High-Dose Folic Acid Use during Pregnancy and Cancer Risk in Mothers and Children With Focus on Maternal Epilepsy

Håkon Magne Vegrim

Thesis for the degree of Philosophiae Doctor (PhD)
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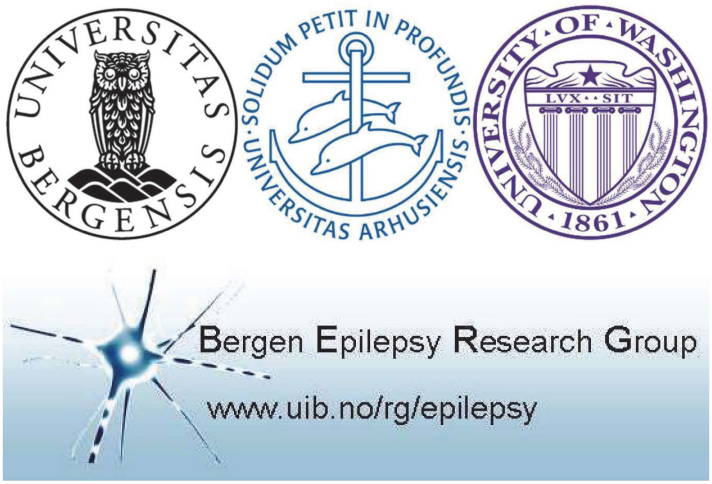
Scientific Collaborations and Environments

This PhD was written at the Department of Clinical Medicine at the University of Bergen as a member of Bergen Epilepsy Research Group (BERG).

BERG is led by Professor and consultant neurologist Marte-Helene Bjørk and primarily focuses on epilepsy-related research and health issues, including epidemiological research, clinical studies, non-epilepsy related register-based research involving headache, myasthenia gravis and multiple sclerosis. They host regular meetings and presentations and have extensive national and international collaborations.

One of BERGs international projects is the SCAN-AED study, a collaborative effort between medical doctors, statisticians, and epidemiologists from all Nordic countries. The SCAN-AED study includes researchers from University of Bergen, Aarhus University, University of Iceland, Institute for Knowledge Brokers in Finland, and Karolinska Institute in Sweden. The goal of this project is to study health issues during pregnancy for individuals treated with antiseizure medications.

During this PhD, I have been a visiting scholar for six months at The National Centre for Register-Based Research at Aarhus University in Denmark, and for three months at the Institute for Health Metrics and Evaluation at the University of Washington in Seattle, USA.



List of Publications

Paper I: Vegrim HM, Dreier JW, Alvestad S, Gilhus NE, Gissler M, Igland J, Leinonen MK, Tomson T, Sun Y, Zoega H, Christensen J, Bjørk MH. "*Cancer Risk in Children of Mothers With Epilepsy and High-Dose Folic Acid Use During Pregnancy*". JAMA Neurology. 2022 Sep 26. DOI:10.1111/epi.16395

Paper II: Vegrim HM, Dreier JW, Igland J, Alvestad S, Gilhus NE, Gissler M, Leinonen MK, Tomson T, Zoega H, Christensen J, Bjørk MH. "*High-Dose Folic Acid Use and Cancer Risk in People Who Have Given Birth: A Register-based Cohort Study*". Submitted 18.12.23 (under review).

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Other related publications as an author not included in my PhD thesis

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Bjørk MH, Vegrim HM, Alvestad S, Bjørke-Monsen AL, Riedel BMI, Gilhus NE, Husebye ESN. "*Pregnancy, Folic Acid, and Antiseizure Medication*". Clinical Epileptology. 2023 Jul 3. DOI: 10.1007/s10309-023-00602-3

Bjørk MH, Tomson Torbjörn, Dreier JW, Alvestad S, Gilhus NE, Gissler M, Igland J, Leinonen MK, Sun Y, Vegrim HM, Zoega H, Christensen J. *"High-Dose Folic Acid and Cancer Risk; Unjustified Concerns by von Wrede and Colleagues Regarding Our Paper – Commentary".* Epilepsia. 2023 Jul 28. DOI: 10.1111/epi.17717.

Abbreviations

aHR	Adjusted hazard ratio
ASM	Antiseizure medication
BERG	Bergen Epilepsy Research Group
BMI	Body Mass Index
CI	95% Confidence interval
DNA	Deoxyribonucleic acid
DAG	Directed acyclic graph
DHF	Dihydrofolate
DHFR	Dihydrofolate reductase
DNA	Deoxyribonucleic acid
DrMedSci	Doctor of Medical Science
DPA	Data Protection Agreement
EEG	Electroencephalography
EURAP	European and International Registry of Antiepileptic Drugs and Pregnancy
GDPR	General data protection regulation
HR	Hazard ratio
ICD	International Classification of Diseases
ILAE	International League Against Epilepsy
IQ	Intelligence quotient
IQR	Interquartile range
JAMA	Journal of the American Medical Association
LMP	Last menstrual period
MD	Medical doctor
MICE	Multivariate imputation by chained equations
MTHF	5-methyltetrahydrofolate
MTHFR	5-methyltetrahydrofolate reductase

MSPH	Master of Science in Public Health
n	Number
NEAD	The Neurodevelopmental Effects of Antiepileptic Drugs Group
p	P-value
PhD	Philosophiae Doctor
RCT	Randomized controlled study
SCAN-AED	Scandinavian multi-registry study of antiepileptic drug teratogenicity
SAM	S-adenosylmethionine
SD	Standard deviation
THF	Tetrahydrofolate
UMFA	Unmetabolized folic acid
US	United states
USA	United States of America
WHO	World Health Organization

English Abstract

Background: Pregnant women taking antiseizure medication (ASM) are recommended higher daily folic acid doses (≥1 mg) than the general population (0.4-0.8 mg) to protect the developing fetus. Folic acid, the synthetic form of folate, is essential for DNA synthesis and repair. Experimental studies have linked exposure to high folic acid doses with increased cancer risk. Clinical research results have been inconclusive. Studies assessing the safety of high-dose folic acid in mothers and children regarding cancer risk have been lacking. Current folic acid guidelines for women on ASM vary internationally.

Aims: Our aims were to investigate the relationship between use of high-dose folic acid during pregnancy and the risk of cancer in both children and their mothers and to provide new evidence on the risks associated with high-dose folic acid use during pregnancy and to describe the current use during pregnancy in mothers with epilepsy.

Materials and methods: Our data source was the SCAN-AED study, a multinational study established in 2017 utilizing key nationwide and mandatory health registers from the Nordic countries. For this research project, we used data from the countries in which folic acid in doses ≥1 mg is a prescription-based supplement: Denmark (1997–2017), Norway (2005–2017), and Sweden (2006–2017). Women were identified in the medical birth registers using unique personal identification numbers, with information linked across the national cancer-, patient-, and prescription registers, as well as leveraging data from the national statistical agencies. Exposure was defined as filled prescription(s) for high-dose folic acid in relation to pregnancy. Outcome was defined as the first occurrence of a malignant cancer, defined in accordance with international cancer classifications. Cancer was defined as malignant based on cancer behavior and assigned with a unique value for each cancer case. When assessing the risk of cancer associated with high-dose folic acid use, we used a Cox proportional hazards model, adjusting for confounders.

Results: We identified 27,784 children born to mothers with epilepsy (0.8% of the total population) in which 21.4% were prenatally exposed to high-dose folic acid. We

identified 64,490 women (4.4% of all women included in study II) who had given birth and used high-dose folic acid during their first pregnancy and/or later during follow-up. Prenatal exposure to high-dose folic acid was associated with an increased risk of cancer in children born to mothers with epilepsy compared to unexposed children to mothers with epilepsy (adjusted hazard ratio (aHR) of 2.7, 95% confidence interval (CI) 1.2–6.3). This risk was not observed in children born to mothers in the general population (aHR 1.1, 95% CI 0.9–1.4), compared to those exposed to those unexposed to high-dose folic acid. In women who had given birth, high-dose folic acid use during pregnancy or later was associated with an increased overall cancer risk compared to unexposed women (aHR of 1.2, 95% CI 1.1–1.2). In the exposed women, we observed a robust association between use of high-dose folic acid and increased risk of non-Hodgkin's lymphoma (aHR 2.0, 95% CI 1.3–2.9). We observed that the use of high-dose folic acid varied widely among pregnant women with epilepsy. It was most common to supplement women with ASM-treated epilepsy in Sweden (74.2%) regardless of type of ASM, and less common in Norway (41.4%) and Denmark (34.4%). More than 40% of the pregnancies in women with ASM-treated epilepsy supplemented with high-dose folic acid, did not fill the first prescription for high-dose folic acid until after the pregnancy had started.

Conclusion and implications: Prescriptions for high-dose folic acid during pregnancy were associated with an increased risk of cancer in children born to mothers with epilepsy compared to children born to mothers with epilepsy who were not supplemented with high-dose folic acid during pregnancy. We also observed an increased risk of cancer in women in the general population who had been exposed to high-dose folic acid during pregnancy compared to unexposed women. The use of high-dose folic acid during pregnancy among women with ASM-treated epilepsy varied across the included countries and was inconsistent. Our findings should be considered when assessing the risks and benefits of folic acid supplements for women, and when discussing the optimal folic acid dosage for high-risk pregnancies in future guidelines. Future studies should explore potential cancer-promoting mechanisms, including the influence of relevant medications such as ASMs and genetic factors.

Norsk abstrakt

Bakgrunn: Gravide kvinner som tar antianfallsmedikasjon (ASM) anbefales høyere daglige doser av folsyre (≥1 mg) enn den generelle befolkningen (0.4–0.8 mg) for å beskytte fosteret. Folsyre, den syntetiske formen av folat, er essensiell for DNA-syntese og reparasjon. Eksperimentelle studier har koblet høydose folsyre med økt kreftfare. Kliniske forskning har vist blandede resultater. Studier som har sett på sikkerheten ved høydose folsyre hos mødre og barn når det gjelder kreftfare har manglet. Gjeldende retningslinjer for folsyrebruk for kvinner som bruker ASM varierer internasjonalt.

