess significance bias, and the 95% prediction intervals excluded the

null value. A highly suggestive association was claimed, if the p -value of the random-effects meta-analysis was below 10^{-6}, the number of cancer cases was greater than 1000, and the largest study in meta-analysis was nominally statistically significant (p <0.05). The criteria for a suggestive association were fulfilled if the p -value of the random-effects meta-analysis was below 10^{-3}, and the number of cancer cases was greater than 1000. All other nominally statistically significant associations (p <0.05) were considered to have weak evidence.

The primary analysis in this umbrella review focused of all studies included in each meta-analysis. Sensitivity analyses were conducted including only the meta-analyses with four or more cohort or nested case-control studies. Analyses were performed using Stata version 14 (College Station, TX) and all p -values were two tailed at a 5% significance level.

Patient involvement

No patients were involved in setting the research question or outcome measures, nor were they involved in developing plans for design or implementation of the study. No patients were asked to advise on interpretation or writing up on results.

RESULTS

Study characteristics

The systematic search process yielded 117 eligible studies (Figure 1) that included 509 environmental factor- and childhood cancer risk- specific meta-analyses. A total of 107 studies were excluded, among which duplicate studies, namely duplicate meta-analyses on the same exposure and outcome pair were identified for 10 associations (Supplementary Table 2). The 117 eligible systematic reviews and meta-analyses summarized evidence from 3873 individual study estimates stemming from 1140 primary studies. The vast majority (84.6%) of original studies were of case-control design (n=964), and the remaining 15.4% were cohort studies (n=176).

The 509 individual meta-analyses of the 117 eligible studies examined associations between environmental factors and 10 different cancer types (all cancer sites, hematological malignancies including overall acute leukemia, acute lymphoblastic [ALL] and acute myeloid [AML] leukemia, CNS tumors, neural tumors/neuroblastoma, sarcomas, bone tumors, retinoblastoma, testicular tumors, Wilms tumors and other solid tumors). The majority of studied outcomes were childhood hematological malignancies (n=374 associations, 73.5%), followed by CNS tumors (n=91 associations, 17.9%) and neural tumors/neuroblastoma (n=18 associations, 3.5%; Table 1). The potential risk factors were assessed during all relevant exposure windows, namely during preconception, pregnancy, delivery and childhood. A median of 11 (range 2-39) study estimates were combined for each meta-analysis. The average number of cancer cases in each meta-analysis was 3688 (range 19-31,610; Table 1). Around two-thirds of the 509 meta-analytical associations (62.9%) were assessed through random effects models, whereas 23.8% of these associations were evaluated only through fixed effects models.

Study quality

Based on the AMSTAR quality assessment tool, the quality of the eligible systematic reviews and meta-analyses ranged from 2 to 10 points, with a median of 5 points (Supplementary Table 3). Most of the included meta-analyses had moderate (n=59, 50.4%) or high (n=34, 29.1%) quality, while the remaining 24 (20.5%) meta-analyses had low quality.

Summary effect size

The summary fixed effects estimates were significant in 257 of 509 meta-analyses (50%), while the summary random effects estimates were significant in 207 associations (41%) at a threshold of$p$ <0.05 (Supplementary Table 4). At a stricter threshold (p <0.001), 114 (22%) and 77 (15%) meta-analyses were statistically significant using fixed and random effects models, respectively. When the p -value was set at 10^{-6}, 43 (8%) fixed effects estimates and 21 (4%) random effects estimates were statistically significant.

