Folic acid in pregnancy and subsequent cancer risk in mothers and their children: An epidemiologic study in Norway, 1999–2010

Jan Helge Seglem Mortensen

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
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Scientific environment

The main scientific environment has been the Research Group for Genetic Epidemiology at the Department of Global Public Health and Primary Care, University of Bergen, Norway.

The Norwegian Cancer Society and the Western Norway Regional Health Authority funded this project. The Faculty of Medicine granted admission to the PhD candidate, who followed the doctoral training and PhD courses at the University of Bergen.

Professor Tone Bjørge from the Research Group for Genetic Epidemiology and the Cancer Registry of Norway was the main supervisor, and Professor Nina Øyen from the Research Group for Genetic Epidemiology and the Department of Medical Genetics, Haukeland University Hospital, was co-supervisor.

Contents

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Abstract

Background:
Observational studies and randomized trials have shown that maternal folic acid supplementation before and during early pregnancy decreases the risk of neural tube defects in offspring. Hence, women of fertile age are advised to use folic acid supplements before and during early pregnancy, and food fortification programs with folic acid have been introduced in many countries.

There is concern about the safety of folic acid supplementation in relation to cancer risk. However, despite this concern, previous studies on cancer are inconsistent and the association between folic acid and cancer is weak.

Norway and many other countries have information campaigns to increase periconceptional folic acid use among women planning pregnancy. Studies have shown an association between maternal social and demographic characteristics and maternal periconceptional folic acid use. However, little is known whether there is an association between social and demographic characteristic of the woman's partner and her folic acid use in the periconceptional period.

Objectives:
To investigate the cancer risk (in total and for specific sites) for the mother after folic acid supplementation before and/or during pregnancy.

To investigate the overall risk for childhood cancer and for major childhood cancer types after *in utero* exposure to maternal supplemental folic acid.

To investigate if selected paternal characteristics are associated with maternal use of folic acid in pregnancy.

Material and methods:
All live-born children in Norway during 1999–2010, as well as their mothers and fathers, were identified by the Medical Birth Registry of Norway and defined as the study populations (687,406 children, 429,004 mothers, 434,686 fathers, and 683,785

childbirths). We identified maternal and childhood cancer cases by linkage to the Cancer Registry of Norway, with follow-up until a cancer diagnosis, emigration, death, or the end of 2010. The study population was also linked to the National Registry, the Norwegian National Education Database, and the Norwegian Labour and Welfare Administration.

Cancer risk among women using folic acid supplements prior to and during one and two or more pregnancies were compared to cancer risk in women not using such supplements. Cancer risk in children exposed in utero to maternal folic acid and/or multivitamin supplements were compared to cancer risk in children whose mothers never used supplements. The associations between folic acid use and cancer risks were estimated as hazard ratios (HRs) with 95% confidence intervals (CIs) using Cox proportional hazard regression models. The association between recommended maternal folic acid use (before and during pregnancy) and paternal age, education, occupation, and country of origin was estimated as relative risks (RRs) with 95% CIs using log-binomial regression with robust error variances.

Results:

From 1999 through 2010, 3,781 mothers developed cancer. The mothers were followed for an average of 7 years (range 0.04–12 years), constituting 2,933,587 person-years. No increased risk was seen for total cancer among women using folic acid in one (HR 1.08; 95% CI 1.00–1.18) or two or more pregnancies (HR 1.06; 95% CI 0.91–1.22) (p_{Trend} = 0.12). No specific subtypes of cancer showed increased risk.

Altogether, 799 children developed cancer during follow-up. The mean follow-up time was 6 years (range 0.04–12 years), constituting 4,052,679 person-years. We found no association between any supplemental folic acid levels and risk of leukemia (e.g., high-level folic acid HR 1.25; 95% CI 0.89–1.76, p_{Trend} 0.20), lymphoma (HR 0.96; 95% CI 0.42–2.21, p_{Trend} 0.51), central nervous system tumors (HR 0.68; 95% CI 0.42–1.10, p_{Trend} 0.32), neuroblastoma (HR 1.05; 95% CI 0.53–2.06, p_{Trend} 0.85), Wilms' tumor (HR 1.16; 95% CI 0.52–2.58, p_{Trend} 0.76), or soft-tissue tumors (HR 0.77; 95% CI 0.34–1.75, p_{Trend} 0.90).

During 1999–2010, the mothers used folic acid supplements before and during pregnancy, as recommended, in about 16% of all births in the study population. Recommended maternal folic acid use was low among mothers whose partners were young (e.g., <20 years RR 0.35; 95% CI 0.28–0.43) or older (e.g., ≥ 40 years RR 0.72; 95% CI 0.71–0.74), had attained a lower educational level (RR 0.69; 95% CI 0.68–0.71), fitted the occupational classes other than "Higher professionals," and originated from low/middle-income countries (RR 0.58; 95% CI 0.56–0.60).

Conclusion:
There was no association between folic acid supplementation before and/or during pregnancy and short-term risk of maternal and childhood cancer. A longer observation of our study population may have increased the statistical power of our analyses.

Despite official recommendations and information campaigns aimed at fertile women in Norway, periconceptional folic acid supplementation is insufficient. Our study demonstrates that recommended periconceptional folic acid use was lower when fathers were among the youngest and oldest, had shorter education, were self-employed or worked in manual occupations, or originated from low/middle-income countries.

List of publications

Mortensen J. H. S., Øyen N., Fomina T., Melbye M., Tretli S., Vollset S. E., Bjørge T. (2015): "Supplemental folic acid in pregnancy and maternal cancer risk." *Cancer Epidemiol*. 2015 Oct 18;39(6):805–811.

Mortensen J. H. S., Øyen N., Fomina T., Melbye M., Tretli S., Vollset S. E., Bjørge T. (2016): "Supplemental folic acid in pregnancy and childhood cancer risk." *Br J Cancer*. 2016 Jan 12;114(1):71–75.

Mortensen J. H. S., Øyen N., Nilsen R. M., Fomina T., Tretli S., Bjørge T. (2018): "Paternal characteristics associated with maternal periconceptional use of folic acid supplementation." *BMC Pregnancy Childbirth*, 18(1):188.

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Abbreviations

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
BMI	Body mass index
CI	Confidence interval
DNA	Deoxyribonucleic acid
FIGLU	Formiminoglutamic acid
HR	Hazard ratio
ICCC-3	International Classification of Childhood Cancer, third edition
ICD-7	International Classification of Diseases, seventh revision
ICD-10	International Classification of Diseases, tenth revision
IVF	In vitro fertilization
MoBa	Norwegian Mother and Child Cohort Study
MTHFR	Methylenetetrahydrofolate reductase
NTD	Neural tube defects
RR	Relative risk
REK	Regional Committee for Medical and Health Research Ethics of Western Norway
RNA	Ribonucleic acid

Summary

	What is already known	*What is added by this study*
Publication I	Randomized and observational studies have shown that maternal periconceptional folic acid supplement use reduces the risk of neural tube defects in the fetus and protects against some neurodevelopmental disorders and pregnancy complications. However, there is still concern about the safety of folic acid supplementation in relation to cancer risk.	Folic acid supplementation before and during pregnancy does not increase the short-term overall maternal cancer risk. Folic acid use in one (HR 1.08; 95% CI 1.00–1.18) or two or more pregnancies (HR 1.06; 95% CI 0.91–1.22) (p_{Trend}= 0.12) was not associated with increased overall cancer risk. Examination of 13 different cancer types revealed no associations between folic acid and cancer.
Publication II	Several case-control studies and an international collaborative study have shown reduced risk of acute lymphoblastic leukemia and acute myeloid leukemia in offspring, and of brain tumors among children exposed to maternal folic acid use. Ecological studies from Canada and the US have shown reduced incidence of Wilms' tumor, primitive neuroectodermal tumors, and neuroblastoma following	Folic acid supplementation was not related to short-term risk of major childhood cancers. Folic acid was not associated with leukemia (HR 1.25; 95% CI 0.89–1.76, p_{Trend} 0.20), lymphoma (HR 0.96; 95% CI 0.42–2.21, p_{Trend} 0.51), central nervous system tumors (HR 0.68; 95% CI 0.42–1.10, p_{Trend} 0.32), neuroblastoma (HR 1.05; 95% CI 0.53–2.06, p_{Trend} 0.85), Wilms' tumor (HR 1.16; 95% CI 0.52–2.58, p_{Trend} 0.76), or soft-tissue tumors (HR

	mandatory folic acid flour fortification.	0.77; 95% CI 0.34–1.75, p_{Trend} 0.90).
Publication III	Maternal predictors of folic acid supplementation are; low maternal age, shorter education, single parenthood, unplanned pregnancy, lower parity, smoking, alcohol use, less physical activity, or originating from a foreign country. However, only a limited number of studies have identified paternal determinants for maternal folic acid intake before and during pregnancy.	Adequate maternal periconceptional folic acid supplementation was lower if the fathers were younger [e.g. < 20 years (RR 0.35; 95% CI 0.28–0.43)], or older than 30–34 years [≥ 40 years (RR 0.72; 95% CI 0.71–0.74)], had shorter education (RR 0.69; 95% CI 0.68–0.71), held manual or self-employed occupations [e.g. class VI Skilled (RR 0.84; 95% CI 0.83–0.86) or class VII Semiskilled and unskilled (RR 0.75; 95% CI 0.73–0.76)], or were born in low/middle-income countries (RR 0.58; 95% CI 0.56–0.60).

1. Introduction

The name folate is derived from the Latin word “folium”, which means large-leafed plant. Humans cannot synthesize folates, and they have to be supplied through the diet. Folate (vitamin B_9) is an essential water-soluble vitamin naturally present in leafy vegetables and some fruits. It is essential for nucleotide biosynthesis, DNA replication, and methyl group supply, and consequently for cell growth and repair (Bailey et al. 2015). Furthermore, folate is involved in the homocysteine metabolism and helps maintain normal levels of this amino acid. Food folates are absorbed in the jejunum and transported to the liver, which contains about 50% of body pool folate (Ohrvik and Witthoft 2011). Inadequate folate intake leads to decreased serum folate, then decreased erythrocyte folate, a rise in homocysteine levels, and megaloblastic changes in the bone marrow (Bailey et al. 2015). Thus, folate deficiency may cause various health problems, such as hyperhomocysteinemia, megaloblastic anemia, and neurological disorders (Bailey et al. 2015, Reynolds 2014).

Folate requirements increase during pregnancy. Several studies report higher folate status (as defined by various measures including blood folate concentrations, folate intake, and/or folic acid intake) before and during early pregnancy than in late pregnancy and after (Cikot et al. 2001, Milman et al. 2006). Short interpregnancy intervals may play a role in preconception folic acid use due to maternal nutrient depletion, and the maternal folate status is likely to worsen with short intervals between multiple pregnancies (Nilsen et al. 2014, van Eijsden et al. 2008).

The role of folate in pregnancy was first reported in 1964 by Brian Hibbard (Hibbard 1964), who performed a study of folate status as urinary excretion of formiminoglutamic acid (FIGLU) in 1,484 low-income obstetric patients from Liverpool, United Kingdom. Hibbard found that abnormal FIGLU excretion was not only related to megaloblastic anemia, placental abruption and spontaneous abortion, but also to premature births, congenital defects and perinatal mortality in previous pregnancies. Thereafter, Hibbard and Smithells proposed that folate deficiency in pregnancy may be associated with central nervous system malformations (Hibbard

and Smithells 1965). This was eventually confirmed in the 1990s by randomized intervention trials (Botto et al. 1999, van der Put et al. 2001).

A large body of evidence from clinical trials and observational studies shows that improving periconceptional folate status before and during early pregnancy can reduce the risk of neural tube defects (NTDs) (Berry et al. 1999, Czeizel and Dudas 1992, Medical Research Council Vitamin Study 1991, Milunsky et al. 1989). Thus, increased folic acid (synthetic form of folate) intake before and during the first three months of pregnancy is recommended in several countries, including Norway (National Council on Nutrition and Physical Activity 1998, Nordic Council of Ministers 2014, Scientific Advisory Committee on Nutrition 2006, U.S. Department of Health and Human Services 1992).

Previous studies have shown that folic acid supplementation is associated with a lower risk of other neurodevelopmental disorders and some severe pregnancy complications like placental abruption, risk of autism spectrum disorders in children, and severe language delay in children (Nilsen et al. 2008, Roth et al. 2011, Suren et al. 2013). However, periconceptional folic acid use is not associatiated with severe congenital heart defects or isolated oral clefts (inverse association between periconceptional folic acid use and oral clefts in combinations with other malformations) (Gildestad et al. 2015, Leirgul et al. 2015), and there is still inconclusive evidence of an association between congenital urinary tract and genital anomalies and folic acid use (Bortolus et al. 2014).

NTDs occur during early embryonic development when the neural tube fails to close completely between 21 and 28 days after conception (Sadler and Thomas 2015). In Norway, the Norwegian Directorate of Health recommends that all women who are planning pregnancy or who are likely to become pregnant should take 0.4 mg of folic acid daily from one month before pregnancy throughout the first 2–3 months of pregnancy (National Council on Nutrition and Physical Activity 1998).

In Norway, the dietary intake of folate alone is not sufficient with regard to minimizing the risk of NTDs (Daltveit et al. 2004, Nilsen et al. 2014). The average

folate intake from foods in Norway is lower than 0.3 mg per day across the overall Norwegian population (Sengpiel et al. 2013).

Numerous countries worldwide have performed campaigns to increase periconceptional supplementation of folic acid among fertile women to reduce the risk of NTDs (National Council on Nutrition and Physical Activity 1998, Nordic Council of Ministers 2014, U.S. Department of Health and Human Services 1992). However, it seems that women tend to start supplementation too late to prevent NTDs (Bitzer et al. 2013, Eichholzer et al. 2006, Nilsen et al. 2006), possibly due to unplanned pregnancies. In order to ensure adequate folate intake among fertile women, several countries, including the US and Canada, have also introduced mandatory food fortification with folic acid (European Food Safety Authority [EFSA] 2009, Food and Drug Administration 1996). In these countries, a reduced risk of neural tube defects has been reported (Castillo-Lancellotti et al. 2013). However, folic acid fortification has been implemented in Moldova and Kosovo (Wald et al. 2018) but not in other European countries including Norway, due to concerns regarding folic acid's potential role in cancer development (European Food Safety Authority [EFSA] 2009).

1.1 Folic acid

The synthetic form of folate is folic acid (pteroyl monoglutamic acid), which is the fully oxidized form of folate. In contrast to folate, folic acid is chemically stable without loss of biochemical activity for months, rendering it very resistant to chemical oxidation (Bailey et al. 2015). Folic acid is used commercially in supplements and fortified foods (Pietrzik et al. 2010), and it has substantially higher bioavailability relative to food folate.

Figure 1 shows the proposed mechanism of folic acid absorption and transportation through the mucosa. The human gut has limited ability to reduce folic acid to 5-methyl tetrahydrofolate (Patanwala et al. 2014). Therefore, a large amount of unaltered folic acid enters the circulation and is taken up by the cells in the liver,

where it is subsequently transformed to tetrahydrofolate, and further to 5-methyl-tetrahydrofolate, by enzymatic processes (Figure 2) (Bailey et al. 2015, Nazki et al. 2014, Patanwala et al. 2014).

Depending on the dose, some folic acid is transported unmetabolized to the peripheral circulation. In contrast to folate, folic acid needs to be reduced to tetrahydrofolate via dihydrofolate by dihydrofolate reductase. The initial step is slow and may be influenced by individual variations in dihydrofolate reductase activity (Bailey et al. 2015).

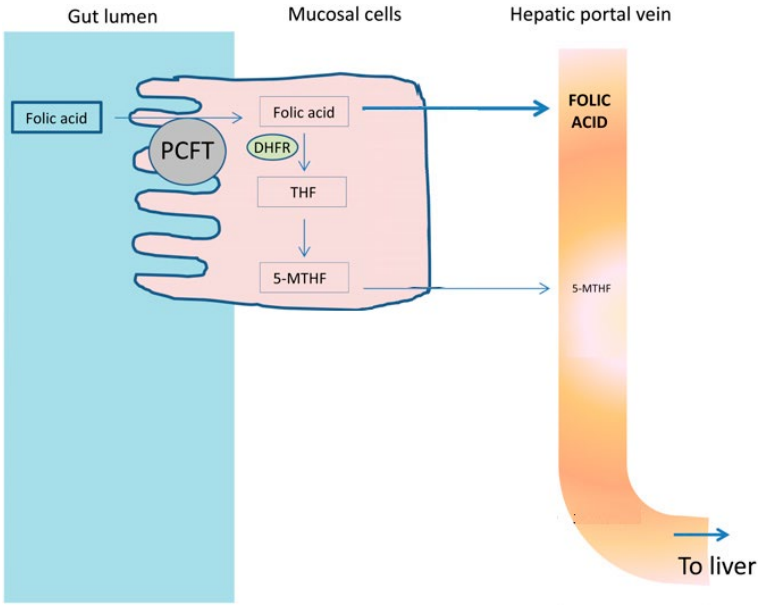


Figure 1: Proposed folate absorption from the gut lumen, metabolism in mucosal cells, and transport out into the hepatic portal vein. DHFR, dihydrofolate reductase; PCFT, proton-coupled folate transporter; THF, tetrahydrofolate; 5-MTHF, 5-methyltetrahydrofolate. Adapted from Patanwala et al. (2014) with permission

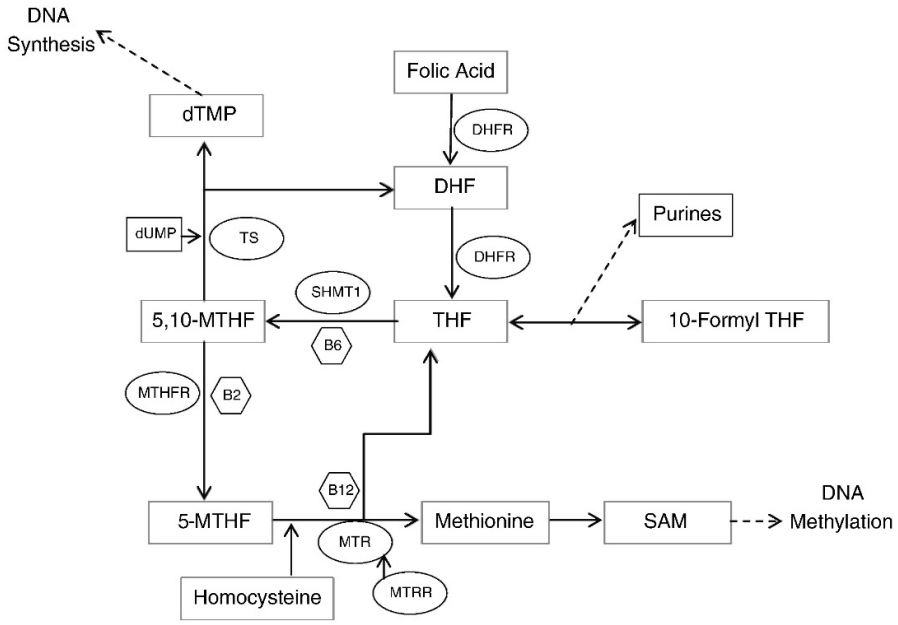


Figure 2: Folic acid metabolism: DHFR, dihydrofolate reductase; SHMT1, serine hydroxymethyl transferase 1; B_6, vitamin B_6; MTHFR, methylenetetrahydrofolate reductase; B_2, vitamin B_2; TS, thymidylate synthase; MTR, methionine synthase; B_{12}, vitamin B_{12}; MTRR, methionine synthase reductase; DHF, dihydrofolate; THF, tetrahydrofolate; 5,10-MTHF, 5,10-methyltetrahydrofolate; 5-MTHF, 5-methyltetrahydrofolate; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidylate monophosphate; 10-Formyl THF, 10-formyl tetrahydrofolate; SAM, S-adenosylmethionine. Adapted from Nazki et al. (2014) with permission

Folate acts as a coenzyme in transferring one-carbon units in the biosynthesis of purine nucleotides and deoxythymidylic acid, which is important in the synthesis of DNA and RNA. Consequently, folate plays an important role in nucleotide synthesis, gene expression and methylation. DNA methylation is essential for cell differentiation and embryonic development. Moreover, DNA methylation plays a role in mediating gene expression, chromatin structure, chromosome stability and inactivation of the X chromosome (Robertson 2005). Folate also remethylates homocysteine to methionine, which is the precursor of S-adenosylmethionine, the primary methyl group donor for most biological methylations, including DNA (Eichholzer et al. 2006, Krishnaswamy and Madhavan Nair 2001, Lucock 2004).

The folate status is affected by variations in folate-dependent enzymes. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folic acid metabolism and DNA methylation reactions. MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is a cosubstrate for the creation of methionine from homocysteine (Trimmer 2013). The MTHFR enzyme is encoded by the *MTHFR* gene (Leclerc et al. 2013). Single-nucleotide polymorphisms in MTHFR coding genes may cause the production of an enzyme with decreased activity that can have an impact on several biochemical processes. Although several MTHFR variants are identified, two polymorphisms have been extensively studied: the C-to-T substitution (677C→T polymorphism) that occurs at locus 677 of the MTHFR gene (Ueland et al. 2001), and the 1298A→C that occurs at locus 1298 (van der Put et al. 1998). Both of these MTHFR polymorphisms (when in the homozygous state) are associated with DNA hypomethylation through their genotypes; however, this is less pronounced for the 1298CC MTHFR genotype (Castro et al. 2004). DNA hypomethylation (commonly seen in solid tumors) as well as DNA hypermethylation in the region of tumor suppressor genes are well-recognised epigenetic changes that occur in human neoplasms (Jones 2005).

1.2 Folic acid and adult cancer

The World Health Organization defines cancer as a group of diseases involving abnormal cell growth with the potential to invade and metastasize to other parts of the body (World Health Organization 2015a). Cancer is a genetic disease resulting from corrupted information in the cellular DNA, leading to abnormal gene expressions and fundamental changes in biological processes within cancer cells (Hanahan and Weinberg 2011, Harrington 2016). Normal genes that control cellular growth, survival and invasion are enhanced, and other genes that suppress growth and invasion are repressed. The genes involved in cancer are divided into two types: oncogenes, and tumor suppressor genes. Activation of oncogenes occurs by specific point mutations within a gene, by multiplied copies of this gene, or by translocation of the gene to a DNA site with high transcription activity or formation of a fusion

gene that codes for proteins with enhanced biological activity (Harrington 2016). This causes uncontrolled cell divisions, enhanced cell survival and enhanced dissemination. A single copy of this mutated gene (proto-oncogene) is sufficient to start cancer disease. Tumor suppressor genes function to inhibit cell proliferation and survival by regulating cell divisions and apoptosis. Inactivation of tumor suppressor genes occurs by mutations that terminate the function of the protein encoded by the gene, or by silencing the promotor of the gene. In contrast to oncogenes, tumor suppressor genes are recessive genes – i.e., both copies of the genes must be affected in order to promote cancer. Thus, they are responsible for inherited cancer syndromes (Harrington 2016). In hereditary cancer syndromes, a germline mutation in one allele of a tumor suppressor gene affects every cell in the body, and it is likely that one of these cells will lose its tumor suppressor function and may progress to cancer early in life (Harrington 2016).

Cancer behavior is defined in terms of eight specific hallmarks: sustaining proliferative signaling, evading growth suppressor, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, the capability to modify, or reprogram cellular metabolism, and allow cancer cells to evade immunological destruction (Hanahan and Weinberg 2011).

About 33% of cancers in Western high-income countries are attributable to factors associated with food, nutrition and physical activity (Wiseman 2008). The etiology for several cancers has been linked to specific environmental factors: e.g. sun exposure and skin cancers, human papilloma virus and uterine cervical cancer, Helicobacter pylori and gastric cancer, viral hepatitis and hepatocellular cancer, and smoking and lung cancer (Wu et al. 2018). Further, there is evidence of a direct association between diet, lifestyle and cancer risk (Baena Ruiz and Salinas Hernandez 2014). Saturated fat intake and alcohol intake seem to increase breast cancer risk (Gonzalez and Riboli 2010), and consumption of red or processed meat cooked at a high temperature may be associated with increased colorectal cancer risk (Pericleous et al. 2013). Conversely, vitamins C and D, some carotenoids, retinol and α-tocopherol, and high intake of dietary fiber and fish reduce the risk of gastric and

colorectal cancer (Gonzalez and Riboli 2010). Further, there is evidence that intake of whole grains, pulses, vegetables and fruits, and low intake of sugary foods, salt, and red and processed meats are associated with lower risk of several cancers (mouth, pharynx, larynx, lung, esophageal, stomach, pancreas, and colorectal) (Norat et al. 2015).

Folate functions as a coenzyme in the one-carbon reactions necessary for purine nucleotide synthesis, thymidylate synthesis and remethylation of homocysteine to methionine. Disruption in the one-carbon metabolic pathway caused by nutritional deficiencies or gene polymorphisms affect DNA synthesis, DNA stability and chromatin methylation (Stover 2011).

Low folate levels in the human body are associated with an ineffective DNA synthesis that affects cell proliferation, cellular physiology and cytological morphology (Kim 1999). Human tissue experiments have shown that folate deficiency causes chromosome breaks that may contribute to increased cancer risk (Blount et al. 1997). Another proposed mechanism may be promotion of cancer cells through *de novo* methylation of tumor suppressor genes with consequent gene inactivation, which leads to tumor progression (Kim 2006). Animal studies on folate and intestinal cancers have shown that inadequate folate intake is an independent risk factor for colorectal cancer (Kim 2003). Similar findings of an association between low folate intake and risk of colorectal cancer has been suggested in humans. A prospective cohort study encompassing 525,488 individuals in the US aged 50–71 years from 1995–96 with mean follow-up of 9.1 years, showed that higher folate intake (> 0.2 mg) was associated with reduced colorectal cancer risk (Gibson et al. 2011). Conversely, there are other studies reporting either no association between folic acid supplementation and colorectal cancer risk (Eussen et al. 2010) or a reduced risk of colorectal cancer among individuals with low folate status (Gylling et al. 2014, J. E. Lee et al. 2012). However, there is support for a potential protective role of folate on colorectal cancer risk. Zacho et al. have performed a cross-sectional study of 5,949 adults and a prospective study of 9,235 adults in Denmark, as well as meta-analyses of 231 studies including 74,671 cases and 93,344 controls (Zacho et al.

2011). They showed that the homozygous MTHFR 677C→T polymorphism (with lifelong hyperhomocysteinemia) compared to non-carriers of the polymorphism was not associated with overall cancer risk. The homozygous MTHFR 677C→T polymorphism was, though, associated with increased risk of esophageal and gastric cancer, and with decreased risk of colorectal cancer. On the other hand, a Norwegian study based on two randomized, double-blind placebo-controlled clinical trials on a total of 6,837 patients (mean age 62.3 years and 23.5% women) with ischemic heart disease, showed that patients taking folic acid supplements (0.8 mg/day combined with other B vitamins) who also had the homozygous MTHFR 677C→T polymorphism, had a greater cancer mortality risk than those who had the 677CC genotype (Ebbing et al. 2009).

To our knowledge, no studies except for the investigation by Charles et al. in 2004, have studied the effect of folic acid supplementation in young, mainly healthy women of fertile age (Charles et al. 2004). Their finding of increased overall cancer mortality and breast cancer mortality among women taking high doses of folic acid (5 mg/day) as compared to placebo, was later criticized because the trial was carried out in the 1960s and did not meet current standards of research, such as double-blind and randomized clinical trials (Bland 2005). The authors themselves and the commentary by Oakley and Mandel pointed out that the reported associations might just be "chance findings" (Oakley and Mandel 2004).

However, findings from epidemiological studies have been inconsistent regarding whether folate poses a cancer risk or not. Two randomized controlled trials (about 14,000 individuals in total) found no protective nor harmful association between folic acid use (in combination with other B vitamins) and cancer risk (overall and site-specific) (Hankey et al. 2012, Zhang et al. 2008). Furthermore, two meta-analyses on folic acid supplementation reported no increased nor decreased incidence of lung cancer (4,390 cases and 6,138 controls from six case-control studies) (Dai et al. 2013), nor overall or site-specific cancer (49,621 participants in 13 randomized trials) (Vollset et al. 2013).

Finally, there seems to be a complex biological association between folic acid and adult cancer that needs cautious interpretation, and further biological and epidemiological research is necessary.

1.3 Folic acid and childhood cancer

Childhood cancer is defined as cancer diagnosed in an individual aged 14 years or younger (before puberty) (Bahadur and Hindmarsh 2000). Childhood and adolescent cancers differ from adult cancers in that they are histologically very diverse. Most adult cancers are carcinomas (Bahadur and Hindmarsh 2000). Childhood and adolescent cancers arise from embryonic cells and originate in developing tissues and organ systems. Embryonal malignancies include neuroblastoma, Wilms' tumor, medulloblastoma, rhabdomyosarcoma, and retinoblastoma (International Agency for Research on Cancer 2014). Some adolescent cancers are more similar to adult cancers – e.g., acute myeloid leukemia, Hodgkin lymphoma, thyroid cancer, and melanoma. Childhood cancers are therefore classified according to the third revision of the 1996 International Classification of Childhood Cancer (ICCC-3) (Steliarova-Foucher et al. 2005). ICCC-3 applies the rules, nomenclature and codes for morphology, topography and behavior according to the International Classification of Diseases for Oncology (ICD-O-3), third revision of 2000. Further, ICCC-3 is a three-level hierarchical classification system, with 12 main groups, 47 subgroups, and the 16 most heterogeneous subgroups are split further into 2–11 divisions in order to study important entities or homogeneous collections of tumors characterized at cytogenetic or molecular level.

Cancer is rare among children. The age-standardized incidence worldwide during 2001–10 was 14 per 100,000 person-years at age 0–14 (Steliarova-Foucher et al. 2017), and in Norway during 2007–16, the incidence was 15 per 100,000 person-years (National Clinical Registry for Childhood Cancer 2016).

The etiology of childhood cancers is generally unknown. Familial or genetic factors are thought to predispose a child to cancer in 10% of cases (Jongmans et al. 2016).

Transplacental exposure to xenobiotics before birth is associated with childhood leukemia, neuroblastoma, brain tumors, hepatoblastoma, and Wilms' tumor through induced genomic, epigenomic and/or non-genomic effects (Fucic et al. 2017). Since the majority of childhood cancers are diagnosed at an early age, a fetal origin has been suspected (Callan and Milne 2009). Furthermore, anthropometric measurements at birth show that fetal growth is positively associated with increased risks of different childhood cancers, supporting the hypothesis that the tumorigenesis manifesting in childhood may start *in utero* (Bjørge et al. 2013).

Mutations are relatively rare in childhood cancers, and various candidate-gene or genome-wide studies show that epigenetic deregulation plays an essential role in childhood cancer development (Yiu and Li 2015). DNA methylation, which is a crucial epigenetic mechanism, is dependent on dietary folate metabolism through the one-carbon pathway. Fetal exposure to folate during pregnancy affects DNA methylation in the offspring during fetal development (Amarasekera et al. 2014), and low maternal folate status corresponds to global DNA hypomethylation in fetuses diagnosed with NTDs (Chang et al. 2011). However, periconceptional folic acid supplementation (0.4 mg/day) is associated with increased methylation at the insulin-like growth factor 2 gene in children aged 17 months (Steegers-Theunissen et al. 2009). Other studies also find an association between prenatal folic acid use and modified DNA methylation in the child (Fryer et al. 2009, Hoyo et al. 2011).

Genetic polymorphisms that code the enzymes involved in the folate pathway regulate the folate metabolism. A recent meta-analysis of *MTHFR* polymorphisms reported that the C677T (677C→T) polymorphism may be associated with decreased risk of ALL (Wang et al. 2012). Protection against childhood cancer through maternal folic acid use before and during pregnancy is biologically plausible because folate and other B vitamins are essential in maintaining genomic stability through DNA methylation, synthesis and repair (Duthie and Hawdon 1998, Kim 1999).

Ecological studies from Canada and the USA, without individual-level information on maternal folic acid intake, have shown reduced incidence of Wilms' tumor,

primitive neuroectodermal tumors, and neuroblastoma among children born after the introduction of folic acid food fortification in 1998. No reduction was found in the incidence of other childhood cancers after initiation of fortification compared to children who were born before fortification was introduced (French et al. 2003, Grupp et al. 2011, Linabery et al. 2012).

In 2001, a case-control study from Western Australia showed reduced risk of childhood acute lymphoblastic leukemia (ALL) among children exposed to supplements containing folic acid or iron (Thompson et al. 2001). Subsequently, a national, population-based, multicenter case-control study performed in Australia between 2003 and 2007 conveyed a weak protective association of self-reported maternal folic acid supplementation before pregnancy with risk of childhood ALL, but no indication of a protective association of folic acid supplementation during pregnancy (Milne et al. 2010). A large international collaborative study, including 6,963 children with ALL, 585 children with acute myeloid leukemia (AML), and 11,635 controls, found reduced risks of ALL and AML after maternal intake of folic acid and other vitamin supplements. The reduced risks of ALL and AML did not vary by time of supplementation exposure (preconception, pregnancy, or pregnancy trimester) (Metayer et al. 2014). In addition, another national, population-based, multicenter case-control study conducted in Australia between 2005 and 2011 reported that folic acid supplementation before and during pregnancy may protect against childhood brain tumors (Milne et al. 2012). However, a population-based case-control study of children born in Sweden between 1975 and 1984 (500 cases and 500 controls) showed no association between maternal use of folic acid supplements and other medications with the risk of childhood brain tumors (Stalberg et al. 2010).

In summary, maternal periconceptional folic acid supplementation seems to be associated with reduced risk of childhood leukaemia, childhood brain tumors and other childhood cancer types. However, there is concern regarding the safety of folic acid in relation to cancer risk and further epidemiological and biological studies may be helpful in informing future risk assessments on a possible association between folic acid supplementation and childhood cancer risk.

1.4 Determinants of periconceptional folic acid use

Low intake of folate from foods is the main cause of low folate status in humans, which is a risk factor of NTDs (Daly et al. 1995, Scientific Advisory Committee on Nutrition 2006). Many countries in Europe have performed information campaigns to increase periconceptional folic acid supplementation among women planning pregnancy (Bower et al. 2005, Daltveit et al. 2004, Staff et al. 2005). In Norway, women planning pregnancy are advised to take a daily supplement of 0.4 mg of folic acid from one month before pregnancy through the first three months of pregnancy (National Council on Nutrition and Physical Activity 1998). However, public health campaigns promoting periconceptional folic acid use have been unsuccessful in many European countries (European Food Safety Authority [EFSA] 2009).

Because the neural tube closes between 21 and 28 days after conception, it is important to start folic acid supplementation before conception (Sadler and Thomas 2015). Many European women of childbearing age are unaware that using folic acid supplements before and during early pregnancy reduces the risk of NTDs (Bitzer et al. 2013). Important predictors of inadequate maternal periconceptional folic acid supplementation are unplanned pregnancies, low socioeconomic levels, and young maternal age (Eichholzer et al. 2006). Low income, parity of more than one, smoking or alcohol use during pregnancy, and a low-income country of origin are also associated with low compliance with recommended folic acid use (Bower et al. 2005, Braekke and Staff 2003, Cueto et al. 2012, Daltveit et al. 2004, Knudsen et al. 2004, Nilsen et al. 2006, Timmermans et al. 2008).