Mål: Målene våre var å undersøke sammenhengen mellom bruk av høydose folsyre under graviditet og risiko for kreft hos barn og deres mødre og dermed gi ny kunnskap om risiko ved bruk av høydose folsyre under svangerskapet samt beskrive bruken av høydose folsyre under svangerskapet hos mødre med epilepsi.

Materialer og metoder: Datakilden for dette prosjektet var SCAN-AED-studien, en internasjonal studie etablert i 2017 som inkluderer data fra en rekke landsdekkende og obligatoriske helseregistre fra de nordiske landene. For dette forskningsprosjektet brukte vi data fra de landene hvor folsyre med doser ≥1 mg er reseptbelagte legemidler: Danmark (1997–2017), Norge (2005–2017) og Sverige (2006–2017). Kvinnene ble identifisert i de medisinske fødselsregistrene ved hjelp av unike personlige identifikasjonsnumre, som videre ble brukt til å koble informasjon på tvers av de nasjonale kreft-, pasient- og reseptregistrene, samt data fra de nasjonale statistiske byråene. Eksponering for folsyre ble definert som uthentede resepter for høydose folsyre i forbindelse med graviditet. Utfallet kreft ble definert som første forekomst av ondartet kreft, definert i samsvar med internasjonale kreftklassifikasjoner basert på kreftatferd angitt for hvert krefttilfelle i kreftregistrene. Vi benyttet en Cox proporsjonal hazards-model justert for konfunderende faktorer for å regne ut risikoestimater for kreft etter høydose folsyrebruk sammenlignet med personer som ikke brukte folsyre i svangerskapet eller var eksponerte for folsyre i fosterlivet.

Resultater: Vi identifiserte 27,784 barn født av mødre med epilepsi (0,8 % av alle barn inkludert i studie I), hvorav 21,4 % barn var eksponert for høydose folsyre før

fødsel. Vi identifiserte 64,490 kvinner (4,4 % av alle kvinner inkludert i studie II) som hadde født barn og som hadde brukt høydose folsyre i løpet av første svangerskap og/eller senere i oppfølgingsperioden. Barn eksponert for høydose folsyre før fødsel og med mor med epilepsi hadde en justert hazard ratio (aHR) på 2.7 (95 % konfidensintervall (CI) 1.2–6.3) for å utvikle kreft sammenlignet med barn født av mødre med epilepsi som ikke var eksponert for høydose folsyre før fødsel. Vi observerte ingen økt risiko hos barn født av mødre i den generelle befolkningen ved å sammenligne dem som var eksponert for høydose folsyre med barn ueksponert for høydose folsyre (aHR 1.1, 95 % CI 0.9–1.4). Blant kvinner inkludert i de skandinaviske fødselsregistrene, var bruk av høydose folsyre assosiert med en økt risiko for å utvikle kreft sammenlignet med kvinner som også hadde født barn, men som ikke hadde brukt høydose folsyre (aHR 1.2, 95 % CI 1.1–1.2). Vi observerte en sterk assosiasjon mellom bruk av høydose folsyre og risikoen for non-Hodgkins lymfom (aHR 2.0, 95 % CI 1.3–2.9). Vi observerte at bruken av høydose folsyre varierte betydelig mellom de inkluderte landene. Bruk var mest vanlig ved ASM-behandlet epilepsi i Sverige (74,2 %) uavhengig av type ASM som ble brukt, og mindre vanlig i Norge (41,4 %) og Danmark (34,4 %). I mer enn 40 % av svangerskapene hos kvinner med ASM-behandlet epilepsi som brukte høydose folsyre, hentet ikke kvinnen den første resepten før etter graviditetsstart.

Konklusjon og konsekvenser: Bruk av høydose folsyre under graviditet var assosiert med en økt risiko for kreft hos barn født av mødre med epilepsi sammenlignet med barn født av mødre med epilepsi som ikke brukte høydose folsyre under graviditet. Vi observerte også en økt kreftrisiko hos kvinner i den generelle befolkningen som hadde brukt høydose folsyre i forbindelse med svangerskap sammenlignet med kvinner som ikke hadde brukt høydose folsyre. Bruken av høydose folsyre under graviditet blant kvinner med ASM-behandlet epilepsi varierte mellom landene, og var til dels uavhengig av type og dose av ASM. Våre funn bør tas med i betraktning når man vurderer risiko og fordeler ved folsyretilskudd for kvinner og når man diskuterer den optimale dosen av folsyre i risikosvangerskap i nye retningslinjer. Fremtidige studier bør utforske mulige årsaksmekanismer for folat-indusert kreftrisiko, inkludert påvirkningen av relevante medikamenter som ASM og genetiske faktorer.

Outline of Thesis

This dissertation consists of eight chapters.

Chapter one gives a brief introduction to the topic and the basis for the scientific work that has been done. Chapter two describes the aims of each of the studies conducted for this PhD. Chapter three provides further background information about epilepsy as a disorder, associated treatments, challenges related to pregnancy among those with epilepsy and/or treated with antiseizure medication, folic acid supplementation, and risk of cancer due to epilepsy, antiseizure medication and folic acid. Chapter four outlines the materials used and our methodological approach. Chapter five presents the summary of the findings from the three included studies for this PhD. Chapter six discusses the results in relation to existing literature, the clinical implications of our findings, and the impact on clinical guidelines and treatment. Chapter seven describes the relevance of our work and suggestions for future research. Chapter eight concludes on the findings included in my PhD thesis.

Chapter 1

Introduction

Epilepsy is a complex and multifaceted neurological disorder affecting around 1% of the global population.[1] It is characterized by recurrent and unpredictable seizures resulting from abnormal and synchronized electrical activity within the brain.[2,3] About 15 million women of childbearing age are diagnosed with epilepsy worldwide.[4,5] This poses a challenge both for the patients and clinicians due to the potential severe health outcomes for both the mother and child, including maternal mortality and congenital anomalies.[6-8] Besides maintaining seizure control during pregnancy, it is crucial to ensure a minimal risk of harm to the child due to epileptic seizures or prenatal medication exposures.

Most clinical guidelines recommend supplementing women with high doses of folic acid (up to 5 mg daily) immediately before and during pregnancy if treated with ASM, to counteract teratogenic risks associated with ASM exposure. This dose is approximately 13 times higher than the folic acid dose recommended for women in the general population.[9] The recommendation for high-dose folic acid supplementation for pregnant women treated with ASM is based on studies reporting a protective effect of high-dose folic acid use against neural tube defects in newborns in high-risk pregnancies in the general population.[10,11] These findings have thus been extrapolated to all groups that are at a higher risk of giving birth to a child with a congenital anomaly. Further, some ASMs have been shown to reduce blood levels of folate.[12] However, due to the lack of evidence on the protectiveness of high-dose folic acid in pregnant people treated with ASMs, the recommendations vary widely concerning what doses of folic acid that should be used, when to start the supplementation, and how long the supplementation should be continued, and if doses should be modified during the pregnancy.

Although supplementation with high-dose folic acid has not yet been shown to protect against congenital anomalies in children prenatally exposed to ASM, supplementation has been associated with other benefits such as lowered risk of autism-spectrum

disorders,[13] improved intelligence quotient scores,[14] and reduced risk of language impairment.[15] However, there has been concerns related to use of folic acid and risk of cancer,[16] and studies assessing the cancer risk in mothers and children exposed to high-dose folic acid have been missing.

Folic acid is the synthetic form of folate, a water-soluble B-vitamin important in the repair and synthesis of DNA, where it acts as a methyl-donor. Due to its key role in DNA metabolism, folate has been investigated extensively in relation to cancer. Some of the first chemotherapies, some still used today (methotrexate), act as anti-folates.[17] Folate and folic acid have been associated with tumor growth, spontaneous DNA mutations, and cellular distress.[18,19] The role of folate in relation to cancer is complex, where both deficiency and excess of folate may stimulate cancer, depending on the individual setting. Remaining concerns related to cancer is an important reason why most Western European countries have not mandatorily fortified foods with folic acid due, unlike most other countries in the world including the USA and the UK.[20-22] While several clinical studies have found no association between the regular low-dose folic acid (0.4 mg daily) and risk of cancer, studies including doses equal to or greater than 1 mg daily have been lacking for pregnant women.[23-25]

To assess we the risk of cancer associated with high-dose folic acid use in relation to pregnancy, we first assessed the risk of cancer in children prenatally exposed to high doses of folic acid born to women with epilepsy. Then we investigated the risk of cancer in women who were supplemented with high-dose folic acid in relation to pregnancy, including the risk of cancer associated with high-dose folic acid use after the first pregnancy. Last, we described the use of high-dose folic acid supplementation in pregnancies to women with ASM-treated epilepsy, comparing the practices of folic acid supplementation in the Scandinavian countries.

Chapter 2

Study Aims

The overall aim of this PhD was to assess the association between the use of high-dose folic acid supplementation in relation to pregnancy and risk of cancer in the mother and child in order to inform clinical guidelines.

Paper I – Prenatal exposure to high-dose folic acid and risk of childhood cancer

The primary aim was to assess the safety of high-dose folic acid supplementation during pregnancy regarding the risk of cancer in children born to mothers with epilepsy. Secondary aims were to study the risk of childhood cancer related to maternal use of different ASMs, to study the impact of potentials mediators on the risk of cancer such as congenital anomalies, and to describe the occurrence of the most common types of childhood cancers due to prenatal exposure to high-dose folic acid.

Paper II – Maternal use of high-dose folic acid and risk of cancer

The primary aim was to assess the safety of high-dose folic acid use regarding the risk of cancer in women included in the Scandinavian medical birth registers. The secondary aim was to assess the risk of different subtypes of cancer associated with high-dose folic acid use.