Of the 21 statistically significant summary random effect sizes at$p$ <10^{-6} (Supplementary Table 4), 13 showed a significant association between high birthweight, paternal smoking, maternal alcohol consumption, exposure to benzene, rural population mixing, as well as residential and occupational exposure to pesticides, especially insecticides and childhood leukemia risk. The magnitude of these effect sizes ranged between 1.19 for high birthweight and 3.30 for maternal occupational pesticide exposure. In addition, four summary random effects estimates found a decreased risk for childhood leukemia at p <10^{-6} in relation to maternal dietary vitamin intake during pregnancy (summary OR: 0.81, 95%CI: 0.74, 0.88), maternal multivitamin supplementation during pregnancy (summary OR: 0.64, 95%CI: 0.52, 0.80), breastfeeding for more than 6 months (summary OR: 0.76, 95%CI: 0.68, 0.84), and high maternal education (summary OR: 0.81, 95%CI: 0.76, 0.87). The remaining 4 out of 21 significant effect sizes found an increased risk for CNS tumors, and especially astrocytomas in relation to high birthweight, as well as for testicular cancer in relation to testicular microlithiasis and isolated cryptorchidism.

The largest study in each meta-analysis was statistically significant in 186 of 509 associations (37%), albeit the effect sizes of the largest studies were in general more conservative than the respective summary random effects estimates (Supplementary Table 4).

Between-study heterogeneity

Between-study heterogeneity ranged from 0 to 98%, and was statistically significant (Q test, p <0.10) in 164 meta-analyses (32%), of which the vast majority (83%) examined the risk of childhood hematological malignancies (Supplementary Table 5). A total of 105 meta-analyses (21%) showed considerable heterogeneity (I^2=50-75%). Substantial heterogeneity (I^2>75%) was found in 29 associations (6%) which examined different risk factors in relation to childhood hematological malignancies, CNS tumors, neuroblastoma and testicular cancer. To further assess the uncertainty of effect estimates, we calculated the 95% prediction intervals, which included the null value in most associations (n=388, 76%).

Small study effects and excess significance bias

Indication for small study effects was evident in 65 meta-analyses (13%) based on the Egger's regression asymmetry test (p [?]0.10), of which only 29 meta-analyses included 10 or more original studies, namely enough power for the Egger's test to identify the presence of small study effects (Supplementary Table 5). The estimation of small study effects was not feasible in 68 meta-analyses (13%) due to the small number of included studies (n=2).

Thirty-seven meta-analyses (7%) had evidence of excess significance bias based on the largest study effect size as the plausible effect size. Of these 37 studies, 29 examined the risk of hematological malignancies and the remaining 8 studies the risk of CNS tumors in relation to various environmental exposures during preconception, pregnancy or childhood (Supplementary Table 5).

Grading the evidence

We graded the evidence regarding the association of environmental risk factors and childhood cancer accounting for the above criteria, namely the p -value of the significant associations, the presence and extent of heterogeneity, as well as the presence of small study effects and excess significance bias (Table 2). Overall, 40% of the 509 meta-analyses were nominally statistically significant, and were thereafter evaluated for strong, highly suggestive, suggestive or weak evidence. A hundred sixty-six of the 509 reported meta-analyses (32.5%) presented weak evidence (p <0.05 for the summary random effects).

We found no association supported by strong evidence. Of note is that the association of isolated cryptorchidism with testicular cancer fulfilled all criteria of strong evidence with the exception of considerable heterogeneity (I^2>50%), and was thus considered as highly suggestive (Table 2).

Overall, 12 meta-analyses (2.4%) were supported by highly suggestive evidence (Figure 2). Among these meta-analyses, two showed a decreased risk, by approximately 20%, of ALL in relation to maternal vitamin supplementation during pregnancy (summary OR: 0.81, 95% CI: 0.74-0.88) and breastfeeding for more than 6

months (summary OR: 0.76, 95% CI: 0.68-0.84). Four meta-analyses supported by highly suggestive evidence found an increased risk for overall leukemia, especially ALL, in relation to paternal smoking during pregnancy , as well as residential exposure to pesticides, especially insecticides during pregnancy or childhood . There was also highly suggestive evidence for the association between high or increased birthweight and overall leukemia, particularly ALL . The remaining 4 out of 12 highly suggestive associations showed increased risk, by 14-22%, for childhood CNS tumors, and especially astrocytoma in relation to high birthweight (>4000 grams), as well as increased risk for testicular cancer in relation to testicular microlithiasis (summary OR: 15.46, 95% CI: 6.93-34.47) and isolated cryptorchidism (summary OR: 2.90, 95% CI: 2.21-3.82; Figure 2).