A Norwegian study with data from 2000–2003 reported that folic acid supplements were used more frequently among women who had higher-educated partners (Nilsen et al. 2006). However, the association of paternal education with maternal folic acid use was weaker than that of maternal education. The study did not assess other paternal factors or combine paternal and maternal factors as to identify inadequate maternal folic acid supplementation. Still, supportive evidence of this finding is that couples tend to exhibit concordant health behaviors for dietary intake, body mass

index (BMI), smoking, alcohol consumption and physical activity (Jackson et al. 2015). Persons living together share the same environment, social network, financial resources, and somewhat, the same health risk; beneficial or negative to health outcomes depending on the health behavior of the partner (Cornelius et al. 2016, Jackson et al. 2015). Moreover, a person within a couple is more likely to make a positive health behavior change if their partner does so (Jackson et al. 2015).

Less is, however, known about how partners may influence the maternal periconceptional use of folic acid. Consequently, it is important to investigate how the partner of the mother can encourage maternal folic acid use.

2. Aim of the thesis

This thesis is part of a larger project which examines the later health consequences for the mother and child after supplemental folic acid in pregnancy.

The specific aims of this thesis were:

1. To assess the cancer risk (in total and for specific sites) for the mother after folic acid supplementation before and/or during pregnancy.
2. To assess the overall risk for childhood cancer and for major childhood cancer types after maternal supplemental folic acid in pregnancy.
3. To evaluate if paternal characteristics are associated with maternal periconceptional use of folic acid in pregnancy.

3. Material and methods

In this section, the study populations and the sample selection criteria used for each publication in the thesis are described.

3.1 Data sources

3.1.1 National Registry

The National Registry of Norway was established in October 1964. It assigns a unique personal identification number to all individuals living, born or immigrated to Norway since the national census in 1960 (Hammer 2002, Statistics Norway 2017). The personal registration number allows a precise linkage between the national registries and other databases in Norway. Further, the National Registry contains demographic data on all residents in Norway since 1960, such as name, date and place of birth, residential address, if the resident is alive, emigrated or dead, citizenship, marital status, country immigrated from or emigrated to and several other information elements. The Norwegian Tax Administration is responsible for ensuring the completeness of the register and keeping the records up to date.

3.1.2 Medical Birth Registry of Norway

The Medical Birth Registry of Norway is a national, population-based health registry containing information on all births in Norway since 1967. It presently holds information on more than 2.8 million births (Norwegian Institute of Public Health 2017). The registry enables identification of women and all their successive births. It holds demographic information on the mother and father, the mother's health before and during pregnancy, including chronic diseases, and complications during pregnancy and delivery. In addition, information on the infant, including birth defects and other perinatal problems, is included. The data enrolled in the Medical Birth Registry of Norway is collected from a notification form that midwives and physicians attending the births are entrusted to complete. All pregnancies ending after

week 16, including stillbirths, are notifiable and must be reported to the Medical Birth Registry of Norway (Irgens 2000, Norwegian Institute of Public Health 2004).

Since December 1998, a revised version of the notification form has been used, including new variables such as maternal dietary supplement intake and whether the mother is a smoker or non-smoker (Appendix 1).

The publications in this thesis were based on data from the revised form only.

3.1.3 Cancer Registry of Norway

The Cancer Registry of Norway was established in 1951 and contains mandatory reported information on all new cancer cases and certain precancerous lesions in Norway (Larsen et al. 2009). Information from clinical notifications, pathological notifications and death certificates are the main reporting sources, providing information about site, histological type and stage of disease at the time of diagnosis.

The coding and classification system at the Cancer Registry of Norway follows international standards (Larsen et al. 2009). In our dataset the International Classification of Diseases version 10 (ICD-10) and the International Classification of Childhood Cancer, version 3, which is based on ICD-O-3, has been the basis for coding, including topography and morphology codes (Cancer Registry of Norway 2016, Steliarova-Foucher et al. 2005, World Health Organization 2015b).

3.1.4 The Norwegian Labour and Welfare Administration

The Norwegian Labour and Welfare Administration was established in 2006 (Norwegian Labour and Welfare Administration 2018). The organization holds information on employment, health status and social benefits of all individuals with residence in Norway since 1992 through the historical event database FD-Trygd (Akselsen and Siverstøl 2013). The occupational code system is based on the International Standard Classification of Occupations (ISCO), revised version from 1988 (Statistics Norway 1998).

3.1.5 Norwegian National Education Database

The Norwegian National Education Database holds information on all individuals' educational theory and practice since 1970, from completion of primary school to doctoral studies (Vangen 2007). All information on educational attainment is reported annually from respective educational institutions to the Norwegian National Education Database (Government of Norway 1998). The database is also a register of the population's highest completed level of education (Statistics Norway 2013, Vangen 2007). The classification is based on a 6-digit coding system of the Norwegian Standard Classification of Education (Statistics Norway 2013, Statistics Norway 2018).

3.2 Study populations

This project is a population-based study. All women living in Norway and giving live births in the period January 1, 1999, to December 31, 2010, including their pregnancies (births), children, and fathers to their children, were defined as our study population. The study cohorts were identified through the Medical Birth Registry of Norway and constituted 429,004 women, 687,406 children, and 683,785 childbirths, including 434,686 fathers, with regard to the study of maternal cancer risk, childhood cancer risk, and paternal determinants of maternal periconceptional folic acid use, respectively (Table 1).

The mothers take part in the study with several pregnancies during follow-up. To retrieve birth-related information, demographic data, maternal education and occupation, and cancer status, the study participants extracted from the Medical Birth Registry of Norway were linked to the National Registry, the Norwegian National Education Database, the Norwegian Labour and Welfare Administration, and the Cancer Registry of Norway, respectively, by using the national identification number (Table 1). For the researcher, the provided data files had the national identification numbers replaced with a project specific serial number.

We extracted information on children born alive during 1999 through 2010, including their registered mothers and fathers, from the Medical Birth Registry of Norway. The children were linked to the Cancer Registry of Norway, and their registered parents were linked to the National Registry, the Norwegian National Education Database and the Norwegian Labour and Welfare Administration by using the unique personal identification number. Since the parents can take part in the study with subsequent births, educational and occupational data at time of childbirth birth were used (Table 1).

Table 1: Inclusion criteria for publications 1 through 3

		Publication		
Inclusion criteria	***Description***	***1***	***2***	***3***
Time period	Year of childbirth	1999–2010	1999–2010	1999–2010
MBRN notification	Information collected at childbirth	Yes	Yes	Yes
CRN notification	Information collected at diagnosis	Yes	Yes	
NUDB	Information collected at childbirth	Yes	Yes	Yes
NAV	Information collected at childbirth[1]	Yes	Yes	Yes
Unit of analysis		Mothers	Children	Births
Initial study sample	Number of subjects before exclusions	442,858	707,495	707,495
Final study sample	Number of subjects included in the analyses	429,004	687,406	683,785

MBRN: Medical Birth Register of Norway. CRN: Cancer Registry of Norway. NUDB: The Norwegian National Education Database. NAV: The Norwegian Labour and Welfare Administration.

In *publication I*, we included 429,004 women with births during 1999 through 2010 registered in the Medical Birth Registry of Norway. Since information on supplemental use has not been registered for induced abortions, 2,491 individual records were excluded. Births to women who emigrated before birth (13,733) or who were diagnosed with cancer before delivery (3,334) were also excluded. We followed each woman from the date of her first childbirth during 1999–2010 until a cancer diagnosis (International Classification of Diseases version 10 [ICD-10]), death, emigration, or end of follow-up at December 31, 2010.

[1] Occupational codes registered in 2003 were applied for births during 1999–2002

The cohort for *publication II* consisted of all live-born children from January 1, 1999 through December 31, 2010, constituting 687,406 children. We excluded 3,371 children with mothers diagnosed with cancer prior to childbirth. The remaining children were followed from date of birth until diagnosis of their first cancer, emigration, death or end of follow-up at December 31, 2010.

Our study population in *publication III* consisted of 683,785 births in Norway from January 1, 1999, through December 31, 2010 (434,154 mothers and 434,686 registered fathers). Because information on folic acid use and paternal identification has not been registered for induced abortions, 2,519 records of births were excluded. Births without a paternal identification number (12,699) or maternal identification number (4,091) were also excluded.

3.3 Exposure

In Norway, folic acid supplements intended for use in pregnancy during 1999–2010 contained 0.4 mg of folic acid, while most multivitamin supplements sold over the counter in all pharmacies contained approximately 0.2 mg of folic acid (Norwegian Scientific Committee for Food Safety 2015). The revised Medical Birth Registry of Norway notification form records information on folic acid and multivitamin supplementation by using checkboxes with the items “no” (no regular dietary supplementation), “folic acid before pregnancy,” “folic acid during pregnancy,” “multivitamins before pregnancy,” and “multivitamins during pregnancy.” Based on the guidelines for electronic notification of births to the Medical Birth Registry of Norway, and the user guide for electronic notification of births (Norwegian Institute of Public Health 2016), the check box referring to no supplementation has to be marked if the check boxes for “folic acid before pregnancy”, “folic acid during pregnancy”, “multivitamins before pregnancy”, and “multivitamins during pregnancy” are left unmarked. On the other hand, if the check box referring to no supplementation is unmarked, at least one of these other check boxes has to be marked in order to complete the form.

In *publication I*, we defined mothers as "folic acid users" if they used folic acid supplements before and/or during pregnancy. Based on this information for each mother in the cohort, we created an exposure variable for maternal folic acid use in successive pregnancies (no use, use in one pregnancy, and use in two or more pregnancies). Similarly, we defined the same mothers as "multivitamin users" if multivitamins were used before and/or during pregnancy. A multivitamin exposure variable for maternal multivitamin use in successive pregnancies was then constructed (no use, use in one pregnancy, and use in two or more pregnancies). Finally, we created a third exposure variable based on the total amount of folic acid from folic acid supplements (0.4 mg) and multivitamin supplements (approximately 0.2 mg) before and/or during pregnancy.

In *publication II*, children were regarded as exposed to folic acid *in utero* if their mothers used folic acid and/or multivitamin supplements before and/or during pregnancy. We categorized the "quantity" of folic acid intake by increasing amounts of folic acid content: no supplement use (0 mg), only multivitamins (approximately 0.2 mg), only folic acid supplements (0.4 mg), or intake of both folic acid supplements and multivitamins (approximately 0.6 mg).

In *publication III*, the following paternal determinants were used in the analyses of adequate maternal folic acid supplementation: paternal age (<20, 20–24, 25–29, 30–34, 35–39, 40+ years); education (compulsory [1–9 years], intermediate [10–12 years], and tertiary [13–19 years]); occupation according to the class scheme of Erikson, Goldthorpe and Portocarero (I Higher professionals, II Lower professionals, IIIa Higher routine, IIIb Lower routine, IV Other self-employed workers, V Technicians, VI Skilled, VII Semiskilled and unskilled, VIIb Agricultural, Unclassified) (Erikson and Goldthorpe 1992); and country of origin according to the classification by the World Health Organization's Health Statistics and Information Systems' estimates for 2000–2012 (Norway, high income countries, and low/middle-income countries) (World Health Organization 2016). Paternal occupation was categorized according to Erikson, Goldthorpe and Portocarero class scheme by means of a manual provided by Flemmen et al. (Flemmen and Andersen 2009).

3.4 Outcomes

In *publication I*, we used the International Classification of Diseases version 10 (ICD-10) to identify incident maternal cancer cases through linkage with the Cancer Registry of Norway (Table 2). Only the first cancer diagnosis was used in our analyses. Subgroups of maternal cancer included the 13 most frequent cancer subgroups in our cohort: colorectal cancer (C18–21), lung cancer (C33–34), melanoma of the skin (C43), non-melanoma skin cancer (C44), breast cancer (C50), cancer of the uterine cervix (C53), ovarian cancer (C56), central nervous system tumors (C70–72, D42–43), thyroid cancer (C73), cancer of the other endocrine glands (C37, C74–75), Hodgkin's lymphoma (C81), non-Hodgkin's lymphoma (C82–85, C96), and leukemia (C91–95, D45–47).

Cancer types with less than 50 cases were categorized as "Other cancers" (C00–17, C22–26, C30–32, C38–41, C45, C47–49, C51–52, C54, C57–58, C64–69, C76, C80, C88, C90).

In *publication II*, we identified the incident childhood cancer cases through linkage with the Cancer Registry of Norway. The first cancer diagnosis for each child was used and categorized according to the International Classification of Childhood Cancer, version 3, which is based on the ICD-O-3 (Steliarova-Foucher et al. 2005) (Table 2).

In *publication III*, adequate maternal folic acid supplement use (folic acid use before and during pregnancy) was the outcome of investigation (Table 2).

3.5 Covariates

Covariates were extracted from the Medical Birth Registry of Norway, the National Registry, the Norwegian National Education Database, and the Norwegian Labour and Welfare Administration. The inclusion of covariates and potential confounders used in the statistical models are described below.

In *publication I*, we chose relevant covariates associated with maternal folic acid use and maternal cancer *a priori*, based on information in previous literature. Maternal year of birth (1949–59, 1960–69, 1970–79, 1980–89, 1990–96), maternal age at first childbirth in the study period (1999–2010) (<20, 20–24, 25–29, 30–34, 35–39, ≥40 years), maternal age at first childbirth (prior to start of follow-up period) (<20, 20–24, 25–29, 30–34, 35–39, ≥40 years), parity (1, 2, 3, ≥4), marital status (unmarried, married/registered partner/cohabitant, divorced/widowed), and smoking habits were collected from the Medical Birth Registry of Norway. Information on maternal smoking was recorded at the start and end of pregnancy (no smoking, sometimes, daily, the number of cigarettes, declined to inform about smoking habits). The smoking data was then categorized into a single variable that contained the maximum cigarette consumption for each woman: never, sometimes, ≤10 cigarettes daily, >10 cigarettes daily, daily smoking–unknown amount. Data on length of maternal education (compulsory [1st–7th class level], intermediate [8th–12th class level], tertiary [13th–20th class level]) and maternal occupation at the time of first childbirth (1999–2010) was collected from the Norwegian National Education Database and the Norwegian Labour and Welfare Administration, respectively. Maternal occupation was categorized according to the International Standard Classification of Occupations, which is divided in 10 major groups: 0. Armed forces and unspecified , 1. Legislators, senior officials and managers, 2. Professionals, 3. Technicians and associate professionals, 4. Clerks, 5. Service workers and shop and market sales workers, 6 Skilled agricultural and fishery workers, 7. Craft and related trades workers, 8. Plant and machine operators and assemblers, 9. Elementary occupations (Table 2).

In *publication II*, we selected covariates associated with maternal folic acid supplementation and childhood cancer risk *a priori* (Table 2). These variables were collected from the Medical Birth Registry of Norway: number of births (1, 2, ≥3), maternal and paternal age (<25, 25–34, ≥35 years), and maternal smoking (never, sometimes, ≤10 cigarettes daily, >10 cigarettes daily, daily smoking of unknown

amount). From the Norwegian National Education Database we retrieved maternal and paternal education data (compulsory, intermediate, tertiary).

In *publication III*, we used subject-matter knowledge and a directed acyclic graph (DAG) approach (Appendix 2) to assess the minimally sufficient adjustment set of variables when studying the association between potential paternal determinants and adequate maternal folic acid supplementation (Shrier and Platt 2008). We constructed a DAG that identified the unconfounded association of paternal determinants with adequate maternal folic acid supplementation (Pearl 2010, Shrier and Platt 2008, Textor et al. 2011). The final adjustment set blocked "non-causal" but not "causal pathways" between paternal determinants and maternal folic acid use, and included year of childbirth (1999– 010), father's age (<20, 20–24, 25–29, 30–34, 35–39, 40+ years), education (compulsory, intermediate, tertiary), and country of origin (Norway, high-income countries, low/middle-income countries) (retrieved from the National Registry) (Appendix 2).

Paternal age, education, and country of origin are related to maternal age, education and country of origin through the custom of marrying with those of the same age and sociological, educational, religious, and ethnic backgrounds (age and educational homogamy, and ethnic endogamy) (Çelikaksoy et al. 2009, Huber and Fieder 2011, van de Putte et al. 2009). Maternal age, education, and country of origin are associated with maternal folic acid use (Nilsen et al. 2006, Timmermans et al. 2008). We therefore included maternal age (<20, 20–24, 25–29, 30–34, 35–39, 40+ years), maternal education (compulsory, intermediate, tertiary), and maternal country of origin (Norway, high-income countries, low/middle-income countries) as potential confounders of the associations between paternal age, education, and country of origin, respectively, and adequate maternal folic acid use in a separate model.

3.6 Statistical analysis

All statistical analyses were performed using STATA versions 13 and 14 (StataCorp. 2013, StataCorp. 2015). For *publications I* and *II,* the statistical package SPSS version 22 was also used.

Data were described as frequencies and risk estimates with confidence intervals (CIs). Potential confounding factors were chosen *a priori* as described above. For *publication III*, directed acyclic graphs were also used to minimize potential bias from intermediate variables when studying the association between potential paternal determinants and adequate maternal folic acid supplementation. Inclusions of confounders in the statistical models are discussed in more detail below, and information about the variables used is presented in Table 2.

3.6.1 Folic acid and maternal cancer risk (publication I)

In our study, the risk of total cancer and subtypes of cancer among women using folic acid in successive pregnancies compared to women using no folic acid were estimated as hazard ratios (HRs) with 95% confidence intervals (CIs) using multivariate, time-dependent Cox proportional hazard regression models (Cox and Oakes 1984). Time since the first childbirth from 1999 through 2010 was used as the time variable. The Cox models were applied when there was an underlying assumption of proportionality. Tests for linear trends over the categories of folic acid supplementation were calculated. All analyses were adjusted for maternal age at first childbirth (age at cohort entry), maternal year of birth, marital status, birth order, education, occupation, and smoking at time of birth. For total cancer and breast cancer only, we also adjusted for age at the woman's first childbirth.

In this publication, we also used multiple imputations on missing smoking status at the time of birth due to 16% of the births missing data on smoking. The imputed analyses were performed following the recommendation by White and Royston (White and Royston 2009).

3.6.2 Folic acid and childhood cancer risk (publication II)

Cancer risk in children exposed to maternal folic acid and/or multivitamin supplements was compared with cancer risk in unexposed children and expressed as HRs with 95% CIs, using Cox proportional hazards regression models when there was an underlying assumption of proportionality. Time since birth was used as the time variable, and all analyses were adjusted for *a priori* selected covariates associated with maternal folic acid use and childhood cancer risk; that is, birth order, maternal smoking, maternal and paternal age, and maternal and paternal education. Maternal periconceptional folic acid and/or multivitamin use was divided into four exposure levels: 0 mg, approximately 0.2 mg, 0.4 mg and approximately 0.6 mg. A test for linear trends was calculated by treating the four exposure levels as continuous in the models (Table 2).

3.6.3 Paternal characteristics associated with maternal periconceptional folic acid supplementation (publication III)

To determine the associations between paternal determinants (age, education, occupation, country of origin) and maternal folic acid supplement use, crude and adjusted relative risks (RRs) with corresponding 95% CIs were calculated by log binomial regression models with the log-link function in STATA version 14 (StataCorp. 2015). RR is the ratio of the risk of adequate maternal folic acid use for different categories of paternal determinants and the risk of adequate maternal folic acid use for the reference category of paternal determinants. Our analyses included robust variance estimation of the 95% CIs with the sandwich estimator, to correct for the intra-individual correlation in women with more than one pregnancy during the study period (Cameron and Miller 2015). All analyses were adjusted according to the description in Table 2. P-values for overall difference between the categories of paternal determinants were calculated using likelihood ratio tests. Effect modification of the association between paternal education and adequate maternal folic acid supplementation by maternal education was evaluated by stratification, and tested with likelihood ratio tests.

Table 2: Overview of the outcomes, exposure variables, adjustment variables, design, statistical models and statistical software used in this study

Publication I	***Publication II***	***Publication III***
	Main outcome	
Maternal cancer (ICD-10)[1]	*Childhood cancer (ICCC-3)*[3]	*Maternal folic acid use*
Total cancer	I. Leukemia, myeloproliferative diseases, and myelodysplastic diseases	Adequate folic acid use
Colorectal cancer (C18–21)		Use before and during pregnancy
Lung cancer (C33–34)		
Melanoma of the skin (C43)		
Non-melanoma skin cancer (C44)	II. Lymphomas and reticuloendothelial neoplasms	
Breast cancer (C50)		
Cancer of the uterine cervix (C53)		
Ovarian cancer (C56)	III. Central nervous system tumors and miscellaneous intracranial and intraspinal neoplasms	
Central nervous system tumors (C70–72, D42–43)		
Thyroid cancer (C73)		
Other endocrine glands[2] (C37, C74–75)	IV. Neuroblastoma and other peripheral nervous cell tumors	
Hodgkin's lymphoma (C81)		
Non-Hodgkin's lymphoma (C82–85, C96)	VI. Renal tumors	
	IX. Soft-tissue and other extraosseous sarcomas	
Leukemia (C91–95, D45–47)		
"Other cancers" (C00–17, C22–26, C30–32, C38–41, C45, C47–49, C51–52, C54, C57–58, C64–69, C76, C80, C88, C90)	"Other cancers"	
	Exposure	
Folic acid (before and/or during pregnancy)	*Total amount of folic acid from multivitamin supplements and folic acid supplements (before and/or during pregnancy)*	*Paternal age*
No use	No use (0 mg)	<20
Use in one pregnancy	Multivitamins only (~ 0.2 mg)	20–24
Use in two or more pregnancies	Folic acid only (0.4 mg)	25–29
	Folic acid and multivitamins (~ 0.6 mg)	30–34
Multivitamins (before and/or during pregnancy)		35–39
No use		≥40
Use in one pregnancy		
Use in two or more pregnancies		*Paternal education*
		Compulsory
Total amount of folic acid from multivitamin and folic acid supplements (before and/or during pregnancy)[4]		Intermediate
		Tertiary
0 mg		
~ 0.2 mg		*Paternal occupation*[5]
0.4 mg		I Higher professionals
~ 0.6 mg		II Lower professionals
		IIIa Higher routine
		IIIb Lower routine
		IV Other self-employed workers
		V Technicians
		VI Skilled
		VII Semiskilled and unskilled
		VIIb Agricultural
		Unclassified

		Paternal country of origin[6]
		Norway
		High-income countries
		Low/middle-income countries
Adjustments (1999–2010)		
Maternal age (1999–2010)	*Maternal age*	*For paternal age*
<20	<25	Year of childbirth
20–24	25–34	Maternal age at childbirth
25–29	≥35	<20
30–34		20–24
35–39	*Paternal age*	25–29
≥40	<25	30–34
	25–34	35–39
Maternal age at the very first childbirth (for total and breast cancer only)	≥35	≥40
	Maternal education	*For paternal education*
<20	Compulsory	Year of childbirth
20–24	Intermediate	Paternal age
25–29	Tertiary	<20
30–34		20–24
35–39	*Paternal education*	25–29
≥40	Compulsory	30–34
	Intermediate	35–39
Maternal year of birth	Tertiary	≥40
1949–59		Paternal country of origin[6]
1960–69	*Birth order*	Norway
1970–79	1	High-income countries
1980–89	2	Low/middle-income countries
1990–96	≥3	Maternal education
		Compulsory
Birth order	*Maternal smoking*	Intermediate
1	Never	Tertiary
2	Sometimes	
3	≤10 cigarettes daily	*For paternal occupation*[5]
≥4	>10 cigarettes daily	Year of childbirth
	Daily smoking, unknown amount	Paternal age
Maternal marital status		< 20
Unmarried		20 – 24
Married/registered partner/cohabitant		25 – 29
Divorced/widowed		30 – 34
		35 – 39
Maternal education		≥ 40
Compulsory		Paternal education
Intermediate		Compulsory
Tertiary		Intermediate
		Tertiary
Maternal occupation[7]		Paternal country of origin[6]
Armed forces or unspecified		Norway
Legislators, senior officials, and managers		High-income countries
Professionals		Low/middle-income countries
Technicians and associate professionals		*For paternal country of origin*
Clerks		Year of childbirth
Service workers and shop and market sales workers		Maternal country of origin[6]
		Norway
		High-income countries
		Low/middle-income countries

Agricultural, forestry, and fishery workers Craft and related trades workers Plant and machine operators and assemblers Elementary occupations		
Maternal smoking Never Sometimes ≤10 cigarettes daily >10 cigarettes daily Daily smoking, unknown amount		
Design		
Population-based cohort study	Population-based cohort study	Population-based cross-sectional study
Statistical models		
Time-dependent Cox proportional hazard regression models	Cox proportional hazard regression models	Log-binomial regression
Statistical software		
SPSS version 22 and STATA version 13	SPSS version 22 and STATA version 13	STATA version 14

[1] International Classification of Diseases, 10th version

[2] Malignant neoplasm of thymus, adrenal gland, and other endocrine glands and related structures excluding endocrine pancreas, ovary, and thyroid.

[3] International Classification of Childhood Cancer, third edition (Steliarova-Foucher et al. 2005)

[4] We have used approximately (~) because multivitamins used in Norway during 1999–2010 initially contained 0 mg of folic acid and later contained 0.2 mg of folic acid. Folic acid supplements contained 0.4 mg of folic acid throughout the study period (Norwegian Scientific Committee for Food Safety 2015).

[5] According to the class scheme of Erikson, Goldthorpe and Portocarero (EGP) (Erikson and Goldthorpe 1992)

[6] According to the classification of the World Health Organization's Health Statistics and Information Systems' estimates for 2000–2012, available from (http://www.who.int/healthinfo/global_burden_disease/estimates/en/index1.html)

[7] Standard Classification of Occupation (STYRK-08 2011) (10 major groups)

3.7 Ethical approval

The study was approved by the Regional Committee for Medical and Health Research Ethics of Western Norway (REK ref. number 2010/3310).

4. Results

4.1 Maternal cancer risk (publication I)

Our study population included 429,004 women, constituting 2,933,587 person-years. The mean age at the start of follow-up was 29 years (range 13–54 years). A total of 3,781 cancer cases were diagnosed during follow-up, and the mean age at cancer diagnosis was 37 years (range 18–56 years). Figure 3 presents the number of cancer cases by maternal age at diagnosis during follow-up (1999–2010).

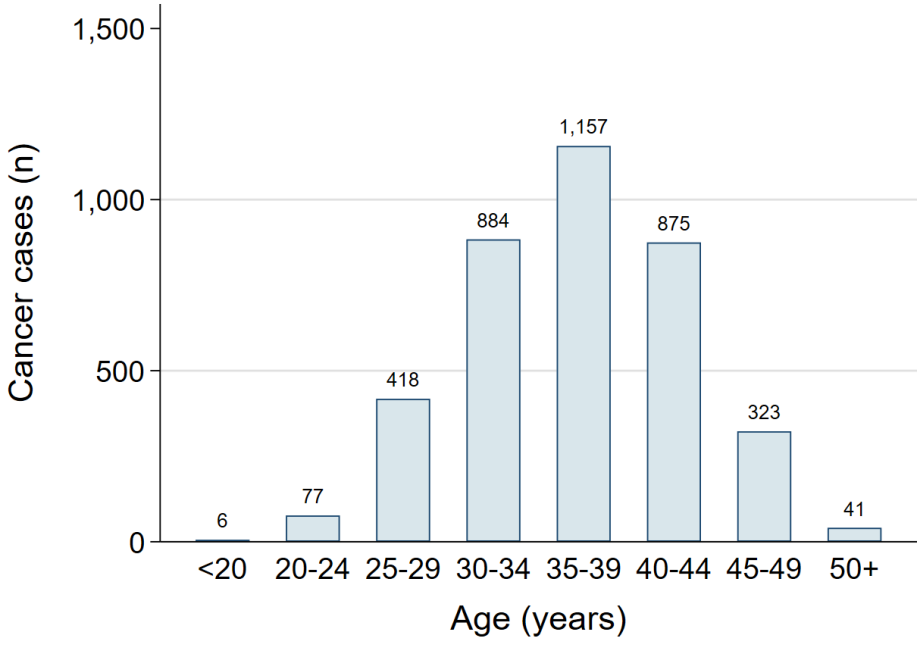


Figure 3: Cancer cases by age at diagnosis among 429,004 women in Norway, 1999–2010

Table 3: Cancer cases registered during follow-up according to cancer types and ICD-10 codes among 429,004 women in Norway, 1999–2010

Cancer types	*ICD-10 codes*	*Cancer cases (N)*
Total cancer		3,781
Colorectal	C18–21	169
Lung & trachea	C33–34	54
Melanoma of the skin	C43	494
Skin, non-melanoma	C44	51
Breast	C50	1,166
Cervix uteri	C53	457
Ovary	C56	74
Central nervous system	C70–72, D42–43	357
Thyroid	C73	252
Other endocrine glands	C37, C74–75	91
Hodgkin's lymphoma	C81	84
Non-Hodgkin's lymphoma	C82–85, C96	93
Leukemia	C91–95, D45–47	96
Other cancers	C00–17, C22–26, C30–32, C38–41, C45, C47–49, C51–52, C54, C57–58, C64–69 C76, C80, C88, C90	343

Mean time between the first birth in the study period and cancer diagnosis was five years (range 0.1–12 years). The most frequent cancer type in the cohort was breast cancer (1,166 cases), followed by melanoma of the skin (494) and cancer of the uterine cervix (457) (Table 3).

Analyses showed no increased overall risk of cancer among women using folic acid in one (HR 1.08; 95% CI 1.00–1.18) or two or more pregnancies (HR 1.06; 95% CI 0.91–1.22) compared to no folic acid use (p_{trend}=0.12). However, subgroup analyses indicated an increased risk of borderline significance for lung cancer (p_{trend}=0.06) and thyroid cancer (p_{trend}=0.05) in relation to folic acid use compared to no use. Analyses of folic acid use in relation to other cancer types showed no effect on the risk estimates compared to no use.

We conducted similar analyses for multivitamin supplementation that showed no increased overall risk of cancer. However, increased risk was found for melanoma of the skin among women using multivitamins in one (HR 1.19; 95% CI: 0.96–1.48) and

two or more pregnancies (HR 1.58; 95% CI: 1.05–2.38) compared to no use (p_{trend}=0.02). Additionally, increased risk of non-Hodgkin's lymphoma was seen among multivitamin users in one (HR 1.54; 95% CI: 0.94–2.53) and two or more pregnancies (HR 2.82; 95% CI: 1.15–6.95) compared to no use (p_{trend}=0.01).

4.2 Childhood cancer risk (publication II)

The study included 687,406 children (born during 1999–2010), and among them, 799 were diagnosed with cancer. In general, childhood cancer occurred more frequently among males (423) than females (376). The average follow-up time was 6 years (range 0.04–12 years), constituting 4,052,679 person-years. Maternal age at childbirth was in the age range of 13 to 55 years.

The proportion of children exposed to perigestational folic acid supplementation increased in the study period from 18% to 69% and multivitamin supplementation increased from 19% to 42%. About 67% of childhood cancer cases were diagnosed within the first 3 years of age (Table 4). Leukemia and central nervous system tumors accounted for 57% of the cancer cases.

No change was found in overall childhood cancer risk for maternal use of multivitamins only (HR 1.05; 95% CI 0.78–1.42), folic acid use only (HR 1.13; 95% CI 0.92–1.38), and combined folic acid and multivitamin use (HR 1.02; 95% CI 0.83–1.25) as compared to no maternal supplement use (p_{trend} 0.60). There was no difference in risk estimates between males and females. Likewise, subgroup analyses of the six most frequent childhood cancer types (leukemia, lymphoma, central nervous system tumors, neuroblastoma, Wilms' tumor, and soft-tissue tumors) showed no change in risk.

4.3 Paternal characteristics associated with maternal periconceptional folic acid supplementation (publication III)

Use of adequate folic acid supplements during 1999–2010 is presented in Figure 4. Adjusted analyses showed that the association between paternal age and adequate

maternal folic acid use was inversely U-shaped; adjusted RRs for adequate use were 0.35 (95% CI 0.28–0.43) and 0.72 (95% CI 0.71–0.74) for paternal age <20 and ≥40 years, respectively, comparing age 30–34 years. Compulsory education (1–9 years) among fathers was compared to tertiary education (14–20 years); the RR was 0.69 (95% CI 0.68–0.71) for adequate use. Occupation classes other than "Higher professionals" were associated with decreased risk of adequate folic acid use, compared with the reference "Lower professionals." Finally, the RR for adequate use was 0.58 (95% CI 0.56–0.60) comparing fathers born in "Low/middle-income countries" with fathers born in Norway.

Stratified analyses of recommended periconceptional folic acid supplementation by maternal and paternal education showed that adequate folic acid use was less likely in births where fathers had compulsory education, regardless of maternal education. The association of paternal compulsory education and recommended folic acid use was weakened by increasing levels of maternal education. However, even when the mother had reached tertiary education, the association of compulsory paternal education with recommended maternal folic acid use was significant (RR 0.75; 95% CI 0.73–0.77), compared to fathers with tertiary education.

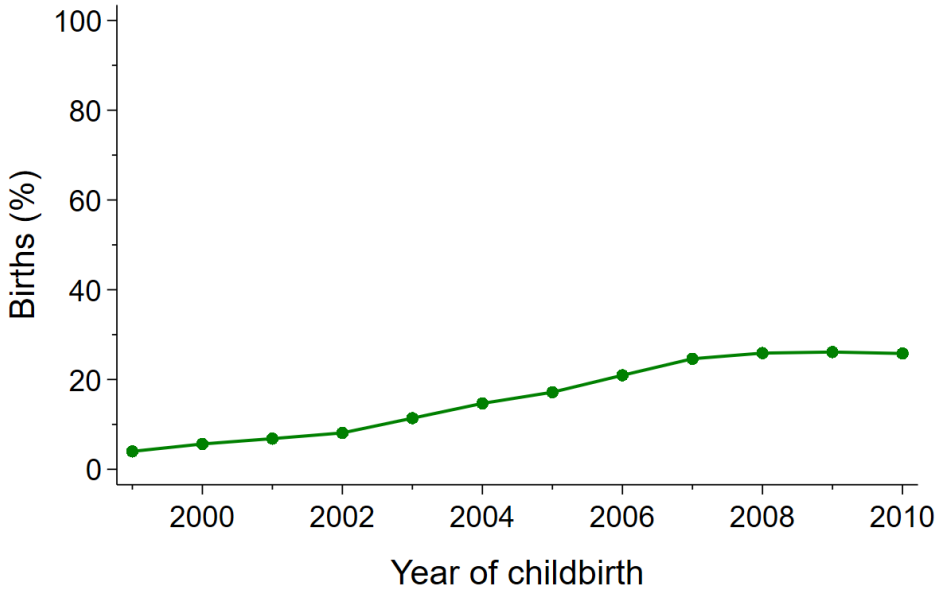


Figure 4: Percentage of adequate folic acid use in 683,785 births, Norway, 1999–2010

5. Discussion

5.1 Methodological considerations

5.1.1 Study designs

The studies on the association between maternal folic acid supplementation in pregnancy and maternal and childhood cancer risk have a population-based cohort design. The study of paternal characteristics of maternal folic acid supplementation has a cross-sectional design. The study populations used in our research were based on a linkage of population-based registries in Norway with comprehensive information for each individual, which makes it possible to produce association measures with high precision and assures generalizability of our results. Furthermore, the Norwegian population is relatively stable and emigration rates are low, and the national registries record statuses of immigration, emigration or deaths continuously for residents of Norway (Statistics Norway 2017).

5.1.2 Internal validity

Internal validity indicates the strength of inference based on the findings for the study population (Rothman et al. 2008i). In other words, it determines the extent to which the association observed in a study is due to independent variables and no other factors. The internal validity is good if the calculated estimate is minimized for systematic errors or biases. The presence of systematic errors that include selection bias, information bias, and confounding may reduce the internal validity of the study (Rothman et al. 2008i).