Paper III – High-dose folic acid use in pregnancies with ASM-treated epilepsy

The primary aim was to characterize the use of high-dose folic acid in women with ASM-treated epilepsy in relation to pregnancy, comparing the supplementation practices in three countries with similar demographic attributes. A secondary aim was to describe the timing of the first high-dose folic acid prescription fill in relation to pregnancy.

Chapter 3

Background

Epilepsy

Epilepsy is a neurological disorder affecting millions of women during childbearing age.[1] It is a noncommunicable disorder, characterized by recurring and unprovoked seizures. It affects people of all ages with similar prevalence in both sexes.[1,26] Seizures can manifest in various forms, potentially involving convulsions, altered consciousness, sensory disturbances, or motor dysfunctions, and are often accompanied by a wide range of associated comorbidities and socio-psychological challenges.[27,28] An isolated seizure occurrence is by itself not necessarily epilepsy, as diagnosis of epilepsy has the following requirements: 1. At least two provoked seizures occurring more than 24 hours apart, 2. One unprovoked seizure and an increased likelihood of additional seizures occurring over the following ten years, comparable to the overall recurrence risk after two unprovoked seizures (60% or more), or 3. A diagnosis of an epilepsy syndrome.[3] It is common to experience a seizure, with as many as 10% of the general population experiencing a seizure during a lifetime.[29]

Epilepsy can be caused by a range of factors, such as genetic mutations, stroke, traumatic brain injuries, brain tumors, metabolic disorders, or infections.[27,30-32] Also psychiatric comorbidities such as depression are associated with an increased risk of epilepsy.[33] However, most causes of epilepsy are unknown, often termed “idiopathic epilepsy”. Most cases of idiopathic epilepsy are thought to be caused by genetic mutations.[34] Epilepsy is typically categorized into two types based on areas of the brain where the seizures originate or starts: Generalized epilepsy and focal epilepsy.[27,30,31,35] Generalized epilepsy involve seizures characterized by abnormal electrical activity throughout the entire brain, whereas focal epilepsy in contrast involve seizure characterized by abnormal electrical activity in specific brain areas. Additionally, seizures that originate from specific brain areas may develop and spread out affecting the entire brain. Generalized and focal epilepsy are equally common in

children, whereas focal epilepsies are more common than generalized epilepsy in the adult population.[36]

Diagnosing epilepsy involves a comprehensive and multi-faceted approach that integrates clinical and paraclinical examinations. A thorough evaluation of the patient's medical history should be followed by a complete description of the patient's seizure episodes, including the frequency of seizures, duration of seizures, and accompanying symptoms. To find any neurological abnormalities, neurological tests are performed, which include assessments of cognitive function, motor function, and sensory perception. To support the clinical observations, clinicians may use electroencephalography (EEG) to capture brainwave patterns during and between seizures, and magnetic resonance imaging and computed tomography scans to look for structural or other abnormalities in the brain. To record and examine seizure occurrences over an extended period, specialist monitoring techniques like ambulatory EEG and video-EEG monitoring can be used. Further, blood tests can be checked to examine metabolic or infectious causes.

The treatment of epilepsy is aimed at reducing the likelihood of seizures to occur, or if possible, eliminating the risk of seizure entirely. Correct assessment of the epilepsy diagnosis is key to obtaining the highest chance of successful treatment. First line of treatment is typically ASM (formerly known as antiepileptic drugs). These drugs are designed to regulate neuronal excitability and are effective in 2 of 3 patients with epilepsy.[37] Surgical procedures should be considered for people whose focal epilepsy is linked to observable anatomical abnormalities in the brain or if the epilepsy is defined as a drug resistant epilepsy with a focus that can be identified.[38] Neuromodulatory procedures like vagus nerve stimulation or responsive neurostimulation are other available international treatment options.[39] Some patients may benefit from newly developed therapies like deep brain stimulation and transcranial magnetic stimulation.[40] By changing brain metabolism, a high-fat, low-carbohydrate ketogenic diet has demonstrated effectiveness in selected patients.[41] The choice of treatment depends on a thorough evaluation of the patient's medical history, seizure type, and underlying causes, and often involves a multidisciplinary approach

with neurologists, neurosurgeons, clinical neurophysiologists, neuroradiologists, and others collaborating to optimize therapeutic outcomes.

Epilepsy and Pregnancy

Approximately 1% of all pregnancies is carried out by women with epilepsy.[42] In contrast to pregnancies in women without epilepsy, those with epilepsy encounter numerous additional risks associated with their condition. Not only does this include a higher risk of stillbirth, fetal growth restriction, congenital anomalies, preeclampsia and hemorrhage,[43,44] but also a higher risk of maternal mortality and morbidity.[6,45-47] Children born to mothers with epilepsy have an increased risk of being diagnosed with epilepsy themselves, regardless of paternal epilepsy, hence often coined as the maternal effect.[48-50] The etiology behind this mechanism is not fully known. However, it is important to emphasize that most pregnancies in women with epilepsy are uncomplicated, and the large majority gives birth to healthy children.[51]

Women with epilepsy are recommended to be carefully followed up by experienced neurologists and obstetricians before, during, and after pregnancy.[7] Treatment of epilepsy during pregnancy is a complex task that requires careful consideration of seizure control and the potential risks associated with ASM use.[7] ASM is needed for most women with epilepsy during pregnancy.[52] As unplanned pregnancies in women with epilepsy are common (around 60% in one study)[53] and associated with increased risk of adverse health outcomes in the child, it is important to establish a safe and effective treatment prior to pregnancy start.[53-55] Most women with epilepsy are able to maintain seizure control throughout pregnancy, although frequent dose adjustment may be required depending on type of ASM used due to altered metabolism during and immediately after the pregnancy.[56] Women with idiopathic generalized epilepsies are more likely to remain seizure-free compared to those with focal epilepsy during pregnancy. In a large study from EURAP, the risk of experiencing generalized tonic-clonic seizures was found to be 15% during pregnancy for women with epilepsy.[56]

The daily dose of ASM during pregnancy is advised to be adjusted to the lowest possible daily dose sufficient to maintain seizure-freedom.[7] This approach also aims to reduce the risk of adverse health outcomes in the child. The eight most used types of ASMs in high-income countries including Europe to treat epilepsy in relation to pregnancy are carbamazepine, lamotrigine, levetiracetam, oxcarbazepine, phenobarbital, phenytoin, topiramate, and valproate.[8] Out of these, valproate has been deemed to be least safe in regards to increased risk of major congenital anomalies and neurodevelopmental impairments.[51,57,58] The risk of congenital anomalies has been shown to increase with higher daily dose levels of valproate, phenobarbital, carbamazepine, and lamotrigine.[8] However, the frequency of congenital anomalies associated with lamotrigine was in the same range as observed in children not prenatally exposed to ASM.[8] Recently, also topiramate has been associated with increased risk of neurodevelopmental disorders.[13,59] Consequently, the European Medicines Agency now advises limiting the use of valproate and topiramate during pregnancy,[60,61] unless the conditions outlined by a "pregnancy prevention programme" established by the European Medicines Agency are adhered to. This program includes and individual assessment of conceptional capacity, regular pregnancy testing during treatment, counseling on valproate and topiramate risks and consistent use of contraception, annual specialist treatment review, and an annual risk acknowledgment form to ensure an appropriate advice comprehension.

Of the currently available ASMs that have been deemed to be safe include lamotrigine, levetiracetam, and oxcarbazepine with the lowest risk of congenital anomalies and cognitive impairments compared to other ASMs.[13,59,62-64] For the newest drugs, the available data are too scarce to evaluate the risk when used during pregnancy. However, lamotrigine has been shown to have poorer seizure control during pregnancy compared to valproate although the mean dose of lamotrigine was increased from the first to third trimesters, resulting that low-dose valproate continues to be used in some pregnancies to maintain seizure control.[8,56] In most women, lamotrigine and levetiracetam are regarded as the safest drugs that at the same time can achieve control seizures during pregnancy.[65]

Use of Folic Acid During Pregnancy

The word folate is derived from Latin “folium”, meaning “leaf”, and is naturally present in foods such as deep leafy green vegetables, legumes eggs, dairy products, grains, seafood, and meat. A sufficient intake of folate during pregnancy is vital to ensure normal fetal growth and development. Pregnancy represents a metabolic state in which the requirement for folate increases. Folate supplementation has got most attention for reducing the risk of neural tube defects in newborns by reducing the chance of folate deficiency during pregnancy.[10,66] Folate deficiency during pregnancy is also associated with other neurodevelopmental disorders in the child such as increased risk of autism spectrum disorders and language impairment.[67] Folic acid supplementation reduces this risk.[68] Use of folic acid in combination with iron supplements can also reduce the risk of anemia in the newborn infants as well as in the mothers.[69,70] Use of folic acid has also been associated with a lowered risk of perinatal depression in a recent meta-analysis.[71] Several studies have addressed whether use of folic acid affects the risk of hypertensive disorders during pregnancy such as eclampsia, but these findings remain inconclusive.[72-75]

Causes of maternal folate deficiency can be inadequate intake of folate through specific and restrictive diets, alcohol misuse, diseases affecting the uptake of folate (such as inflammatory bowels disease or bariatric surgery), mutations in the folate activating enzymes affecting the conversion of folate, anti-folate medications such as ASMs or methotrexate, or an increased need for folate such as pregnancy, cancer and thyreotoxicosis.[76]

Folic acid is the synthetic form of folate used in fortified foods or in dietary supplements. Folic acid is converted into folate upon absorption in the intestines.[77] The World Health Organization (WHO) recommends a daily intake of 0.4-0.8 mg folic acid daily for pregnant women, which can be achieved through a combination of dietary sources and using folic acid supplements.[9,78] Folic acid supplementation is recommended from the timepoint when pregnancy is actively planned and should be continued at least through the first trimester of pregnancy. In an effort to reduce the

incidence of neural tube defects in the general population, most countries in the world mandatorily fortify foods such as wheat, grains and cereals with folic acid.[22] The US is among these countries which has fortified foods with folic acid since 1998 with the mean daily consumption of folic acid estimated to be 0.14 mg daily due to such fortification.[79] However, it has been questioned whether this effort has reduced the incidence of neural tube defects in the US.[80] Similar findings have been reported in a Cochrane systematic review, where only one of the included studies (a non-RCT) indicated that folic acid fortification in wheat or maize flour may be associated with a decreased risk of neural tube defects.[81]