A total of 26 meta-analyses (5.1%) were supported by suggestive evidence (Figure 2). Three associations showed a significant inverse association of maternal folic acid supplementation during pregnancy and daycare attendance with ALL, as well as between breastfeeding for more than 6 months and overall acute leukemia. The remaining 23 associations showed increased risk for childhood hematological malignancies related to various exposures (use of assisted reproductive technologies, alcohol and coffee consumption during pregnancy, home exposure to pesticides, especially herbicides and insecticides during childhood, high exposure to traffic density, high benzene exposure, petrol station/repair garage proximity, fetal loss history, paternal ever smoking, birthweight increase and preterm birth) , as well as increased risk for CNS tumors in relation to birthweight increase and indoor pesticide exposure during preconception or childhood .

Sensitivity analyses including only systematic reviews and meta-analyses with [?]4 cohort or nested-case-control studies (n=28 meta-analyses) yielded statistically significant results for nine (32.1%) associations which were all supported by weak evidence. These associations concerned medically assisted reproduction, high birthweight and advanced paternal age in relation to increased risk of hematological and other solid cancers (results not shown).

DISCUSSION

Principal findings

The present large-scale umbrella review explored the strength and validity of evidence in 509 meta-analyses of environmental exposures and risk of developing 10 different types of childhood cancer. None of the 509 meta-analyses were supported by strong evidence, whereas only 2.4% of the associations were considered to have highly suggestive evidence inferred by strongly statistically significant results. These associations were mainly confined to increased risk of overall leukemia, especially ALL, in relation to high birthweight, paternal smoking and exposure to pesticides, particularly insecticides, as well as decreased risk of ALL related to maternal vitamin supplementation and breastfeeding (>6 months). There was also highly suggestive evidence for increased risk of CNS tumors, and especially astrocytoma in relation to high birthweight, as well as increased risk of testicular cancer in relation to testicular microlithiasis and isolated cryptorchidism.

Despite the abundance of published evidence on environmental exposures and childhood cancer risk, 40% of the included meta-analyses reported a nominally statistically significant summary effect estimate. Moreover, though the reported associations seem biologically plausible, our study showed that inherent biases might overestimate the suggested associations, which is consistent with the results of previous umbrella reviews on cancer epidemiology . When decreasing the threshold at $p < 10^{-6}$, only 4% of the summary random effects estimates remained statistically significant. Considerable or substantial heterogeneity (>50%) was reported in 26% of meta-analyses, albeit the 95% prediction intervals which further account for heterogeneity included the null value in most associations (76%). There was indication for small study effects in 13% of the meta-analyses, while 7% of the associations had evidence of excess significance bias. Lastly, most of the included meta-analyses had a moderate- and high-quality rating based on the AMSTAR quality assessment tool.

Previous literature

Our study aimed to address the open question whether childhood cancer is a preventable disease by implementing interventions on candidate risk factors . Our results are in line with those reported by recent studies and support the hypothesis that the origins of childhood cancer seem to be influenced not only by

genetics, but also by the perturbation of the normal developmental processes following exposure to exogenous stressors . Currently, no primary preventive measures for childhood cancer have been established. The main reasons include the lack of causative determination on the level of available evidence, the lack of determination of the effectiveness of interventions at population level, as well as our current inability to specify the hazardous conditions in terms of exposure level . Acknowledging the challenges in determining causation and in quantifying the need for preventive measures to be undertaken in the field of environmental health, we aimed to stringently appraise the validity of evidence and potential biases in the context of an umbrella review, namely a critical integration of evidence stemming from currently published systematic reviews and meta-analyses. Our grading of evidence largely conforms to systematic analyses of the literature performed by the World Cancer Research Fund (WCRF) and the International Agency for Research on Cancer (IARC), as well as to the protocols proposed by the US Preventive Services Task Force (USPSTF) .