Selection bias

Selection bias is a distortion that occurs when the selection of subjects for a study, or the likelihood of them being retained in the study, leads to a result that differs from what would happen if the entire target population was enrolled (Rothman et al. 2008h). Common causes of selection bias in a cohort study are missing data, exclusion of study subjects, or short follow-up.

In *publications I* and *II*, information on maternal smoking was missing for 16% of the births. We performed analyses with and without maternal smoking as a covariate in the models. We found the HR estimates of maternal cancer and childhood cancer were similar when analyzing with and without the covariate maternal smoking. Furthermore, multiple imputation analyses (*publication I*) on missing smoking status at time of birth showed no substantial changes in the HR estimates. Further, in *publication III*, we lacked information on the father for about 2% of the births. These represented births with fathers unreported by the pregnant woman or fathers without an identification number assigned by the National Registry. Since the percentage of missing fathers was less than 10%, there was no reason to perform multiple imputation analyses, and removal of the missing cases was a reasonable solution (Cismondi et al. 2013). As information was missing for 4.4% of the births on paternal education and 7.7% of the births on paternal occupation, it was also reasonable to remove these missing cases from the analyses.

Our study is based on population-based register data that are compulsory, and loss to follow-up or selective reporting is minimal. The three study populations included the entire population of women giving birth in Norway during 1999–2010, live children born between 1999 and 2010, and all registered births in Norway from 1999 through 2010. This is unlikely to cause serious selection bias, and it is thus assumed to have no influence on the results of our research.

Our analyses may be affected by short follow-up, which causes selection bias where the time-period of cohort follow-up is too short for an outcome to appear (Encyclopedia of Epidemiology 2008). A challenge regarding *publications I and II* is the relatively short follow-up time of our study population. In *publication I*, the follow-up time was, on average, 7 years. The mean time between the first birth in the study period and cancer diagnosis was 5 years. Further, the average follow-up time in *publication II* was 6 years, and the mean time from birth to childhood cancer diagnosis was 3 years. Cancer is a heterogenic disease and may develop over decades. Thus, it is important that an observational study has appropriate follow-up to identify

cancer cases. A longer follow-up would probably detect more cancer cases and increase the strength of our analyses.

Information bias

Information bias is an error that results from incorrect classification or measurement of the exposure or outcome of interest (Rothman et al. 2008d). Classification errors of exposure or outcome that depend on the values of other variables are defined as differential misclassification. Classification errors of exposure or outcome that *do not* depend on the values of other variables are defined as non-differential misclassification.

Differential misclassification is systematic errors initiated by differences in the exactness or completeness of memory recollections reported by study participants concerning an event (recall bias) or systematic errors initiated because of selective revealing or suppression of information by individuals (reporting bias) (Rothman et al. 2008b). Such errors exist if an individual who has the outcome of interest has better recall of the exposing factor than those who do not have the outcome of interest. Selective revealing or suppression of information by individuals in the present study is not likely because our data were collected from compulsory population-based registries, though independent of each other. Concerning *publications I, II* and *III*, details of all folic acid exposures were collected retrospectively during hospitalization at the time of birth. Although this registration may be susceptible to recall bias, it is unlikely that vitamin supplements have been recalled differently among the mothers. Relating to *publications I* and *II*, information on supplement use was recorded before a cancer diagnosis, thus precluding recall bias. Most childbearing women know the adverse health effects of smoking, and they may be subject to social or medical disapproval. Self-reported smoking status may reduce the reliance of smoking data in our study. However, the smoking habits were documented before a possible cancer diagnosis. Furthermore, a validation study of self-reported smoking in Norway confirms the high validity of self-reported smoking in population-based studies (Kvalvik et al. 2012).

Non-differential misclassification means that the frequency of errors is equally distributed in the groups being compared. Non-differential misclassification of exposure may occur when records are incomplete due to incorrect registration, incorrect coding of disease diagnosis or errors in interpreting information from records. This leads to an underestimate of the risk and an incorrect conclusion (Sorahan and Gilthorpe 1994). When the exposure variables are dichotomous, non-differential misclassification tends to produce analysis estimates toward the null value (Rothman et al. 2008f). In other words, if there is an association, non-differential misclassification will minimize the association.

Registration of births in the Medical Birth Registry of Norway is performed by health personnel in attendance during hospitalization. Folic acid and multivitamin supplementation may have been registered incorrectly or underreported by the hospitals. Moreover, the health personnel in attendance may have provided incorrect information or failed to obtain information from the mothers. A possible misclassification of folic acid supplementation (independent of cancer risk) would bias risk estimates toward the null value and, in theory, may conceal an association between folic acid intake and maternal or childhood cancer risk.

Folic acid supplement use registered in the Medical Birth Registry of Norway has previously been validated against data from the Norwegian Mother and Child Cohort Study (MoBa) (Nilsen et al. 2009). Participants in the MoBa study (N = 73,579) were compared with women registered with births in the Medical Birth Registry of Norway (N = 398,849) during 2000–2006. The prevalence of folic acid use was substantially higher among MoBa participants. However, examination of differences in association measures for eight exposure-outcome associations showed no statistical relative difference for folic acid supplementation between women registered in the Medical Birth Registry of Norway and participants of the MoBa study. This finding demonstrates according to Nilsen et al. no bias in exposure–outcome associations, and the authors consider the Medical Birth Registry of Norway as a reliable data source of maternal supplement use when studying measures of association, but not for prevalence studies.

Registration of malignant tumors is compulsory in Norway, and the Cancer Registry of Norway registers all incident cancers in Norway. Health professionals and pathology laboratories involved in the diagnosis, treatment, and monitoring of cancer patients must report to the registry. Although the validity of the cancer registration is good, underreporting or misclassification of a cancer diagnosis may occur. Estimation by the capture/recapture method showed that the overall completeness of the Cancer Registry of Norway data during 2001–2005 was 98.8%, and the overall completeness 5 years after time of diagnosis was 97.8% (Larsen et al. 2009). The difference in completeness between the Cancer Registry of Norway data during 2001–2005 and the overall completeness 5 years after the time of diagnosis may represent under-reporting of malignancies or delayed notifications of cancer cases.

Confounding

Confounding implies that a "third" variable adversely affects the relation between the exposure of interest and the outcome (Rothman et al. 2008a). When confounding is present, the confounding variable has to be associated with the exposure in the source population, as well as being a risk factor of the outcome. This variable must not be an intermediate step in the causal pathway (Rothman et al. 2008a). In epidemiologic research, adjustments may increase bias through overadjustment or unnecessary adjustment (Schisterman et al. 2009). Unnecessary adjustment may be defined as control for a variable that does not affect the causal relation between exposure and outcome, but can affect its precision. Contrary to unnecessary adjustment, lack of important data may lead to unknown or unmeasured confounding (residual confounding) (Rothman et al. 2008e). Since confounding is a distortion that causes an outcome to be falsely attributed to another variable, it is important to address it in order to make valid causal inferences from observational data. Directed acyclic graphs (DAGs) give a visual representation of causal assumptions and help to identify the presence of confounding for a causal model (Pearl 2010, Shrier and Platt 2008, Textor et al. 2011).

In *publications I* and *II*, the potential confounding variables were chosen *a priori* and examined according to their influence on the risk estimates. In *publication III,*

subject-matter knowledge and DAGs (Appendix 2) were used to identify the unconfounded association of paternal determinants with adequate maternal folic acid supplementation (Pearl 2010, Shrier and Platt 2008, Textor et al. 2011).

In *publication I*, the models were adjusted for maternal age at first childbirth (age at cohort entry) during 1999–2010, maternal year of birth, parity, marital status, education, occupation, and smoking status at the time of birth. Age in our study relates to cancer because cancer incidence increases with age (White et al. 2014). Women who have their first full-term pregnancy at a young age have a lower risk of developing hormone receptor positive breast cancer later in life than women who have their first child later in life (at 30 years or more) (Bernstein 2002). Since breast cancer was the most frequent cancer type in our cohort, we therefore adjusted risks for breast cancer and total cancer for maternal age at first childbirth (including first birth before cohort entry in 1999).

We had no information on maternal weight and height, physical activity, diet or use of alcohol. High BMI, low physical activity and high alcohol use are associated with the risk of some cancer types (Bagnardi et al. 2001, Bhaskaran et al. 2014, I. M. Lee et al. 2012). Alcohol use, known to antagonize folate absorption and metabolism, is unlikely to be an important confounder as the consumption of alcohol during pregnancy is generally low in Norway. In our study, we could not control for health-related covariates other than smoking. Therefore, confounding from other risk factors is possible. However, adjustment for smoking and other existing covariates showed no change in the risk estimates, suggesting reduced likelihood of residual confounding.

In *publication II*, the final models included birth order, maternal smoking, maternal and paternal age, and maternal and paternal education.

We had no information on dietary folate. However, residual confounding by dietary folate is less likely. Maternal plasma levels of serum folate are strongly related to folic acid supplementation (Bjorke-Monsen et al. 2013), and two other studies of maternal periconceptional folic acid supplement use and offspring oral clefts and

autism risks have shown that adjustment for dietary folate did not change overall risk estimates (Suren et al. 2013, Wilcox et al. 2007).

Information on the mother's weight and height, physical activity, diet, use of alcohol, or use of contraceptive pills were not available in our data. Consequently, confounding from other risk factors may occur. Maternal height and weight before pregnancy are, however, available in the Medical Birth Registry of Norway from 2007 and onwards, but are currently reported from only 40% of the birth clinics (T. Nilsen et al. 2016).

In *publication III*, the models were adjusted for year of childbirth, father's age, education, and country of origin. In addition, we included maternal age, education, and country of origin as possible confounders of the associations between paternal age, education, or country of origin, and maternal adequate folic acid use.

We had no information on pregnancy planning, maternal physical activity or maternal use of alcohol. Since these covariates are related to maternal periconceptional folic acid use, confounding from other risk factors is possible.

Maternal smoking was not considered a potential confounder of the association between paternal determinants and adequate maternal folic acid supplementation. A cross-sectional study showed that maternal smoking before pregnancy was not an independent determinant of preconception folic acid use (R. M. Nilsen et al. 2016).

Effect modification

Effect modification is distinct from confounding in that it occurs when an exposure has different effects among different subgroups, and it is only associated with the outcome but not the exposure (Greenland et al. 2008).

We evaluated effect modification by stratification and by including an interaction term in the regression models.

The rationale for evaluating maternal education as an effect modifier was that maternal education seems to be positively associated with paternal education (van de Putte et al. 2009).

In *publication III*, the relationship of compulsory paternal education and recommended maternal folic use was reduced by increasing level of maternal education. However, even when the mother had tertiary education, the relationship of compulsory education among fathers on recommended folic acid supplementation was significant, thus suggesting an interaction between them.

5.1.3 External validity

Generalizability or external validity refers to the extent to which the results and conclusions of a study can be generalized to the world at large (Rothman et al. 2008c). Internal validity is a prerequisite for external validity because a study must demonstrate that the exposure is the cause of variation in the outcome before one can generalize that the exposure causes the outcome (Juul 2013).

To achieve high external validity of our results, we need the study population to be representative of other, larger populations in other places and at other times. Since our study is a population-based study, external validity would apply to populations where welfare, education and healthcare are similar to Norway.

In *publication I*, our finding of no association between folic acid supplementation and cancer risk is in accordance with several prospective cohort studies and meta-analyses (populations geographically and ethnically different from ours) showing no overall or site-specific relation between folic acid use and cancer risk (Hankey et al. 2012, Qin et al. 2013, Vollset et al. 2013, Zhang et al. 2008). In *publication II*, we found no relationship between periconceptional folic acid supplementation and major childhood cancers. This is in discordance with a large international collaborative study, including 47,000 children with acute leukemia and 11,000 controls, finding reduced risks of ALL and acute myeloid leukemia (AML) after maternal intake of folic acid supplements (Metayer et al. 2014). Moreover, our results in *publication II*

can only be generalized to younger children since our cohort was followed for an average of 6 years.

5.1.4 Precision

Precision in epidemiologic measurements refers to the extent or reduction of random errors (Rothman et al. 2008g). In general, we can improve precision by increasing the study size or modifying the study design.

Our data is large and nationwide with balanced groups (i.e., folic acid exposure, no folic acid exposure, with cancer, without cancer) which produces more precise estimates (Rothman et al. 2008g). We used 95% confidence interval (i.e., 5% possibility that the observed association is the result of chance) and the width of these intervals reflects the precision.

Despite our large study population, the sub-analyses had smaller numbers of events. In *publication I,* the association between folic acid supplementation prior to and during one or two or more pregnancies and the relatively low number of subtypes of maternal cancer cases, compared with a large sample size with no supplement use, yielded less precise estimates, as is indicated by the widths of the confidence intervals. Additional stratification of exposure into "use before pregnancy" and "use during pregnancy" was consequently not feasible. In *publication II,* the association measures between supplemental folic acid in pregnancy and childhood cancer, as compared to no supplement use, generated estimates of reduced precision due to a small number of children with cancer. Further stratification into preconceptionally use and use during pregnancy was also not reasonable in this publication. In *publication III,* the proportion of mothers using folic acid supplements and the distribution of fathers across paternal age, education, occupation, and country of origin may affect the precision of the study estimates. However, as shown by the relatively narrow confidence intervals, the association measures between paternal determinants and adequate maternal folic acid use yielded precise estimates. Nevertheless, a small number of individuals in some subgroups gave wider confidence intervals.

The categorization of our data may also affect precision. In *publication I*, we categorized the cancer cases according to ICD-10 into 13 main groups that were studied in detail. Among all maternal cancer cases, 1% had taken folic acid before, 15% during, and 9% before and during pregnancy. Taking into account that the mothers were studied in one pregnancy or two or more pregnancies, the number of cancer cases decreased substantially for each following birth: 1,214 cancer cases among mothers with only one pregnancy, and 298 cancer cases among mothers with two or more pregnancies. Even among the most frequent cancer types, the number of folic acid users and cancer cases dropped substantially.

In *publication II*, we divided the childhood cancer cases according to the ICCC-3 into six categories (leukemia, lymphoma, central nervous system tumors, neuroblastoma, Wilms' tumor, and soft-tissue tumors). In order to account for the low number of childhood cancer cases and the number of children exposed to supplements, only the major childhood cancer groups were evaluated in the analyses.

In conclusion, precision of the estimates in our study is generally not a problem when inferences are based on the overall results.

5.2 Discussion of results

Our nation-wide cohort study of all live births in Norway during 1999–2010, showed no association between maternal periconceptional folic acid supplementation and maternal cancer. Similarly, maternal folic acid supplementation in pregnancy was not associated with risk of major childhood cancers in offspring.

Our population-based cross-sectional study showed that paternal characteristics; fathers who were younger and older at their infant's birth, had achieved a lower level of education, had a manual or self-employed occupation, or originated from low/middle-income countries, were associated with inadequate maternal folic acid use in Norway from 1999 through 2010.

5.2.1 Maternal cancer risk (publication I)

Our findings of no relation between maternal periconceptional folic acid supplementation and overall or site-specific cancer are in accordance with a meta-analysis of 13 randomized controlled trials, comprising about 50,000 participants who were allocated to folic acid or to a placebo, and observed for 5 years. The authors reported no association between folic acid supplementation and cancer incidence (overall or subtypes of cancers) compared to the placebo (Vollset et al. 2013). Further, during 2000–2004, about 8,000 patients from 10 countries who had suffered a recent stroke or transient ischemic attack were randomly assigned to receive B vitamins (2 mg of folic acid, 25 mg of vitamin B_6, and 0.5 mg vitamin B_{12}) or a placebo. Consistent with our results, the study found no relation between participants allocated to B vitamins compared to the placebo and cancer incidence (Hankey et al. 2012). Moreover, in compliance with our findings, a randomized controlled trial comprising approximately 5,000 female health workers aged 42 years or older with a preexisting cardiovascular disease or having coronary risk factors in the US during 1998–2005, reported no association between combined folic acid, vitamin B_6, and vitamin B_{12} treatment and overall cancer or breast cancer risk compared to the placebo (Zhang et al. 2008).

In line with our results, a case-control study encompassing 2,491 women with breast cancer and 2,521 matched controls in Europe (Denmark, Italy except Naples, the Netherlands, Norway, Spain, Sweden and UK) reported no relationship between plasma concentration levels of folate and vitamin B_{12} and breast cancer risk (Matejcic et al. 2017). However, in contrast to our finding of no association between folic acid supplementation and breast cancer risk, a prospective cohort study including 25,400 postmenopausal women in the US showed that high folate intake (>0.8 mg) was associated with increased risk of breast cancer compared to no use (Stolzenberg-Solomon et al. 2006). The authors commented that their observed results might be due to chance or uncontrolled confounding.

In our study, use of folic acid supplements was not associated with lung cancer risk. This finding is concurrent to the observations from the Vitamin Lifestyle cohort study encompassing 77,118 participants in the age range of 50 to 76 years during 2000–2002 in the US that reported no association between supplemental folate, vitamin B_6 or vitamin B_{12} and lung cancer risk among women (Brasky et al. 2017). However, the authors observed increased lung cancer risk among men using vitamin B_6 and B_{12}, but not among women.

Contrary to our finding of no relationship between folic acid supplement use and colorectal cancer, a randomized controlled multi-center trial in China, comprising 791 participants randomized to receive either 1 mg/day folic acid supplement or treated without folic acid and followed for 3 years, showed a decreased risk of colorectal cancer (Gao et al. 2013). However, similar to our results, a meta-analysis of nested prospective case-control studies on circulating folate and colorectal cancer (3,477 cases and 7,039 controls) showed no association between circulating folate concentrations and colorectal cancer risk (Chuang et al. 2013). Moreover, the authors observed an inverse association between circulating folate and colorectal cancer risk in case-control studies that measured folate by radioimmunoassay compared to case-control studies using microbiological assay. According to the authors, this finding could reflect differences in cohort and study design rather than assay performance (Chuang et al. 2013). Furthermore, prospective studies of natural food folates and folic acid intake suggest that high folate intake may reduce the risk of colorectal cancer (Kennedy et al. 2011, Lee et al. 2011, Stevens et al. 2011). As opposed to these findings, results from prospective studies on blood folate levels are inconsistent and cannot confirm a proposed protective role for colorectal cancer (Eussen et al. 2010, J. E. Lee et al. 2012, Takata et al. 2014, van Guelpen et al. 2006, Weinstein et al. 2008). A population-based case-control study from Sweden, comprising 226 colorectal cancer cases and 437 matched controls with median age about 60 years, showed a “bell-shaped” association between plasma folate concentrations and colorectal cancer risk (van Guelpen et al. 2006). The authors observed a doubling of colorectal cancer risk among individuals in the middle compared to lowest plasma

folate quintile. Another population-based, nested case-control study from Sweden, with 331 cases, 662 matched controls and followed for median 10.8 years, revealed that low plasma folate concentrations were related to low colorectal cancer risk in a population with low folate status (Gylling et al. 2014). The authors proposed that low folate status might have a protective role for colorectal cancer or suppress progression of preneoplastic or neoplastic lesions. A potential explanation for these discordant results might be that studies assessing folate exposure by dietary intake seem to encompass larger cohorts than studies using blood folate concentrations. Consequently, studies of blood folate concentrations could have less statistical power to detect a potential protective association between folate and colorectal cancer. On the other hand, blood folate concentration reflects the intake of natural folate through diet as well as ingested folic acid supplements. Further, folate intake is measured either as dietary folate from foods or as total folate from both dietary folate and folic acid supplements (Park et al. 2013). In some epidemiologic studies, it is not always clear whether intake from folic acid supplements has been included in the dietary assessments. On the other hand, the dietary sources of folate from fruits, vegetables and cereals contain other important vitamins, fibers and minerals that may have independent cancer-protective influence (Larsson et al. 2006, Song et al. 2015).

Interestingly, Kim has proposed a potential dual modulatory role of folate on colorectal cancer based on animal studies (Kim 2007). In normal colorectal mucosa, folate deficiency appears to stimulate the initial stages of carcinogenesis, and supplementation of moderate doses of folic acid may suppress, whereas high doses of folic acid may enhance the development of cancer (Kim 2007). On the other hand, folic acid supplementation may enhance the growth of cancer cells in established neoplastic foci, whereas folate deficiency may inhibit progression of established colorectal neoplasms (Kim 2007). Similarly, Ulrich and Potter suggest that folate administration before the presence of preneoplastic lesions may prevent neoplastic development, whereas folate supplementation may increase tumor formation once early neoplastic lesions are established (Ulrich and Potter 2007). A potential dual role of serum folate in the development and progression of colorectal cancer in humans is

supported by findings in a case-control study by Chiang et al. and the nested case-control study by Gylling et al. (Chiang et al. 2014, Gylling et al. 2014).

We found no association between multivitamin supplementation and overall cancer risk. This is also supported by other studies reporting weak or no associations between multivitamin use and total cancer risk (Neuhouser et al. 2009, Park et al. 2011). A cohort study comprising about 180,000 participants (aged 45–75 years) in Hawaii and California between 1993 and 1996 showed no association between multivitamin supplementation (information based on mailed questionnaire) and cancer risk, overall or for subtypes of cancer, compared to no use (Park et al. 2011). An observational study comprising about 160,000 postmenopausal women (aged 50–79 years) in the US between 1993 and 1998 analyzed health and risk factors for cancer, and other illnesses, in relation to multivitamin use. Multivitamin use was collected at baseline and at follow-up time points during in-person clinic visits, and the women were followed for 8 years. The authors found no association between multivitamin use and cancer risk, compared to no use (Neuhouser et al. 2009).

Similar to our findings of increased risk of malignant melanoma among women using multivitamins compared to no supplement use, the authors of a randomized, double-blinded, placebo-controlled trial comprising 7,876 women (aged 35–60 years) and 5,141 men (aged 45–60 years) in France suggested that antioxidant supplementation increases the incidence of skin cancers, including melanomas, in women but not in men (Hercberg et al. 2007). However, their findings have been criticized because of flaws in their study methods and in the interpretation of the results (Green et al. 2008). Though, our finding of increased risk of malignant melanoma is in discordance with a previous prospective cohort study, comprising 69,671 men and women, on self-reported antioxidant supplement use and melanoma risk in the US, showing no increased melanoma risk (Asgari et al. 2009).

Our finding of increased risk of non-Hodgkin’s lymphoma among women using multivitamin supplements compared to no supplement use is in concordance with a pooled cohort study (involving 88,410 women and 47,336 men in the US) on the use

of individual vitamin and multivitamin supplements and the risk of non-Hodgkin's lymphoma (Zhang et al. 2001). This study showed that multivitamin use was related to a higher risk of non-Hodgkin's lymphoma among women, but not among men, and the authors concluded that their observed findings were the results of chance. However, most non-Hodgkin's lymphoma cases occur in individuals older than 60 years (Zhang et al. 2011), which is considerable older than the mean age at cancer diagnosis in our cohort (37 years).

Several possible mechanisms have been proposed by which folate or the bioactive form of folic acid may modulate cancer risk. Low folate status causes uracil incorporation in DNA and DNA strand breaks (Berger et al. 2008). The DNA breaks lead to fragmentation and rearrangement of the chromosomes that causes generation of cells with aberrant karyotypes and altered genes, which may promote cancer development (Duthie 2011). Further, a common single nucleotide polymorphism in the MTHFR gene (MTHFR 677C→T polymorphism) is connected to reduced MTHFR enzyme activity and function that is important for the methylation and nucleotide pathways (Figueiredo et al. 2013, Liew and Gupta 2015). Moreover, the MTHFR 677C→T polymorphism is related to colorectal cancer, uterine cervical cancer (Liew and Gupta 2015), and breast, esophageal, gastric and pancreatic cancers (Boccia et al. 2008, Ericson et al. 2009, Larsson et al. 2006). Further, a meta-analysis comprising 360 thyroid cancer cases and 900 controls reported that the MTHFR 677C →T polymorphism was associated with increased thyroid cancer risk (Yang et al. 2014). We had no information on MTHFR polymorphisms in our study, but we observed an increased risk of borderline significance of thyroid cancer among women using folic acid supplements in one or two or more pregnancies compared to no supplement use.

Our study of maternal cancer risk and folic acid supplementation was limited by a lack of records on frequency, or precise duration of folic acid and multivitamin use throughout pregnancy. Individual maternal data on MTHFR polymorphism status and blood folate level would have been beneficial to our study. An additional concern in our study is latency in cancer development. A longer follow-up period would have

increased the size of our study population, and more cancer cases may have been detected, which would have strengthened the statistical power of our analyses.

Presently, there is no evidence to support a significant causal effect of folic acid on cancer because there are insufficient data available (Moussa et al. 2016, Scientific Advisory Committee on Nutrition 2017). In our study, we found no association between maternal periconceptional folic acid supplementation and cancer risk (overall and site-specific). However, we could not assess the long-term risk of folic acid use in pregnancy for maternal cancer.

5.2.2 Childhood cancer risk (publication II)

Our analyses of estimated maternal intake of folate from folic acid supplements, multivitamins, and combined use of these supplements showed no association with leukemia, lymphoma, childhood brain tumors, neuroblastoma, Wilms' tumor, or soft-tissue tumors (ICCC-3). In line with our finding of no relation between folic acid and leukemia, a case-control study from the US (357 cases and 405 matched controls from the Northern California Childhood Leukemia Study during 1995–2002) reported no association between birth folate concentrations and risk of childhood leukemia (Chokkalingam et al. 2013).

However, our results are in discordance with previous case-control studies that showed an inverse association between self-reported folic acid supplementation and ALL or central nervous system tumors (Metayer et al. 2014, Milne et al. 2012, Milne et al. 2010, Thompson et al. 2001). A case-control study from Western Australia (83 cases and 166 matched controls during 1984–1992) reported a strong inverse association between maternal folic acid supplementation during pregnancy and ALL (Thompson et al. 2001). However, this study was not designed to investigate the association between self-reported folic acid supplementation and ALL, and the findings were unexpected. Because of these findings, two studies followed: A prospective, national, population-based, multicenter case-control study (416 cases and 1,361 matched controls during 2003–2007) on maternal use of folic acid and other vitamins before and during pregnancy, and a meta-analysis based on the results from

previous studies (Milne et al. 2010). The authors found some evidence of an inverse association between preconceptional folic acid use and ALL, including a weak, inverse dose-response relationship, but no association between folic acid supplementation use during pregnancy and ALL. A possible protective role of folic acid against ALL has been suggested in an international case-control study that included 7,000 children with ALL and 11,000 controls (during 1980–2012), which reported reduced risk of ALL and acute myeloid leukemia after maternal intake of folic acid supplements and other vitamins before or during pregnancy (Metayer et al. 2014). Similarly, high intake of folic acid and other one-carbon metabolism nutrients from food and supplements combined was also associated with reduced ALL risk in a case-control study in the US comprising 681 ALL cases, 103 AML cases, and 1,076 matched controls (Singer et al. 2016).

An Australian case-control study of childhood brain tumors was performed between 2005 and 2011 (327 cases from 10 pediatric oncology centers, and 867 matched control children) to investigate a proposed association between self-reported folic acid use (before and during pregnancy) and childhood brain tumors. Contrary to our findings, the authors found an inverse association of childhood brain tumors and maternal folic acid use before and possibly during pregnancy (Milne et al. 2012). The strengths of this study were the large sample size for main and subgroup analyses, information about preconception and postconception exposures, and sufficient data to conduct sensitivity analyses. Weaknesses were possible recall and selection biases, and lack of specificity in type and dose of vitamins and other minerals. Similarly, a recent multinational case-control study on childhood brain tumors in Denmark, Sweden, Norway and Switzerland (CEFALO), which included interviews with 352 mothers of eligible cases and 646 population-based controls with data from birth registries, showed an inverse association between self-reported maternal vitamin intake during pregnancy and childhood brain tumor risk (Vienneau et al. 2016).

Due to limited statistical power, we could not stratify our exposure data into preconceptional use and use during pregnancy. However, in line with our findings of no relation between maternal folic acid use and childhood brain tumors, a population-

based case-control study from France (510 cases and 3,102 matched controls aged under 15 years) found no association between folic acid supplement intake and childhood brain tumor risk (Bailey et al. 2017). However, a weakness of this study was low use of folic acid supplements before conception (5.3% for cases and 7.8% for controls).

Our analyses were further limited by a relatively short follow-up period. A longer observation time would have increased the number of study participants and the number of childhood cancer cases, thus increasing the statistical power of our analyses. The exposure information regarding folic acid intake during pregnancy that was available to us could have been more detailed regarding dose, frequency, and precise duration of use. However, we combined information on multivitamin and folic acid supplement use to establish exposure categories of increasing folic acid intake (0, ~0.2 mg, 0.4 mg, ~0.6 mg).

Maternal smoking information was missing for 16% of the pregnancies. However, the association between periconceptional folic acid exposure and childhood cancer risk where essentially similar when the variable for maternal smoking was not included in our models. A similar finding is supported by a cohort study comprising 801,867 children in Denmark, showing no association between maternal smoking during pregnancy and overall childhood cancer risk (Momen et al. 2016).

Childhood cancer is a heterogenic group of rare malignancies that is different from adult cancers, and seems to originate *in utero* (Bjørge et al. 2013, Stiller 2004). Even when they are apparently similar to adult cancers like leukemia, lymphomas, brain tumors and other solid tumors, childhood cancer differ in underlying pathology, behavior and treatment outcome compared to the commonly occurring cancers of middle and old age (Murphy et al. 2013). Compared to adults, where carcinomas are the most common type, usually occurring during middle or old age, childhood cancers are predominantly embryonal tumors, and some sarcomas and germ-cell tumors, appearing at a young age (Steliarova-Foucher et al. 2005). The etiology is largely unknown, and a complex interplay of genetic, nutritional, hormonal, and

environmental factors has been associated with some childhood cancer types (Callan and Milne 2009).

Based on the substantial role of folate in DNA methylation and nucleotide biosynthesis, dysregulation of DNA methylation *in utero* and in the early postnatal period may be one of many underlying mechanisms by which maternal periconceptional and postnatal nutrition can modulate childhood cancer risk (Kim 2016, Ly et al. 2012). It is hypothesized that during the embryonic stage, DNA methylation is reprogrammed and maintained during the postnatal period, rendering it susceptible to the *in utero* and early postnatal nutritional environment (Kim 2016, Ly et al. 2012). Moreover, a study showed that blood folate concentration is inversely associated with DNA methylation, and that low folate at conception may act as a limiting factor on DNA methylation, but only below a certain threshold (200 µg/d) (Gonseth et al. 2015). The authors hypothesize that early-onset cancers like ALL may be triggered by low folate status through DNA methylation mechanisms (Gonseth et al. 2015).

Ecological studies from countries with mandatory folic acid food fortification have shown a reduced incidence of certain childhood cancers, and several case-control studies suggest a possible protective association of periconceptional maternal folic acid use in relation to major childhood cancer types. Our population-based study of maternal periconceptional folic acid use and childhood cancer risk showed no relationship between maternal folic acid supplementation and major childhood cancers. On the other hand, we cannot assess the long-term childhood cancer risk after folic acid exposure *in utero.*

5.2.3 Paternal characteristics associated with maternal periconceptional folic acid supplementation (publication III)

To our knowledge, few studies have identified paternal determinants for maternal folic acid intake before and during pregnancy. Our analyses showed that fathers at younger and older age, compared to those aged 30–34 years at childbirth were associated with inadequate folic acid supplementation. There was a relationship

between the father completing only compulsory education, compared to tertiary education, and inadequate folic acid supplementation. Fathers in a manual or self-employed occupation compared with the reference "Lower professionals," and fathers who were born in low/middle-income countries compared to fathers born in Norway, were associated with inadequate folic acid supplementation.

While our study focuses on paternal determinants of adequate maternal folic acid use, numerous studies have shown that younger maternal age is a significant determinant of inadequate folic acid use (R. M. Nilsen et al. 2016, Nilsen et al. 2006, Timmermans et al. 2008, Tort et al. 2013). This is consistent with our findings of younger paternal age as a risk factor for inadequate folic acid use. In contrast to these studies, we found low maternal use of folic acid among fathers at advanced age. Men are able to reproduce later in life than women; available data from the Nordic countries, Australia, England, Wales, and France, suggest that about 10% of fathers commence fatherhood in their 40s, and a smaller proportion have children after 50 years of age (Schmidt et al. 2012). Concurrent to these data, the fathers were 40 years and older in 12% of the births in our study population.

A previous study comprising about 27% of the births registered in the Medical Birth Registry of Norway during 2000–03 on the relationship between recommended maternal periconceptional supplementation and partner's education align well with our results (Nilsen et al. 2006). The study found a positive association between paternal education and recommended periconceptional folic acid use. Nilsen and colleagues showed that in pregnancies with fathers having university or college education, the adjusted relative risk (RR) of periconceptional maternal folic acid use was 1.4 (95% CI 1.1–1.8) compared to pregnancies with fathers having primary education only (Nilsen et al. 2006). However, the association was weaker than for maternal education. Similar results to ours have also been found among married Pakistani women, showing that the educational status of the mother's husband is associated with maternal intake of iron and folic acid supplements (Nisar et al. 2014). Inadequate supplement use was strongly related to low paternal education.

A cross-sectional study (5,153 women extracted from the National Birth Defects Prevention Study in the US) of folic acid use, prenatal care, smoking, and drinking in early pregnancy by maternal occupation identified several occupational groups with increased prevalence of high-risk maternal behavior (smoking, alcohol consumption, or not attending prenatal care) or low use of folic acid supplements during pregnancy (Agopian et al. 2012). The occupational groups in which the women were less likely to use folic acid were "healthcare support," "protective service," "food preparation/serving-related," "building and grounds cleaning/maintenance," "sales and related," "farming/fishing/forestry," "production," and "transportation/material moving". These findings seem to be in line with our findings that occupational classes other than "higher professionals" among fathers were related to decreased risk of adequate folic acid supplement use.

Studies on the relationship between women's ethnic background and recommended periconceptional folic acid supplementation have been performed in Norway and other European countries (Netherlands, Belgium, Ireland and the United Kingdom) (Baraka et al. 2011, Brough et al. 2009, Kinnunen et al. 2017, McGuire et al. 2010, Timmermans et al. 2008). These studies have similar findings to our own, showing a low risk of adequate maternal periconceptional folic acid use among ethnic minority groups compared to the native population.

Our investigation showed that periconceptional folic acid supplement use was still infrequent in Norway. During 1999–2010, the mothers used the recommended folic acid supplements for only 16% of the births in our study. Similar results have been found in Denmark. A cross-sectional study consisting of 22,000 pregnant women (primiparious and multiparous) during 2000–2002 showed that only 14% of them used folic acid as recommended (Knudsen et al. 2004). However, by 2012, only 10.4% of 462 women attending a nuchal translucency scan in Demark used folic acid as recommended (Friberg and Jorgensen 2015).

Education is the basis for labor achievements, and if such achievements are relevant to health, education may also increase the ability to act in healthy ways based on

knowledge of health (Pampel et al. 2010). A population-based register study of all-cause mortality in Norway during 1980–2003 for all Norwegian men and women born between 1950 and 1973 (aged 30–53 years) showed that mortality is lower among those who have, or have had, a well-educated partner than among those with a less educated partner, although one's own education is more important (Kravdal 2008). Similar findings are reported in a cross-sectional study of the association between spousal education and self-rated health among married men and women in the US, constituting 337,846 married individuals aged 25 years and older (Brown et al. 2014). Spousal education attenuated the association between individuals' own education and self-rated health among married men and women. The results suggest that husbands' level of education is more important for wives' self-rated health than wives' level of education is for husbands' self-rated health. Further, a Dutch cross-sectional study of 40,000 individuals aged 25–74 years on the importance of partner status and education, showed that women appear to be more dominated by their partner's educational level than men are with regard to healthy behavior (Monden et al. 2003). However, the evidence for male dominance in their study was weak.