A higher daily folic acid intake, ranging from 1–5 mg daily, is usually recommended for people who are at higher risk of giving birth to a child with a congenital anomaly.[82] The WHO recommends that the daily dose of folic acid should be individualized,[83] suggesting that measurements of folate concentration could aid in determining the optimal folic acid supplementation. Studies have shown that a red blood cell folate concentration >906 nmol/L or serum folate concentration >25.5 nmol/L is sufficient to reduce the risk of neural tube defects in the child.[68,84,85] The WHO recommends maintaining a red blood folate concentration be above this threshold for all women of reproductive age.[86] Such levels are typically reached after four weeks of supplementation using 0.8 mg folic acid daily.[87] Whereas red cell folate indicates the long-term folate status, serum folate indicates a more recent folate intake.[86] If serum folate is used rather than red cell folate, the correlation between serum folate and red cell folate should be measured and established to ensure that the threshold of 25 nmol/L is maintained throughout pregnancy. However, in patients with vitamin B12 deficiency, a higher threshold may be required to maintain sufficient levels of folate throughout pregnancy.[85] These studies did not address whether there should be an upper limit for serum folate or red blood cell concentration or if a higher threshold for serum folate concentration should be established for women diagnosed with epilepsy.

Folate Metabolism

Folic acid is used rather than folate as a dietary supplementation since folate has polyglutamate side chains, requiring oxidation and hydrolysis before absorption and exists therefore in a reduced and unstable state. Folic acid on the other hand contains oxidized pteroylmonoglutamate, which makes it more stable and readily bioavailable.[88] The bioavailability of dietary folate is influenced by several factors such as presence of alcohol, malabsorption disorders, and the intestinal pH level. Folate metabolism involves several enzymatic reactions that convert dietary folate or folic acid into its active form, 5-methyltetrahydrofolate (MTHF), which represents the main marker to assess serum folate status.[89] MTHF is together with cobalamin (vitamin B_{12}) necessary to remethylate homocysteine to produce methionine, which is an important methyl donor. Because of this reaction, homocysteine is a useful metabolic marker for folate status. The homocysteine concentration will increase if MTHF falls below 25-27 nmol/L.[90]

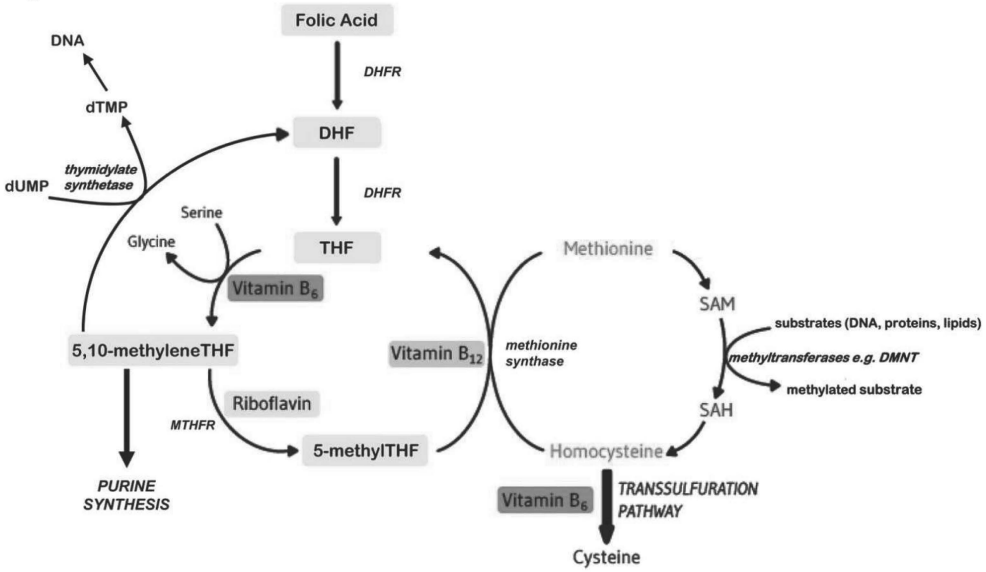
The initial step in folate metabolism is the reduction of dietary folate or folic acid to dihydrofolate (DHF) by dihydrofolate reductase (DHFR) and further reduction to tetrahydrofolate (THF) (Figure 1).[91] THF is the active form of folate involved in one-carbon transfer reactions, essential for the synthesis of nucleic acids, which are the building blocks for DNA, RNA, and proteins.[89] THF is converged to 5,10-MTHF, which is further catalyzed by methylenetetrahydrofolate reductase (MTHFR) to 5-MTHF.[92] 5-MTHF represents the primary circulating form of folate in the body and is required for the remethylation of homocysteine to methionine.[92] In the case of folate deficiency, 5-MTHF will decrease while homocysteine levels will build up in the blood. Methionine is necessary for the synthesizes of S-adenosylmethionine (SAM), a universal methyl donor involved in DNA methylation and in many other methylation reactions.[89]

Folate metabolism is tightly regulated to maintain the balance between folate availability and utilization in the body. The regulation of folate metabolism is influenced by various factors, including dietary intake, folic acid supplements, genetic variations in the activity of folate-related enzymes, and the presence of other nutrients

such as vitamin B12.[89] Individual factors such as body mass index, socioeconomic factors, country of origin, and race/ethnicity may affect folate concentrations.[83] Folate deficiency has been linked to several health conditions, including macrocytic anemia,[93] increased risk of cardiovascular disease through elevated levels of homocysteine,[94] and cognitive impairments such as depression and dementia.[95] Conversely, elevated blood levels of folate in the presence of low vitamin B12 levels have been associated with a higher likelihood of insulin resistance and obesity.[96] Low levels of vitamin B12 may mimic folate deficiency due to biochemical interdependence in which a vitamin B12 deficiency impairs the conversion of THF leading to a functional folate deficiency as folate gets trapped in a form which is not useful to the body (Figure 1).

Furthermore, folate status depends in part on genetic factors, and some mutations that increase the risk for folate deficiency are common in the general population. This includes single-nucleotide polymorphisms that regulate the one-carbon metabolism. The most important single-nucleotide polymorphism is the 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C→T genotype, which results in a 50% reduced enzymatic activity in homozygous persons, leading to a low concentration of folate and also homocysteinemia.[97]

Figure 1: Folic acid and related b-vitamins in one-carbon metabolism



Adapted from McNulty et al[66]. Key abbreviations listed in "Abbreviations", page 9-10.

The Interplay Between Folic Acid and ASM

The relationship between folic acid and ASM is complex. Some ASMs are known to interact with folic acid metabolism so that they reduce serum levels of folate. This is true especially for ASMs which stimulate the cytochrome P450 enzymes which are important for clearance of various medications.[12,98] Carbamazepine, phenobarbital, phenytoin, and primidone are all associated with enhancing folate catabolism leading to inhibition of the conversion of homocysteine into methionine, subsequently leading to elevated homocysteine levels. Valproate, topiramate, gabapentin, oxcarbazepine, and levetiracetam have also been associated with low folate levels and high homocysteine concentrations.[99] However, the mechanisms by which these ASMs affect folate metabolism are not fully understood. Lamotrigine was originally synthesized to work as an antifolate drug and has been associated with reduced serum levels of folate metabolites compared to their untreated counterparts.[100,101] Further, increased ASM concentrations have been correlated with higher levels of unmetabolized folic acid (UMFA) and inactive folate metabolites in pregnant women with epilepsy. This is coupled with a diminished ratio between active 5-methyltetrahydrofolate (MTHF) and its inactive forms, indicating an increased rate of folate catabolism.[98]

Several ASMs have been shown to reduce placental absorption of folate especially valproate.[102] Carbamazepine, lamotrigine, levetiracetam, and lacosamide, have also been linked with reduced absorption of folate, but no to the same extent as valproate.[103-107]

Recommendations on Folic Acid Supplementation During Pregnancy for Women With Epilepsy

The appropriate folic acid supplementation dose and timing in relation to pregnancy in women with epilepsy remains for a large part unknown. Therefore, it is not surprising that clinical guidelines provide a widely varying set of recommendations for folic acid supplementation for women treated with ASM during pregnancy, ranging from 0.4–5 mg daily being recommended (Table 1).