A strong level of evidence was not supported by the present study primarily due to the presence of biases (i.e. significant between-study heterogeneity). However, beyond these limitations, our results provided highly suggestive evidence for some specific and potentially preventable risk factors of childhood cancer, which supports those reported by recently published studies, systematic reviews and pooled analyses from large consortia .

Birthweight

Birthweight is one of the most commonly studied perinatal risk factor of childhood cancer. High birthweight as a proxy of fetal macrosomia has been found to increase the risk of several cancers, including leukemia, CNS tumors, neuroblastoma, colorectal and breast cancer. Both genetics/epigenetics and environmental factors have been proposed to underlie these associations, whereas growth factors (IGF-1 and IGF-2) seem to play a crucial role in carcinogenesis by stimulating mitogenesis and cell cycle progression, while inhibiting apoptosis. Thus, IGFs can lead to an increase in the number of hematopoietic stem cells and to the promotion of malignant transformation of some of them, which may subsequently expand to pre-leukemic and then to overt leukemic. Moreover, it is well known that fetal macrosomia is associated with increased rates of cesarean delivery. An increased risk of leukemia in children born by cesarean section has been reported after controlling for birthweight, which has been attributed to altered microbiota colonization or/and to the lack of increased cortisol levels that could eliminate preleukemic and leukemic cells (the "adrenal hypothesis"). Furthermore, given the global increase in the incidence of obesity and the effect of overweight/obesity during pregnancy on high birthweight, the potential association between fetal macrosomia and childhood cancer risk merits further investigation. It would be interesting to explore whether primary prevention of overweight/obesity may indirectly reduce the incidence of childhood cancer.

Pesticides

IARC has classified different pesticides as definite, probable or possible carcinogens to humans. Studies in experimental animals, in vitro test systems and humans occupationally exposed to pesticides have shown DNA damage (oxidative DNA damage, DNA strand breaks) or chromosomal damage (micronuclei), indicating the potential genotoxicity of these chemicals even though regulatory genetic toxicity tests are generally negative. The biological plausibility of the intrauterine exposure to pesticides lies in their effect on oxidative stress, which may cause DNA single- and double-strand breaks in fetal hematopoietic stem and progenitor cells at specific cleavage sites. These lesions, if mis-repaired may lead to chromosomal translocations involved in the regulation of early hematopoiesis. More recent studies on epigenetics have also shown that pesticide exposure during the critical period of pregnancy may detrimentally alter the epigenome and gene expression profile of stem cells, positioning these cells for malignant transformation. Our results are in line with recent systematic reviews and meta-analyses which have shown that pesticide, especially insecticide exposure during pregnancy increases the risk of childhood leukemia, particularly among infants. Yet, we should acknowledge that pesticides have mainly been studied as groups, sometimes divided into insecticides, herbicides and fungicides, but still lumping chemically distinct active ingredients and formulations, which likely range from harmful to harmless. More research is needed to identify those products within pesticides that are associated with an increased risk of different cancer types.