A cross-sectional study of data from the 2011–2013 National Survey of Family Growth in the US (2,089 pregnant women with a partner aged 18–49 years) examined the relationship between paternal pregnancy intention and breastfeeding duration. They found that unwanted pregnancy, specifically among fathers aged 18–24 years, was associated with no breastfeeding or shorter breastfeeding duration (Wallenborn et al. 2017). This result suggests a lack of support from fathers who do not want a pregnancy. Similar associations of paternal support and breastfeeding outcomes have been found in other studies (Bronte-Tinkew et al. 2007, Tohotoa et al. 2009). Moreover, previous research on men's birth intentions in the 2006–2010 National Survey of Family Growth study in the US reported that 63% of pregnancies were wanted by the father (Lindberg and Kost 2014). Further, a study on rapid repeat pregnancies in the US reported that the odds of having a rapid pregnancy after a previous one was higher when the father intended pregnancy but not the mother, and lower if the father did not intend pregnancy but the mother did (Cha et al. 2016). The

relevance of these findings is that men's intention status of births influences their parental involvement and attitudes toward fertility-related behaviors (Lindberg et al. 2017).

In summary, these studies demonstrate the importance of the partner's influence on maternal reproductive health and family planning. Our findings may support the importance of the father's influence on maternal periconceptional folic acid supplementation.

6. Conclusions

In this thesis, the access to data from population-based registries enabled us to study the association between folic acid and multivitamin supplementation in pregnancy and cancer risk in presumably healthy women and their children. Furthermore, we studied several potential paternal indicators of maternal periconceptional folic acid supplementation. Based on the results of our study, we reached the following conclusions:

Folic acid supplementation does not increase the short-term overall cancer risk or risk of particular types of cancer among women using folic acid before and/or during pregnancy.

Maternal folic acid supplementation use before and/or during pregnancy was not associated with risk of childhood leukemia, lymphomas, central nervous system tumors, neuroblastoma, Wilms' tumor, or soft-tissue tumors. Although our cohort was observed over an average period of 6 years, the association between periconceptional folic acid exposure in utero and childhood cancer can only be addressed for young children.

In general, this study highlights how the partner's age, education, occupation and country of birth influences a woman's use of recommended folic acid supplements. We found low maternal compliance to recommended periconceptional folic acid use in pregnancies when the fathers were among the youngest and oldest, had a shorter education, worked in a manual or self-employed occupation, or were born in low/middle-income countries.

7. Future perspectives

Although our analyses address the short-term relationship between folic acid supplementation and cancer, we cannot assess the long-term effect of folic acid use on maternal or childhood cancer risk.

The role of folates in cancer biology remains to be clearly defined. There is evidence that various adult and childhood cancers have different associations with folates. In order to determine the long-term risk of folic acid supplementation, a cohort study with extended follow-up is necessary to determine the risk and rate of progression from exposure to cancer for total and subgroups of adult and childhood cancers. When stratification of exposure data is not feasible due to the limited statistical power of the analyses, increasing the overall number of individuals will reduce data sparseness and improve precision. In order to achieve this in future studies, a considerably larger study population than ours is needed.

A limitation of *publications I* and *II* is that there could be misclassification of maternal folic acid and multivitamin use. The information collected about folic acid use should be as detailed as possible, and thus future studies should include a renewed validation study. Moreover, multivitamins sold over the counter during our study period contained 0.0–0.2 mg of folic acid. A future study should also have information on folic acid doses in multivitamins.

Lastly, studies highlight paternal influence in reproductive decisions, and couples tend to postpone parenthood. A future study of men's birth intentions could focus on advanced paternal age (more than 35 years), socioeconomic status, maternal periconceptional folic acid use and adverse perinatal outcomes.

8. Errata

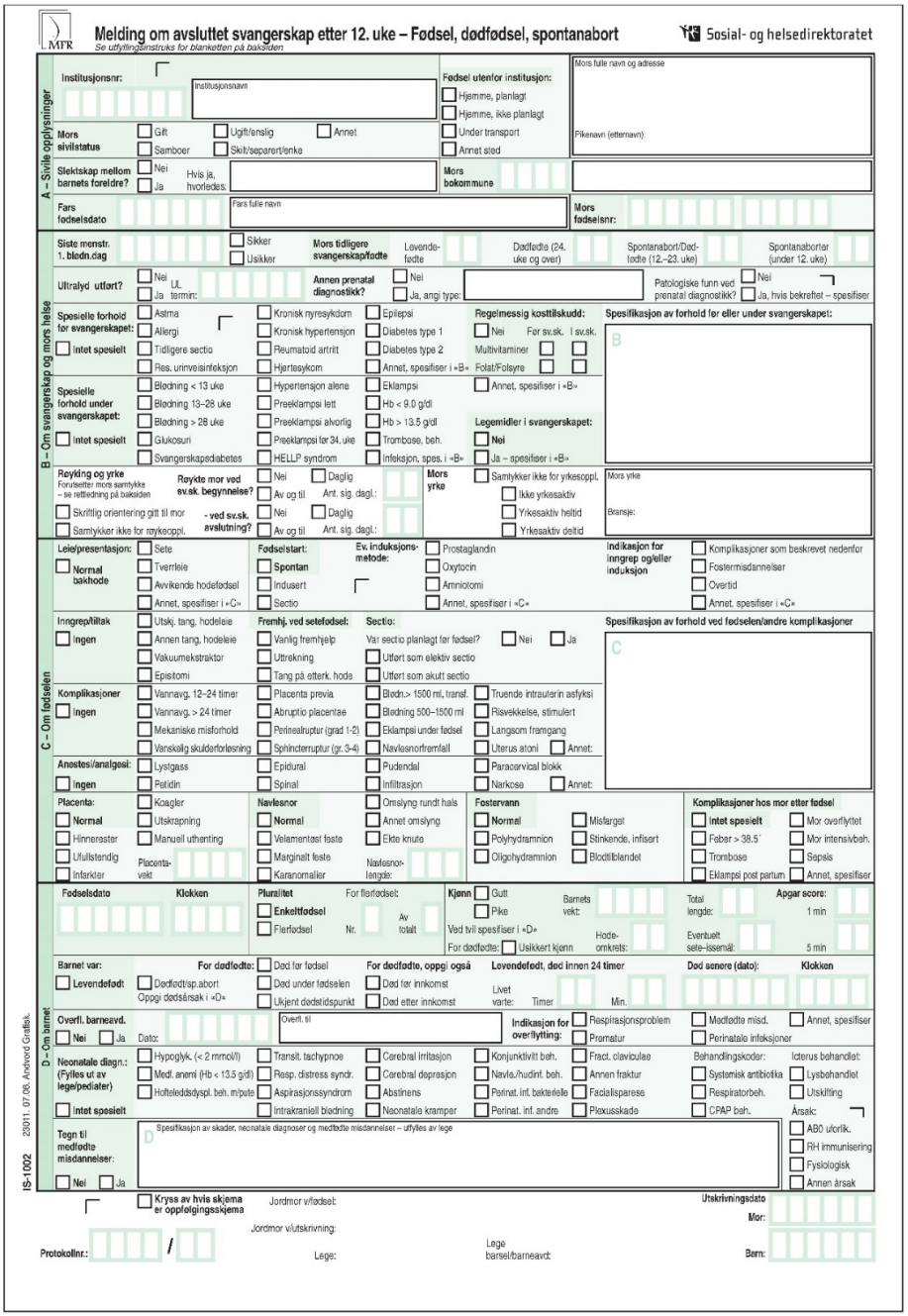
Publication I:

The fourth sentence in 2.2 Exposure (Material and methods) on page 806: *Furthermore, the mothers were defined as multivitamin users if folic acid were used before and/or during pregnancy*. Should read: *Furthermore, the mothers were defined as multivitamin users if multivitamins were used before and/or during pregnancy.*

9. Appendix

9.1 Appendix 1. Notification form for birth registration

Notification form for MBRN (1998 -)



Melding om avsluttet svangerskap etter 12. uke – Fødsel, dødfødsel, spontanabort

Se utfyllingsinstruks for blanketten på baksiden

Sosial- og helsedirektoratet

A – Sivile opplysninger

Institusjonsnr: | Institusjonsnavn | Fødsel utenfor institusjon: Hjemme, planlagt; Hjemme, ikke planlagt; Under transport; Annet sted | Mors fulle navn og adresse | Pikenavn (etternavn:)

Mors sivilstatus: Gift; Samboer; Ugift/enslig; Skilt/separert/enke; Annet

Slektskap mellom barnets foreldre? Nei; Ja. Hvis ja, hvorledes:

Mors bokommune

Fars fødselsdato | Fars fulle navn | Mors fødselsnr:

B – Om svangerskap og mors helse

Siste menstr. 1. blødn.dag | Sikker; Usikker

Mors tidligere svangerskap/fødte: Levendefødte | Dødfødte (24. uke og over) | Spontanabort/Dødfødte (12.–23. uke) | Spontanaborter (under 12. uke)

Ultralyd utført? Nei; Ja. UL termin:

Annen prenatal diagnostikk? Nei; Ja, angi type:

Patologiske funn ved prenatal diagnostikk? Nei; Ja, hvis bekreftet – spesifiser

Spesielle forhold før svangerskapet: Intet spesielt; Astma; Allergi; Tidligere sectio; Res. urinveisinfeksjon; Kronisk nyresykdom; Kronisk hypertensjon; Reumatoid artritt; Hjertesykom; Epilepsi; Diabetes type 1; Diabetes type 2; Annet, spesifiser i «B»

Regelmessig kosttilskudd: Nei; Før sv.sk.; I sv.sk.; Multivitaminer; Folat/Folsyre

Spesifikasjon av forhold før eller under svangerskapet: B

Spesielle forhold under svangerskapet: Intet spesielt; Blødning < 13 uke; Blødning 13–28 uke; Blødning > 28 uke; Glukosuri; Svangerskapsdiabetes; Hypertensjon alene; Preeklampsi lett; Preeklampsi alvorlig; Preeklampsi før 34. uke; HELLP syndrom; Eklampsi; Hb < 9.0 g/dl; Hb > 13.5 g/dl; Trombose, beh.; Infeksjon, spes. i «B»; Annet, spesifiser i «B»

Legemidler i svangerskapet: Nei; Ja – spesifiser i «B»

Røyking og yrke (Forutsetter mors samlykke – se rettledning på baksiden): Skriftlig orientering gitt til mor; Samlykker ikke for røykeoppl.

Røykte mor ved sv.sk. begynnelse? Nei; Daglig; Av og til; Ant. sig. dagl.:

– ved sv.sk. avslutning? Nei; Daglig; Av og til; Ant. sig. dagl.:

Mors yrke: Samlykker ikke for yrkesoppl.; Ikke yrkesaktiv; Yrkesaktiv heltid; Yrkesaktiv deltid | Mors yrke | Bransje:

C – Om fødselen

Leie/presentasjon: Normal bakhode; Sete; Tverrleie; Avvikende hodefødsel; Annet, spesifiser i «C»

Fødselstart: Spontan; Indusert; Sectio

Ev. induksjonsmetode: Prostaglandin; Oxytocin; Amniotomi; Annet, spesifiser i «C»

Indikasjon for inngrep og/eller induksjon: Komplikasjoner som beskrevet nedenfor; Fostermisdannelser; Overtid; Annet, spesifiser i «C»

Inngrep/tiltak: Ingen; Utskj. tang, hodeleie; Annen tang, hodeleie; Vakuumekstraktor; Episitomi

Fremhj. ved setefødsel: Vanlig fremhjelp; Uttrekning; Tang på etterk. hode

Sectio: Var sectio planlagt før fødsel? Nei; Ja. Utført som elektiv sectio; Utført som akutt sectio

Spesifikasjon av forhold ved fødselen/andre komplikasjoner: C

Komplikasjoner: Ingen; Vannavg. 12–24 timer; Vannavg. > 24 timer; Mekaniske misforhold; Vanskelig skulderforløsning

Placenta previa; Abruptio placentae; Perinealruptur (grad 1-2); Sphincterruptur (gr. 3-4); Blødn.> 1500 ml, transf.; Blødning 500–1500 ml; Eklampsi under fødsel; Navlesnorfremfall; Truende intrauterin asfyksi; Risvekkelse, stimulert; Langsom framgang; Uterus atoni; Annet:

Anestesi/analgesi: Ingen; Lystgass; Petidin

Epidural; Spinal; Pudendal; Infiltrasjon; Paracervical blokk; Narkose; Annet:

Placenta: Normal; Hinneresster; Ufullstendig; Infarkter; Koagler; Utskrapning; Manuell uthenting; Placenta-vekt

Navlesnor: Normal; Velamentast feste; Marginalt feste; Karanomalier; Omslyng rundt hals; Annet omslyng; Ekte knute; Navlesnor-lengde:

Fostervann: Normal; Polyhydramnion; Oligohydramnion; Misfarget; Stinkende, infisert; Blodtiblandet

Komplikasjoner hos mor etter fødsel: Intet spesielt; Føbar > 38.5°; Trombose; Eklampsi post partum; Mor overflyttet; Mor intensivbeh.; Sepsis; Annet, spesifiser

D – Om barnet

Fødselsdato | Klokken

Flerfødsel: Pluralitet: Enkeltfødsel; Flerfødsel. For flerfødsel: Nr. Av totalt

Kjønn: Gutt; Pike; Ved tvil spesifiser i «D»; For dødfødte: Usikkert kjønn

Barnets vekt: | Hode-omkrets:

Total lengde: | Eventuelt sete-issenål:

Apgar score: 1 min; 5 min

Barnet var: Levendefødt; Dødfødt/sp.abort. Oppgi dødsårsak i «D»

For dødfødte: Død før fødsel; Død under fødselen; Ukjent dødstidspunkt

For dødfødte, oppgi også: Død før innkomst; Død etter innkomst

Levendefødt, død innen 24 timer: Livet varte: Timer Min.

Død senere (dato): | Klokken

Overfl. barneavd.? Nei; Ja. Dato:

Overfl. til

Indikasjon for overflytting: Respirasjonsproblem; Prematur; Medfødte misd.; Perinatale infeksjoner; Annet, spesifiser

Neonatale diagn.: (Fylles ut av lege/pediater) Intet spesielt

Hypoglyk. (< 2 mmol/l); Medl. anemi (Hb < 13.5 g/dl); Hofteleddsdyspl. beh. m/pute

Transit. tachypnoe; Resp. distress syndr.; Aspirasjonssyndrom; Intrakraniell blødning

Cerebral irritasjon; Cerebral depresjon; Abstinens; Neonatale kramper

Konjunktivitt beh.; Navle-/hudinf. beh.; Perinat. inf. bakterielle; Perinat. inf. andre

Fract. claviculae; Annen fraktur; Facialisparese; Plexusskade

Behandlingskoder: Systemisk antibiotika; Respiratorbeh.; CPAP beh.

Icterus behandlet: Lysbehandlet; Utskifting

Årsak: AB0 uforlik.; Rh immunisering; Fysiologisk; Annen årsak

Tegn til medfødte misdanneiser: Nei; Ja

Spesifikasjon av skader, neonatale diagnoser og medfødte misdanneiser – utfylles av lege: D

IS-1002 2011. 07.06. Arvtrod Grafisk.

Kryss av hvis skjema er oppfølgingsskjema

Jordmor v/fødsel:

Jordmor v/utskrivning:

Lege:

Lege barsel/barneavd:

Utskrivningsdato Mor:

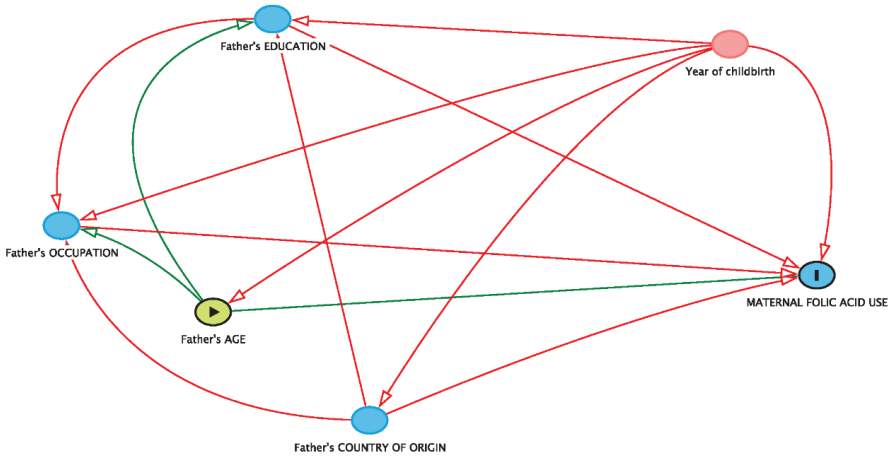
Barn:

Protokollnr.:

9.2 Appendix 2. Directed acyclic graphs (DAGs) (Publication III)

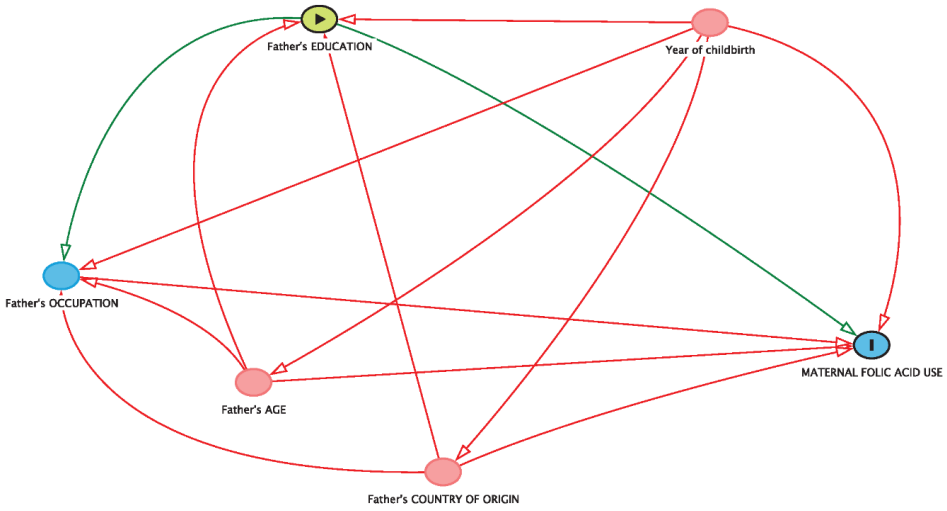
Paternal health-related factors as exposition and maternal periconceptional folic acid use as an outcome.

Association between paternal age and maternal periconceptional folic acid use



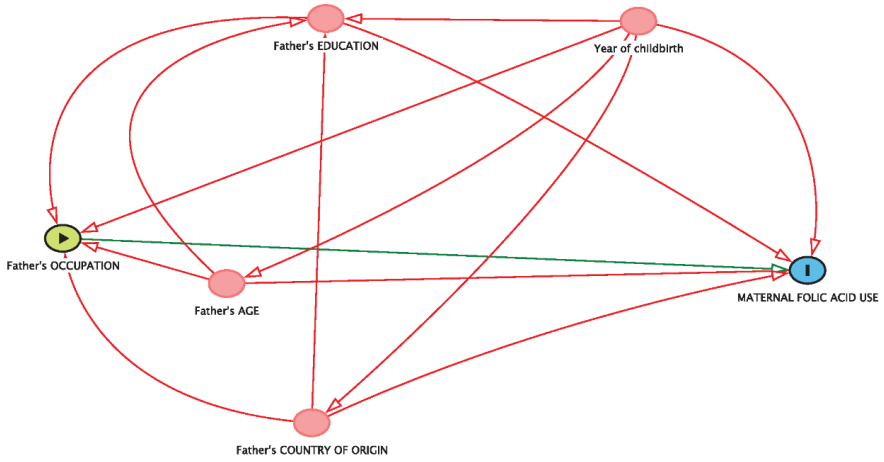
We claim an association between “Father’s age” and “Maternal folic acid use” that is represented by an open “causal” path in green. Moreover, father’s age is related to father’s education, father’s occupation, and maternal folic acid use by open paths in green. Father’s education is also related to father’s occupation and maternal folic acid use by biasing paths in red. Father’s country of origin is related to father’s occupation, father’s education, and maternal folic acid use by biasing paths in red. Year of childbirth is related to father’s education, father’s occupation, father’s age, father’s country of origin, and maternal folic acid use by biasing paths in red. By using DAGitty (Textor et al. 2011), we identify year of childbirth as the minimal sufficient adjustment variable.

Association between paternal education and maternal periconceptional folic acid use



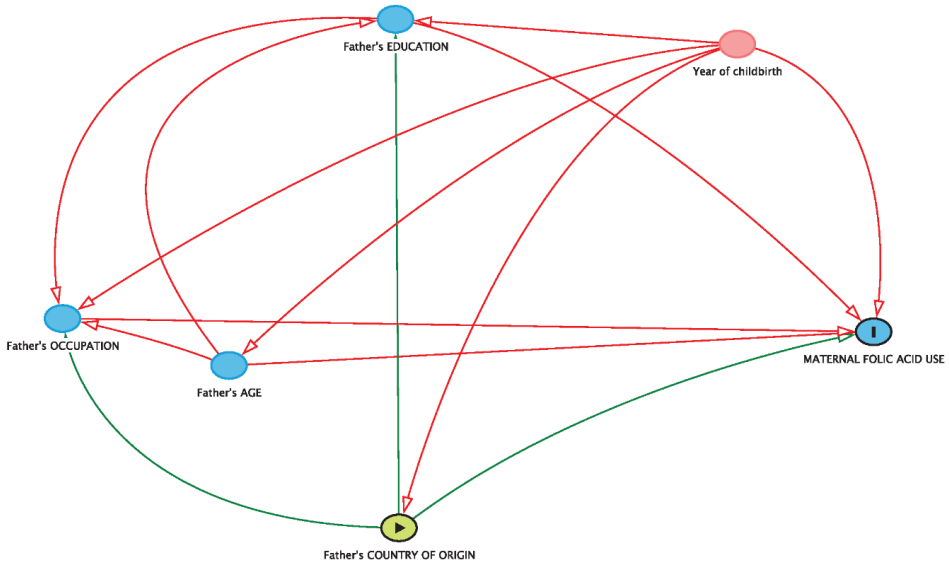
We claim an association between "Father's education" and "Maternal folic acid use," which is represented by an open "causal" path in green. Father's education is further related to father's occupation by an open path in green. Father's age is related to father's education, father's occupation, and maternal folic acid use by biased paths in red. Further, father's country of origin is related to father's occupation, father's education, and maternal folic acid use by biasing paths in red. Year of childbirth is related to father's occupation, father's age, father's country of origin, and maternal folic acid use by biasing paths in red. Using DAGitty (Textor et al. 2011), we identify father's age, father's country of origin, and year of childbirth as the minimal sufficient adjustment variables.

Association between paternal occupation and maternal periconceptional folic acid use



We claim an association between “Father’s occupation” and “Maternal folic acid use,” which is represented by an open “causal” path in green. Father’s education is further related to father’s occupation and maternal folic acid use by biased paths in red. Father’s age is further related to father’s education, father’s occupation, and maternal folic acid use by biased paths in red. Moreover, father’s country of origin is related to father’s occupation, father’s education, and maternal folic acid use by biasing paths in red. Year of childbirth is further related to father’s occupation, father’s age, father’s country of origin, and maternal folic acid use by biasing paths in red. Using DAGitty (Textor et al. 2011), we identify father’s age, father’s education, father’s country of origin, and year of childbirth as the minimal sufficient adjustment variables.

Association between paternal country of origin and maternal periconceptional folic acid use



We claim an association between “Father’s country of origin” and “Maternal folic acid use,” which is represented by an open “causal” path in green. Moreover, father’s country of origin is related to father’s occupation, father’s education, and maternal folic acid use by open paths in green. Father’s education is further related to father’s occupation and maternal folic acid use by biased paths in red. Father’s age is further related to father’s education, father’s occupation, and maternal folic acid use by biased paths in red. Year of childbirth is further related to father’s occupation, father’s age, father’s country of origin, and maternal folic acid use by biasing paths in red. By using DAGitty (Textor et al. 2011), we identify year of childbirth as the minimal sufficient adjustment variable.

10. References

Agopian, A. J., Lupo, P. J., Herdt-Losavio, M. L., Langlois, P. H., Rocheleau, C. M., Mitchell, L. E. and the National Birth Defects Prevention Study (2012) Differences in folic acid use, prenatal care, smoking, and drinking in early pregnancy by occupation. *Prev Med, 55*(4), pp. 341–5.

Akselsen, A. and Siverstøl, S. L. (2013) FD-Trygd, List of variables. *2018*, p. 59.

Amarasekera, M., Martino, D., Ashley, S., Harb, H., Kesper, D., Strickland, D., Saffery, R. and Prescott, S. L. (2014) Genome-wide DNA methylation profiling identifies a folate-sensitive region of differential methylation upstream of ZFP57-imprinting regulator in humans. *FASEB J, 28*(9), pp. 4068–76.

Asgari, M. M., Maruti, S. S., Kushi, L. H. and White, E. (2009) Antioxidant supplementation and risk of incident melanomas: results of a large prospective cohort study. *Arch Dermatol, 145*(8), pp. 879–82.

Baena Ruiz, R. and Salinas Hernandez, P. (2014) Diet and cancer: risk factors and epidemiological evidence. *Maturitas, 77*(3), pp. 202–8.

Bagnardi, V., Blangiardo, M., La Vecchia, C. and Corrao, G. (2001) A meta-analysis of alcohol drinking and cancer risk. *Br J Cancer, 85*(11), pp. 1700–5.

Bahadur, G. and Hindmarsh, P. (2000) Age definitions, childhood and adolescent cancers in relation to reproductive issues. *Hum Reprod., 15*(1), p. 227.

Bailey, H. D., Rios, P., Lacour, B., Guerrini-Rousseau, L., Bertozzi, A. I., Leblond, P., Faure-Conter, C., Pellier, I., Freycon, C., Michon, J., Puget, S., Ducassou, S., Orsi, L. and Clavel, J. (2017) Factors related to pregnancy and birth and the risk of childhood brain tumours: The ESTELLE and ESCALE studies (SFCE, France). *Int J Cancer, 140*(8), pp. 1757–69.

Bailey, L. B., Stover, P. J., McNulty, H., Fenech, M. F., Gregory, J. F., 3rd, Mills, J. L., Pfeiffer, C. M., Fazili, Z., Zhang, M., Ueland, P. M., Molloy, A. M., Caudill, M. A., Shane, B., Berry, R. J., Bailey, R. L., Hausman, D. B., Raghavan, R. and Raiten, D. J. (2015) Biomarkers of Nutrition for Development-Folate Review. *J Nutr, 145*(7), pp. 1636S–80S.

Baraka, M. A., Steurbaut, S., Leemans, L., Foulon, W., Laubach, M., Coomans, D., Jansen, E. and Dupont, A. G. (2011) Determinants of folic acid use in a multi-ethnic population of pregnant women: a cross-sectional study. *J Perinat Med, 39*(6), pp. 685–92.

Berger, S. H., Pittman, D. L. and Wyatt, M. D. (2008) Uracil in DNA: consequences for carcinogenesis and chemotherapy. *Biochem Pharmacol, 76*(6), pp. 697–706.

Bernstein, L. (2002) Epidemiology of endocrine-related risk factors for breast cancer. *J Mammary Gland Biol Neoplasia., 7*(1), pp. 3–15.

Berry, R. J., Li, Z., Erickson, J. D., Li, S., Moore, C. A., Wang, H., Mulinare, J., Zhao, P., Wong, L. Y., Gindler, J., Hong, S. X. and Correa, A. (1999) Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. *N Engl J Med., 341*(20), pp. 1485–90.

Bhaskaran, K., Douglas, I., Forbes, H., dos-Santos-Silva, I., Leon, D. A. and Smeeth, L. (2014) Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5.24 million UK adults. *Lancet, 384*(9945), pp. 755–65.

Bitzer, J., von Stenglin, A. and Bannemerschult, R. (2013) Women's awareness and periconceptional use of folic acid: data from a large European survey. *Int J Womens Health, 5*, pp. 201–13.

Bjorke-Monsen, A. L., Roth, C., Magnus, P., Midttun, O., Nilsen, R. M., Reichborn-Kjennerud, T., Stoltenberg, C., Susser, E., Vollset, S. E. and Ueland, P. M. (2013) Maternal B vitamin status in pregnancy week 18 according to reported use of folic acid supplements. *Mol Nutr Food Res., 57*(4), pp. 645–52.

Bjørge, T., Sørensen, H. T., Grotmol, T., Engeland, A., Stephansson, O., Gissler, M., Tretli, S. and Troisi, R. (2013) Fetal growth and childhood cancer: a population-based study. *Pediatrics, 132*(5), pp. e1265–75.

Bland, J. M. (2005) Taking folate in pregnancy and risk of maternal breast cancer: what's in a name? *BMJ., 330*(7491), pp. 600–1.

Blount, B. C., Mack, M. M., Wehr, C. M., MacGregor, J. T., Hiatt, R. A., Wang, G., Wickramasinghe, S. N., Everson, R. B. and Ames, B. N. (1997) Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc Natl Acad Sci U S A., 94*(7), pp. 3290–5.

Boccia, S., Hung, R., Ricciardi, G., Gianfagna, F., Ebert, M. P., Fang, J. Y., Gao, C. M., Gotze, T., Graziano, F., Lacasana-Navarro, M., Lin, D., Lopez-Carrillo, L., Qiao, Y. L., Shen, H., Stolzenberg-Solomon, R., Takezaki, T., Weng, Y. R., Zhang, F. F., van Duijn, C. M., Boffetta, P. and Taioli, E. (2008) Meta- and pooled analyses of the methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and gastric cancer risk: a huge-GSEC review. *American Journal of Epidemiology, 167*(5), pp. 505–16.

Bortolus, R., Blom, F., Filippini, F., van Poppel, M. N., Leoncini, E., de Smit, D. J., Benetollo, P. P., Cornel, M. C., de Walle, H. E., Mastroiacovo, P., Italian and Dutch folic acid trial study, g. (2014) Prevention of congenital malformations and other adverse pregnancy outcomes with 4.0 mg of folic acid: community-based randomized clinical trial in Italy and the Netherlands. *BMC Pregnancy Childbirth, 14*, p. 166.

Botto, L. D., Moore, C. A., Khoury, M. J. and Erickson, J. D. (1999) Neural-tube defects. *N Engl J Med., 341*(20), pp. 1509–19.

Bower, C., Miller, M., Payne, J. and Serna, P. (2005) Promotion of folate for the prevention of neural tube defects: who benefits? *Paediatr Perinat Epidemiol., 19*(6), pp. 435–44.

Braekke, K. and Staff, A. C. (2003) Periconceptional use of folic acid supplements in Oslo. *Acta Obstet Gynecol Scand., 82*(7), pp. 620–7.

Brasky, T. M., White, E. and Chen, C. L. (2017) Long-Term, Supplemental, One-Carbon Metabolism-Related Vitamin B Use in Relation to Lung Cancer Risk in the Vitamins and Lifestyle (VITAL) Cohort. *J Clin Oncol, 35*(30), pp. 3440–8.

Bronte-Tinkew, J., Ryan, S., Carrano, J. and Moore, K. A. (2007) Resident fathers' pregnancy intentions, prenatal behaviors, and links to involvement with infants. *Journal of Marriage and Family, 69*(4), pp. 977–90.

Brough, L., Rees, G. A., Crawford, M. A. and Dorman, E. K. (2009) Social and ethnic differences in folic acid use preconception and during early pregnancy in the UK: effect on maternal folate status. *J Hum Nutr Diet, 22*(2), pp. 100–7.

Callan, A. C. and Milne, E. (2009) Involvement of the IGF system in fetal growth and childhood cancer: an overview of potential mechanisms. *Cancer Causes Control, 20*(10), pp. 1783–98.

Cameron, A. C. and Miller, D. L. (2015) A Practitioner's Guide to Cluster-Robust Inference. *J Hum Resour., 50*(2), pp. 317–73.

Cancer Registry of Norway (2016) Cancer in Norway 2015. *Cancer incidence, mortality, survival and prevalence in Norway*.

Castillo-Lancellotti, C., Tur, J. A. and Uauy, R. (2013) Impact of folic acid fortification of flour on neural tube defects: a systematic review. *Public Health Nutr, 16*(5), pp. 901–11.

Castro, R., Rivera, I., Ravasco, P., Camilo, M. E., Jakobs, C., Blom, H. J. and de Almeida, I. T. (2004) 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C→T and 1298A→C mutations are associated with DNA hypomethylation. *J Med Genet., 41*(6), pp. 454–8.

Çelikaksoy, A., Nekby, L. and Rashid, S. (2009) *Assortative mating by ethnic background and education in Sweden: The role of parental composition on partner choice*. Stockholm, Sweden: The Stockholm University Linnaeus Center for Integration Studies (SULCIS).

Cha, S., Chapman, D. A., Wan, W., Burton, C. W. and Masho, S. W. (2016) Discordant pregnancy intentions in couples and rapid repeat pregnancy. *Am J Obstet Gynecol, 214*(4), pp. 494e1–e12.

Chang, H., Zhang, T., Zhang, Z., Bao, R., Fu, C., Wang, Z., Bao, Y., Li, Y., Wu, L., Zheng, X. and Wu, J. (2011) Tissue-specific distribution of aberrant DNA methylation associated with maternal low-folate status in human neural tube defects. *J Nutr Biochem, 22*(12), pp. 1172–7.

Charles, D., Ness, A. R., Campbell, D., Davey Smith, G. and Hall, M. H. (2004) Taking folate in pregnancy and risk of maternal breast cancer. *BMJ., 329*(7479), pp. 1375–6.

Chiang, F. F., Huang, S. C., Wang, H. M., Chen, F. P. and Huang, Y. C. (2014) High serum folate might have a potential dual effect on risk of colorectal cancer. *Clin Nutr.*, pp. 1–5.

Chokkalingam, A. P., Chun, D. S., Noonan, E. J., Pfeiffer, C. M., Zhang, M., Month, S. R., Taggart, D. R., Wiemels, J. L., Metayer, C. and Buffler, P. A. (2013) Blood levels of folate at birth and risk of childhood leukemia. *Cancer Epidemiol Biomarkers Prev, 22*(6), pp. 1088–94.

Chuang, S. C., Rota, M., Gunter, M. J., Zeleniuch-Jacquotte, A., Eussen, S. J., Vollset, S. E., Ueland, P. M., Norat, T., Ziegler, R. G. and Vineis, P. (2013) Quantifying the dose-response relationship between circulating folate concentrations and colorectal cancer in cohort studies: a meta-analysis based on a flexible meta-regression model. *American Journal of Epidemiology, 178*(7), pp. 1028–37.

Cikot, R. J., Steegers-Theunissen, R. P., Thomas, C. M., de Boo, T. M., Merkus, H. M. and Steegers, E. A. (2001) Longitudinal vitamin and homocysteine levels in normal pregnancy. *Br J Nutr., 85*(1), pp. 49–58.

Cismondi, F., Fialho, A. S., Vieira, S. M., Reti, S. R., Sousa, J. M. and Finkelstein, S. N. (2013) Missing data in medical databases: impute, delete or classify? *Artif Intell Med, 58*(1), pp. 63–72.

Cornelius, T., Desrosiers, A. and Kershaw, T. (2016) Spread of health behaviors in young couples: How relationship power shapes relational influence. *Soc Sci Med., 165*, pp. 46–55.

Cox, D. R. and Oakes, D. (1984) *Analysis of survival data,* London: Chapman and Hall Ltd.