However, it is generally recommended that women with epilepsy treated with ASM should be supplemented with folic acid during pregnancy, as illustrated by a global survey conducted by the International League Against Epilepsy (ILAE) which revealed that 52 out of 57 ILAE chapters had incorporated folate recommendations into their standard recommendation protocols. While the majority advocated a daily intake of 4 mg of folic acid, some proposed daily doses ranging from 1 to 4 mg, and some recommended low-dose folic acid of 0.4 mg folic acid daily. Recommendations from a consensus report published by the ILAE as well as Norwegian Health Authorities suggest a daily 0.4 mg folic acid supplement for all women of reproductive age using ASMs irrespective of planning pregnancy or not. This because of the high occurrence of unintended pregnancies among women with epilepsy.[53]

The recommendations of high-dose folic acid supplementation during pregnancy for women treated with ASM have been extrapolated from studies involving pregnant women who were at an increased risk of congenital abnormalities for their children.[108] An initial RCT study including 3012 women aimed at quantifying the effects of higher doses of folic acid than 0.4 mg daily on the risk of neural tube defects in women who have previously given birth to a child with a neural tube defects, because previous studies had failed to show protectiveness of 0.4 mg folic daily in this group of women. This study compared the use of 4 mg daily of folic acid to other multivitamins without folic acid, and to no supplementation.[108] Women with epilepsy were excluded from the study in case supplementation adversely affected the treatment during pregnancy. They revealed that 72% of neural tube defects were prevented in women who took 4 mg of folic acid. However, the authors acknowledged that a lower daily dose of folic acid could be sufficient to achieve protectiveness against neural tube defects. Yet, a later study by the same research group went forth and reported that a 5 mg tablet of folic acid reduced the risk of neural tube defects even more than 4 mg daily, concluding on recommending that the general population should use 5 mg daily instead of 0.4 mg daily of folic acid during pregnancy.[109] The same study did not acknowledge that there could be any adverse effects, and studies indicating side-effects did not exist at the time. At that time, the only known health risk due to use of

high-dose folic acid, vitamin B12 deficiency, was not considered a relevant health risk according to the author's personal opinion. These results have later then been extrapolated to other groups of pregnant women with an increased risk of having children with congenital anomalies. However, the original study was not designed to adequately compare high and low-dose folic acid supplementation. A multicenter, randomized controlled trial (RCT) involving 1,060 women planning pregnancies found that folic acid supplementation at daily doses of 4.0 mg compared with 0.4 mg gave a lower risk of preterm birth, spontaneous abortion, and children being small for gestational age.[110] The women were included if they planned pregnancy within the next year and randomized to take either 4 mg or 0.4 mg folic acid from time of randomization and until the 12th week of gestation if pregnancy occurred. Outcomes were obtained through interview at 16 weeks and 24 weeks of gestation, and post-delivery. However, the study did not find a lower rate of congenital anomalies with 4 mg. Neither of these studies assessed the effect of high-dose folic acid during pregnancy in women with epilepsy or in women using ASM.

The precise recommendations regarding folic acid supplementation before and during pregnancy vary internationally. Below is a selection of Nordic, European, American, and international guidelines on folic acid supplementation during pregnancy for people with epilepsy or who are treated with ASM. This variation illustrates the lack of evidence regarding the optimal dose and timing for folic acid supplementation for this group of women.

Table 1: Folic acid guidelines for women with epilepsy or on ASM during pregnancy.

Clinical recommendations	Year of publication	Dose recommendation
Nordic recommendations		
Danish Health Authority [111]	2005 (Valid since 1998)	5 mg daily if treated with "older types" of antiseizure medications: phenobarbital, phenytoin, ethosuximide, carbamazepine, valproate, clonazepam, clobazam.
The Danish Neurological Society	2022	5 mg daily if treated with valproate or an enzyme inducing ASM during first trimester. 0.4 mg daily is recommended for everyone planning pregnancy.
Current Care Guidelines, Finland [112]	2020	No recommendation of high-dose folic acid if treated with antiseizure medication.
The National University Hospital of Iceland [113]	2019	5 mg daily at least during the three first months of pregnancy regardless of type of antiseizure medication used.
The Norwegian Medical Association [114]	2018	4-5 mg daily if treated with any ASM from pregnancy start and until the second trimester, thereafter 0.4 mg daily.
The Norwegian National Centre for Epilepsy [115]	2022	4 mg daily immediately before pregnancy and throughout the three first months of pregnancy if treated with valproic acid or with another antiseizure medication with unknown fetotoxicity. After three first months of pregnancy, 0.4 mg daily.
Swedish Medical Products Agency [116]	2019	No recommendation of high-dose folic acid if treated with antiseizure medication.
American recommendations		
American Academy of Neurology [117]	2009	At least 0.4 mg daily before and during pregnancy.
American College of Obstetricians and Gynecologists [118]	2017	0.4 mg folic acid if treated with antiseizure medication.
Society of Obstetricians and Gynecologists of Canada [68]	2022	1 mg daily before and during the first trimester, 0.4 mg thereafter unless serum folate <28-30 nmol/L or red blood cell folate <907 nmol/L.
European recommendations		
The National Institute for Health and Care Excellence, The United Kingdom [119]	2022	No specific recommendation of high-dose folic acid if treated with antiseizure medication.
The Scottish Intercollegiate Guidelines Network [120]	2015	5 mg daily before and during pregnancy regardless of antiseizure medication used.
The German Neurological Association[121]	2023	0.4-0.8 mg daily during pregnancy.
The Swiss League Against Epilepsy [122]	2023	1-3 mg daily before and during pregnancy regardless of antiseizure medication used. Higher doses can be considered based on serum folate levels or red blood cell folate levels.
International recommendations		
International League Against Epilepsy [7]	2019	At least 0.4 mg daily, and 4-5 mg daily depending on type of antiseizure medication used (not specified).
UpToDate [123]	2022	4 mg daily if treated with carbamazepine or valproate, 1 mg daily if treated with any other type of antiseizure medication.

Table developed from Bjørk et al[124]. Guidelines that recommend daily supplementation of folic acid (0.4 mg daily) during all fertile years: The Norwegian Medical Association (2018) and International League Against Epilepsy (2019).

Benefits, Harms, and Unknown Effects of High-Dose Folic Acid Use in Women With Epilepsy and Their Children.

Although folic acid was originally recommended in the general pregnant population to reduce the risk of neural tube defects in the child, and with a higher dose being recommended to pregnant women with epilepsy due to the risks associated with ASMs, it is still not known whether supplementation of folic acid reduces the risk of congenital anomalies in children born to a mother with ASM-treated epilepsy. As previously mentioned, folate is crucial for DNA methylation, which in turn influences gene regulation.[125] Folate deficiency can result in epigenetic changes with long-term consequences for the child.[126,127] Folic acid supplementation can therefore have benefits beyond the primary goal of reducing the risk of congenital anomalies.

Benefits

The use of folic acid during pregnancy in mothers with ASM-treated epilepsy has been associated with a reduced risk of neurodevelopmental impairments. In the Neurodevelopmental Effects of Antiepileptic Drugs (NEAD) study,[14,128] improved cognitive outcomes in children at age 3 and 6 years were demonstrated in children prenatally exposed to ASM monotherapy (carbamazepine, lamotrigine, phenytoin, or valproate) comparing children to mothers supplemented with folic acid during pregnancy to children to mothers who did not take folic acid supplementation This was true for most ASMs, but not if prenatally exposed to valproate. In the NEAD study, more than half of the pregnant people used folic supplements in a dose higher than 0.4 mg daily. Building upon the foundation of the NEAD study, the MONEAD (Maternal Outcomes and Neurodevelopmental Effects of Antiepileptic Drugs) study which included maternal outcomes related to epilepsy and its treatment during pregnancy, the authors reported no significant effect of periconceptional use of folate on cognitive outcomes in children at three years of age.[129] In a 2023 study also performed by the MONEAD study, periconceptional folic acid was not associated with improved adaptive functioning, a measure of overall adaptive behavior, in children of mothers

with epilepsy at four and a half years of age.[130] Studies by Husebye et al., utilizing the Norwegian Mother, Father and Child (MoBa) study, reported improved neurodevelopmental outcomes in children who were prenatally exposed to ASM with better language outcomes.[15,131] These improvements were observed in children where the mother took folic acid supplements during pregnancy, compared to cases with no supplementation. Increasing plasma folate levels in pregnant women with epilepsy during gestational week 18 exhibited an inverse relationship with autistic features in children prenatally exposed to ASM.[132] However, results on whether use folic acid in relation to pregnancy reduces the risk of neurodevelopmental impairments is conflicting with studies also reporting no difference in mean IQ in children to mothers with epilepsy treated with ASM.[133-135] It has been questioned whether some mothers actually might have been over-supplemented.[136] High levels of UMFA in the umbilical cord has been associated with an increased risk of impaired psychomotor development during the first year of life as well as an increased risk of autism spectrum disorders.[137-140] However, UMFA exposure in utero has not been associated to increase the risk of autistic traits in children to mothers with ASM-treated epilepsy.[141]

Further, mothers with epilepsy who were treated with ASM during pregnancy was a lower risk of preterm birth compared to mothers with ASM-treated epilepsy who did not take folic acid supplementation.[55]

Harms

There may be harm due to excessive folic acid intake. Use of folic acid, especially daily doses equal to or greater than 1 mg daily, may mask a vitamin B12 deficiency. Such deficiency can over time cause megaloblastic anemia.[142] The precise mechanism how excess levels of folate interfere with vitamin B12 status remain largely unknown.

High levels of UMFA in umbilical cord-blood has been associated with an increased risk of autism spectrum disorders in a prospective cohort study on cord blood folate in the US[137] and the use of 0.4 mg folic acid daily or greater during pregnancy was

associated with increased risk of impaired psychomotor development in another prospective multicenter cohort study conducted in Spain.[140]

It has been suggested that a U-shaped association regarding harmfulness might exist, and studies have shown that both folate deficiency and excess folate has been associated with an increased risk of gestational diabetes,[143] and in halting the cytotoxicity of natural killer cells.[144] Natural killer cells are important part of our immune systems by protecting tissue from viral infections and tumor cells.