Tobacco smoking

Smoking is one of the best-established carcinogenic hazards. Of note is that previous studies on childhood cancer epidemiology have mostly shown null associations between maternal smoking and risk of childhood cancer, which is in line with the results of the present umbrella review. By contrast, paternal smoking during pregnancy has been more consistently reported as potential risk factor of childhood cancer, especially ALL, which was also supported by the herein results; the magnitude of this effect was calculated at 1.20 summary random effects estimate. Our study also found suggestive evidence for paternal ever smoking, even before pregnancy in relation to childhood leukemia. IARC considered the evidence from different studies as sufficient to suggest a causal link between paternal smoking and childhood cancers. Exposure to mutagenic polycyclic aromatic compounds from tobacco smoke can increase the formation of DNA adducts in sperm. Smoking also induces the generation of reactive oxygen species and reduces levels of antioxidant cellular defenses, thus contributing to oxidative stress and DNA damage. Oxidative DNA damage is not randomly distributed in mature human spermatozoa but occurs preferentially in unpackaged, protein-free regions of the genome in close proximity to the nuclear membrane, which are especially vulnerable to oxidative stress. Overall, tobacco smoke may cause genetic changes through the germline that may make children more susceptible to developing cancer[NO_PRINTED_FORM]. Indeed, paternal smoking may contribute up to 1.3 million extra cases of aneuploid pregnancies per generation due to smoking-induced *de novo* germline mutations transmitted from fathers to offspring. In addition, recent epigenome-wide association studies (EWASs) suggest that tobacco affects the genomic DNA methylation profiles. Smoking-related cytosine-phosphate-guanine (CpG) sites have been identified in various genes, such as *AHRR* , *F2RL3* and *GPR15* , which could be used as predictors of smoking-related health risks. Tobacco-related products may induce specific differences in the spermatozoal microRNA content, which subsequently mediate pathways vital for healthy sperm and normal embryo development, particularly cell death and apoptosis. Smoking may also alter genomic imprinting due to DNA hypomethylation and reduce sperm cytosine methyl transferase messenger RNA levels, which may, in turn, lead to the expression of normally silent paternal alleles.

Vitamin supplementation and breastfeeding

Previous studies have shown a protective role of maternal supplementation with B-vitamins and folic acid for childhood cancers, such as ALL, neuroblastoma, CNS tumors and germ cell tumors. Folate is important for cell division because of its role in de novo purine and pyrimidine synthesis, and also in the DNA repair mechanism. The rapid turnover of cancer cells entails greater DNA synthesis which, in turn, increases folate requirements to maintain this high rate of cell proliferation. Folate and other B-vitamins involved in 1-carbon metabolism are essential for the high-fidelity synthesis of DNA and activated methyl groups that are required for DNA methylation and regulation of chromatin structure. Genetic mouse models have shown that impaired 1-carbon metabolism interferes with genome integrity, which might explain the folate- and vitamin-related pathologies including the risk of childhood cancer, especially leukemia. In the present study, maternal vitamin supplementation during pregnancy was inversely associated with decreased risk, by around 20%, of childhood ALL, which is congruent with the results of large studies and the general recommendations that a healthy maternal diet around conception and early pregnancy based on the intake of B-vitamins (B9, B6, B12) is protective. Consistent with other studies, the present results also showed a decreased risk, by 20%, of ALL in children breastfed for more than 6 months. Breast milk contains immunologically active components, exosomes and exosomal RNAs, as well as anti-inflammatory defense mechanisms that influence the development and maturation of the immune system. It also contains antibodies that have a prebiotic effect and promote a more favorable infant gut microbiome. The more mature immune system of breastfed infants involves a greater abundance of natural-killer and stem cells compared to formula-fed infants. Encouraging breastfeeding into clinical practice seems to be a cost-efficient and beneficial public health measure.

Testicular conditions

Testicular microlithiasis and cryptorchidism have both been associated with increased risk of testicular cancer, which is in line with the highly suggestive evidence stemming from the present study. The majority of preadolescent boys with testicular microlithiasis and cancer have other predisposing conditions, such as

cryptorchidism. Testicular microlithiasis is a relatively uncommon condition characterized by the presence of calcifications in the testicular parenchyma, possibly due to dysgenesis of the testis with slough of degenerated cells inside an obstructed seminiferous tubule. The mechanisms that may explain how these calcifications may be involved in the pathogenesis of testicular cancer remain unclear; in particular, it is far less known whether and when a patient with microlithiasis would develop testicular cancer. It is also possible that testicular microlithiasis may be associated with rather than being a risk for future development of testicular tumor, because the follow-up of patients with incidental microlithiasis have shown a low risk of developing testicular cancer. Despite this debate, our results are in line with previous studies supporting the general recommendations of regular screening for testicular cancer in case of testicular microlithiasis. Although the association between cryptorchidism and testicular cancer has been well-studied, the underlying mechanisms remain poorly understood. Cryptorchidism is one of the strongest risk factors for infertility and testicular germ cell tumors. Although corrective surgery largely reduces the risk of this tumor, in some cases the affected testis becomes cancerous. This finding is suggestive of permanent epigenetic changes as differences in promoter methylations and corresponding gene expression of several genes have been reported in testicular germ cell tumors. Gene expression in cryptorchid testes and animal models has shown deregulation of growth factors important for the balance of self-renewal and the proliferation of germ cells . The open question is whether the risk of malignant malformation is the result of a genetic predisposition or is due to the maldescent testis, which is more prone to dysplasia and malformation. In cases of isolated cryptorchidism, the 10% increased risk of developing cancer in the contralateral normally descended testis has provided indications that genetic factors may also play significant role by inducing aberrant gonadocyte development in fetal life.