Cueto, H. T., Riis, A. H., Hatch, E. E., Wise, L. A., Rothman, K. J. and Mikkelsen, E. M. (2012) Predictors of preconceptional folic acid or multivitamin supplement use: a cross-sectional study of Danish pregnancy planners. *Clin Epidemiol., 4*, pp. 259–65.

Czeizel, A. E. and Dudas, I. (1992) Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med., 327*(26), pp. 1832–5.

Dai, W. M., Yang, B., Chu, X. Y., Wang, Y. Q., Zhao, M., Chen, L. and Zhang, G. Q. (2013) Association between folate intake, serum folate levels and the risk of lung cancer: a systematic review and meta-analysis. *Chin Med J (Engl). 126*(10), pp. 1957–64.

Daltveit, A. K., Vollset, S. E., Lande, B. and Oien, H. (2004) Changes in knowledge and attitudes of folate, and use of dietary supplements among women of reproductive age in Norway 1998-2000. *Scand J Public Health, 32*(4), pp. 264–71.

Daly, L. E., Kirke, P. N., Molloy, A., Weir, D. G. and Scott, J. M. (1995) Folate levels and neural tube defects. Implications for prevention. *JAMA, 274*(21), pp. 1698–702.

Duthie, S. and Hawdon, A. (1998) DNA instability (strand breakage, uracil misincorporation, and defective repair) is increased by folic acid depletion in human lymphocytes in vitro. *The FASEB Journal, 12*(14), pp. 1491–7.

Duthie, S. J. (2011) Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis. *J Inherit Metab Dis, 34*(1), pp. 101–9.

Ebbing, M., Bonaa, K. H., Nygard, O., Arnesen, E., Ueland, P. M., Nordrehaug, J. E., Rasmussen, K., Njolstad, I., Refsum, H., Nilsen, D. W., Tverdal, A., Meyer, K. and Vollset, S. E. (2009) Cancer incidence and mortality after treatment with folic acid and vitamin B12. *JAMA., 302*(19), pp. 2119–26.

Eichholzer, M., Tonz, O. and Zimmermann, R. (2006) Folic acid: a public-health challenge. *Lancet, 367*(9519), pp. 1352–61.

Encyclopedia of Epidemiology (2008) Screening. In Sarah Boslaugh, (ed.) *Encyclopedia of Epidemiology*,S: Sage Publication Inc. pp. 942–3.

Ericson, U. C., Ivarsson, M. I., Sonestedt, E., Gullberg, B., Carlson, J., Olsson, H. and Wirfalt, E. (2009) Increased breast cancer risk at high plasma folate concentrations among women with the MTHFR 677T allele. *Am J Clin Nutr, 90*(5), pp. 1380–9.

Erikson, R. and Goldthorpe, J. H. (1992) *The constant flux: a study of class mobility in industrial societies,* Oxford University Press, USA.

European Food Safety Authority [EFSA] (2009) Folic acid: an update on scientific developments. *EFSA Supporting Publications, 6*(8), pp. 9–216.

Eussen, S. J., Vollset, S. E., Igland, J., Meyer, K., Fredriksen, A., Ueland, P. M., Jenab, M., Slimani, N., Boffetta, P., Overvad, K., Tjonneland, A., Olsen, A., Clavel-Chapelon, F., Boutron-Ruault, M. C., Morois, S., Weikert, C., Pischon, T., Linseisen, J., Kaaks, R., Trichopoulou, A., Zilis, D., Katsoulis, M., Palli, D., Berrino, F., Vineis, P., Tumino, R., Panico, S., Peeters, P. H., Bueno-de-Mesquita, H. B., van Duijnhoven, F. J., Gram, I. T., Skeie, G., Lund, E., Gonzalez, C. A., Martinez, C., Dorronsoro, M., Ardanaz, E., Navarro, C., Rodriguez, L., Van Guelpen, B., Palmqvist, R., Manjer, J., Ericson, U., Bingham, S., Khaw, K. T., Norat, T. and Riboli, E. (2010) Plasma folate, related genetic variants, and colorectal cancer risk in EPIC. *Cancer Epidemiol Biomarkers Prev., 19*(5), pp. 1328–40.

Figueiredo, J. C., Levine, A. J., Crott, J. W., Baurley, J. and Haile, R. W. (2013) Folate-genetics and colorectal neoplasia: what we know and need to know next. *Mol Nutr Food Res, 57*(4), pp. 607–27.

Flemmen, M. and Andersen, P. L. (2009) Implementering av EGP klasseskjema for norske registerdata [Implementation of the class scheme of Erikson, Goldthorpe and

Portocarero on Norwegian register data]. In,Oslo: Institutt for sosiologi og samfunnsgeografi, Universitetet i Oslo.

Food and Drug Administration (1996) Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid. Final Rule. 21 CFR Parts 136, 137, and 139. In CFR, (ed.): Fed. Regist. pp. 8781–9.

French, A. E., Grant, R., Weitzman, S., Ray, J. G., Vermeulen, M. J., Sung, L., Greenberg, M. and Koren, G. (2003) Folic acid food fortification is associated with a decline in neuroblastoma. *Clin Pharmacol Ther, 74*(3), pp. 288–94.

Friberg, A. K. and Jorgensen, F. S. (2015) Few Danish pregnant women follow guidelines on periconceptional use of folic acid. *Dan Med J, 62*(3), pp. 1–4.

Fryer, A. A., Nafee, T. M., Ismail, K. M., Carroll, W. D., Emes, R. D. and Farrell, W. E. (2009) LINE-1 DNA methylation is inversely correlated with cord plasma homocysteine in man: a preliminary study. *Epigenetics, 4*(6), pp. 394–8.

Fucic, A., Guszak, V. and Mantovani, A. (2017) Transplacental exposure to environmental carcinogens: Association with childhood cancer risks and the role of modulating factors. *Reprod Toxicol, 72*, pp. 182–90.

Gao, Q. Y., Chen, H. M., Chen, Y. X., Wang, Y. C., Wang, Z. H., Tang, J. T., Ge, Z. Z., Chen, X. Y., Sheng, J. Q., Fang, D. C., Yu, C. G., Zheng, P. and Fang, J. Y. (2013) Folic acid prevents the initial occurrence of sporadic colorectal adenoma in Chinese older than 50 years of age: a randomized clinical trial. *Cancer Prev Res (Phila), 6*(7), pp. 744–52.

Gibson, T. M., Weinstein, S. J., Pfeiffer, R. M., Hollenbeck, A. R., Subar, A. F., Schatzkin, A., Mayne, S. T. and Stolzenberg-Solomon, R. (2011) Pre- and postfortification intake of folate and risk of colorectal cancer in a large prospective cohort study in the United States. *Am J Clin Nutr, 94*(4), pp. 1053–62.

Gildestad, T., Bjorge, T., Vollset, S. E., Klungsoyr, K., Nilsen, R. M., Haaland, O. A. and Oyen, N. (2015) Folic acid supplements and risk for oral clefts in the newborn: a population-based study. *Br J Nutr, 114*(9), pp. 1456–63.

Gonseth, S., Roy, R., Houseman, E. A., de Smith, A. J., Zhou, M., Lee, S. T., Nussle, S., Singer, A. W., Wrensch, M. R., Metayer, C. and Wiemels, J. L. (2015) Periconceptional folate consumption is associated with neonatal DNA methylation modifications in neural crest regulatory and cancer development genes. *Epigenetics, 10*(12), pp. 1166–76.

Gonzalez, C. A. and Riboli, E. (2010) Diet and cancer prevention: Contributions from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Eur J Cancer., 46*(14), pp. 2555–62.

Government of Norway (1998) Act of 17 July 1998 no. 61 relating to Primary and Secondary Education and Training (the Education Act). In Ministry of Education and Research, (ed.),Oslo.

Green, A. C., Hughes, M. C., Ibiebele, T. I., Williams, G. M., van der Pols, J. C. and Ortonne, J. P. (2008) Antioxidant supplementation and risk of skin cancers. *J Nutr, 138*(5), pp. 978–79.

Greenland, S., Lash, T. L. and Rothman, K. J. (2008) Concept of interaction. In *Modern Epidemiology*, chap. 5, Philadelphia, PA, USA: Lippincot Williams & Wilkins (Wolters Kluwer Health). pp. 71–84.

Grupp, S. G., Greenberg, M. L., Ray, J. G., Busto, U., Lanctot, K. L., Nulman, I. and Koren, G. (2011) Pediatric cancer rates after universal folic acid flour fortification in Ontario. *J Clin Pharmacol., 51*(1), pp. 60–5.

Gylling, B., Van Guelpen, B., Schneede, J., Hultdin, J., Ueland, P. M., Hallmans, G., Johansson, I. and Palmqvist, R. (2014) Low folate levels are associated with reduced risk of colorectal cancer in a population with low folate status. *Cancer Epidemiol Biomarkers Prev, 23*(10), pp. 2136–44.

Hammer, H. (2002) [The central population registry in medical research]. *Tidsskr. Nor. Laegeforening., 122*(26), p. 2550.

Hanahan, D. and Weinberg, R. A. (2011) Hallmarks of cancer: the next generation. *Cell, 144*(5), pp. 646–74.

Hankey, G. J., Eikelboom, J. W., Yi, Q., Lees, K. R., Chen, C., Xavier, D., Navarro, J. C., Ranawaka, U. K., Uddin, W., Ricci, S., Gommans, J., Schmidt, R. and Vitamins to Prevent Stroke Trial Study Group (2012) Treatment with B vitamins and incidence of cancer in patients with previous stroke or transient ischemic attack: results of a randomized placebo-controlled trial. *Stroke., 43*(6), pp. 1572–7.

Harrington, K. J. (2016) The biology of cancer. *Medicine, 44*(1), pp. 1–5.

Hercberg, S., Ezzedine, K., Guinot, C., Preziosi, P., Galan, P., Bertrais, S., Estaquio, C., Briancon, S., Favier, A., Latreille, J. and Malvy, D. (2007) Antioxidant

supplementation increases the risk of skin cancers in women but not in men. *J Nutr, 137*(9), pp. 2098–105.

Hibbard, B. M. (1964) The Role of Folic Acid in Pregnancy; with Particular Reference to Anaemia, Abruption and Abortion. *J Obstet Gynaecol Br Commonw, 71*(71), pp. 529–42.

Hibbard, E. D. and Smithells, R. W. (1965) Folio acid metabolism and human embryopathy. *Lancet, 1*, p. 1254.

Hoyo, C., Murtha, A. P., Schildkraut, J. M., Jirtle, R. L., Demark-Wahnefried, W., Forman, M. R., Iversen, E. S., Kurtzberg, J., Overcash, F., Huang, Z. and Murphy, S. K. (2011) Methylation variation at IGF2 differentially methylated regions and maternal folic acid use before and during pregnancy. *Epigenetics, 6*(7), pp. 928–36.

Huber, S. and Fieder, M. (2011) Educational homogamy lowers the odds of reproductive failure. *PLoS One, 6*(7), p. e22330.

International Agency for Research on Cancer (2014) *World Cancer Report 2014,* 150 cours Albert Thomas, 69372 Lyon Cedex 08, France: International Agency for Research on Cancer.

Irgens, L. M. (2000) The Medical Birth Registry of Norway. Epidemiological research and surveillance throughout 30 years. *Acta Obstet Gynecol Scand., 79*(6), pp. 435–9.

Jackson, S. E., Steptoe, A. and Wardle, J. (2015) The influence of partner's behavior on health behavior change: the English Longitudinal Study of Ageing. *JAMA Intern Med., 175*(3), pp. 385–92.

Jones, P. A. (2005) Overview of cancer epigenetics. *Semin Hematol, 42*(3 Suppl 2), pp. S3–8.

Jongmans, M. C., Loeffen, J. L., Waanders, E., Hoogerbrugge, P. M., Ligtenberg, M. J., Kuiper, R. P. and Hoogerbrugge, N. (2016) Recognition of genetic predisposition in pediatric cancer patients: An easy-to-use selection tool. *Eur J Med Genet, 59*(3), pp. 116–25.

Juul, S. (2013) Generalisering (ekstern validitet) [Generalisation (external validation)]. In Østergaard, B., (ed.) *Epidemiologi og evidens, 2. udg.*,Kap. 7,København: Munksgaard. pp. 134–5.

Kennedy, D. A., Stern, S. J., Moretti, M., Matok, I., Sarkar, M., Nickel, C. and Koren, G. (2011) Folate intake and the risk of colorectal cancer: a systematic review and meta-analysis. *Cancer Epidemiol., 35*(1), pp. 2–10.

Kim, Y. I. (1999) Folate and carcinogenesis: evidence, mechanisms, and implications. *J Nutr Biochem., 10*(2), pp. 66–88.

Kim, Y. I. (2003) Role of folate in colon cancer development and progression. *J Nutr., 133*(11 Suppl 1), pp. 3731S–9S.

Kim, Y. I. (2006) Folate: a magic bullet or a double edged sword for colorectal cancer prevention? *Gut., 55*(10), pp. 1387–9.

Kim, Y. I. (2007) Folate and colorectal cancer: an evidence-based critical review. *Mol Nutr Food Res., 51*(3), pp. 267–92.

Kim, Y. I. (2016) Current Status of Folic Acid Supplementation on Colorectal Cancer Prevention. *Current Pharmacology Reports, 2*(1), pp. 21–33.

Kinnunen, T. I., Sletner, L., Sommer, C., Post, M. C. and Jenum, A. K. (2017) Ethnic differences in folic acid supplement use in a population-based cohort of pregnant women in Norway. *BMC Pregnancy Childbirth, 17*(1), p. 143.

Knudsen, V. K., Orozova-Bekkevold, I., Rasmussen, L. B., Mikkelsen, T. B., Michaelsen, K. F. and Olsen, S. F. (2004) Low compliance with recommendations on folic acid use in relation to pregnancy: is there a need for fortification? *Public Health Nutr., 7*(7), pp. 843–50.

Kravdal, O. (2008) A broader perspective on education and mortality: are we influenced by other people's education? *Soc Sci Med, 66*(3), pp. 620–36.

Krishnaswamy, K. and Madhavan Nair, K. (2001) Importance of folate in human nutrition. *Br J Nutr., 85 Suppl 2*, pp. S115–24.

Kvalvik, L. G., Nilsen, R. M., Skjaerven, R., Vollset, S. E., Midttun, O., Ueland, P. M. and Haug, K. (2012) Self-reported smoking status and plasma cotinine concentrations among pregnant women in the Norwegian Mother and Child Cohort Study. *Pediatr Res, 72*(1), pp. 101–7.

Larsen, I. K., Smastuen, M., Johannesen, T. B., Langmark, F., Parkin, D. M., Bray, F. and Moller, B. (2009) Data quality at the Cancer Registry of Norway: an overview of

comparability, completeness, validity and timeliness. *Eur J Cancer, 45*(7), pp. 1218–31.

Larsson, S. C., Giovannucci, E. and Wolk, A. (2006) Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis. *Gastroenterology, 131*(4), pp. 1271-83.

Leclerc, D., Sibani, S. and Rozen, R. (2013) Molecular biology of methylenetetrahydrofolate reductase (MTHFR) and overview of mutations/polymorphisms. In *Madame Curie Bioscience Database*, Austin (TX): Landes Bioscience. pp. 2000–13.

Lee, I. M., Shiroma, E. J., Lobelo, F., Puska, P., Blair, S. N., Katzmarzyk, P. T. and the Lancet Physical Activity Series Working Group (2012) Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet, 380*(9838), pp. 219–29.

Lee, J. E., Wei, E. K., Fuchs, C. S., Hunter, D. J., Lee, I. M., Selhub, J., Stampfer, M. J., Willett, W. C., Ma, J. and Giovannucci, E. (2012) Plasma folate, methylenetetrahydrofolate reductase (MTHFR), and colorectal cancer risk in three large nested case-control studies. *Cancer Causes Control, 23*(4), pp. 537–45.

Lee, J. E., Willett, W. C., Fuchs, C. S., Smith-Warner, S. A., Wu, K., Ma, J. and Giovannucci, E. (2011) Folate intake and risk of colorectal cancer and adenoma: modification by time. *Am J Clin Nutr, 93*(4), pp. 817–25.

Leirgul, E., Gildestad, T., Nilsen, R. M., Fomina, T., Brodwall, K., Greve, G., Vollset, S. E., Holmstrom, H., Tell, G. S. and Oyen, N. (2015) Periconceptional Folic Acid Supplementation and Infant Risk of Congenital Heart Defects in Norway 1999-2009. *Paediatr Perinat Epidemiol., 29*(5), pp. 391–400.

Liew, S. C. and Gupta, E. D. (2015) Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: epidemiology, metabolism and the associated diseases. *Eur J Med Genet, 58*(1), pp. 1–10.

Linabery, A. M., Johnson, K. J. and Ross, J. A. (2012) Childhood cancer incidence trends in association with US folic acid fortification (1986-2008). *Pediatrics, 129*(6), pp. 1125–33.

Lindberg, L. D. and Kost, K. (2014) Exploring U.S. men's birth intentions. *Matern Child Health J., 18*(3), pp. 625–33.

Lindberg, L. D., Kost, K. and Maddow-Zimet, I. (2017) The Role of Men's Childbearing Intentions in Father Involvement. *J Marriage Fam, 79*(1), pp. 44–59.

Lucock, M. (2004) Is folic acid the ultimate functional food component for disease prevention? *BMJ., 328*(7433), pp. 211–4.

Ly, A., Hoyt, L., Crowell, J. and Kim, Y. I. (2012) Folate and DNA methylation. *Antioxid Redox Signal, 17*(2), pp. 302–26.

Matejcic, M., de Batlle, J., Ricci, C., Biessy, C., Perrier, F., Huybrechts, I., Weiderpass, E., Boutron-Ruault, M. C., Cadeau, C., His, M., Cox, D. G., Boeing, H., Fortner, R. T., Kaaks, R., Lagiou, P., Trichopoulou, A., Benetou, V., Tumino, R., Panico, S., Sieri, S., Palli, D., Ricceri, F., Bueno-de-Mesquita, H. B., Skeie, G., Amiano, P., Sanchez, M. J., Chirlaque, M. D., Barricarte, A., Quiros, J. R., Buckland, G., van Gils, C. H., Peeters, P. H., Key, T. J., Riboli, E., Gylling, B., Zeleniuch-Jacquotte, A., Gunter, M. J., Romieu, I. and Chajes, V. (2017) Biomarkers of folate and vitamin B12 and breast cancer risk: report from the EPIC cohort. *Int J Cancer, 140*(6), pp. 1246–59.

McGuire, M., Cleary, B., Sahm, L. and Murphy, D. J. (2010) Prevalence and predictors of periconceptional folic acid uptake – prospective cohort study in an Irish urban obstetric population. *Hum Reprod, 25*(2), pp. 535–43.

Medical Research Council Vitamin Study (1991) Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group. *Lancet, 338*(8760), pp. 131–7.

Metayer, C., Milne, E., Dockerty, J. D., Clavel, J., Pombo-de-Oliveira, M. S., Wesseling, C., Spector, L. G., Schuz, J., Petridou, E., Ezzat, S., Armstrong, B. K., Rudant, J., Koifman, S., Kaatsch, P., Moschovi, M., Rashed, W. M., Selvin, S., McCauley, K., Hung, R. J., Kang, A. Y. and Infante-Rivard, C. (2014) Maternal supplementation with folic acid and other vitamins and risk of leukemia in offspring: a Childhood Leukemia International Consortium study. *Epidemiology, 25*(6), pp. 811–22.

Milman, N., Byg, K. E., Hvas, A. M., Bergholt, T. and Eriksen, L. (2006) Erythrocyte folate, plasma folate and plasma homocysteine during normal pregnancy and postpartum: a longitudinal study comprising 404 Danish women. *Eur J Haematol., 76*(3), pp. 200–5.

Milne, E., Greenop, K. R., Bower, C., Miller, M., van Bockxmeer, F. M., Scott, R. J., de Klerk, N. H., Ashton, L. J., Gottardo, N. G., Armstrong, B. K. and the Aus. C. B. T. Consortium (2012) Maternal use of folic acid and other supplements and risk of childhood brain tumors. *Cancer Epidemiol Biomarkers Prev., 21*(11), pp. 1933–41.

Milne, E., Royle, J. A., Miller, M., Bower, C., de Klerk, N. H., Bailey, H. D., van Bockxmeer, F., Attia, J., Scott, R. J., Norris, M. D., Haber, M., Thompson, J. R., Fritschi, L., Marshall, G. M. and Armstrong, B. K. (2010) Maternal folate and other vitamin supplementation during pregnancy and risk of acute lymphoblastic leukemia in the offspring. *Int J Cancer., 126*(11), pp. 2690–9.

Milunsky, A., Jick, H., Jick, S. S., Bruell, C. L., MacLaughlin, D. S., Rothman, K. J. and Willett, W. (1989) Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. *JAMA, 262*(20), pp. 2847–52.

Momen, N. C., Olsen, J., Gissler, M. and Li, J. (2016) Exposure to maternal smoking during pregnancy and risk of childhood cancer: a study using the Danish national registers. *Cancer Causes Control, 27*(3), pp. 341–9.

Monden, C. W., van Lenthe, F., de Graaf, N. D. and Kraaykamp, G. (2003) Partner's and own education: does who you live with matter for self-assessed health, smoking and excessive alcohol consumption? *Soc Sci Med., 57*(10), pp. 1901–12.

Moussa, H. N., Hosseini Nasab, S., Haidar, Z. A., Blackwell, S. C. and Sibai, B. M. (2016) Folic acid supplementation: what is new? Fetal, obstetric, long-term benefits and risks. *Future Sci OA, 2*(2), p. FSO116.

Murphy, M. F., Bithell, J. F., Stiller, C. A., Kendall, G. M. and O'Neill, K. A. (2013) Childhood and adult cancers: contrasts and commonalities. *Maturitas, 76*(1), pp. 95-8.

National Clinical Registry for Childhood Cancer (2016) The Norwegian Childhood Cancer Registry Annual report, 2016. In Cancer Registry of Norway, (ed.) *[Årsrapport 2016 med resultater og forbedringstiltak fra Nasjonalt kvalitetsregister for barnekreft]*, Oslo: Cancer Registry of Norway. pp. 18–9.

National Council on Nutrition and Physical Activity (1998) Recommendations and means to increase folate intake among women in fertile age [Anbefalinger og virkemidler for økt folatinntak blant kvinner i fertil alder]. Report no 1/1998. In,Oslo: National Council on Nutrition and Physical Activity [Statens Ernæringsråd].

Nazki, F. H., Sameer, A. S. and Ganaie, B. A. (2014) Folate: metabolism, genes, polymorphisms and the associated diseases. *Gene, 533*(1), pp. 11–20.

Neuhouser, M. L., Wassertheil-Smoller, S., Thomson, C., Aragaki, A., Anderson, G. L., Manson, J. E., Patterson, R. E., Rohan, T. E., van Horn, L., Shikany, J. M., Thomas,

A., LaCroix, A. and Prentice, R. L. (2009) Multivitamin Use and Risk of Cancer and Cardiovascular Disease in the Women's Health Initiative Cohorts. *Arch Intern Med., 169*(3), pp. 294–304.

Nilsen, R. M., Leoncini, E., Gastaldi, P., Allegri, V., Agostino, R., Faravelli, F., Ferrazzoli, F., Finale, E., Ghirri, P., Scarano, G. and Mastroiacovo, P. (2016) Prevalence and determinants of preconception folic acid use: an Italian multicenter survey. *Ital J Pediatr, 42*(1), p. 65.

Nilsen, R. M., Mastroiacovo, P., Gunnes, N., Alsaker, E. R., Bjorke-Monsen, A. L., Eussen, S. J., Haugen, M., Johannessen, A., Meltzer, H. M., Stoltenberg, C., Ueland, P. M. and Vollset, S. E. (2014) Folic acid supplementation and interpregnancy interval. *Paediatr Perinat Epidemiol., 28*(3), pp. 270–4.

Nilsen, R. M., Vollset, S. E., Gjessing, H. K., Magnus, P., Meltzer, H. M., Haugen, M. and Ueland, P. M. (2006) Patterns and predictors of folic acid supplement use among pregnant women: the Norwegian Mother and Child Cohort Study. *Am J Clin Nutr., 84*(5), pp. 1134–41.

Nilsen, R. M., Vollset, S. E., Gjessing, H. K., Skjaerven, R., Melve, K. K., Schreuder, P., Alsaker, E. R., Haug, K., Daltveit, A. K. and Magnus, P. (2009) Self-selection and bias in a large prospective pregnancy cohort in Norway. *Paediatr Perinat Epidemiol., 23*(6), pp. 597–608.

Nilsen, R. M., Vollset, S. E., Rasmussen, S. A., Ueland, P. M. and Daltveit, A. K. (2008) Folic acid and multivitamin supplement use and risk of placental abruption: a population-based registry study. *Am J Epidemiol., 167*(7), pp. 867–74.

Nilsen, T., Magnus, P. and Ørstavik, R. (2016) Maternal body composition in relation to twinning. *Norsk Epidemiologi, 26*(1–2), pp. 77–81.

Nisar, Y. B., Dibley, M. J. and Mir, A. M. (2014) Factors associated with non-use of antenatal iron and folic acid supplements among Pakistani women: a cross sectional household survey. *BMC Pregnancy Childbirth, 14*, p. 305.

Norat, T., Scoccianti, C., Boutron-Ruault, M. C., Anderson, A., Berrino, F., Cecchini, M., Espina, C., Key, T., Leitzmann, M., Powers, H., Wiseman, M. and Romieu, I. (2015) European Code against Cancer 4th Edition: Diet and cancer. *Cancer Epidemiol, 39 Suppl 1*, pp. S56–66.

Nordic Council of Ministers (2014) *Nordic Nutrition Recommendations 2012: Integrating nutrition and physical activity,* Nordic Council of Ministers [online], available: https://doi.org/10.6027/Nord2014-002 [Accessed Aug. 2018].

Norwegian Institute of Public Health (2004) *Annual report 2001 and 2002*. Fødsler i Norge. Bergen, Norway: Norwegian Institute of Public Health and University of Bergen.

Norwegian Institute of Public Health (2016) *Notification of birth to the Medical Birth Registry of Norway*, Available: https://www.fhi.no/en/hn/health-registries/medical-birth-registry-of-norway/notification-of-birth-to-the-medical-birth-registry-of-norway/ [Accessed Aug. 2018]].

Norwegian Institute of Public Health (2017) *Medisinsk fødselsregister og abortregisteret – statistikkbanker [Medical birth registry and abortion registry – statistical database]*, Available: http://statistikk.fhi.no/mfr/ [Accessed Aug. 2018].

Norwegian Labour and Welfare Administration (2018) *About NAV*, Available: https://www.nav.no/en/Home/About+NAV/What+is+NAV [Accessed Aug. 2018].

Norwegian Scientific Committee for Food Safety (2015) Risk assessment of folic acid in food supplements. Scientific opinion of the Panel on Nutrition, Dietetic Products, Novel Food and Allergy. *VKM Report 2015: 05*, (August), p. 13.

Oakley, G. P. and Mandel, J. S. (2004) Folic acid fortification remains an urgent health priority. *BMJ, 329*(7479), p. 1376.

Ohrvik, V. E. and Witthoft, C. M. (2011) Human folate bioavailability. *Nutrients, 3*(4), pp. 475–90.

Pampel, F. C., Krueger, P. M. and Denney, J. T. (2010) Socioeconomic Disparities in Health Behaviors. *Annu Rev Sociol, 36*, pp. 349–70.

Park, J. Y., Vollset, S. E., Melse-Boonstra, A., Chajes, V., Ueland, P. M. and Slimani, N. (2013) Dietary intake and biological measurement of folate: a qualitative review of validation studies. *Mol Nutr Food Res, 57*(4), pp. 562–81.

Park, S. Y., Murphy, S. P., Wilkens, L. R., Henderson, B. E. and Kolonel, L. N. (2011) Multivitamin use and the risk of mortality and cancer incidence: the multiethnic cohort study. *American Journal of Epidemiology, 173*(8), pp. 906-14.

Patanwala, I., King, M. J., Barrett, D. A., Rose, J., Jackson, R., Hudson, M., Philo, M., Dainty, J. R., Wright, A. J., Finglas, P. M. and Jones, D. E. (2014) Folic acid handling by the human gut: implications for food fortification and supplementation. *Am J Clin Nutr, 100*(2), pp. 593–9.

Pearl, J. (2010) An introduction to causal inference. *Int J Biostat., 6*(2), pp. 1–59.

Pericleous, M., Mandair, D. and Caplin, M. E. (2013) Diet and supplements and their impact on colorectal cancer. *J Gastrointest Oncol, 4*(4), pp. 409–23.

Pietrzik, K., Bailey, L. and Shane, B. (2010) Folic acid and L-5-methyltetrahydrofolate: comparison of clinical pharmacokinetics and pharmacodynamics. *Clin Pharmacokinet, 49*(8), pp. 535–48.

Qin, X., Cui, Y., Shen, L., Sun, N., Zhang, Y., Li, J., Xu, X., Wang, B., Xu, X., Huo, Y. and Wang, X. (2013) Folic acid supplementation and cancer risk: a meta-analysis of randomized controlled trials. *Int J Cancer, 133*(5), pp. 1033–41.

Reynolds, E. H. (2014) The neurology of folic acid deficiency. *Handb Clin Neurol., 120*, pp. 927-43.

Robertson, K. D. (2005) DNA methylation and human disease. *Nat Rev Genet, 6*(8), pp. 597–610.

Roth, C., Magnus, P., Schjolberg, S., Stoltenberg, C., Suren, P., McKeague, I. W., Davey Smith, G., Reichborn-Kjennerud, T. and Susser, E. (2011) Folic acid supplements in pregnancy and severe language delay in children. *JAMA., 306*(14), pp. 1566–73.

Rothman, K. J., Greenland, S. and Lash, T. L. (2008a) Confounding. In *Modern Epidemiology, 3rd ed.*, chap. 9, Philadelphia, PA, USA: Lippincot (Wolters Kluwer Health). pp. 129–33.

Rothman, K. J., Greenland, S. and Lash, T. L. (2008b) Differential misclassification. In *Modern Epidemiology, 3rd ed.*, chap. 9, Philadelphia, PA, USA: Lippincot (Wolters Kluwer Health). pp. 138–9.

Rothman, K. J., Greenland, S. and Lash, T. L. (2008c) Generalizability. In *Modern Epidemiology, 3rd ed.*, chap. 9, Philadelphia, PA, USA: Lippincot (Wolters Kluwer Health),. pp. 146–7.

Rothman, K. J., Greenland, S. and Lash, T. L. (2008d) Information bias. In *Modern Epidemiology, 3rd ed.*, chap. 9, Philadelphia, PA, USA: Lippincot (Wolters Kluwer Health). pp. 137–9.

Rothman, K. J., Greenland, S. and Lash, T. L. (2008e) Misclassification of confounders. In *Modern Epidemiology, 3rd ed.*, chap. 9, Philadelphia, PA, USA: Lippincot (Wolters Kluwer Health). pp. 144–5.

Rothman, K. J., Greenland, S. and Lash, T. L. (2008f) Nondifferential misclassification. In *Modern Epidemiology, 3rd ed.*, chap. 9, Philadelphia, PA, USA: Lippincot (Wolters Kluwer Health). pp. 139–44.

Rothman, K. J., Greenland, S. and Lash, T. L. (2008g) Precision and Statistics in Epidemiologic Studies. In *Modern Epidemiology, 3rd ed.*, chap. 10, Philadelphia, PA, USA: Lippincot (Wolters Kluwer Health). pp. 148–67.

Rothman, K. J., Greenland, S. and Lash, T. L. (2008h) Selection bias. In *Modern Epidemiology, 3rd ed.*, chap. 9, Philadelphia, PA, USA: Lippincot (Wolters Kluwer Health), p. 134.

Rothman, K. J., Greenland, S. and Lash, T. L. (2008i) Validity of estimation In *Modern Epidemiology, 3rd ed.*, chap. 9, Philadelphia, PA, USA: Lippincot (Wolters Kluwer Health). pp. 128–9.

Sadler, T. W. and Thomas, W. (2015) Embryonic Development in Days. In *Langman's medical embryology,* 13th ed.,United States: Philadelphia : Wolters Kluwer Lippincott Williams & Wilkins. pp. ii.

Schisterman, E. F., Cole, S. R. and Platt, R. W. (2009) Overadjustment bias and unnecessary adjustment in epidemiologic studies. *Epidemiology, 20*(4), pp. 488-95.

Schmidt, L., Sobotka, T., Bentzen, J. G., Nyboe Andersen, A., Eshre Reproduction and Society Task Force (2012) Demographic and medical consequences of the postponement of parenthood. *Hum Reprod Update, 18*(1), pp. 29–43.

Scientific Advisory Committee on Nutrition (2006) *Folate and Disease Prevention,* TSO (The Stationery Office), London: Scientific Advisory Committee on Nutrition.

Scientific Advisory Committee on Nutrition (2017) *Update on folic acid,* [online], available: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/637111/SACN_Update_on_folic_acid.pdf [Accessed November 2018].

Sengpiel, V., Bacelis, J., Myhre, R., Myking, S., Pay, A. D., Haugen, M., Brantsaeter, A. L., Meltzer, H. M., Nilsen, R. M., Magnus, P., Vollset, S. E., Nilsson, S. and Jacobsson, B. (2013) Folic acid supplementation, dietary folate intake during pregnancy and risk for spontaneous preterm delivery: a prospective observational cohort study. *BMC Pregnancy Childbirth, 13*, p. 160.

Shrier, I. and Platt, R. W. (2008) Reducing bias through directed acyclic graphs. *BMC Med Res Methodol., 8*(1), p. 70.

Singer, A. W., Selvin, S., Block, G., Golden, C., Carmichael, S. L. and Metayer, C. (2016) Maternal prenatal intake of one-carbon metabolism nutrients and risk of childhood leukemia. *Cancer Causes Control, 27*(7), pp. 929–40.

Song, M., Garrett, W. S. and Chan, A. T. (2015) Nutrients, foods, and colorectal cancer prevention. *Gastroenterology, 148*(6), pp. 1244–60 e16.

Sorahan, T. and Gilthorpe, M. S. (1994) Non-differential misclassification of exposure always leads to an underestimate of risk: an incorrect conclusion. *Occup Environ Med, 51*(12), pp. 839–40.

Staff, A. C., Loken, E. B., Holven, K., Sygnestveit, K., Vollset, S. E. and Smeland, S. (2005) [Effects of public initiatives aimed at reducing neural tube defects with folic acid supplementation]. *Tidsskr. Nor. Laegeforen., 125*(4), pp. 435–7.

Stalberg, K., Haglund, B., Stromberg, B. and Kieler, H. (2010) Prenatal exposure to medicines and the risk of childhood brain tumor. *Cancer Epidemiol, 34*(4), pp. 400-4.

StataCorp. (2013) Stata Statistical Software: Release 13. In: College Station, TX: StataCorp LP.

StataCorp. (2015) Stata Statistical Software: Release 14. In: College Station, TX: StataCorp LLC

Statistics Norway (1998) Standard Classification of Occupation.

Statistics Norway (2013) *About The National Education Database (NUDB)*, Available: http://www.ssb.no/a/english/mikrodata_en/datasamling/nudb/nudb_20130607-en.html [Accessed Aug. 2018].

Statistics Norway (2017) *Statistics Norway*, Available: https://www.ssb.no/en [Accessed Aug. 2018].

Statistics Norway (2018) *Norwegian Standard Classification og Education*, Available: https://www.ssb.no/en/utdanning/norwegian-standard-classification-of-education [Accessed Aug. 2018].