Unknown Effects of High-Dose Folic Acid Supplementation During Pregnancy

It is not fully understood whether supplementation with ≥1 mg daily folic acid during pregnancy reduces the risk of giving birth to a child with congenital anomalies in children born to mothers treated with ASM during pregnancy, compared to children born to mothers not treated with ASMs. Furthermore, it is unclear if the risk differs when comparing different types of ASMs, with or without such folic acid supplementation. One study identified an increased risk of congenital anomalies in children of mothers with epilepsy treated with ASM during pregnancy who were supplemented with folic acid, compared to children born to mothers with ASM-treated epilepsy who did not supplement their diet.[8] However, a protective effect of folic acid could still not be ruled out due to confounding by indication, meaning that the increased risk of congenital anomaly may be influenced by the concomitant treatment with ASM during pregnancy. Periconceptional use of folic acid was not found to change the risk of congenital anomalies in a US prospective and observational cohort study which included folic acid doses up to 4 mg daily or more.[145] In the general population, it has been suggested that 1 mg daily of folic acid is sufficient for women at risk of recurrent neural tube defects in their offspring, as studies have reported no further reduction in risk with folic acid doses above 1 mg daily.[146]

Cancer

Cancer is a heterogeneous disease characterized by uncontrolled cell growth and is the second leading cause of mortality in the world after cardiovascular disease.[147] Cancer has an immense impact on individuals and societies, with over 100 subtypes identified, each characterized by specific cell types and behaviors. At a cellular level, the cause of cancer is rooted in genetic mutations that disrupt the normal mechanisms of cell division and growth regulation.[148] These mutations change methylation patterns of DNA, leading to an alteration in the nucleosome positioning and causing histone modifications.[149] Mutations can be induced by various set of factors, such as exposure to chemical and environmental carcinogens including smoking and radiation,[150,151] genetic predisposition,[152,153] and random errors during DNA replication.[154,155] Some mutations lead to changes in specific genes that regulate normal cell growth and cell divisions, called oncogenes and tumor suppressor genes.[156] Tumors can be benign, remaining localized and non-invasive, or malignant and capable of invading surrounding tissues and spreading to distant sites, causing metastasis.[157]

Cancer occurs across all age groups, sexes and socioeconomic levels.[158] However, around 70% of cancers are diagnosed above 75 years of age, with lower incidence rate in females compared to males.[159,160] By avoiding tobacco and alcohol, ensuring sufficient and regular physical activity, avoiding excess body mass, reducing environmental pollution, reduce the level of occupational carcinogens, avoiding radiation such as ultraviolet radiation, it has been claimed that it is possible to avoid as much as 50% of all cancers globally.[160,161] In the Nordic countries, the age-standardized annual incidence rate was 570 cancer cases per 100,000 person-years in 2021, steadily increasing since 1965.[162] Standard treatments of cancers include chemotherapy, surgery, radiation, and often in combination.[163] More recently, immunotherapy, stem cell therapy, use of nanoparticles, etc., have been included as new cancer therapies, illustrating the importance of developing treatments that targets pathways leading to cancer.[163-165]

Childhood cancer is defined by the WHO as any cancer that is diagnosed before 20 years of age.[166] The general survival of cancer in children is approximately 80% in high-income countries.[167] However, more than 90% of all cancer cases in children occur in low- and middle-income countries.[167] It is the most frequent cause of death in children living in high-income countries, and with an increasing incidence rate during the last decades.[166] However, the risk of being diagnosed with childhood cancer is small. The total risk of being diagnosed with cancer before 20 years of age is around 0.35%.[168] The three most common types of childhood cancers are leukemia, cancer in the CNS, and lymphomas.[166] The incidence of cancer during childhood is U-shaped, with a drop in incidence from birth and until five years of age, before the incidence is slowly increasing again.[169] There are only a few known risk factors for childhood cancer; ionizing radiation, chemotherapy, and increasing maternal age. It is a challenge to identify risk factors for childhood cancer due to difficulties in obtaining enough cases to assess associations between a rare disease and exposures.

Cancer intertwines with reproductive health in women during their fertile years.[170,171] Chemotherapy and radiation, while effective against cancer cells, can damage healthy tissues, leading to premature ovarian failure and infertility.[172,173] Fertility preservation techniques such as egg or embryo freezing before cancer treatment have become increasingly important.[174] Pregnancy during or after cancer treatment presents complex scenario.[171] Balancing the mother's health with that of the developing fetus requires careful consideration. Some cancer treatments pose a risk to fetal development, necessitating a multidisciplinary approach involving oncologists, obstetricians, and other specialists to determine the best course of action.[175] Additionally, some cancer and cancer treatments can influence the hormonal balance and thereby affect menstrual cycles and fertility.[175,176]

Associations Between Epilepsy, ASM and Cancer

The relationship between epilepsy and the risk of cancer is intricate and influenced by ASMs and associated medications, socioeconomic factors linked to the disease, and

potentially genetic characteristics inherent to specific types of epilepsy.[177,178] Studies on whether epilepsy by itself increases the risk of cancer have been conflicting. A Danish study including 8000 hospitalized patients with epilepsy reported that epilepsy was associated with an increased risk of cancer, specifically cancer in the central nervous system but also lung cancer and non-Hodgkin's lymphoma.[179] However, they did not account for the bidirectional association between epilepsy and CNS cancer and did not have information on important lifestyle factors such as smoking habits. The risk of non-Hodkin's lymphoma was non-significant with small numbers of observations (n=16). Epilepsy was further found to be associated with and cancer in a recent Danish report (preprint) from 2023 that found an increased risk of cancer in children born to mothers with epilepsy as well as in mothers using ASMs.[180] These studies did not account for concomitant use of folic acid. Other clinical studies have not found any association between epilepsy and risk of cancer.[181,182] Incident epilepsy is a common first symptom of cancer, and approximately 20% of all patients with systemic cancers develop epilepsy.[183] Up to 70% of patients with primary brain tumors experience seizures, and up to 40% of patients with cancer have metastases to the brain.[184] To identify any environmental factors that alter the risk of cancer is challenging. Lifestyle factors associated with chronic epilepsy, as well as the diagnostic procedures and treatments for epilepsy, may theoretically contribute to an increased cancer risk.[181] Surveillance bias, in which the diagnosis of epilepsy prompts extensive medical examination leads to the detection of an occult cancer, may contribute to a higher cancer detection rate in patients with epilepsy.[185]

Results regarding the association between the use of ASM and cancer prevalence have been conflicting.[181,186] Several of the most commonly used ASMs such as valproate, oxcarbazepine, lamotrigine, levetiracetam, and lacosamide, have been proposed to have been theorized to have potential anticancerous properties and suggested to be investigated for drug repurposing for cancer treatment.[187] Valproate has been of particular interest regarding cancer risk as this ASM possesses histone deacetylase inhibitory activity, which reduces to cancer cell growth arrest, differentiation, and apoptosis.[188-191] The epigenetic changes induced by valproate through altered DNA

methylation and histone acetylation, intertwine with similar effects caused by folate.[188] However, a population-based case-control study found that long-term use of valproate was associated with a slightly increased risk of certain cancers, including lung, colorectal, and prostate cancer.[192] Only for lung cancer the association was significant with an adjusted odds ratio of 2.32 (95% CI 1.12-4.79). Valproate was in an observational cohort study of approximately 3,000 patients with epilepsy in UK associated with an increased risk of colon cancer.[193] However, this was not shown in a similar study in Germany with no significant increase in cancer incidence among epilepsy patients treated with ASM, including valproate.[178] Further, valproate (and levetiracetam, carbamazepine, oxcarbazepine, lamotrigine, and phenytoin) was in a Norwegian observational cohort study not found to alter the survival of patients with glioblastoma.[194] Other clinical studies have indicated that ASM use may increase the risk of certain cancers such as cancer in liver, mouth, throat, and respiratory tract, [181] while other studies have found no significant associations between ASM use and cancer risk.[178,181] Further, as several ASMs are known to be photosensitizing, a Danish case-control study found an association between carbamazepine and lamotrigine and an increased risk of squamous cell carcinoma.[195]

Folic Acid and Risk of Cancer

Folate has been studied extensively for several decades for its theoretical role in carcinogenesis and for the treatment of cancer, linked to its role in DNA synthesis and repair as described previously.[17,196,197] One of the first cancer treatments developed act as an anti-folate, as do several anticancer drugs still used today, such as methotrexate.[198,199] The exact mechanisms on how folate and folic acid affect carcinogenesis are complex and not fully understood. The effects of folic acid on cancer risk seem to be inconsistent and are most likely affected by individual factors.[196]

Altered DNA methylation has been associated with an increased risk of several cancer types, including colorectal cancer, breast cancer, and prostate cancer.[200,201] The risk of

cancer can be influenced by both folate insufficiency and excess folate levels, indicating that a U-shaped association might exist.[196,202-204] Folate insufficiency may result in unstable DNA and a lack of resources for DNA repair, whereas excess folate may provide ample resources for growth and development in latent tumor tissue. Any potential risks associated with excess UMFA remain largely unknown, but prenatal exposure to 5 mg of folic acid daily has been shown to induce DNA changes through altered methylation, including genes important in normal embryonic development and cellular proliferation.[205]

In the folic acid metabolism pathway, methionine is converted to SAM (Figure 1). Insufficient levels of SAM due to lack of folate or cobalamin can result in lowered methylation of CpG islands found in DNA which can alter gene transcription and affect how both tumor suppressor genes and proto-oncogenes are expressed.[206,207] Furthermore, folate deficiency may halt the conversion of deoxyuridine monophosphate which is crucial for the synthesis and repair of nucleotides, thus resulting in DNA strand breaks and reduced DNA repair capacity.[206,207] Excess of folic acid on the other hand may result in the oversaturation of DHFR, suggested to increase the risk of cancer.[196] Oversaturation is theoretically reached by a daily folic acid dose above 0.4 mg daily.[208,209] Recently, a study observed that exposure to high-dose folic acid promoted cancer development in mice with hepatocellular carcinoma, most likely due to an acceleration of the methionine cycle in cancer tissues.[210]

The enzyme that is important in activating folate, MTHR, has several known gene polymorphisms, with C677T and A1298C being best characterized.[211] Both variants are common in the general population, with around 25% being carriers of either allele. They are both associated with reduced enzyme activity.[211,212] The C677T variant has previously been shown to be associated with an increased risk of cancer.[213] Caucasians being homozygous for C677TT have an increased risk of non-Hodgkin's lymphoma.[214] In persons of Asian ethnicity, the homozygous variant of A1298CC is associated with an increased risk of non-Hodgkin's lymphoma and leukemia.[215]