Methodological considerations

We acknowledge that the present study integrated data from different systematic reviews and meta-analyses that used non-uniform literature search criteria and different analytical approaches; thus, the potential of missing some studies cannot be excluded. In addition, an inherent limitation of the present umbrella review, as of any review, is that it is liable to become out of date almost as soon as it is finished; of note is that eight systematic reviews and meta-analyses have been published after the end of the literature search up to the submission of the present study . To overcome this limitation, we are planning to develop our work to a living umbrella review. Moreover, summarizing the evidence from large pooled analyses and several large cohort studies on environmental risk factors and childhood cancer was beyond the scope of the present umbrella review, which integrated data only on systematic reviews and meta-analyses. The combination of systematic reviews, meta-analyses, pooled analyses and cohort studies under the scope of a future "umbrella review" is being planned. In addition, though we included all study-specific associations reported in each meta-analysis, we have missed some additional subgroup meta-analyses due to insufficient study-specific estimates. Of note is that age-specific or sex-specific subgroup meta-analyses (i.e. sub-analyses on infant leukemia) were not retrieved due to the lack of study-specific effect estimates. The only available age-specific meta-analyses examined the association of environmental risk factors with early-onset leukemia (<5 years) and CNS tumors (<10 years). Thus, we could not further address at this stage the potentially differential effect of these factors in males and females, or the differential effect of these factors on infant leukemia, which is a distinct disease entity. Moreover, the assessment of the quality of the primary studies included in each meta-analysis was beyond the scope of this study; however, the AMSTAR tool allowed us to assess some trajectories of the quality of primary studies. However, though the fully adjusted meta-analysis-specific effect estimates were used whenever available, some meta-analyses were based on unadjusted primary study-specific estimates; the definition of the fully adjusted model also varied greatly from study to study and from exposure to exposure. Thus, issues of confounding may have hampered the results presented herein. In addition, owing to the rarity of childhood cancer, primary cohort studies represented a minority of this literature with case-control studies accounting for the overwhelming majority of primary studies included in each meta-analysis. Case-control evidence is prone to selection bias and is thus less robust to provide support to the causality of associations. Indeed, sensitivity analyses including only cohort or nested case-control studies provided weak level of evidence about specific exposures and childhood cancer risk. Moreover, though we examined the presence and extent of biases, the statistical tests implemented were not definitive in determining the

exact source of these biases . Lastly, some inherent limitations of these tests, i.e., the possibility of false-positive results from the Egger's test, should be taken account, although the effect magnitude is usually not substantial and the primary studies are not randomized controlled trials of interventions.

Conclusions

The association of environmental factors and childhood cancer risk has been extensively studied, albeit yielding inconclusive so far results. Our exhaustive literature search on these factors and risk of 10 different childhood cancer types showed that only half of the reported associations reached a nominally statistical significance level, while only 2.4% of published meta-analyses were supported by highly suggestive evidence. Beyond any limitations and biases that may affect the summary effect estimates, the present findings, supported by mechanistic information, provide highly suggestive evidence that exposure to seven maternal/neonatal factors (including prenatal exposure to environmental chemicals or intake of B-vitamins) significantly affects -either increases or decreases- the risk of childhood leukemia, CNS tumors and testicular cancer. Considerable uncertainty remains for other stressors and outcomes. Further evidence from sufficiently powered studies and large consortia with uniform reporting of analyses is needed to allow firmer conclusions to be drawn. Given the increasing trend in the westernization of habits, evidence of the strength of the associations between lifestyle influences and childhood cancer may allow finer identification of people at high risk, who could be selected for individual-based primary prevention strategies.