Steegers-Theunissen, R. P., Obermann-Borst, S. A., Kremer, D., Lindemans, J., Siebel, C., Steegers, E. A., Slagboom, P. E. and Heijmans, B. T. (2009) Periconceptional maternal folic acid use of 400 microg per day is related to increased methylation of the IGF2 gene in the very young child. *PLoS One, 4*(11), p. e7845.

Steliarova-Foucher, E., Colombet, M., Ries, L. A. G., Moreno, F., Dolya, A., Bray, F., Hesseling, P., Shin, H. Y., Stiller, C. A. and IICC-3 contributors (2017) International incidence of childhood cancer, 2001-10: a population-based registry study. *Lancet Oncol, 18*(6), pp. 719–31.

Steliarova-Foucher, E., Stiller, C., Lacour, B. and Kaatsch, P. (2005) International Classification of Childhood Cancer, third edition. *Cancer., 103*(7), pp. 1457-67.

Stevens, V. L., McCullough, M. L., Sun, J., Jacobs, E. J., Campbell, P. T. and Gapstur, S. M. (2011) High levels of folate from supplements and fortification are not associated with increased risk of colorectal cancer. *Gastroenterology, 141*(1), pp. 98–105.

Stiller, C. A. (2004) Epidemiology and genetics of childhood cancer. *Oncogene, 23*(38), pp. 6429-44.

Stolzenberg-Solomon, R. Z., Chang, S. C., Leitzmann, M. F., Johnson, K. A., Johnson, C., Buys, S. S., Hoover, R. N. and Ziegler, R. G. (2006) Folate intake, alcohol use, and postmenopausal breast cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Am J Clin Nutr., 83*(4), pp. 895–904.

Stover, P. J. (2011) Polymorphisms in 1-carbon metabolism, epigenetics and folate-related pathologies. *J Nutrigenet Nutrigenomics, 4*(5), pp. 293–305.

Suren, P., Roth, C., Bresnahan, M., Haugen, M., Hornig, M., Hirtz, D., Lie, K. K., Lipkin, W. I., Magnus, P., Reichborn-Kjennerud, T., Schjolberg, S., Davey Smith, G., Oyen, A. S., Susser, E. and Stoltenberg, C. (2013) Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. *JAMA., 309*(6), pp. 570–7.

Takata, Y., Shrubsole, M. J., Li, H., Cai, Q., Gao, J., Wagner, C., Wu, J., Zheng, W., Xiang, Y. B. and Shu, X. O. (2014) Plasma folate concentrations and colorectal cancer risk: a case-control study nested within the Shanghai Men's Health Study. *Int J Cancer, 135*(9), pp. 2191–8.

Textor, J., Hardt, J. and Knuppel, S. (2011) DAGitty: a graphical tool for analyzing causal diagrams. *Epidemiology, 22*(5), p. 745.

Thompson, J. R., Gerald, P. F., Willoughby, M. L. and Armstrong, B. K. (2001) Maternal folate supplementation in pregnancy and protection against acute lymphoblastic leukaemia in childhood: a case-control study. *Lancet, 358*(9297), pp. 1935-40.

Timmermans, S., Jaddoe, V. W., Mackenbach, J. P., Hofman, A., Steegers-Theunissen, R. P. and Steegers, E. A. (2008) Determinants of folic acid use in early pregnancy in a multi-ethnic urban population in The Netherlands: the Generation R study. *Prev Med., 47*(4), pp. 427–32.

Tohotoa, J., Maycock, B., Hauck, Y. L., Howat, P., Burns, S. and Binns, C. W. (2009) Dads make a difference: an exploratory study of paternal support for breastfeeding in Perth, Western Australia. *Int Breastfeed J, 4*, p. 15.

Tort, J., Lelong, N., Prunet, C., Khoshnood, B. and Blondel, B. (2013) Maternal and health care determinants of preconceptional use of folic acid supplementation in France: results from the 2010 National Perinatal Survey. *BJOG, 120*

Trimmer, E. E. (2013) Methylenetetrahydrofolate reductase: biochemical characterization and medical significance. *Curr Pharm Des, 19*(14), pp. 2574–93.

U.S. Department of Health and Human Services (1992) Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. *MMWR., 41*(RR-14), pp. 1–7.

Ueland, P. M., Hustad, S., Schneede, J., Refsum, H. and Vollset, S. E. (2001) Biological and clinical implications of the MTHFR C677T polymorphism. *Trends Pharmacol Sci., 22*(4), pp. 195–201.

Ulrich, C. M. and Potter, J. D. (2007) Folate and cancer – timing is everything. *JAMA., 297*(21), pp. 2408–9.

van de Putte, B., van Poppel, F., Vanassche, S., Sanchez, M., Jidkova, S., Eeckhaut, M., Oris, M. and Matthijs, K. (2009) The Rise of Age Homogamy in 19th Century Western Europe. *Journal of Marriage and Family, 71*(5), pp. 1234-53.

van der Put, N. M., Gabreels, F., Stevens, E. M., Smeitink, J. A., Trijbels, F. J., Eskes, T. K., van den Heuvel, L. P. and Blom, H. J. (1998) A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am J Hum Genet., 62*(5), pp. 1044–51.

van der Put, N. M., van Straaten, H. W., Trijbels, F. J. and Blom, H. J. (2001) Folate, homocysteine and neural tube defects: an overview. *Exp Biol Med (Maywood), 226*(4), pp. 243–70.

van Eijsden, M., Smits, L. J., van der Wal, M. F. and Bonsel, G. J. (2008) Association between short interpregnancy intervals and term birth weight: the role of folate depletion. *Am J Clin Nutr., 88*(1), pp. 147–53.

van Guelpen, B., Hultdin, J., Johansson, I., Hallmans, G., Stenling, R., Riboli, E., Winkvist, A. and Palmqvist, R. (2006) Low folate levels may protect against colorectal cancer. *Gut., 55*(10), pp. 1461–6.

Vangen, T. (2007) *Nasjonal utdanningsdatabase NUDB. Dokumentasjonsrapport: datavarehus for utdanningsdata, 1970–2006 [National education database NUDB. Documentation report: The Education Data Warehouse, 1970–2006],* Statistics Norway [online], available: http://www.ssb.no/emner/04/90/notat_200754/notat_200754.pdf [Accessed Aug 2018].

Vienneau, D., Infanger, D., Feychting, M., Schuz, J., Schmidt, L. S., Poulsen, A. H., Tettamanti, G., Klaeboe, L., Kuehni, C. E., Tynes, T., Von der Weid, N., Lannering, B. and Roosli, M. (2016) A multinational case-control study on childhood brain tumours, anthropogenic factors, birth characteristics and prenatal exposures: A validation of interview data. *Cancer Epidemiol, 40*, pp. 52–9.

Vollset, S. E., Clarke, R., Lewington, S., Ebbing, M., Halsey, J., Lonn, E., Armitage, J., Manson, J. E., Hankey, G. J., Spence, J. D., Galan, P., Bønaa, K. H., Jamison, R., Gaziano, J. M., Guarino, P., Baron, J. A., Logan, R. F. A., Giovannucci, E. L., den Heijer, M., Ueland, P. M., Bennett, D., Collins, R. and Peto, R. (2013) Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: meta-analyses of data on 50 000 individuals. *The Lancet, 381*(9871), pp. 1029–36.

Wald, N. J., Morris, J. K. and Blakemore, C. (2018) Public health failure in the prevention of neural tube defects: time to abandon the tolerable upper intake level of folate. *Public Health Rev, 39*(1), pp. 2–11.

Wallenborn, J. T., Masho, S. W. and Ratliff, S. (2017) Paternal Pregnancy Intention and Breastfeeding Duration: Findings from the National Survey of Family Growth. *Matern Child Health J, 21*(3), pp. 554–61.

Wang, H., Wang, J., Zhao, L., Liu, X. and Mi, W. (2012) Methylenetetrahydrofolate reductase polymorphisms and risk of acute lymphoblastic leukemia-evidence from an updated meta-analysis including 35 studies. *BMC Med Genet, 13*, pp. 77.

Weinstein, S. J., Albanes, D., Selhub, J., Graubard, B., Lim, U., Taylor, P. R., Virtamo, J. and Stolzenberg-Solomon, R. (2008) One-carbon metabolism biomarkers and risk of colon and rectal cancers. *Cancer Epidemiol Biomarkers Prev, 17*(11), pp. 3233–40.

White, I. R. and Royston, P. (2009) Imputing missing covariate values for the Cox model. *Stat Med, 28*(15), pp. 1982–98.

White, M. C., Holman, D. M., Boehm, J. E., Peipins, L. A., Grossman, M. and Henley, S. J. (2014) Age and cancer risk: a potentially modifiable relationship. *Am J Prev Med, 46*(3 Suppl 1), pp. S7–15.

Wilcox, A. J., Lie, R. T., Solvoll, K., Taylor, J., McConnaughey, D. R., Abyholm, F., Vindenes, H., Vollset, S. E. and Drevon, C. A. (2007) Folic acid supplements and risk of facial clefts: national population based case-control study. *BMJ, 334*(7591), p. 464.

Wiseman, M. (2008) The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. *Proc Nutr Soc, 67*(3), pp. 253–6.

World Health Organization (2015a) *Cancer. Fact sheet N°297*, Available: http://www.who.int/mediacentre/factsheets/fs297/en/ [Accessed Aug. 2018].

World Health Organization (2015b) *International statistical classification of diseases and related health problems, 10th revision, Fifth edition, 2016,* World Health Organization [online], available: http://www.who.int/iris/handle/10665/246208 [Accessed December 2018].

World Health Organization (2016) Global Health Estimates 2015: Disease burden by Cause, Age, Sex, by Country and by Region, 2000–2015. In, Geneva: World Health Organization.

Wu, S., Zhu, W., Thompson, P. and Hannun, Y. A. (2018) Evaluating intrinsic and non-intrinsic cancer risk factors. *Nat Commun, 9*(1), p. 3490.

Yang, Y. M., Zhang, T. T., Yuan, L. and Ren, Y. (2014) The association between the C677T polymorphism in MTHFR gene and the risk of thyroid cancer: a meta-analysis. *Eur Rev Med Pharmacol Sci, 18*(15), pp. 2097–101.

Yiu, T. T. and Li, W. (2015) Pediatric cancer epigenome and the influence of folate. *Epigenomics, 7*(6), pp. 961–73.

Zacho, J., Yazdanyar, S., Bojesen, S. E., Tybjaerg-Hansen, A. and Nordestgaard, B. G. (2011) Hyperhomocysteinemia, methylenetetrahydrofolate reductase c.677C>T polymorphism and risk of cancer: cross-sectional and prospective studies and meta-analyses of 75,000 cases and 93,000 controls. *Int J Cancer., 128*(3), pp. 644–52.

Zhang, S. M., Cook, N. R., Albert, C. M., Gaziano, J. M., Buring, J. E. and Manson, J. E. (2008) Effect of combined folic acid, vitamin B6, and vitamin B12 on cancer risk in women: a randomized trial. *JAMA., 300*(17), pp. 2012–21.

Zhang, S. M. M., Giovannucci, E. L., Hunter, D. J., Rimm, E. B., Ascherio, A., Colditz, G. A., Speizer, F. E. and Willett, W. C. (2001) Vitamin supplement use and the risk of non-Hodgkin's lymphoma among women and men. *American Journal of Epidemiology, 153*(11), pp. 1056–63.

Zhang, Y., Dai, Y., Zheng, T. and Ma, S. (2011) Risk Factors of Non-Hodgkin Lymphoma. *Expert Opin Med Diagn, 5*(6), pp. 539–50.

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Supplemental folic acid in pregnancy and maternal cancer risk

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ABSTRACT

Background: There is evidence that increased intake of folate protects against the development of several types of cancer. Some studies have, however, raised concern about the safety of folate in relation to cancer risk. Here we examined the risk of maternal cancer after intake of supplemental folic acid in pregnancy.
Methods: This is a population-based cohort study comprising 429,004 women with data from the Medical Birth Registry of Norway, the Cancer Registry of Norway, and other national registries from 1999 to 2010. Altogether 3781 cancer cases were identified during follow-up (average 7 years). Cox proportional hazards regression models were used to estimate hazard ratios of maternal cancer according to folic acid use prior to and during one or two or more pregnancies as compared to no supplement use.
Results: Folic acid supplementation use had no overall effect on cancer risk in women using folic acid supplementation in one (HR 1.08; 95% CI 1.00–1.18) or two or more pregnancies (HR 1.06; 95% CI 0.91–1.22) ($p_{trend} = 0.12$). Analyses of 13 cancer types revealed no associations between folic acid and cancer.
Conclusion: Folic acid supplementation before and during pregnancy had no overall effect on maternal cancer risk.
Impact: Folic acid substitution before and/or during pregnancy does not increase the short-term overall maternal cancer risk.

1. Introduction

Pre-gestational intake of folic acid prevents neural tube defects (NTDs) [1–3], and in many countries health authorities recommend women planning pregnancy to take folic acid supplementation before and during pregnancy [2]. Mandatory food fortification with folic acid has been implemented in many countries but remains controversial in others, with issues concerning cancer risk [4–7]. At present, there is no mandatory folic acid food fortification in Norway. The Norwegian National Nutrition Council recommends that all women who are planning pregnancy or are likely to become pregnant use 400 μg folic acid daily from one month before pregnancy throughout the first three months of pregnancy [8].

Folates are a group of B-vitamins important in DNA synthesis, replication, and genomic stability [9,10]. Folic acid is the synthetic form of folate with a substantially higher bioavailability relative to food folate [11]. Data from human studies suggests that consumption of high doses of folic acid, or with the highest blood folate concentrations, have a significantly reduced risk of developing colon polyps or cancer [12]. However, an entirely protective role for folate against carcinogenesis has been questioned. Based on human and animal evidence Kim proposed that folic acid supplementation may enhance colorectal carcinogenesis in neoplastic foci whereas folate deficiency may have an inhibitory effect [13]. Further, supraphysiologic doses of folic acid may enhance the development of cancer in normal colorectal mucosa, modest doses of folic acid may suppress, whereas folate deficiency may predispose the normal mucosa to neoplastic transformation [13]. So far, findings from epidemiologic studies have not been consistent on the subject of folate and cancer risk. A 2013 meta-analysis of 13 randomized trials including

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50,000 individuals comparing folic acid use versus placebo to prevent complications in cardiovascular disease, showed no statistically significant association with total cancer or sub-types of cancer [14].

No studies on periconceptional folic acid supplementation and maternal cancer risk have previously been conducted except for a randomized, double-blind study published in 2004 that later was criticized for the statistical approach and study design [15,16].

In the Medical Birth Registry of Norway, folic acid supplementation use has been registered since 1998. The aim of this study was to examine the subsequent risk of maternal cancer after intake of supplemental folic acid in pregnancy.

2. Material and methods

2.1. Data sources

Using the unique personal identification number given to citizens living in Norway, data was retrieved from the Norwegian Central Population Registry (NCPR) with linked data from the Medical Birth Registry of Norway (MBRN) [17], the Cancer Registry of Norway (CRN) [18], the Norwegian Labour and Welfare Administration (NAV) and the Norwegian National Education Database (NUDB). MBRN is a population-based registry containing information on all births in Norway since 1967 [17]. It is based on compulsory notification of all deliveries from gestational week 16 (since 2002 from week 12). CRN was established in 1951 and contains information on all new cancer cases and certain precancerous lesions in Norway. NAV was established in 2006 after governmental reorganization of the Directorate of Labour in Norway (founded in 1945), and holds information on employment, health status and social benefits of all individuals with residence in Norway since 1992. Since 1970, NUDB has registered information on all individuals' education since completed primary school and as far as doctoral studies in one database.

2.2. Exposure

The MBRN's notification form from December 1998 onwards has recorded information on folic acid and multivitamin supplementation by using checkboxes with the items "folic acid before pregnancy", "folic acid during pregnancy", "multivitamins before pregnancy", and "multivitamins during pregnancy". In Norway, folic acid supplements intended for use in pregnancy contained 0.4 mg folic acid, while most multivitamin supplements contained 0.0–0.2 mg of folic acid. The mothers were defined as folic acid users if folic acid were used before and/or during pregnancy. Furthermore, the mothers were defined as multivitamin users if folic acid were used before and/or during pregnancy. Based on the above information, we created two exposure variables of folic acid use, and one exposure variable of multivitamin use; the use in successive pregnancies (no use, use in one pregnancy, and use in two or more pregnancies), and the total amount of folic acid from multivitamin supplements (approximately 0.2 mg) and folic acid supplements (0.4 mg).

2.3. Outcome

Incident cancer cases (International Classification of Diseases version 10 (ICD-10)) were identified through linkage with CRN. For each mother, only the first cancer diagnosis was used. The 13 most frequent cancer sub-groups in our cohort were chosen. Sub-groups of cancers included colorectal cancer (C18–21), lung cancer (C33–34), melanoma of the skin (C43), non-melanoma skin cancer (C44), breast cancer (C50), and cancer of the uterine cervix (C53), ovary (C56), central nervous system (C70–72, D42–43), thyroid (C73), and other endocrine glands (C37, C74–75), Hodgkin's lymphoma (C81), non-Hodgkin's lymphoma (C82–85, C96), and leukemia (C91–95, D45–47). Cancer sites with less than 50 cases were combined in the group "Other cancers" (C00–17, C22–26, C30–32, C38–41, C45, C47–49, C51–52, C54, C57–58, C64–69, C76, C80, C88, C90).

2.4. Confounders

Data on maternal year of birth, maternal age at first childbirth in the study period (1999–2010), maternal age at first childbirth (prior to start of follow-up period), parity, marital status, and smoking habits was collected from the MBRN. Information on maternal smoking was recorded at start and end of pregnancy (no smoking, sometimes, daily, number of cigarettes, declined to inform about smoking habits). The smoking data was then combined into a single variable that contained the maximum cigarette consumption for each woman. Information on length of education and occupation at time of childbirth was collected from NUDB and NAV, respectively.

2.5. Study cohort

All women living in Norway and giving birth in the period January 1, 1999 to December 31, 2010 (429,004 women and 679,484 pregnancies) constituted our study cohort. Induced abortions (2491) were excluded since information on vitamin use has not been registered. Pregnancies to women who emigrated before birth (13,733) or women who were diagnosed with cancer before delivery (3334) were also excluded. The women were followed from the date of their first birth during 1999-2010 until a cancer diagnosis, death, emigration, or end of follow-up at December 31, 2010.

2.6. Statistical analysis

Hazard ratios (HRs) of cancer with 95% confidence intervals (95% CIs), among women using folic acid in successive pregnancies compared to women using no folic acid, were estimated using time-dependent Cox proportional hazard regression models [19]. Time since the first childbirth during 1999–2010 was used as time variable. Tests for linear trend over the categories of folic acid supplementation were conducted.

Similar time-dependent Cox proportional hazard regression analyses were also conducted for multivitamin use in successive pregnancies compared to women using no multivitamins.

The Cox models were adjusted for maternal age at first childbirth (age at cohort entry) during 1999–2010 ($<$20, 20–24, 25–29, 30–34, 35–39, $\geq$40 years), maternal year of birth (1949–59, 1960–69, 1970–79, 1980–89, 1990–96), and parity (1, 2, 3, $\geq$4), marital status (unmarried, married/registered partner/cohabitant, divorced/widowed), education (compulsory (1st–7th class level), intermediate (8th–12th class level), tertiary (14th–20th class level)), occupation (10 main groups), and smoking status at the time of birth (never, sometimes, $\leq$10 cigarettes daily, $>$10 cigarettes daily, daily smoking–unknown amount). For total cancer and breast cancer, we also adjusted for maternal age at very first childbirth ($<$20, 20–24, 25–29, 30–34, 35–39, $\geq$40 years).

For the years 2003–2010 occupational codes were available. Occupational codes registered in 2003 were applied for births during 1999–2002.

Since 16% of the study population had missing smoking information, we performed multiple imputation on missing smoking status at the time of birth according to White and Royston [20], and Sterne and colleagues [21]. Time-dependent Cox

Table 1
Characteristics of the study population at start of follow-up, Norway, 1999–2010.

Maternal characteristics	Cohort	Person-years	%	Cancer cases (*n*)	%
Mothers	429,004	2,933,587	100	3781	100
Maternal year of birth					
1949–59	3158	31,900	1	119	3
1960–69	102,284	920,987	31	1813	48
1970–79	227,841	1,621,811	55	1640	43
1980–89	92,535	355,060	12	208	6
1990–96	3186	3829	0	1	0
Maternal age at first childbirth in 1999–2010					
<20 years	15,119	92,407	3	45	1
20–24 years	79,225	504,471	17	344	9
25–29 years	146,380	1,013,239	35	984	26
30–34 years	124,835	890,856	30	1361	36
35–39 years	52,931	365,858	12	823	22
≥40	10,514	66,755	2	224	6
Maternal age at first childbirth[a]					
<20 years	33,626	249,038	8	256	7
20–24 years	122,114	885,507	30	916	24
25–29 years	150,240	1,054,830	36	1317	35
30–34 years	78,821	497,201	17	822	22
35–39 years	21,343	120,523	4	275	7
≥40 years	3,105	15,631	1	59	2
Missing data	19,755	110,857	4	136	4
Folic acid use in pregnancy[b]					
No use[c]	252,620	2,002,547	68	2579	68
Before pregnancy	5082	31,153	1	32	1
During pregnancy	112,874	622,961	21	801	21
Before and during pregnancy	58,428	276,926	9	369	10
Multivitamin use in pregnancy[b,d]					
No use[c]	298,543	2,187,443	75	2,826	75
Before pregnancy	5,890	33,067	1	53	1
During pregnancy	79,995	467,255	16	564	15
Before and during pregnancy	44,576	245,821	8	338	9
Education[b]					
Compulsory (1st–7th class level)	86,530	604,712	21	692	18
Intermediate (8th–12th class level)	149,530	1,093,481	37	1418	38
Tertiary (13th–20th class level)	174,222	1,133,166	39	1571	42
Missing data	18,722	102,227	3	100	3
Occupation[b,e]					
Armed forces and unspecified	144,109	1,154,409	39	1516	40
Legislators, senior officials and managers	11,397	75,386	3	124	3
Professionals	21,543	118,501	4	192	5
Technicians and associate professionals	61,202	353,946	12	464	12
Clerks	27,314	192,749	7	268	7
Service workers and shop and market sales workers	97,772	605,065	21	639	17
Agricultural, forestry and fishery workers	1443	9766	0	8	0
Craft and related trades workers	4175	26,800	1	46	1
Plant and machine operators and assemblers	8732	59,862	2	63	2
Elementary occupations	19,164	127,270	4	114	3
Missing data	32,153	209,833	7	347	9
Parity[b]					
1	278,438	1,631,675	56	1751	46
2	86,528	750,522	26	1110	29
3	45,168	391,057	13	644	17
≥4	18,870	160,333	5	276	7
Marital status[b]					
Unmarried	33,345	200,844	7	214	6
Married/partnership	385,481	2,644,365	90	3449	91
Divorced	2322	16,564	1	24	1
Missing data	7856	71,814	2	94	2
Smoking[b]					
Never	275,462	1,885,522	64	2416	64
Sometimes	12,245	85,200	3	115	3
≤10 cigarettes daily	49,956	367,012	13	520	14
>10 cigarettes daily	18,304	117,721	4	151	4
Daily, unknown amount	4061	27,608	1	23	1
Missing data	68,976	450,524	15	556	15

[a] Including births before 1999.
[b] At start of follow-up.
[c] No information on use.
[d] Multivitamins used in Norway contain on average 0.2 mg folic acid.
[e] Occupational codes registered in 2003 were applied for births during 1999–2002.

Table 2
Cancer cases registered during follow-up (1999–2010) according to age at diagnosis and calendar year among 429,004 women in Norway.

	Cancer cases (n)	%
Age at primary cancer diagnosis (years)		
<20	6	0
20–24	77	2
25–29	418	11
30–34	884	23
35–39	1157	31
≥40	1239	33
Year of primary cancer diagnosis		
1999–2001	199	5
2002–2004	630	17
2005–2007	1194	32
2008–2010	1758	46
Total	3781	100

proportional hazard regression analyses were then conducted on the imputed data set.

The statistical analyses were carried out with the statistical packages SPSS version 22 and STATA version 13 [22,23].

3. Results

The women were followed for an average of 7 years (range 0.04–12 years), constituting 2,933,587 person-years. The mean age at start of follow-up was 29 years (range 13–54 years). Characteristics of the study population at start of follow-up are presented in Table 1.

During follow-up, 3781 cancer cases were diagnosed. The mean age at diagnosis was 37 years (range 18–56 years). Mean time between the first birth in the study period and cancer diagnosis was five years (range 0.1–12 years). Breast cancer was the most frequent cancer type in the cohort (1166 cases). A total of 343 cancer cases were grouped into the "Other" category when the cancer site frequency was less than 50 cases. Table 2 shows maternal age and year of primary cancer diagnosis.

Fig. 1 shows the use of supplements (folic acid, multivitamins) and smoking related to pregnancy from 1999 to the end of the study period in 2010. In 1999, only 18% of the women used folic acid in pregnancy compared to 71% in 2010. Multivitamin use increased from 19% in 1999 to 42% in 2010. Daily and intermittent smoking registered among women in our cohort decreased from 26% in 1999 to 20% in 2010.

The adjusted HRs of cancer (total and sub-types) with 95 % CIs by folic acid use (before and/or during pregnancy) in one and two or more pregnancies compared to no folic acid use during the study period are presented in Table 3. No increased risk was seen for total cancer among women using folic acid in one (HR 1.08; 95% CI 1.00–1.18) or two or more pregnancies (HR 1.06; 95% CI 0.91–1.22) (p_{trend} = 0.12), and other sub-types of cancer, except for an increased risk for lung and trachea cancer (p_{trend} = 0.06) and thyroid cancer (p_{trend} = 0.05) of borderline significance.

Further adjustments for multivitamin use (in the analyses of folic acid use) showed no substantial changes in the risk estimates, neither for total cancer nor specific sub-types (data not shown). Analyses of total dose of folic acid (continuous variable) ingested from multivitamin supplements (0.2 mg folic acid) and folic acid supplements (0.4 mg folic acid) revealed no substantial changes in the risk estimates, neither for total cancer nor specific sub-types (data not shown).

Multivitamin use (before and/or during pregnancy) in one, or two or more pregnancies compared to no multivitamin use were not associated with increased risk of total cancers. However, increased risk was seen for melanoma of the skin among women using multivitamins in one (HR 1.19; 95% CI: 0.96–1.48), and two or more pregnancies (HR 1.58; 95% CI: 1.05–2.38) (p_{trend} = 0.02). Additionally, increased risk of non-Hodgkin's lymphoma was seen among multivitamin users in one (HR 1.54; 95% CI: 0.94–2.53) and two or more pregnancies (HR 2.82; 95% CI: 1.15–6.95) (p_{trend} = 0.01).

Imputed analyses (on missing smoking data) showed no substantial changes in the risk estimates, neither for total cancer nor specific sub-types (data not shown).

4. Discussion

The aim of this study was to evaluate the association between the recommended folic acid supplementation use and cancer risk. Our population-based cohort study comprising 429,004 women with data from the national registries in Norway, showed no significant relationship between periconceptional folic acid use and cancer risk.

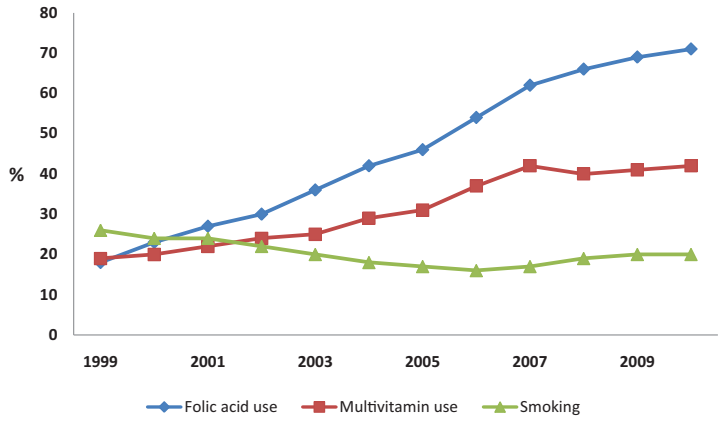

Fig. 1. Percentage folic acid (before and/or during pregnancy), multivitamin (before and/or during pregnancy) and cigarette use (intermittent or daily before/during pregnancy) among 679,484 pregnancies in Norway, 1999–2010.

Table 3
Hazard ratios (HR) of cancer with 95% confidence intervals (95% CI) by folic acid supplementation in one, or two or more pregnancies among 429,004 women in Norway, 1999–2010.

Cancer types	ICD-10 codes	Number of pregnancies with folic acid use	Cancer cases (*N*)	Model 1			Model 2		
				HR	CI95%	p_{trend}	HR	CI95%	p_{trend}
Total cancer		0	2269	1.00	Reference		1.00	Reference	
		1	1214	1.09	1.01–1.17		1.08	1.00–1.18	
		≥2	298	1.04	0.92–1.17	0.08	1.06	0.91–1.22	0.12
Colorectal	C18–21	0	98	1.00	Reference		1.00	Reference	
		1	52	1.11	0.79–1.56		0.91	0.60–1.38	
		≥2	19	1.75	1.06–2.90	0.06	1.96	1.10–3.50	0.16
Lung and trachea	C33–34	0	31	1.00	Reference		1.00	Reference	
		1	17	1.20	0.66–2.17		1.69	0.82–3.48	
		≥2	6	1.80	0.74–4.39	0.21	2.41	0.83–7.01	0.06
Melanoma of the skin	C43	0	275	1.00	Reference		1.00	Reference	
		1	164	1.18	0.97–1.43		1.08	0.87–1.35	
		≥2	55	1.52	1.13–2.04	0.00	1.35	0.96–1.89	0.11
Skin, non-melanoma	C44	0	34	1.00	Reference		1.00	Reference	
		1	16	0.98	0.54–1.78		0.79	0.39–1.63	
		≥2	1	0.26	0.04–1.92	0.31	0.29	0.04–2.17	0.20
Breast	C50	0	728	1.00	Reference		1.00	Reference	
		1	356	1.07	0.94–1.21		1.10	0.94–1.28	
		≥2	82	0.95	0.75–1.20	0.80	0.96	0.73–1.27	0.62
Cervix uteri	C53	0	269	1.00	Reference		1.00	Reference	
		1	151	1.09	0.89–1.33		1.06	0.83–1.34	
		≥2	37	0.93	0.66–1.32	0.85	0.93	0.63–1.39	0.99
Ovary	C56	0	48	1.00	Reference		1.00	Reference	
		1	23	0.95	0.58–1.56		1.04	0.58–1.86	
		≥2	3	0.57	0.17–1.84	0.43	0.90	0.26–3.10	0.99
Central nervous system	C70-72, D42-43	0	208	1.00	Reference		1.00	Reference	
		1	121	1.15	0.92–1.44		1.13	0.86–1.47	
		≥2	28	1.04	0.70–1.56	0.40	0.97	0.61–1.53	0.72
Thyroid	C73	0	138	1.00	Reference		1.00	Reference	
		1	84	1.17	0.89–1.54		1.36	0.99–1.86	
		≥2	30	1.57	1.05–2.35	0.03	1.41	0.88–2.26	0.05
Other endocrine glands	C37, C74–75	0	59	1.00	Reference		1.00	Reference	
		1	28	0.92	0.59–1.45		0.80	0.46–1.38	
		≥2	4	0.48	0.17–1.34	0.22	0.54	0.19–1.58	0.20
Hodgkin's lymphoma	C81	0	44	1.00	Reference		1.00	Reference	
		1	34	1.46	0.93–2.28		1.34	0.81–2.23	
		≥2	6	1.00	0.42–2.38	0.34	0.78	0.29–2.10	0.79
Non-Hodgkin's lymphoma	C82–85, C96	0	54	1.00	Reference		1.00	Reference	
		1	34	1.18	0.77–1.82		1.24	0.75–2.05	
		≥2	5	0.90	0.36–2.29	0.72	1.00	0.37–2.67	0.61
Leukaemia	C91–95, D45–47	0	60	1.00	Reference		1.00	Reference	
		1	31	1.05	0.68–1.62		1.19	0.71–2.00	
		≥2	5	0.74	0.29–1.86	0.76	0.65	0.19–2.18	0.96
Other cancers	C00–17, C22–26, C30–32, C38–41, C45, C47–49, C51–52, C54, C57–58, C64–69 C76, C80, C88, C90	0	223	1.00	Reference		1.00	Reference	
		1	103	0.92	0.73–1.17		1.07	0.81–1.41	
		≥2	17	0.65	0.39–1.07	0.11	0.92	0.53–1.58	0.93

Model 1: Adjusted for maternal age (<20, 20–24, 25–29, 30–34, 35–39, ≥40 years) at first childbirth in the study period 1999–2010.
Model 2: Further adjusted for maternal year of birth (1949–59, 1960–69, 1970–79, 1980–89, 1990–96), parity (1, 2, 3, ≥4), marital status (unmarried, married/registered partner/cohabitant, divorced/widowed), education (compulsory [1st–7th class level], intermediate [8th–12th class level], tertiary [14th–20th class level]), occupation (armed forces/unspecified, legislators, senior officials/managers, professionals, technicians/associate professionals, clerks, service workers/shop workers/market sales workers, agricultural/forestry/fishery workers, craft/ related trades workers, plant/machine operators, assemblers/elementary occupations), and smoking (never, intermittent, ≤10 cigarettes daily, >10 cigarettes daily, daily smoking–unknown number of cigarettes). For total cancer and breast cancer the model was also adjusted for maternal age at first childbirth (<20, 20–24, 25–29, 30–34, 35–39, ≥40 years) prior to start of follow-up.

4.1. Comparisons with the literature

Our results are in accordance with several prospective cohort studies and meta-analyses showing no overall or site-specific association between folic acid use and cancer risk [14,24–26]. However, there are inconsistencies in the literature regarding folate status and the risk of cancer. Some prospective cohort studies have found an inverse association between dietary folate intake or blood folate concentrations and risk of cancer of the colon, breast, ovary, and pancreas [27–32]. Contrary to these observational studies, two randomized controlled trials found no protective association between folic acid use (in combination with other B-vitamins) and cancer risk (overall and site-specific) [24,25].

Several potential mechanisms have been proposed by which folate or the bioactive form of folic acid may increase the risk of cancer. Folate is important for the synthesis of DNA, methylation, and repair [9,10]. An imbalance in these three functions might play a role in carcinogenesis. Unmetabolized folic acid may compromise the immunological defense against cancer and augment the growth of established cancer cells [33]. Other reports suggest that folic acid supplementation may promote cancer cells in already established neoplastic foci through de novo methylation of tumor suppressor genes with consequent gene inactivation, leading to tumor progression [5,34].

Interestingly, a potential dual modulatory role of folate on colorectal cancer has been proposed by Kim [13]. Folic acid may enhance the growth of cancer cells in established neoplastic foci whereas folate deficiency may inhibit progression of established colorectal neoplasms. On the other hand, in normal colorectal mucosa, folate deficiency may stimulate the initial stages of carcinogenesis in the colon and rectum, moderate doses of folic acid use may suppress, whereas high doses of folic acid may enhance the development of cancer [13].

The complex relationship between folate intake and colorectal cancer risk may be further modulated by genetic variants of folate metabolism enzymes. The enzyme methylenetetrahydrofolate reductase (MTHFR) is involved in the folate metabolism necessary for both DNA methylation and DNA synthesis. A common polymorphism in the *MTHFR* gene (MTHFR 677C→T polymorphism) is connected to reduced MTHFR enzyme activity and function that is important for the nucleotide and methylation pathways [35]. However, the MTHFR 677C→T polymorphism appears to decrease the risk of several adult cancer types (colorectal, liver, uterine cervical and acute lymphocytic leukemia) [35]. The MTHFR 677 TT genotype seems, however, to increase the risk of esophageal, gastric and pancreatic cancer [36].