Experimental studies have linked use of folic acid to enhanced tumor growth,[19,216] severe oxidative stress,[217] increased risk of mammary tumors in rat offspring,[218] and increased risk of spontaneous DNA mutations.[18] However, exposure to folic acid has also been shown to reduce tumor growth in breast cancer tissue in the presence of progesterone, implicating that folic acid interacts with other metabolites regarding the effect on cancer development.[219] An increased risk of cancer due to exposure to folic acid has also been reported in several studies,[220-223] including an increased risk of colorectal cancer and prostate cancer.[221,224] The concern of an increased cancer risk with high folate levels was further raised a RCT study from the US identified a borderline significant increased risk of colorectal adenoma in patients using 1 mg daily of folic acid compared to placebo.[93] An increased risk of cancer in patients using 1 mg of folic acid daily or greater was also identified in a combined analysis of two Norwegian RCT studies with a observational posttrial follow-up.[221] However, the goal of these to studies was not to assess the risk of cancer, but were primarily designed to investigate if folic acid supplementation could decrease cardiovascular disease risk by lowering methionine levels. Conversely, a protective effect against colorectal cancer in patients with inflammatory bowels disease has been suggested,[225,226] and also that folic acid might protect against melanoma skin cancer,[227,228] although results regarding protection against skin cancers are inconclusive.[229,230]

Most clinical studies have not found any increased risk of cancer, including no increased risk of maternal or childhood cancers after folic acid use in pregnancy.[23-25,216,220,231,232] However, there has up to this PhD not been any study assessing the risk of cancer in mothers taking folic acid doses $\geq$1 mg daily or in children prenatally exposed to such folic acid doses except for the risk of breast cancer in exposed mothers.[233] Prior studies on cancer risk in humans have typically face the same limitations: They often compare different or unspecified doses of folic acid on cancer risk, typically relying on self-reported folic acid usage, include few participants with short follow-up time, and they have been unable to include non-elderly patients as the study population.

Chapter 4

Materials and methods

Data Material

SCAN-AED

The data used in this PhD study were derived from the SCAN-AED project, led by Professor Marte-Helene Bjørk and funded by NordForsk. The project's primary goal is to determine the best selection of ASMs and optimal folic acid treatment for pregnant women. Moreover, the project's objectives include evaluating potential health risks for children exposed to various ASMs during pregnancy, whether these medications are used individually or in combination. Additionally, the study seeks to determine the safety and effectiveness of the higher doses of folic acid administered to this group of women before and during pregnancy to mitigate the risks associated with ASM treatment during pregnancy. The SCAN-AED project was established in 2017 and represents a collaboration between research teams specializing in epilepsy and registry-based research at University of Bergen (Norway), Aarhus University (Denmark), the Finnish Institute for Health and Welfare, Karolinska Institutet (Sweden), and The University Hospital of Iceland. These five countries are similar regarding population demographics and health care services. They also have similar national population registers that can be interconnected through personal identification numbers.

In total, the registers encompass nearly 27 million individuals from the Nordic countries of Denmark, Finland, Iceland, Norway and Sweden, in which 120,000 individuals will have epilepsy. In the SCAN-AED, we have access to around 4.5 million pregnancies whom 11,000 mothers were diagnosed with epilepsy. By integrating individual-level data from medical birth records with information from other registers, utilizing the unique personal identification numbers, we acquired a comprehensive dataset with data from all registers from the different countries harmonized together by using a common data model. This dataset includes details on medication drug prescriptions from prescription registers, socioeconomic data from

national statistical agencies, cancer information from national cancer registers, and ICD codes for both mothers and their offspring from patient registers. The SCAN-AED dataset is substantial in size, providing high statistical power to perform analyses for the group with epilepsy and allows for the adjustment of crucial confounding factors. Consequently, this project can comprehensively explore associations between prenatal exposure to various ASMs, various folic acid supplementation regimens and health outcomes for both the child and the mother. These associations have not previously been properly investigated due to lack of statistical power. Studies published using the SCAN-AED material include "*Association of Prenatal Exposure to Antiseizure Medication With Risk of Autism and Intellectual Disability*" by Bjørk et al JAMA Neurology in 2022,[13] and "*Prenatal Exposure to Antiseizure Medication and Incidence of Childhood- and Adolescence-Onset Psychiatric Disorder*" by Dreier et al JAMA Neurology in 2023.[59]

As Finland does not include folic acid as a prescription-based drug even in high dose, and there was not sufficient information on cancer from Iceland in the SCAN-AED project, the papers included in this PhD were conducted based on data from Denmark, Norway, and Sweden. The data used from the SCAN-AED project contained register-data collected until the end of 2017.

More information on the SCAN-AED project can be accessed at www.scanaed.org.

Logo for the SCAN-AED collaboration (adapted from www.scanaed.org). Copyright: The affiliated members.

National Registers and Management of Nordic Health Collaboration Data

In the Nordic countries, including Denmark, Norway, and Sweden, the establishment of nationwide health registers marks a significant milestone in public healthcare and medical research worldwide.[234-236] These registers are characterized by their all-encompassing scope and mandatory data submission that are collected in a prospective manner, making them invaluable and readily available repositories of healthcare-related information. As the Nordic countries have created and run their nationwide registers in a similar manner, the data can be merged into large databases able to investigate associations between rare exposures and outcomes that are hardly possible anywhere else. Upon merging such nationwide data from several Nordic countries, the data are exported to, stored, and managed at Statistics Denmark, as Danish Health authorities do not allow sending microdata abroad, but can conversely receive such data from other countries. Statistics Denmark allows remote digital access to data.

Below are the nationwide registers utilized in this PhD thesis and their content described in further detail.

Medical Birth Registers

The national medical birth registers are government-mandated healthcare databases that systematically compile comprehensive data on all pregnancies within the respective countries. They represent the source registers for the definition of the study population for the studies in this PhD. The medical birth registers collect a wide range of demographic and clinical information, including parental identification numbers, parental age, parity, maternal smoking habits, pregnancy and delivery complications, medical interventions, birth outcomes, gestational age, birth weight, and specific maternal comorbidities including as epilepsy. Denmark does not include specific information on maternal diagnosis in the medical birth register. The Norwegian Medical Birth Register collect information about folic acid supplementation, which is self-reported as “yes” or “no”, i.e., without any information on dose and timing.

The origins of the Nordic medical birth registers trace back to 1967 in Norway,[237,238] with Denmark and Sweden following suit in 1973,[239,240] and were created due to the thalidomide incident which led to over 10,000 limb deformities globally, the registers aims for early detection of perinatal health issues through epidemiological surveillance.[238]

Prescription Registers

The national prescription registers collect and store information on all filled medication prescriptions that have been dispensed in pharmacies. They offer detailed information such as associated Anatomical Therapeutic Chemical classification (ATC) code, drug strength, total defined daily dose, package size, and dispensing date.[241] However, the registers do not encompass information on over-the-counter medicines or medications administered during in-patient hospital nor nursing home stays. The Danish Registry of Medicinal Product Statistics was established in 1995,[242] the Norwegian Prescription Database in 2004, and the Swedish Prescribed Drug Register in July 2005. The Norwegian and Danish Prescription Databases also include information on the indication of treatment or the diagnosis for which medications were covered and reimbursed.

Cancer Registers

The national cancer registers collect detailed information about cancer in addition to the registered ICD-10 diagnosis, such as tumor topography (localization) and morphology (tissue structure), tumor behavior, screening and treatment procedures, patient-related outcomes, date of death and tumor characteristics.[243] The cancer registers are among the oldest national registers in the Nordic countries, with the Danish Cancer Register established in 1941,[244] The Norwegian Cancer Register in 1951,[245,246] and the Swedish Cancer Register in 1958.[247] All three registers are considered close to 100% coverage of call cancer cases and to be of high validity.[248]

Patient Registers

The national patient registers in Norway, Denmark, and Sweden contain data related to both public and private hospital admissions. These registers include, but are not limited to, diagnoses categorized in accordance with the ICD-10 codes, various treatments administered during hospital stays, dates of admittance, and the duration of each patient's hospitalization. The Danish National Hospital Register was established in 1977,[249] The Swedish National Patient Register in 1987,[250] and the Norwegian Patient Register in 2008.[251]

National Statistical Agencies

The national statistical agencies in Denmark (1850), Sweden (1858), and Norway (1876) register socioeconomic and demographic data. This includes information on household income, educational level, emigration records, and mortality statistics. Variables were digitized and standardized in alignment with internationally recognized standards, such as the ISCED 2011 classification, by us in the SCAN-AED project to harmonize country-wise information for educational levels. This harmonization made cross-country comparisons possible.

Study Population and Study Design

The study population was identified in the medical birth registers in all three studies included in this PhD. The length of follow-up was restricted by the availability of data from the national prescription registers, with the longest time of follow-up in SCAN-AED being Danish data from January 1st, 1997, to December 31st, 2017.

The same study design was applied in paper I and II in this PhD, in which we assessed the association between use of high-dose folic acid ($\geq$1 mg daily) and risk of cancer in the children (paper I), and in the mothers (paper II). Both studies are defined as observational cohort studies. They are deemed observational as we have not been involved in any intervention or manipulation regarding the exposure but have relied on the mandatory collected data. They are defined as cohort studies, meaning that the

study groups examined shared common characteristics and were followed over a defined period to investigate specific outcomes related to a shared exposure. In our context, the outcome was cancer, and the exposure was filling a prescription of high-dose folic acid from a pharmacy. Both studies rely on data that were collected prospectively as information in the registers was collected chronologically over time, without regard to any exposures or outcomes.

In paper III, we did not follow the study population until a specific and predefined outcome. Rather, we described the use of the exposure, high-dose folic acid, within a specific time period. We chose to describe the use of high-dose folic acid before and during pregnancy in women with ASM-treated epilepsy. We chose a time period when we had full coverage in the prescription- and patient registers in all three countries, i.e., 2006-2017.

In paper I, the study population was children, and they were followed from date of birth and up until 20 years of life. In paper II, the study population were women, followed from their first pregnancy and until end of our follow up period. In paper III, the study population (or unit of analysis) was pregnancies occurring in women who received treatment for their epilepsy with an ASM during the pregnancy. Statistical details on the handling of the data collected during follow up are described in the statistical methods section.