Contributors : MK and EEN conceived and designed the study. MK, GM, CT, AK, XT and EEN acquired and collected the data. MK and GM analyzed the data. MK drafted the initial version of the manuscript. GM, CT, AK, XT, KT, LG, JS, A-BH, TS, AH, EP and EEN drafted and critically revised the manuscript for important intellectual content and gave final approval of the version to be published. EEN is the guarantor.

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Competing Financial Interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: Not required.

Data sharing: No additional data available.

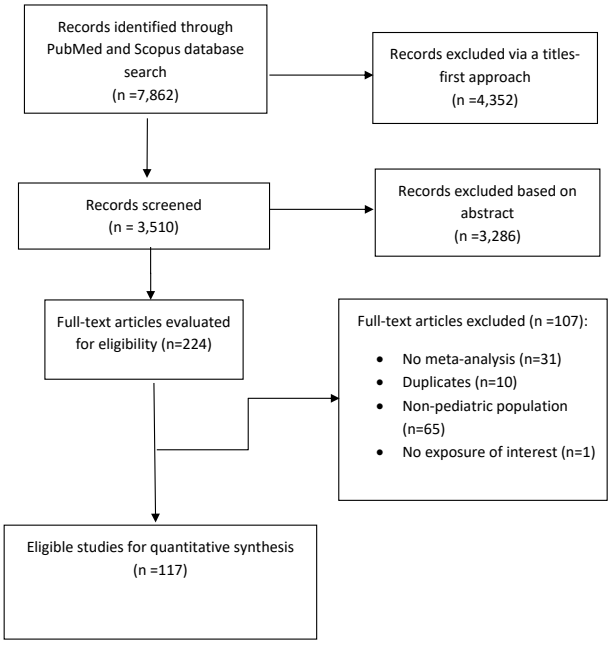
Transparency : The lead author (MK) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

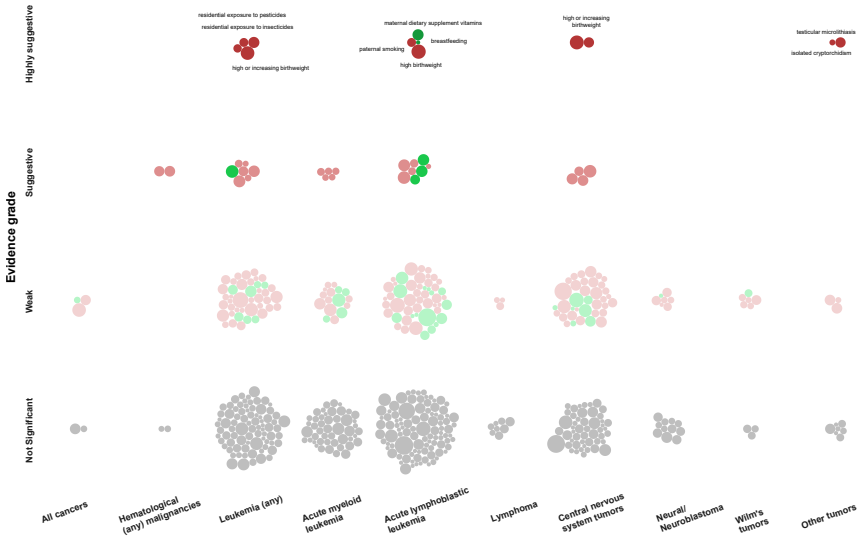
REFERENCES

Figure legends

Figure 1. Flowchart of literature search process

Figure 2 . Bubble plot visualization of the grading of evidence on the associations between environmental factors and risk of developing childhood cancer





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