We also evaluated the association between multivitamin use and risk of cancer. Our finding of no association between multivitamin use and total cancer risk is supported by other studies, reporting little or no influence from multivitamin use on total risk of cancer, including colorectal cancer [37,38]. Though, in sub-group analyses we found an increased risk of malignant melanoma and non-Hodgkin's lymphoma. These findings are in discrepancy with a large prospective cohort study on antioxidant supplementation that did not show increased melanoma risk [39]. However, a study by Zhang et al. in 2001 showed that multivitamin use was associated with a higher risk of non-Hodgkin's lymphoma among women, but not among men, and the authors concluded that their observed findings were the results of chance [40].

4.2. Strengths and limitations

To our knowledge, this is the largest study on cancer risk and folic acid use among pregnant women to date. The strengths of our study are the large cohort consisting mainly of healthy women in fertile age and the use of population-based registries covering the entire Norwegian population, assuring generalizability of our results. The loss to follow-up was minimal.

A limitation of this study is no records on dose, frequency, or precise duration of folic acid or multivitamin use throughout pregnancy. However, supplemental folic acid and multivitamin use as recorded in the MBRN, has also been used in other epidemiological studies [41,42]. In this study, we could not control for other health behaviours than smoking. Consequently, there could be confounding from other risk factors.

Altogether 16% of the pregnancies included in the study lacked smoking data, but imputation of missing values for smoking did not change our estimates. Most childbearing women know the adverse health effects of smoking to the foetus, which could reduce the reliance of self-reported smoking habits. On the other hand, smoking habits were documented before a possible cancer diagnosis.

Breast cancer was the most frequent cancer type in our cohort. Since young women at first full-term pregnancy have a decreased risk of developing hormone receptor positive breast cancer later in life [43], we also adjusted risks of breast cancer and total cancer for maternal age at her very first birth (including first birth before cohort entry in 1999). Adjustments for other potentially confounding factors (age at first childbirth in the study period, maternal year of birth, marital status, occupation, and smoking) showed minor changes in estimates, which reduced the likelihood of residual confounding. But, we could not adjust for other potential confounders, such as body mass index (BMI), physical activity, diet, alcohol intake, use of NSAIDs, exogenous hormones, and familial cancer syndromes, because these covariates were not available. Alcohol use, known to antagonise folate absorption and metabolism, is unlikely an important confounder, as the consumption of alcohol during pregnancy is generally low in Norway [44].

5. Conclusion

Overall, we found no association between folic acid supplementation and cancer risk. Our study cannot, however, assess the long-term impact of folic acid supplementation on cancer risk. The complex biological relation between folate and cancer needs cautious interpretation, and additional epidemiological research is warranted.

Ethical approval

The study was approved by the Regional Committee for Medical and Health Research Ethics of Western Norway.

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Conflict of interest

The authors have no conflicts of interest to disclose.

References

[1] MRC Vitamin Study Research Group, Prevention of neural tube defects: results of the Medical Research Council Vitamin Study, Lancet 338 (1991) 131–137.
[2] SACN, Folate and Disease Prevention, in: Nutrition SACo (Ed.), TSO (The Stationery Office), London, 2006.
[3] L.D. Botto, C.A. Moore, M.J. Khoury, J.D. Erickson, Neural-tube defects, N. Engl. J. Med. 341 (1999) 1509–1519.

[4] B.F. Cole, J.A. Baron, R.S. Sandler, R.W. Haile, D.J. Ahnen, R.S. Bresalier, et al., Folic acid for the prevention of colorectal adenomas: a randomized clinical trial, JAMA 297 (2007) 2351–2359.
[5] Y.I. Kim, Will mandatory folic acid fortification prevent or promote cancer? Am. J. Clin. Nutr. 80 (2004) 1123–1128.
[6] J.B. Mason, A. Dickstein, P.F. Jacques, P. Haggarty, J. Selhub, G. Dallal, et al., A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis, Cancer Epidemiol. Biomarkers Prev. 16 (2007) 1325–1329.
[7] A.D. Smith, Y.I. Kim, H. Refsum, Is folic acid good for everyone, Am. J. Clin. Nutr. 87 (2008) 517–533.
[8] Statensernæringsråd Anbefalinger og virkemidler for økt folatinntak blant kvinner i fertil alder. The Norwegian Directorate of Health (1998).
[9] Y.I. Kim, Folate and carcinogenesis: evidence, mechanisms, and implications, J. Nutr. Biochem. 10 (1999) 66–88.
[10] S.W. Choi, J.B. Mason, Folate status: effects on pathways of colorectal carcinogenesis, J Nutr. 132 (2002) 2413S–2418S.
[11] P. Sanderson, H. McNulty, P. Mastroiacovo, I.F. McDowell, A. Melse-Boonstra, P. M. Finglas, et al., Folate bioavailability: UK Food Standards Agency workshop report, Br. J. Nutr. 90 (2003) 473–479.
[12] S.J. Duthie, Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis, J. Inherit. Metab. Dis. 34 (2011) 101–109.
[13] Y.I. Kim, Folate and colorectal cancer: an evidence-based critical review, Mol. Nutr. Food Res. 51 (2007) 267–292.
[14] S.E. Vollset, R. Clarke, S. Lewington, M. Ebbing, J. Halsey, E. Lonn, et al., Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: meta-analyses of data on 50 000 individuals, Lancet 381 (2013) 1029–1036.
[15] D. Charles, A.R. Ness, D. Campbell, G. Davey Smith, M.H. Hall, Taking folate in pregnancy and risk of maternal breast cancer, BMJ 329 (2004) 1375–1376.
[16] J.M. Bland, Taking folate in pregnancy and risk of maternal breast cancer: what's in a name? BMJ 330 (2005) 600 author reply -1.
[17] L.M. Irgens, The Medical Birth Registry of Norway. Epidemiological research and surveillance throughout 30 years, Acta Obstet. Gynecol. Scand. 79 (2000) 435–439.
[18] I.K. Larsen, M. Smastuen, T.B. Johannesen, F. Langmark, D.M. Parkin, F. Bray, et al., Data quality at the Cancer Registry of Norway: an overview of comparability, completeness, validity and timeliness, Eur. J. Cancer 45 (2009) 1218–1231.
[19] D.R. Cox, D. Oakes, Analysis of survival data, Chapman and Hall Ltd, London, 1984.
[20] I.R. White, P. Royston, Imputing missing covariate values for the Cox model, Stat. Med. 28 (2009) 1982–1998.
[21] J.A. Sterne, I.R. White, J.B. Carlin, M. Spratt, P. Royston, M.G. Kenward, et al., Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls, BMJ 338 (2009) b2393.
[22] I.B.M. Corporation, IBM SPSS Statistics for Windows, Version 19.0, IBM Corp., Armonk, NY, 2010.
[23] STATA, Stata Statistical Software: Release 14, StataCorp LP, College Station, TX, 2015.
[24] S.M. Zhang, N.R. Cook, C.M. Albert, J.M. Gaziano, J.E. Buring, J.E. Manson, Effect of combined folic acid, vitamin B6, and vitamin B12 on cancer risk in women: a randomized trial, JAMA 300 (2008) 2012–2021.
[25] G.J. Hankey, J.W. Eikelboom, Q. Yi, K.R. Lees, C. Chen, D. Xavier, et al., Treatment with B vitamins and incidence of cancer in patients with previous stroke or transient ischemic attack: results of a randomized placebo-controlled trial, Stroke 43 (2012) 1572–1577.
[26] X. Qin, Y. Cui, L. Shen, N. Sun, Y. Zhang, J. Li, et al., Folic acid supplementation and cancer risk: a meta-analysis of randomized controlled trials, Int. J. Cancer 133 (2013) 1033–1041.
[27] E. Giovannucci, M.J. Stampfer, G.A. Colditz, D.J. Hunter, C. Fuchs, B.A. Rosner, et al., Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study, Ann. Intern. Med. 129 (1998) 517–524.
[28] D.A. Kennedy, S.J. Stern, M. Moretti, I. Matok, M. Sarkar, C. Nickel, et al., Folate intake and the risk of colorectal cancer: a systematic review and meta-analysis, Cancer Epidemiol. 35 (2011) 2–10.
[29] U. Ericson, E. Sonestedt, B. Gullberg, H. Olsson, E. Wirfalt, High folate intake is associated with lower breast cancer incidence in postmenopausal women in the Malmo Diet and Cancer cohort, Am. J. Clin. Nutr. 86 (2007) 434–443.
[30] C.S. Fuchs, W.C. Willett, G.A. Colditz, D.J. Hunter, M.J. Stampfer, F.E. Speizer, et al., The influence of folate and multivitamin use on the familial risk of colon cancer in women, Cancer Epidemiol. Biomarkers Prev. 11 (2002) 227–234.
[31] S.C. Larsson, E. Giovannucci, A. Wolk, Dietary folate intake and incidence of ovarian cancer: the Swedish Mammography Cohort, J. Natl. Cancer Inst. 96 (2004) 396–402.
[32] S.C. Larsson, N. Hakansson, E. Giovannucci, A. Wolk, Folate intake and pancreatic cancer incidence: a prospective study of Swedish women and men, J. Natl. Cancer Inst. 98 (2006) 407–413.
[33] A.M. Troen, B. Mitchell, B. Sorensen, M.H. Wener, A. Johnston, B. Wood, et al., Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women, J. Nutr. 136 (2006) 189–194.
[34] Y.I. Kim, Nutritional epigenetics: impact of folate deficiency on DNA methylation and colon cancer susceptibility, J. Nutr. 135 (2005) 2703–2709.
[35] Y.I. Kim, Role of the MTHFR polymorphisms in cancer risk modification and treatment, Future Oncol. 5 (2009) 523–542.
[36] S.C. Larsson, E. Giovannucci, A. Wolk, Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis, Gastroenterology 131 (2006) 1271–1283.
[37] S.Y. Park, S.P. Murphy, L.R. Wilkens, B.E. Henderson, L.N. Kolonel, Multivitamin use and the risk of mortality and cancer incidence: the multiethnic cohort study, Am. J. Epidemiol. 173 (2011) 906–914.
[38] M.L. Neuhouser, S. Wassertheil-Smoller, C. Thomson, A. Aragaki, G.L. Anderson, J.E. Manson, et al., Multivitamin use and risk of cancer and cardiovascular disease in the Women's Health Initiative cohorts, Arch. Intern. Med. 169 (2009) 294–304.
[39] M.M. Asgari, S.S. Maruti, L.H. Kushi, E. White, Antioxidant supplementation and risk of incident melanomas: results of a large prospective cohort study, Arch. Dermatol. 145 (2009) 879–882.
[40] S.M.M. Zhang, E.L. Giovannucci, D.J. Hunter, E.B. Rimm, A. Ascherio, G.A. Colditz, et al., Vitamin supplement use and the risk of non-Hodgkin's lymphoma among women and men, Am. J. Epidemiol. 153 (2001) 1056–1063.
[41] R.M. Nilsen, S.E. Vollset, S.A. Rasmussen, P.M. Ueland, A.K. Daltveit, Folic acid and multivitamin supplement use and risk of placental abruption: a population-based registry study, Am. J. Epidemiol. 167 (2008) 867–874.
[42] S.E. Vollset, H.K. Gjessing, A. Tandberg, T. Ronning, L.M. Irgens, V. Baste, et al., Folate supplementation and twin pregnancies, Epidemiology 16 (2005) 201–205.
[43] L. Bernstein, Epidemiology of endocrine-related risk factors for breast cancer, J. Mammary Gland Biol. Neoplasia 7 (2002) 3–15.
[44] M.C. Magnus, L.A. DeRoo, S.E. Haberg, P. Magnus, P. Nafstad, W. Nystad, et al., Prospective study of maternal alcohol intake during pregnancy or lactation and risk of childhood asthma: the Norwegian Mother and Child Cohort Study, Alcohol. Clin. Exp. Res. 38 (2014) 1002–1011.

Publication 2

FULL PAPER

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Supplemental folic acid in pregnancy and childhood cancer risk

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Background: We investigated the association between supplemental folic acid in pregnancy and childhood cancer in a nation-wide study of 687 406 live births in Norway, 1999–2010, and 799 children diagnosed later with cancer.

Methods: Adjusted hazard ratios (HRs) compared cancer risk in children by approximated periconceptional folic acid levels (folic acid tablets and multivitamins (0.6 mg), only folic acid (0.4 mg), only multivitamins (0.2 mg)) and cancer risk in unexposed.

Results: Any folic acid levels were not associated with leukemia (e.g., high-level folic acid HR 1.25; 95% CI 0.89–1.76, P_{Trend} 0.20), lymphoma (HR 0.96; 95% CI 0.42–2.21, P_{Trend} 0.51), central nervous system tumours (HR 0.68; 95% CI 0.42–1.10, P_{Trend} 0.32), neuroblastoma (HR 1.05; 95% CI 0.53–2.06, P_{Trend} 0.85), Wilms' tumour (HR 1.16; 95% CI 0.52–2.58, P_{Trend} 0.76), or soft-tissue tumours (HR 0.77; 95% CI 0.34–1.75, P_{Trend} 0.90).

Conclusions: Folic acid supplementation was not associated with risk of major childhood cancers.

Health authorities in many countries recommend women planning pregnancy to take folic acid before and during pregnancy to reduce offspring risk of neural tube defects (SACN, 2006). A large number of countries also fortify flour with folic acid (CDC, 2008). Mandatory food fortification with folic acid is debated in some countries because of the suggested cancer risk in adults (Kim, 2004; Mason *et al*, 2007; Smith *et al*, 2008). However, in case–control studies on children, cancer risks (leukemia, brain tumours) were reduced if the mother had been exposed to perigestational maternal folic acid supplementation (Thompson *et al*, 2001; Milne *et al*, 2010; Milne *et al*, 2012; Metayer *et al*, 2014). And, in ecological studies from Canada and the United States of America, the childhood cancer incidence (Wilms' tumour, primitive neuroectodermal tumours, neuroblastoma) has been reduced after mandatory folic acid flour fortification (French *et al*, 2003; Grupp *et al*, 2011; Linabery *et al*, 2012).

The aim of our study was to investigate the association between maternal intake of folic acid supplementation in pregnancy and offspring risk of childhood cancer in a nation-wide cohort study in Norway.

MATERIALS AND METHODS

Data sources. The unique personal identification number assigned to all Norwegian residents enabled linkage of information between the Medical Birth Registry of Norway (MBRN) (Irgens, 2000), the Cancer Registry of Norway (CRN) (Larsen *et al*, 2009), and the Norwegian National Education Database that holds information on all individuals' education (Kinge *et al*, 2015).

Folic acid and multivitamin supplementation exposure. Folic acid and multivitamin supplementation use has been registered in

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the MBRN since December 1998. The registration form uses check boxes with the items 'folic acid before pregnancy', 'folic acid during pregnancy', 'multivitamins before pregnancy', and 'multivitamins during pregnancy'. During the study period, the folic acid content was 0.4 mg in folic acid supplements and approximately 0.2 mg in multivitamin supplements. Children were defined as exposed to folic acid if their mothers used folic acid supplements and/or multivitamins before and/or during pregnancy. Maternal folic acid intake was categorised by increasing folic acid content; no supplement use (0 mg), only multivitamins (approximately 0.2 mg), only folic acid supplements (0.4 mg), or intake of both folic acid supplements and multivitamins (approximately 0.6 mg).

Childhood cancer. Childhood cancer cases were identified through linkage with CRN. For each child, the first cancer diagnosis was used. The childhood cancers were categorised according to the International Classification of Childhood Cancer, version 3, which is based on ICD-O-3 (Steliarova-Foucher *et al*, 2005).

Study cohort. The study cohort consisted of all live births in Norway, 1 January 1999 through 31 December 2010 (excluding children with mothers with a prebirth cancer diagnosis (3371)), with follow-up until a cancer diagnosis, emigration, death, or 31 December 2010.

Statistical analysis. Risk of childhood cancers in children exposed to maternal folic acid and/or multivitamin supplements was compared with cancer risk in unexposed children and estimated with hazard ratios (HRs) using Cox proportional hazards regression models with time since birth as the time variable, adjusting for *a priori* selected covariates associated with maternal folic acid use and childhood cancer risk; that is, birth order (1, 2, ⩾3), maternal smoking (never, sometimes, ⩽10 cigarettes daily, >10 cigarettes daily, daily smoking of unknown amount), maternal and paternal age (<25, 25–34, ⩾35 years), and maternal and paternal education (compulsory, intermediate, tertiary). *P*-values for linear trend were calculated for folic acid exposure levels (0 mg, 0.2 mg, 0.4 mg, 0.6 mg). Statistical analyses were performed in STATA version 14 (STATA, 2015).

Ethics. The Regional Committee for Medical and Health Research Ethics of Western Norway approved the study.

RESULTS

Among 687 406 children included in the study, 799 developed cancer. The mean follow-up time was 6 years (range 0.04–12 years), constituting 4 052 679 person-years (Table 1). Among all births, 4% were multiple births, and 2% were born after assisted reproductive technology. Mean maternal age at childbirth was 29 years (range 13–55 years). The proportion of children exposed to perigestational supplementation increased in the study period, 1999–2010; intake of folic acid changed from 18% to 69% and multivitamins from 19% to 42%.

About 67% of all cancers were diagnosed within the first 3 years of life (Table 2). Leukemia and central nervous system (CNS) tumours accounted for 57% of the cases. We performed analyses for the six most frequent childhood cancer types (leukemia, lymphoma, CNS tumours, neuroblastoma, Wilms' tumour, soft tissue tumours) (Table 3). There was no change in childhood leukemia risk by maternal use of multivitamins only (HR 1.23; 95% CI 0.75–2.01), folic acid use only (HR 1.13; 95% CI 0.79–1.63), or combined folic acid and multivitamin use (HR 1.25; 95% CI 0.89–1.76), as compared with no supplement use (P_{Trend} 0.20). Similarly, there were no associations between CNS tumours and different levels of maternal folic acid intake; multivitamins only (HR 1.08; 95% CI 0.60–1.94), folic acid use only (HR 1.18; 95% CI 0.78–1.78), or combined folic acid and multivitamin use (HR 0.68; 95% CI 0.42–1.10), as compared with no supplement use (P_{Trend} 0.32). The HRs of the other frequent childhood cancer types (lymphoma, neuroblastoma, Wilms' tumour, soft tissue tumours) did not change for different levels of folic acid exposure. Adding birth year to adjustment models showed no substantial

Table 1. Characteristics of the study population of 687 406 live births, Norway, 1999–2010

Characteristics	Cohort (*n*)	Person-years	%	Cancer cases (*n*)
Children	687 406	4 052 679	100	799
Sex				
Boys	352 604	2 077 322	51	423
Girls	334 802	1 975 357	49	376
Gestational age (weeks)				
<37	46 682	271 770	7	60
37–41	587 197	3 447 416	85	670
⩾42	48 830	307 613	8	62
Missing	4697	25 881	1	7
Birth weight (g)				
<2500	33 804	191 809	5	39
2500–3999	516 075	3 008 163	74	587
⩾4000	136 760	847 264	21	173
Missing	767	5443	0	0
Birth order				
1	284 468	1 651 442	41	339
2	244 834	1 446 964	36	281
⩾3	158 104	954 274	24	179
Maternal age at child birth, years				
<25	117 065	697 604	17	133
25–34	452 481	2 709 049	67	539
⩾35	117 860	646 026	16	127
Paternal age at child birth, years				
<25	52 776	312 202	8	65
25–34	396 496	2 406 027	59	468
⩾35	231 836	1 307 428	32	257
Missing	6298	27 023	1	9
Maternal education[a]				
Compulsory	128 452	782 418	19	148
Intermediate	232 745	1 475 123	36	288
Tertiary	299 871	1 662 622	41	340
Missing	26 338	132 516	3	23
Paternal education[a]				
Compulsory	129 537	779 208	19	142
Intermediate	301 918	1 842 424	45	373
Tertiary	227 910	1 297 762	32	251
Missing	28 041	133 286	3	33
Maternal smoking				
Did not smoke	459 617	2 678 139	66	529
Smoked sometimes	17 222	106 380	3	15
Smoked ⩽10 cigarettes daily	69 270	455 935	11	103
Smoked >10 cigarettes daily	25 210	144 005	4	30
Smoked daily, unknown amount	5331	33 502	1	4
Missing	110 756	634 718	16	118
Maternal supplementation[b]				
No use	325 706	2 307 683	57	424
Multivitamins only	46 598	309 597	8	61
Folic acid only	145 856	675 461	17	154
Folic acid and multivitamin use	169 246	759 938	19	160

[a]Compulsory education length was 9 years until 1996 and 10 years from 1997 onwards.
[b]Maternal supplement intake before and/or during pregnancy, categorised by folic acid content: No use; multivitamins (approximately 0.2 mg); folic acid supplements (0.4 mg); and folic acid and multivitamins (approximately 0.6 mg).

Table 2. Children with first-time childhood cancer ($n = 799$) by age at diagnosis, year of diagnosis, and major cancer types (ICCC-3), identified among 687 406 livebirths, Norway, 1999–2010

	Cancer cases	%
Age at cancer diagnosis (years)		
<2	326	41
2–3	211	26
4–5	150	19
⩾6	112	14
Year of cancer diagnosis		
1999–2001	59	7
2002–2004	172	22
2005–2007	239	30
2008–2010	329	41
Cancer types (ICCC-3)		
I Leukemias, myeloproliferative diseases, and myelodysplastic diseases	268	34
Lymphoid leukemia	208	
Acute myeloid leukemias	45	
II Lymphomas and reticuloendothelial neoplasms	42	5
III CNS and miscellaneous intracranial and intraspinal neoplasms	185	23
Ependymoma	26	
Astrocytoma	79	
Intracranial and intraspinal embryonal tumours	50	
IV Neuroblastoma and other peripheral nervous cell tumours	72	9
Neuroblastoma and ganglioneuroblastoma	71	
VI Renal tumours	53	7
Wilms' tumour	52	
IX Soft tissue and other extraosseous sarcomas	64	8
Rhabdomyosarcoma	24	
Other specified soft tissue sarcomas	28	
Other cancers	115	14
Total	799	100

Abbreviations: CNS = central nervous system; ICCC-3 = International Classification of Childhood Cancer, third edition (Steliarova-Foucher *et al*, 2005).

changes in the risk estimates for neither cancer types. And excluding 867 children with Down syndrome from the analyses did not change the HR estimates for specific cancers.

DISCUSSION

In a nation-wide cohort study of all live births, estimated maternal intakes of multivitamins, folic acid, or combined intake of these supplements were not associated with childhood cancer.

Our results of no association between periconceptional folic acid supplementation and major childhood cancers are in discordance with case–control studies showing inverse associations between self-reported folic acid use and acute lymphoblastic leukemia (ALL) (Thompson *et al*, 2001; Milne *et al*, 2010; Metayer *et al*, 2014) and CNS tumours (Milne *et al*, 2012).

A recent large international collaborating study, including >7000 children with acute leukemia and 11 000 controls, found reduced risks of ALL and acute myeloid leukemia (AML) after maternal intake of folic acid supplements. And these reduced risks of ALL and AML did not vary by timing of the supplementation exposure (preconception, pregnancy, or pregnancy trimester) (Metayer *et al*, 2014). However, an Australian study found weak evidence of a reduced risk of ALL from folate supplementation before pregnancy, but no reduced risk from use during pregnancy (Milne *et al*, 2010). Also, another Australian study reported on an inverse association of childhood brain tumours and folic acid supplementation before and possibly also during pregnancy (Milne *et al*, 2012). In our study, a further stratification of the exposure data into preconceptional use and use during pregnancy was not feasable due to the limited statistical power of the analyses.

The strengths of our study include using comprehensive data from population-based registries covering the entire Norwegian population. To our knowledge, Norway is the only country where individual-level information on periconceptional folic acid and multivitamin intake has been collected for the entire birth population since 1999. All incident cancer cases have been reported to the Cancer Registry of Norway since 1952 (Larsen *et al*, 2009). And information on supplement use was collected before cancer diagnosis precluding recall bias.

Table 3. Hazard ratios (HRs) with 95% confidence intervals (95% CI) of childhood cancer by perigestational supplementation of folic acid and/or multivitamins, among 687 406 children, Norway, 1999–2010

Cancer types	Supplements[a]	Cancer cases	HR[b]	95% CI	P_{Trend}
All cancers	No supplements	424	1.00	Reference	
	Multivitamins only	61	1.05	0.78–1.42	
	Folic acid only	154	1.13	0.92–1.38	
	Folic acid and multivitamins	160	1.02	0.83–1.25	0.60
I Leukemias, myeloproliferative diseases, and myelodysplastic diseases					
	No supplements	135	1.00	Reference	
	Multivitamins only	21	1.23	0.75–2.01	
	Folic acid only	50	1.13	0.79–1.63	
	Folic acid and multivitamins	62	1.25	0.89–1.76	0.20
(a) Lymphoid leukemia					
	No supplements	100	1.00	Reference	
	Multivitamins only	16	1.30	0.75–2.27	
	Folic acid only	42	1.30	0.87–1.95	
	Folic acid and multivitamins	50	1.31	0.89–1.94	0.12
(b) Acute myeloid leukemia					
	No supplements	28	1.00	Reference	
	Multivitamins only	3	0.97	0.29–3.27	
	Folic acid only	5	0.59	0.22–1.60	
	Folic acid and multivitamins	9	0.96	0.43–2.17	0.67

Table 3. (Continued)

Cancer types	Supplements[a]	Cancer cases	HR[b]	95% CI	P_{Trend}
II Lymphomas and reticuloendothelial neoplasms					
	No supplements	25	1.00	Reference	
	Multivitamins only	3	0.55	0.13–2.33	
	Folic acid only	5	0.40	0.12–1.34	
	Folic acid and multivitamins	9	0.96	0.42–2.21	0.51
III CNS and miscellaneous intracranial and intraspinal neoplasms					
	No supplements	107	1.00	Reference	
	Multivitamins only	14	1.08	0.60–1.94	
	Folic acid only	37	1.18	0.78–1.78	
	Folic acid and multivitamins	27	0.68	0.42–1.10	0.32
(b) Astrocytoma					
	No supplements	44	1.00	Reference	
	Multivitamins only	8	1.57	0.72–3.40	
	Folic acid only	15	1.31	0.70–2.45	
	Folic acid and multivitamins	12	0.86	0.43–1.73	0.97
(c) Intracranial and intraspinal embryonal tumours					
	No supplements	28	1.00	Reference	
	Multivitamins only	2	0.61	0.14–2.59	
	Folic acid only	12	1.28	0.60–2.76	
	Folic acid and multivitamins	8	0.69	0.27–1.74	0.69
IV Neuroblastoma and other peripheral nervous cell tumours					
(a) Neuroblastoma and ganglioneuroblastoma					
	No supplements	37	1.00	Reference	
	Multivitamins only	5	0.99	0.35–2.82	
	Folic acid only	15	1.08	0.54–2.15	
	Folic acid and multivitamins	14	1.05	0.53–2.06	0.85
VI Renal tumours					
(a) Wilms' tumour					
	No supplements	28	1.00	Reference	
	Multivitamins only	5	1.60	0.60–4.25	
	Folic acid only	9	1.01	0.42–2.40	
	Folic acid and multivitamins	10	1.16	0.52–2.58	0.76
IX Soft tissue and other extraosseous sarcomas					
	No supplements	32	1.00	Reference	
	Multivitamins only	5	1.12	0.39–3.22	
	Folic acid only	18	1.72	0.90–3.29	
	Folic acid and multivitamins	9	0.77	0.34–1.75	0.90

Abbreviation: CNS = central nervous system.
[a]Maternal supplement intake before and/or during pregnancy, categorised by folic acid content: No use; multivitamins (approximately 0.2 mg); folic acid supplements (0.4 mg); and folic acid and multivitamins (approximately 0.6 mg).
[b]Hazard ratios (HR) with 95% confidence intervals (95% CI) adjusted for birth order (1, 2, ⩾3), smoking (never, sometimes, ⩽10 cigarettes daily, >10 cigarettes daily, daily smoking of unknown amount), maternal and paternal age (<25, 25–34, ⩾35 years), and maternal and paternal education (compulsory, intermediate, tertiary) comparing cancer risk in children exposed to periconceptional folic acid (multivitamins, folic acid, folic acid and multivitamins) and cancer risk in children without perigestational folic acid exposure (reference).

The study had some limitations. Even though our cohort was large, the numbers of several childhood cancer types were relatively low, which may limit the statistical power of our findings. The follow-up time of study participants were on average 6 years, and our results could only be generalised to younger children. Maternal folic acid intake could have been misclassified; in the beginning of the study period, folic acid users were under-reported to the MBRN (Nilsen *et al*, 2009). A possible misclassification of folic acid dose (independent of cancer risk) would bias risk estimates towards the null value and, in theory, could have concealed an association between folic acid intake and childhood cancer risk. Information on maternal smoking was missing for 16% of the births; however, HR estimates adjusting for maternal smoking were similar to HRs without smoking adjustments. Although we did not have information on dietary folate, residual confounding by dietary folate is less likely. In pregnant women, maternal plasma levels of serum folate is strongly related to intake of folic acid supplements (Bjorke-Monsen *et al*, 2013). And in other studies of maternal intake of folic acid supplements and offspring outcomes (oral clefts, autism), adjustment for dietary folate did not change overall risk estimates (Wilcox *et al*, 2007; Suren *et al*, 2013). We could not adjust for mother's weight and height, physical activity, diet, use of alcohol, or use of contraceptive pills, as these covariates were not available in the MBRN.

In conclusion, we found no association between maternal supplemental folic acid intake before and/or during pregnancy and risk of leukemia, lymphomas, CNS tumours, neuroblastoma, Wilms' tumour, or soft tissue tumours among younger children.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Bjorke-Monsen AL, Roth C, Magnus P, Midttun O, Nilsen RM, Reichborn-Kjennerud T, Stoltenberg C, Susser E, Vollset SE, Ueland PM (2013) Maternal B vitamin status in pregnancy week 18 according to reported use of folic acid supplements. *Mol Nutr Food Res* **57**(4): 645–652.

CDC (2008) Trends in wheat-flour fortification with folic acid and iron—worldwide, 2004 and 2007. *MMWR Morb Mortal Wkly Rep* **57**: 8–10.

French AE, Grant R, Weitzman S, Ray JG, Vermeulen MJ, Sung L, Greenberg M, Koren G (2003) Folic acid food fortification is associated with a decline in neuroblastoma. *Clin Pharmacol Ther* **74**(3): 288–294.

Grupp SG, Greenberg ML, Ray JG, Busto U, Lanctot KL, Nulman I, Koren G (2011) Pediatric cancer rates after universal folic acid flour fortification in Ontario. *J Clin Pharmacol* **51**(1): 60–65.

Irgens LM (2000) The Medical Birth Registry of Norway. Epidemiological research and surveillance throughout 30 years. *Acta Obstet Gynecol Scand* **79**(6): 435–439.

Kim YI (2004) Will mandatory folic acid fortification prevent or promote cancer? *Am J Clin Nutr* **80**(5): 1123–1128.

Kinge JM, Steingrimsdottir OA, Moe JO, Skirbekk V, Naess O, Strand BH (2015) Educational differences in life expectancy over five decades among the oldest old in Norway. *Age Ageing* **44**(6): 1040–1045.

Larsen IK, Smastuen M, Johannesen TB, Langmark F, Parkin DM, Bray F, Moller B (2009) Data quality at the Cancer Registry of Norway: an overview of comparability, completeness, validity and timeliness. *Eur J Cancer* **45**(7): 1218–1231.

Linabery AM, Johnson KJ, Ross JA (2012) Childhood cancer incidence trends in association with US folic acid fortification (1986-2008). *Pediatrics* **129**(6): 1125–1133.

Mason JB, Dickstein A, Jacques PF, Haggarty P, Selhub J, Dallal G, Rosenberg IH (2007) A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. *Cancer Epidemiol Biomarkers Prev* **16**(7): 1325–1329.

Metayer C, Milne E, Dockerty JD, Clavel J, Pombo-de-Oliveira MS, Wesseling C, Spector LG, Schuz J, Petridou E, Ezzat S, Armstrong BK, Rudant J, Koifman S, Kaatsch P, Moschovi M, Rashed WM, Selvin S, McCauley K, Hung RJ, Kang AY, Infante-Rivard C (2014) Maternal supplementation with folic acid and other vitamins and risk of leukemia in offspring: a Childhood Leukemia International Consortium study. *Epidemiology* **25**(6): 811–822.

Milne E, Greenop KR, Bower C, Miller M, van Bockxmeer FM, Scott RJ, de Klerk NH, Ashton LJ, Gottardo NG, Armstrong BK, Aus CBTC (2012) Maternal use of folic acid and other supplements and risk of childhood brain tumors. *Cancer Epidemiol Biomarkers Prev* **21**(11): 1933–1941.

Milne E, Royle JA, Miller M, Bower C, de Klerk NH, Bailey HD, van Bockxmeer F, Attia J, Scott RJ, Norris MD, Haber M, Thompson JR, Fritschi L, Marshall GM, Armstrong BK (2010) Maternal folate and other vitamin supplementation during pregnancy and risk of acute lymphoblastic leukemia in the offspring. *Int J Cancer* **126**(11): 2690–2699.

Nilsen RM, Vollset SE, Gjessing HK, Skjaerven R, Melve KK, Schreuder P, Alsaker ER, Haug K, Daltveit AK, Magnus P (2009) Self-selection and bias in a large prospective pregnancy cohort in Norway. *Paediatr Perinat Epidemiol* **23**(6): 597–608.

SACN (2006) Folate and Disease Prevention. TSO (The Stationery Office), Scientific Advisory Committe on Nutrition: London, UK.

Smith AD, Kim YI, Refsum H (2008) Is folic acid good for everyone? *Am J Clin Nutr* **87**(3): 517–533.

STATA (2015) *Stata Statistical Software: Release 14*. StataCorp LP: College Station, TX, USA.

Steliarova-Foucher E, Stiller C, Lacour B, Kaatsch P (2005) International Classification of Childhood Cancer, third edition. *Cancer* **103**(7): 1457–1467.

Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, Lie KK, Lipkin WI, Magnus P, Reichborn-Kjennerud T, Schjolberg S, Davey Smith G, Oyen AS, Susser E, Stoltenberg C (2013) Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. *JAMA* **309**(6): 570–577.

Thompson JR, Gerald PF, Willoughby ML, Armstrong BK (2001) Maternal folate supplementation in pregnancy and protection against acute lymphoblastic leukaemia in childhood: a case-control study. *Lancet* **358**(9297): 1935–1940.

Wilcox AJ, Lie RT, Solvoll K, Taylor J, McConnaughey DR, Abyholm F, Vindenes H, Vollset SE, Drevon CA (2007) Folic acid supplements and risk of facial clefts: national population based case-control study. *BMJ* **334**(7591): 464.

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RESEARCH ARTICLE Open Access

Paternal characteristics associated with maternal periconceptional use of folic acid supplementation

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Abstract

Background: Maternal predictors of folic acid (FA) supplementation use to reduce offspring risk of neural tube defects are well known, while paternal determinants for maternal FA use are less known. Such knowledge is important to increase women's compliance to recommended periconceptional FA use.

Methods: In a nation-wide study of 683,785 births registered in the Medical Birth Registry of Norway during 1999–2010, the associations between paternal characteristics (age, education, occupation, country of origin) and maternal FA use were estimated by relative risks (RR) with 95% confidence intervals (CI), using log-binomial regression.

Results: Maternal FA use before and during pregnancy (adequate FA use) was found in 16% of the births. The association between paternal age and adequate FA use was inversely U-shaped; adjusted RRs for adequate FA use were 0.35 (95% CI 0.28–0.43) and 0.72 (95% CI 0.71–0.74) for paternal age < 20 and ≥ 40 years, respectively, comparing age 30–34 years. Compulsory education (1–9 years) among fathers was compared to tertiary education; the RR was 0.69 (95% CI 0.68–0.71) for adequate FA use. The lower risk of adequate FA use for paternal compulsory education was present in all categories of maternal education. Occupation classes other than "Higher professionals" were associated with decreased risk of adequate FA use, compared with the reference "Lower professionals". RR for adequate FA use was 0.58 (95% CI 0.56–0.60) comparing fathers from "Low/middle-income countries" with fathers born in Norway.

Conclusion: Adequate FA use in the periconceptional period was lower when fathers were younger or older than 30–34 years, had shorter education, had manual or self-employed occupations, or originated from low/middle-income countries. Partners may contribute to increase women's use of periconceptional FA supplementation.

Keywords: Pregnancy, Supplement use, Folic acid, Norway

Background

Folate is necessary in foetal development, and folic acid (FA) supplementation is widely acknowledged to reduce the risk of neural tube defects (NTDs) [1–5]. FA is the synthetic form of the B-vitamin folate, which is essential in the synthesis of DNA, methylation, and DNA repair [6]. Start of FA supplementation prior to conceiving is important in order to reduce the risk of NTDs because the neural tube closes between 21 and 28 days after conception [7].

Randomized clinical trials and non-randomized intervention trials have demonstrated that periconceptional FA use reduces the risk of NTDs [1–3]. Recent studies have reported that FA is associated with protection against other neurodevelopmental disorders and some severe pregnancy complications [8–10]. The protective effect of FA on NTDs has led health authorities

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in several countries, including Norway, to recommend women to take FA supplements before pregnancy and in early pregnancy [11–13].

Many countries in Europe, including Norway, have performed information campaigns to increase the use of periconceptional FA supplementation among women planning pregnancy [14–16]. Presently there is no mandatory folic acid food fortification in Norway [17]. Official Norwegian guidelines from 1998, states that all women planning their pregnancy should use 0.4 mg FA daily from 1 month before pregnancy and throughout the first 2–3 months of pregnancy to reduce the risk of NTDs [12]. However, the proportion of preconception FA supplementation use in Norway is still too low [18] and by 2015 it was 33% [19].

Previous studies have identified maternal factors associated with inadequate FA in the periconceptional period, such as low maternal age, shorter education, single parenthood, unplanned pregnancy, lower parity, smoking, alcohol use, less physical activity, or originating from a foreign country [15, 16, 20–24].

Since couples tend to exhibit concordant health behaviour's for dietary intake, smoking, alcohol consumption, physical activity, and body mass index (BMI) [25–27], a woman's partner may contribute to her use of periconceptional FA supplements. In fact, in an early report from the Norwegian Mother and Child Cohort Study (MoBa), 2000–2003, counting 22,500 women, FA supplements were used more frequently among women with partners with a higher education [22]. However, the study did not assess other paternal factors or combined paternal and maternal factors as to identify women with inadequate FA use.

Taking advantage of the Medical Birth Registry of Norway that to our knowledge is the only national registry with information on periconceptional use of FA supplements [28], we updated parent information with data from national registries to investigate whether paternal factors (age, education, occupation, country of origin) was associated with mothers' intake of recommended FA in pregnancy.

Methods

Data-sources

Maternal FA use before and/or during pregnancy was collected from the Medical Birth Registry of Norway (MBRN) [28]. Paternal and maternal demographic data came from the National Registry (NR). Information on paternal and maternal occupation originated from the Norwegian Labour and Welfare Administration (NAV), and we retrieved paternal and maternal educational data from the Norwegian National Education Database (NUDB) [29].

MBRN is a population-based registry containing information on all births in Norway since 1967 [28]. The registry holds demographic information on the mother and the father, the mother's health before and during pregnancy, including chronic diseases, information on in vitro fertilization (IVF), complications during pregnancy and delivery as well as information on the infant, including birth defects and other perinatal problems. Midwives and physicians attending the deliveries register the data. Since 1967, there has been mandatory reporting of all live and stillbirths from the 16 gestational week to MBRN.

NR contains demographic information on all residents in Norway since 1960, including the date of birth, country of origin, and the dates of immigration, emigration, or death [30]. NR assigns a unique personal identification number to all individuals born or immigrated to Norway, enabling accurate record linkages.

NAV was established in 2006 after governmental reorganization of the Directorate of Labour in Norway (founded in 1945), and has registered information on occupation, health status, and social benefits of all individuals with residence in Norway since 1992. The Norwegian occupational code system is based on the International Standard Classification of Occupations (ISCO), revised version from 1988 [31].

Since 1970, NUDB holds information on all individuals' education history from primary school up to doctoral studies in one database [32]. The classification is based on the Norwegian Standard Classification of Education.

Maternal FA supplement use

We constructed a binary variable for intake of FA supplement use (0.4 mg/day) (regardless of concomitant multivitamin use) registered in the MBRN since December 1998 onwards; adequate FA use (recommended FA supplementation before and during pregnancy), and inadequate FA use (FA supplementation only before pregnancy, or only during pregnancy, or no record of FA use).

MBRN also registers multivitamin use, but our investigation focused on periconceptional FA use as such intake was according to official guidelines.

Paternal characteristics

We used the following paternal variables in our analyses of adequate FA supplementation; paternal age (< 20, 20–24, 25–29, 30–34, 35–39, 40+ years); education (Compulsory (1–9 years), Intermediate (10–12 years), Tertiary (13–19 years)); occupation according to the class scheme of Erikson, Goldthorpe, and Portocarero (I Higher professionals, II Lower professionals, IIIa Higher routine, IIIb Lower routine, IV

Other self-employed workers, V Technicians, VI Skilled, VII Semiskilled and unskilled, VIIb Agricultural, Unclassified) (EGP) [33]; and country of origin according to the classification by World Health Organization, Health statistics and information systems, Estimates for 2000–2012 (Norway, High income countries, Low/middle-income countries) [34].

Covariates

We used directed acyclic graphs (DAGs) and subject-matter knowledge to select a minimally sufficient adjustment set of variables that identify the unconfounded association of paternal characteristics on adequate maternal FA supplementation use [35–37].

The potential confounders of the paternal characteristics and maternal FA use relationship included year of childbirth (continuous), paternal age (< 20, 20–24, 25–29, 30–34, 35–39, 40+ years), education (Compulsory, Intermediate, Tertiary), or country of origin (Norway, High-income countries, Low/middle-income countries).

Furthermore, we included maternal age (< 20, 20–24, 25–29, 30–34, 35–39, 40+ years), maternal education (Compulsory, Intermediate, Tertiary), and maternal country of origin (Norway, High-income countries, Low/middle-income countries) as possible confounders of the associations between paternal age, education, or country of origin, and maternal adequate FA use.

Maternal smoking was not included in the final models because smoking was not considered a confounder of the association of paternal characteristics on maternal FA supplementation use [38].

Study population

During 1999–2010, 716,021 births were registered in MBRN. We excluded births (induced abortions) without information on FA or multivitamin supplementation use (2519) and births without maternal identification number (4091). For multiple births, we included data for the first birth and excluded 12,927 next born individuals. Among the remaining 696,484 births, we excluded 12,699 births without paternal identification number, leaving 683,785 live births and stillbirths for analyses.

Statistical analysis

Associations between paternal characteristics (age, education, occupation, country of origin) and maternal FA use were estimated as relative risks (RRs) with 95% confidence intervals (CIs) by log-binomial regression, using the log-link function in Stata version 15 [39]. The 95% CIs were based on robust variance estimation with the sandwich estimator to correct for the intra-individual correlation in women with more than one pregnancy during the study period [40]. Births with missing data on covariates were excluded from the analyses. *P*-values for overall difference between the categories of paternal characteristics were calculated using likelihood ratio tests. We evaluated and tested the potential effect modification of the association between paternal education and maternal FA use by stratification and likelihood ratio test.

Results

Our study included 683,785 births during 1999–2010. Table 1 presents the characteristics of the parents. The median ages of the fathers and mothers at childbirth were 33 and 30 years, respectively. For about 41% of the births, the mothers were primiparous, and about 2% of the births were conceived after in vitro fertilization (IVF). The majority of the births were of Norwegian-born parents (84% of the fathers and 83% of the mothers). For about 34% of the births, the fathers had tertiary education, and for about 19% of the births, the fathers had compulsory education only. The paternal educational level varied by his country of origin. Fathers originating from low/middle-income countries generally had lower educational level compared to fathers originating from Norway and other high-income countries (not shown). Occupation classified as "Lower professionals," accounted for 22% of all the births. For about 14% of the births, the women smoked daily at the start of pregnancy, about 3% smoked intermittently, and 67% did not smoke. Nearly 17% of the smoking data were missing.

For about 16% of all births in the study population, the mothers were assigned to the category adequate FA supplementation users. However, during 1999 through 2010, the proportion of adequate FA supplementation use increased from 4% at the start of the study period (1999) to 26% in 2010.

Table 2 presents crude and adjusted RRs for adequate maternal periconceptional FA use by paternal variables (determinants). Adjusted analyses showed an inverse "U-shaped" relationship between paternal age and adequate maternal FA supplement use where the smallest RRs were found for paternal age below 20 years (RR 0.35 (95% CI 0.28–0.43)), 20–24 years (RR 0.68 (95% CI 0.66–0.71)), and 40 years and above (RR 0.72 (95% CI 0.71–0.74)) compared to paternal age 30–34 years. Paternal compulsory education was associated with reduced risk of adequate FA use (RR 0.69 (95% CI 0.68–0.71)) compared to paternal tertiary education. All paternal occupation classes were associated with reduced risk of adequate FA use except for "I Higher professionals", when compared to "II Lower professionals", in particular "VII Semiskilled and unskilled" (RR 0.75 (95% CI 0.73–0.76)), and "VIIb Agricultural" (RR 0.73 (95% CI 0.69–0.78)).

Table 1 Paternal and maternal characteristics in 683,785 births in Norway, 1999–2010

	Births			
	Fathers	%	Mothers	%
Number of births	683,785	100.0	683,785	100.0
Age				
< 20	4401	0.6	15,464	2.3
20–24	48,448	7.1	100,016	14.6
25–29	162,671	23.8	223,480	32.7
30–34	235,401	34.4	228,203	33.4
35–39	151,540	22.2	99,727	14.6
40+	81,324	11.9	16,895	2.5
Education				
Compulsory education (1–9 years)	130,953	19.2	125,479	18.4
Intermediate (10–12 years)	302,384	44.2	230,320	33.7
Tertiary education (13–19 years)	229,818	33.6	298,036	43.6
Missing data	20,630	3.0	29,950	4.4
Occupational class [a]				
I Higher professionals	86,635	12.7	50,650	7.4
II Lower professionals	152,781	22.3	122,804	18.0
IIIa Higher routine	77,540	11.3	197,174	28.8
IIIb Lower routine	40,070	5.9	114,795	16.8
IV Other self-employed workers	358	0.1	119	0.0
V Technicians	5550	0.8	1492	0.2
VI Skilled	108,755	15.9	15,961	2.3
VII Semiskilled and unskilled	111,584	16.3	73,994	10.8
VIIb Agricultural	7663	1.1	2720	0.4
Unclassified	52,824	7.7	51,264	7.5
Missing data	40,025	5.9	52,812	7.7
Country of origin [b]				
Norway	574,602	84.0	567,241	83.0
High income countries	33,487	4.9	30,920	4.5
Low/middle-income countries	75,497	11.0	85,597	12.5
Missing data	199	0.0	27	0.0
Marital status				
Unmarried			37,057	5.4
Married/Partnership			634,283	92.8
Divorced			3417	0.5
Missing data			9028	1.3
In vitro *fertilization (IVF)*				
No			669,024	97.8
Yes			14,761	2.2
Birth order				
1			280,178	41.0
2			244,532	35.8
≥3			159,075	23.3
Maternal chronic disease [c]				
No			623,817	91.2
Yes			59,968	8.8
Maternal smoking before pregnancy				
Non-smoker			456,797	66.8
Intermittent			18,518	2.7
Daily			93,662	13.7
Missing data			114,808	16.8
Maternal folic acid use in pregnancy				
No use			371,820	54.4
Only before			8930	1.3
Only during			192,169	28.1
Before and during			110,866	16.2

[a]Categorized according to the class scheme of Erikson, Goldthorpe and Portocarero (EGP) [33]
[b]Categorized according to the classification by World Health Organization, Health statistics and information systems, Estimates for 2000–2012 [34]
[c]Asthma, hypertension, kidney disease, chronic urinary infection, rheumatoid arthritis, heart disease, epilepsy, diabetes mellitus (type I or II), and thyroid disease

Mothers whose children's father originated from low/middle-income countries had also a reduced risk of adequate FA use (RR 0.58 (95% CI 0.56–0.60)) compared to fathers originating from Norway.

Table 3 presents crude and adjusted RRs with 95% CIs of adequate FA use by maternal and paternal education. Adjusted analyses showed that adequate FA use was less likely in births were fathers had compulsory education, regardless of maternal education. The association of paternal compulsory education and recommended FA use was weakened by increasing level of maternal education. However, even when the mother had tertiary education, the association of compulsory paternal education on adequate maternal FA use was significant (RR 0.75 (95% CI 0.73–0.77)), compared to fathers with tertiary education.

Discussion

The present population-based study (683,785 births during 1999–2010) showed that recommended maternal FA use was low among fathers who were young or older at their children's birth, had achieved shorter education, held a manually or self-employed occupation, or originated from low/middle-income countries. Even among mothers who had achieved higher education, recommended periconceptional maternal FA use was low among less educated fathers.

Several studies have investigated the association between maternal socio-demographic, reproductive, and medical characteristics and adherence to recommended intake of periconceptional FA. A common feature among mothers is that young age, low educational level, low

Table 2 Relative risks (RRs) with 95% confidence intervals (95% CIs) of adequate maternal periconceptional folic acid supplement use (before and during pregnancy) by paternal characteristics, in 683,785 births, Norway, 1999–2010

	Folic acid supplementation use		Unadjusted		Adjusted for paternal factors [a b]		Further adjusted for maternal factors [a c]	
Characteristics	Yes	No	RR*	95% CI	RR*	95% CI	RR*	95% CI
Paternal age (years)								
< 20	86	4315	0.11	0.09–0.13	0.10	0.08–0.13	0.35	0.28–0.43
20–24	3325	45,123	0.37	0.36–0.39	0.37	0.36–0.38	0.68	0.66–0.71
25–29	22,886	139,785	0.76	0.75–0.78	0.77	0.76–0.79	0.94	0.93–0.96
30–34	43,348	192,053	1.00	Reference	1.00	Reference	1.00	Reference
35–39	28,488	123,052	1.02	1.01–1.03	0.97	0.96–0.99	0.90	0.89–0.91
40+	12,733	68,591	0.85	0.83–0.87	0.80	0.78–0.81	0.72	0.71–0.74
Paternal education								
Compulsory (1–10 years)	11,694	119,259	0.40	0.39–0.40	0.52	0.51–0.53	0.69	0.68–0.71
Intermediate (11–13 years)	45,411	256,973	0.67	0.66–0.67	0.75	0.74–0.76	0.87	0.85–0.88
Tertiary (14–20 years)	51,894	177,924	1.00	Reference	1.00	Reference	1.00	Reference
Missing data	1867	18,763						
Paternal occupational class [d]								
I Higher professionals	19,957	66,678	1.05	1.04–1.07	1.05	1.03–1.06		
II Lower professionals	33,366	119,415	1.00	Reference	1.00	Reference		
IIIa Higher routine	12,292	65,248	0.73	0.71–0.74	0.89	0.88–0.91		
IIIb Lower routine	5352	34,718	0.61	0.59–0.63	0.85	0.83–0.87		
IV Other self-employed workers	60	298	0.77	0.61–0.97	0.83	0.65–1.05		
V Technicians	836	4714	0.69	0.65–0.74	0.89	0.84–0.95		
VI Skilled	15,174	93,581	0.64	0.63–0.65	0.84	0.83–0.86		
VII Semiskilled and unskilled	11,643	99,941	0.48	0.47–0.49	0.75	0.73–0.76		
VIIb Agricultural	908	6755	0.54	0.51–0.58	0.73	0.69–0.78		
Unclassified	7575	45,249	0.66	0.64–0.67	0.96	0.94–0.99		
Missing data	3703	36,322						
Paternal country of origin [e]								
Norway	99,339	475,263	1.00	Reference	1.00	Reference	1.00	Reference
High income countries	6535	26,952	1.13	1.10–1.16	1.06	1.04–1.09	1.06	1.03–1.08
Low-middle-income countries	4975	70,522	0.38	0.37–0.39	0.35	0.34–0.36	0.58	0.56–0.60
Missing data	17	182						

[a]All RRs for adequate folic acid supplementation adjusted for year of childbirth (continuous)
[b]RRs by paternal age, no other adjustment for paternal factors; RRs by paternal education adjusted for paternal age (< 20, 20–24, 25–29, 30–34, 35–39, 40+), paternal country of origin (Norway, high-income countries, low/middle-income countries); RRs by paternal occupation adjusted for paternal age, fathers country of origin, fathers education (compulsory, intermediate, tertiary); and RR by paternal origin of country, no other adjustment for paternal factors
[c]RRs by paternal age, further adjusted for maternal age (< 20, 20–24, 25–29, 30–34, 35–39, 40+); RRs by paternal education, further adjusted for maternal education (compulsory, intermediate, tertiary); RRs by paternal occupation, no further adjustment for maternal factors; RRs by paternal country of origin adjusted for maternal country of origin (Norway, High-income countries, Low/middle-income countries)
[d]Categorized according to the class scheme of Erikson, Goldthorpe and Portocarero (EGP) [33]
[e]Categorized according to the classification by World Health Organization, Health statistics and information systems, Estimates for 2000–2012 [34]
*p-value for difference between categories of paternal characteristics was < 0.001 using likelihood ratio test

socioeconomic status, unplanned pregnancy, higher parity, smoking, single marital status, and non-western birthplace is the most important determinants for inadequate FA supplementation use [20–23]. Furthermore, maternal chronic diseases and IVF were positively associated with adequate periconceptional FA supplementation use [22, 38].

In Denmark, a cross-sectional study consisting of 22,000 pregnant women (primiparious and multiparous) showed that only 14% of the women used FA as recommended and compliance was positively associated with being primiparous, older than 25 years and non-smoker [21]. Similarly, for about 16% of the births in our study, the mothers had followed the

Table 3 Relative risks (RRs) with 95% confidence intervals (95% CI) of adequate maternal periconceptional folic acid supplement use (before and during pregnancy) by combining maternal and paternal education in 683,785 pregnancies, Norway, 1999–2010

		Adequate folic acid use		Unadjusted		Adjusted [a]	
Maternal education	Paternal education	Yes	No	RR*	95% CI	RR*	95% CI
Compulsory education	Compulsory education	2557	48,326	0.46	0.43–0.50	0.53	0.50–0.57
	Intermediate education	4758	52,693	0.77	0.72–0.81	0.76	0.72–0.81
	Tertiary education	1292	10,651	1.00	Reference	1.00	Reference
	Missing information	208	4994				
Intermediate education	Compulsory education	5079	43,708	0.60	0.57–0.62	0.66	0.64–0.68
	Intermediate education	18,055	116,378	0.77	0.75–0.79	0.81	0.79–0.83
	Tertiary education	7477	35,269	1.00	Reference	1.00	Reference
	Missing information	426	3928				
Tertiary education	Compulsory education	3805	17,985	0.70	0.67–0.72	0.75	0.73–0.77
	Intermediate education	22,150	80,373	0.86	0.85–0.87	0.88	0.87–0.89
	Tertiary education	42,541	126,896	1.00	Reference	1.00	Reference
	Missing information	747	3539				
Missing data (maternal education)		28,179	1771				

[a]Adjusted for paternal age (< 20, 20–24, 25–29, 30–34, 35–39, 40+), year of childbirth (1999–2010 (continuous)), paternal country of origin (Norway, high income countries, low/middle income countries), stratified by maternal education

*p values for interaction between maternal and paternal education were calculated by likelihood-ratio tests (unadjusted p value < 0.001; adjusted p value < 0.001)

national guidelines of FA use in the study period (1999–2010).

In Norway, a publication from the Norwegian Mother and Child Cohort Study (MoBa), comprising 27% of the births registered in MBRN during 2000–2003, showed similar results to ours [22]. They found a positive association between paternal education and recommended periconceptional FA use. In pregnancies with fathers having university or college education the adjusted relative risk (RR) of periconceptional maternal FA use was 1.4 (95% CI 1.1–1.8) compared to pregnancies with fathers with primary education. However, the association was weaker than for maternal education. When paternal *tertiary* education was compared to paternal *compulsory* education (reference) in our analyses, we found a similar result for adjusted RR of 1.45 (95% CI 1.42–1.48).

Couples who live together share the same environment, social network, financial resources, and to some extent, the same health risk; beneficial or negative to health outcomes depending on the health behaviour of the spouses [25, 27]. Furthermore, a Dutch study of 40,000 individuals aged 25–74 years showed that women seems more affected by their partner's educational level than men are with regard to healthy behaviour [41].

In accordance with our findings, a cross-sectional household survey conducted in Pakistan (comprising 6266 women), showed that maternal intake of iron and FA supplements was positively associated with the educational status of the mothers' husband [42].

The association of ethnic background and maternal periconceptional FA use have been studied in Norway and other European countries (Netherlands, Belgium, Ireland and the United Kingdom) [23, 43–46]. These studies show that supplement use is less common among most ethnic minority groups than among the comparison groups. We have similar findings in our study, showing a lower risk of adequate maternal FA use among fathers originating from low/middle-income countries.

The strengths of our study included use of comprehensive data from population-based registries in Norway that assures generalizability of our results, and registration of individual-level information on periconceptional FA intake for all births in Norway since 1999 (except for terminated pregnancies).

Our study had some limitations. Maternal FA intake could have been misclassified; in the beginning of the study period, FA users were underreported to the MBRN [47]. Our results may therefore be somewhat weaker than the true associations. Furthermore, we could not adjust for pregnancy planning, maternal physical activity or maternal use of alcohol [16, 18, 20–24], as these potential confounders/covariates were not available in our dataset. However, a recent longitudinal study during 2014 on men's pregnancy planning comprising about 800 participants in Sweden, showed that 81% of the pregnancies were planned and the level of paternal education was positively associated with pregnancy planning [48]. Moreover, data from 22,500 mothers in the MoBa study with deliveries recorded in 2000–2003 showed that 78% of the mothers had planned their pregnancy [22]. However, MoBA is not entirely representative of the

total pregnant population in Norway, since the participants are somewhat better educated, slightly older at delivery, and with a lower percentage of smokers than the overall pregnant population.

Information about fathers was not available in 12,699 births (2% of all births in the study population) and were excluded from the study population. They represent births with fathers unreported by the pregnant woman or fathers without identification number from the NR. Among the excluded births (missing father information), 10% of the mothers had adequate periconceptional FA supplementation (16% in the study population) with an RR of 0.63 (95% CI 0.60–0.66) for adequate maternal FA use comparing births with unregisterd fathers with births having registered fathers.

Adjusting for maternal confounders (maternal age, education, or country of origin) in our analysis reduced the strength of the associations between paternal determinants (age, education or country of origin) and adequate maternal periconceptional FA use. This suggest that paternal factors are important, but targeting maternal demographic and socioeconomic conditions and other factors related to low use is still important. However, our findings have implications for public health practice. Recent research on men's birth intentions has shown that 63% of pregnancies were intended (wanted) by the father [49]. Further, our study demonstrates the importance of the partner's impact on maternal reproductive health and family planning through shared decision-making.

Conclusions

In conclusion, our study supports the importance of father's prenatal role in their children's health. In order to improve maternal periconceptional FA supplementation use, information and knowledge about the importance of FA's preventive potential needs to be directed to both men and women. Furthermore, our findings show that women having partners originating from low/middle-income countries, partners at age < 30 and > 34 years, having compulsory education only, and having occupations other than "higher professional", compared to "lower professionals", are particularly susceptible to low periconceptional FA use. Therefore, campaigns for improved FA supplementation use should focus particularly on these groups.

Abbreviations

BMI: Body mass index; DAGs: Directed acyclic graphs; IVF: In vitro fertilization; MBRN: Medical Birth Registry of Norway; MoBa: Norwegian Mother and Child Cohort Study; NAV: Norwegian Labour and Welfare Administration; NTDs: Neural tube defects; NUDB: Norwegian National Education Database

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Availability of data and materials

Principal investigator, prof. Nina Øyen, sought and obtained all permissions to access data from the Medical Birth Registry of Norway (MBRN), the National Registry (NR), the Norwegian Labour and Welfare Administration, and the Norwegian National Education Database (NUDB). The datasets analysed during the current study are not freely available due to national regulations.

Authors' contributions

JHM conceived the study, performed all analyses, and led the writing. NØ and TB conceived the study and participated in manuscript preparation and writing. RMN participated in the analyses and writing. TF participated in the statistical analyses. ST participated in manuscript preparation. All authors helped to conceptualize ideas, interpret findings, and review drafts of the manuscript. All authors read and approved the final manuscript. No conflicts of interest are declared.

Ethics approval and consent to participate

The study was approved by the Regional Committee for Medical and Health Research Ethics of Western Norway (REK ref. number 2010/3310).

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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References

1. MRC. Prevention of neural tube defects: results of the Medical Research Council vitamin study. MRC vitamin study research group. Lancet. 1991; 338(8760):131–7.
2. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med. 1992;327(26): 1832–5.
3. Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H, Mulinare J, Zhao P, Wong LY, Gindler J, et al. Prevention of neural-tube defects with folic acid in China. China-U.S. collaborative project for neural tube defect prevention. N Engl J Med. 1999;341(20):1485–90.
4. Scientific Advisory Committee on Nutrition. Folate and Disease Prevention. TSO (The Stationery Office), vol. 2006. London: Scientific Advisory Committee on Nutrition.
5. De-Regil LM, Pena-Rosas JP, Fernandez-Gaxiola AC, Rayco-Solon P. Effects and safety of periconceptional oral folate supplementation for preventing birth defects. Cochrane Database Syst Rev. 2015;12:CD007950.
6. Wagner C. Biochemical role of folate in cellular metabolism (reprinted from folate and health disease, pgs 23-42, 1995). Clin Res Regul Aff. 2001;18(3): 161–80.
7. Sadler TWTW, Langman J. Langman's medical embryology, 11th ed. / T.W. Sadler; original illustrations by Jill Leland; computer illustrations by Susan L. Sadler-Redmond; scanning electron micrographs by Kathy Tosney; ultrasound images by Nancy Chescheir and Hytham Imseis. United States: Philadelphia: Wolters Kluwer Lippincott Williams & Wilkins; 2010.

8. Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, Lie KK, Lipkin WI, Magnus P, Reichborn-Kjennerud T, et al. Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. JAMA. 2013;309(6):570–7.
9. Roth C, Magnus P, Schjolberg S, Stoltenberg C, Suren P, McKeague IW, Davey Smith G, Reichborn-Kjennerud T, Susser E. Folic acid supplements in pregnancy and severe language delay in children. JAMA. 2011;306(14): 1566–73.
10. Nilsen RM, Vollset SE, Rasmussen SA, Ueland PM, Daltveit AK. Folic acid and multivitamin supplement use and risk of placental abruption: a population-based registry study. Am J Epidemiol. 2008;167(7):867–74.
11. Centers for disease control. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR. 1992;41(RR-14):1–7.
12. National Council on Nutrition and Physical Activity. Recommendations and means to increase folate intake among women in fertile age [Anbefalinger og virkemidler for økt folatinntak blant kvinner i fertil alder]. Report no 1/ 1998. Oslo: National Council on Nutrition and Physical Activity [Statens Ernæringsråd]; 1998.
13. Nordic Nutrition Recommendations 2012. In: Integrating nutrition and physical activity. Edited by 2014 NCoM. 5th ed. Copenhagen; 2014. http://dx.doi.org/10.6027/Nord2014-002.
14. Lumley J, Watson L, Watson M, Bower C. Periconceptional supplementation with folate and/or multivitamins for preventing neural tube defects. Cochrane Database Syst Rev. 2001;(3):CD001056. https://doi.org/10.1002/14651858.CD001056.
15. Daltveit AK, Vollset SE, Lande B, Oien H. Changes in knowledge and attitudes of folate, and use of dietary supplements among women of reproductive age in Norway 1998-2000. Scand J Public Health. 2004;32(4): 264–71.
16. Bower C, Miller M, Payne J, Serna P. Promotion of folate for the prevention of neural tube defects: who benefits? Paediatr Perinat Epidemiol. 2005;19(6): 435–44.
17. Ministry of Health and Care Services. [National action plan for a better diet (2017–2021)] Nasjonal handlingsplan for bedre kosthold (2017–2021). In: Edited by omsorgsdepartementet MoHaCSH-o: Departementenes sikkerhets- og serviceorganisasjon; 2017. p. 57.
18. Nilsen RM, Mastroiacovo P, Gunnes N, Alsaker ER, Bjorke-Monsen AL, Eussen SJ, Haugen M, Johannessen A, Meltzer HM, Stoltenberg C, et al. Folic acid supplementation and interpregnancy interval. Paediatr Perinat Epidemiol. 2014;28(3):270–4.
19. Medical birth registry and abortion registry - statistical database [Medisinsk fødselsregister og abortregisteret - statistikkbanker] [http://statistikk.fhi.no/mfr/]. Accessed 23 May 2018.
20. Cueto HT, Riis AH, Hatch EE, Wise LA, Rothman KJ, Mikkelsen EM. Predictors of preconceptional folic acid or multivitamin supplement use: a cross-sectional study of Danish pregnancy planners. Clin Epidemiol. 2012;4:259–65.
21. Knudsen VK, Orozova-Bekkevold I, Rasmussen LB, Mikkelsen TB, Michaelsen KF, Olsen SF. Low compliance with recommendations on folic acid use in relation to pregnancy: is there a need for fortification? Public Health Nutr. 2004;7(7):843–50.
22. Nilsen RM, Vollset SE, Gjessing HK, Magnus P, Meltzer HM, Haugen M, Ueland PM. Patterns and predictors of folic acid supplement use among pregnant women: the Norwegian mother and child cohort study. Am J Clin Nutr. 2006;84(5):1134–41.
23. Timmermans S, Jaddoe VW, Mackenbach JP, Hofman A, Steegers-Theunissen RP, Steegers EA. Determinants of folic acid use in early pregnancy in a multi-ethnic urban population in the Netherlands: the generation R study. Prev Med. 2008;47(4):427–32.
24. Braekke K, Staff AC. Periconceptional use of folic acid supplements in Oslo. Acta Obstet Gynecol Scand. 2003;82(7):620–7.
25. Jackson SE, Steptoe A, Wardle J. The influence of partner's behavior on health behavior change: the English longitudinal study of ageing. JAMA Intern Med. 2015;175(3):385–92.
26. Desrosiers A, Thompson A, Divney A, Magriples U, Kershaw T. Romantic partner influences on prenatal and postnatal substance use in young couples. J Public Health (Oxf). 2016;38(2):300–7.
27. Cornelius T, Desrosiers A, Kershaw T. Spread of health behaviors in young couples: how relationship power shapes relational influence. Soc Sci Med. 2016;165:46–55.
28. Irgens LM. The medical birth registry of Norway. Epidemiological research and surveillance throughout 30 years. Acta Obstet Gynecol Scand. 2000; 79(6):435–9.
29. Kinge JM, Steingrimsdottir OA, Moe JO, Skirbekk V, Naess O, Strand BH. Educational differences in life expectancy over five decades among the oldest old in Norway. Age Ageing. 2015;
30. Hammer H. The central population registry in medical research. *Tidsskr Nor Laegeforening*. 2002;122(26):2550.
31. STYRK-08: [Standard Classification of Occupations (STYRK-08)] Standard for yrkesklassifisering (STYRK-08). In: Notater. Oslo–Kongsvinger: Statistics Norway; 2011. ISSN 1891-5906. https://www.ssb.no/en/.
32. Vangen T: Nasjonal utdanningsdatabase NUDB. 2007.
33. Erikson R, Goldthorpe JH. The constant flux: a study of class mobility in industrial societies: Oxford University Press, USA; 1992.
34. Global Health Estimates 2015: Disease burden by Cause, Age, Sex, by Country and by Region, 2000-2015. Geneva: World Health Organization; 2016.
35. Shrier I, Platt RW. Reducing bias through directed acyclic graphs. BMC Med Res Methodol. 2008;8(1):70.
36. Textor J, Hardt J, Knuppel S. DAGitty: a graphical tool for analyzing causal diagrams. Epidemiology. 2011;22(5):745.
37. Pearl J. An introduction to causal inference. Int J Biostat. 2010;6(2):Article 7.
38. Nilsen RM, Leoncini E, Gastaldi P, Allegri V, Agostino R, Faravelli F, Ferrazzoli F, Finale E, Ghirri P, Scarano G, et al. Prevalence and determinants of preconception folic acid use: an Italian multicenter survey. Ital J Pediatr. 2016;42(1):1–10.
39. StataCorp. Stata Statistical Software: Release 15. College Station. TX: StataCorp LLC; 2017.
40. Cameron AC, Miller DL. A Practitioner's guide to cluster-robust inference. J Hum Resour. 2015;50(2):317–73.
41. Monden CW, van Lenthe F, de Graaf ND, Kraaykamp G. Partner's and own education: does who you live with matter for self-assessed health, smoking and excessive alcohol consumption? Soc Sci Med. 2003;57(10):1901–12.
42. Nisar YB, Dibley MJ, Mir AM. Factors associated with non-use of antenatal iron and folic acid supplements among Pakistani women: a cross sectional household survey. BMC Pregnancy Childbirth. 2014;14:305.
43. Kinnunen TI, Sletner L, Sommer C, Post MC, Jenum AK. Ethnic differences in folic acid supplement use in a population-based cohort of pregnant women in Norway. BMC Pregnancy Childbirth. 2017;17(1):143.
44. Baraka MA, Steurbaut S, Leemans L, Foulon W, Laubach M, Coomans D, Jansen E, Dupont AG. Determinants of folic acid use in a multi-ethnic population of pregnant women: a cross-sectional study. J Perinat Med. 2011;39(6):685–92.
45. McGuire M, Cleary B, Sahm L, Murphy DJ. Prevalence and predictors of periconceptional folic acid uptake–prospective cohort study in an Irish urban obstetric population. Hum Reprod. 2010;25(2):535–43.
46. Brough L, Rees GA, Crawford MA, Dorman EK. Social and ethnic differences in folic acid use preconception and during early pregnancy in the UK: effect on maternal folate status. J Hum Nutr Diet. 2009;22(2):100–7.
47. Nilsen RM, Vollset SE, Gjessing HK, Skjaerven R, Melve KK, Schreuder P, Alsaker ER, Haug K, Daltveit AK, Magnus P. Self-selection and bias in a large prospective pregnancy cohort in Norway. Paediatr Perinat Epidemiol. 2009; 23(6):597–608.
48. Bodin M, Kall L, Tyden T, Stern J, Drevin J, Larsson M. Exploring men's pregnancy-planning behaviour and fertility knowledge:a survey among fathers in Sweden. Ups J Med Sci. 2017;122(2):127–35.
49. Lindberg LD, Kost K. Exploring U.S. men's birth intentions. Matern Child Health J. 2014;18(3):625–33.

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