Outcomes and Exposures

Outcomes

Cancer

Childhood cancer (paper I)

The first occurrence of cancer in children was set as the main outcome in paper I and was identified in the national cancer registers. To distinguish malignancies to be included in our study from benign or borderline tumors, we applied the definitions used by the International Classification of Diseases for Oncology, 3rd edition (ICD-O/3), which includes a comprehensive compilation of data concerning cancer site (topography) and structural attributes (morphology).[157] The criteria for classifying

cancers as malignant were based on a behavioral code value equal to or exceeding 3.[157] Our classification of childhood cancers adhered to the guidelines outlined in the International Classification of Childhood Cancer, Third Edition.[252] The cancer registers also contain information on when the diagnosis was made. We did not include non-melanoma skin cancer in our paper as this cancer type is not considered a fatal type of cancer. Furthermore, for this tumor type there is variation in registration practices over the years of our study.[253]

Maternal Cancer (paper II)

The national cancer registers were used to identify any cancer in women who had given birth, referencing ICD-O/3 to define the cancer and cancer subtypes. We applied the ICD-O/3 behavioral values $\geq$3 to define cancers as malignant. Tumors in the CNS were defined as a separate category including all tumors regardless of behavioral value, as tumors occurring within the skull are regarded as hazardous from localization alone. We also included tumors in the urinary tract allowing for behavioral values $\geq$2 for the same reason that there is little room to expand and obstructing urine transport from the kidneys. We did not include non-melanoma skin cancers for the same reason as described for defining childhood cancer.

Information on maternal cancer was also used in paper I but as a covariate, and defined in a similar way as described above, but only including cancer cases with an ICD-O/3 value $\geq$3 occurring before pregnancy, not making separate variables for cancer in the CNS or urinary tract. In paper I, we conducted a sensitivity analysis by excluding all cases of maternal cancer diagnosed before pregnancy. We excluded maternal cancer to reduce the risk that the association between prenatal exposure to high-dose folic acid and childhood cancer could not be attributed to genetic factors.

Exposures

High-Dose Folic Acid

In Denmark, Norway, and Sweden, doses of folic acid ≥1 mg are defined as prescription-based drug. The national prescription registers store information about the dosage strength of folic acid per tablet in mg, the quantity of tablets prescribed, and the date the prescription was filled.

In the three included countries, clinicians can prescribe tablets containing either 1 mg or 5 mg of folic acid. Both these dose strengths are readily available in Denmark and Sweden, while 5 mg is not marketed in Norway. The prescribing doctor must provide documentation to the Norwegian Medical Products Agency explaining the rationale for using the 5 mg folic acid dosage. The ATC code of BB03BB01 was used to identify prescription fills of both 1 and 5 mg in the prescription registers.

Definition of High-Dose Folic Acid in Paper I

Prenatal exposure to high-dose folic acid was defined as at least one prescription fill for 1 mg or 5 mg from 90 days prior to the last menstrual period (LMP) and until the date of birth. The average daily dose of folic acid in mg was determined based on the number of pills and dose prescribed per day from 90 days prior to LMP and until the date of birth.

Definition of High-Dose Folic Acid in Paper II

Maternal use of high-dose folic acid was defined as at least one filled prescription for either 1 mg or 5 mg of folic acid from the start of follow-up until date of cancer diagnosis, date of death, date of emigration, or end of the follow-up time, whichever came first. We included use of high-dose folic acid use from two years prior to LMP and until start of the 22nd week of pregnancy, allowing for capturing pre-pregnancy use of high-dose folic acid.

In this paper, high-dose folic acid was considered a time-varying exposure. This means that the status of exposure could change from "not exposed" to "exposed" throughout

the follow-up period. This approach allowed us to assess how a continuous and cumulative use of high-dose folic acid influenced the risk of cancer over time. Details on the time-varying exposure design is described in the statistical methods section.

Definition of High-Dose Folic Acid in Paper III

Exposure to high-dose folic acid was defined as having filled at least one prescription for either 1 mg or 5 mg of folic acid within 90 days before the date of LMP up to the date of birth in pregnancies to women with epilepsy who had also filled a prescription for ASM between the LMP and the date of birth. We evaluated the timing of the first folic acid prescription fill by applying a one-year lookback period.

Over-the-counter folic acid (Paper III)

The Medical Birth Register of Norway collects information about self-reported use of folic acid during pregnancy, which is not recorded in the medical birth registers of Denmark or Sweden. In Paper III, we estimated the consumption of over-the-counter folic acid (usually in doses of 0.4 mg or 0.8 mg per day) among pregnant women treated for epilepsy with ASMs. This was done by subtracting the number of pregnancies in which the mother had filled prescriptions for either 1 mg or 5 mg of folic acid from 90 days prior to the last menstrual period up to delivery, from the total number of pregnancies to mothers who reported using folic acid during pregnancy.

Covariables

Covariates were chosen based on known and clinically probable confounders of the association between high-dose folic acid exposure and risk of cancer. Directed-acyclic graphs were used in our discussion about which covariates to select in each study to obtain minimal sufficient adjustment sets.[254]

Antiseizure Medication

ASM can only be obtained through a licensed doctor's prescription in all three countries, and all ASMs can be identified in the prescription registers by using the following ATC codes: N03, N05BA09, and S01EC01.

In paper I, we defined exposure to ASM as any prescription fills from 90 days prior to LMP and until the date of birth. In this paper, we excluded ASM prescriptions that were not maintained from the date of the LMP and until the date of birth. This exclusion was done because some women discontinue the ASM medication when they become pregnant. By this exclusion, we reduce the likelihood of misclassifying women who are not using ASM during their pregnancy. We examined carbamazepine, lamotrigine, levetiracetam, and valproate as separate monotherapies since these were the most commonly used. All other monotherapies were grouped together under the category "other monotherapies." ASM polytherapy was defined as more than one prescription fill of more than one type of ASM from LMP and until date of birth.

In paper II, ASM exposure was defined as any prescription fills between two years prior to LMP and until the date of birth. Use of ASM during the first pregnancy was assumed to be the indication for prescription fills for high-dose folic acid throughout the follow-up.

In paper III, exposure to ASM was defined as any ASM. The most used ASM monotherapies with more than 100 observations were examined separately. Other ASM monotherapies were compiled into "other monotherapies", and included if the prescription was filled between LMP and date of birth. ASM polytherapy was defined in the same way as in paper I.

Child Comorbidities

Congenital Anomalies and Chromosomal Abnormality (paper I)

Congenital anomalies and chromosomal abnormalities were identified in the patient registers. Congenital anomaly was defined as any registration of ICD-10 codes Q00–Q89, and chromosomal abnormality was defined as any registration of ICD-10 codes

Q90–99, both diagnosed at any time after date of birth. Congenital anomaly or chromosomal abnormality was included as a covariate as these conditions are associated with an increased risk of childhood cancer.[255,256]

Maternal comorbidities

Maternal Diabetes Mellitus (paper I and II)

Maternal diabetes mellitus (type I and II) was identified using the patient registers and medical birth registers (except for Denmark) and included as a confounder between the use of high-dose folic acid and the risk of cancer, both childhood cancer and maternal cancer.[257,258]

For study I, maternal diabetes mellitus was defined as any registration of ICD-10 codes E10–E14 (type I or II diabetes) or O24.4 or O24.9 (gestational diabetes) in the patient registers at any time from one year prior to LMP and until the date of birth, or checkbox marked as "yes" for maternal diabetes in the medical birth registers.

For study II, maternal diabetes was defined as any registration of ICD-10 codes E10–E14 (type I or II diabetes) in the patient registers or checkbox marked "yes" for maternal diabetes in the medical birth registers. A diagnosis of maternal diabetes mellitus was assumed to be the indication for high-dose folic acid use after the first pregnancy throughout follow-up.

Maternal Inflammatory Bowel Disease (paper II)

Maternal inflammatory bowel disease was identified in the national patient registers, defined as any registration of ICD-10 codes K50–51, at any time before the date of delivery for the first pregnancy and until the date of birth. Inflammatory bowel disease was added as a covariate as it is associated with use of high-dose folic acid in pregnancy and increases the risk of intestinal cancer.[82,259]

Maternal Epilepsy (paper I and III)

Maternal epilepsy was identified in the patient registers and defined as any registration of ICD-10 codes of G40 or G41 prior to the date of giving birth, or in the medical birth registers (except for Denmark) ("yes"/"no").

Maternal Body Mass Index (paper I and II)

Maternal body mass index (BMI) was identified in the medical birth registers at the start of pregnancy and used to categorize obesity. Obesity was defined as BMI equal to or higher than 35 kg/m^2 ("yes"/"no"), as well as missing information on BMI.

For paper II, we used information obtained during the first pregnancy only.

Maternal Smoking Status (paper I and II)

The medical birth registers were used to retrieve information on maternal smoking status during the beginning of pregnancy and categorized into "yes" or "no" as well as missing information. For paper II, we used information recorded in the first pregnancy.

Maternal Hospital Admissions (paper I and II)

The number of hospital admissions prior to date of birth was retrieved from the patient registers and used as a proxy for maternal comorbidity. We categorized them into 0 admissions, 1 admission, or $\geq$2 admissions. In paper I, we included hospitalizations occurring within one year prior to the date of birth. In paper II, we included hospitalizations occurring within two years prior to the date of birth.

Maternal Tuberous Sclerosis (paper I)

Maternal tuberous sclerosis was defined as any registration of ICD-10 code Q85.1 prior to date of delivery and identified in the national patient registers. Maternal tuberous sclerosis was adjusted for as a potential confounder between the association between exposure to high-dose folic acid and childhood cancer.[260,261]

Demographical and Other Covariates

Maternal Educational Level (paper I and II)

The highest completed education at the date of giving birth was identified in the national statistical agencies. Completed educational level was categorized into compulsory, pre-university, college/university, and postgraduate, as well as missing information.

Parity (paper II)

The number of deliveries during the follow-up period was identified in the medical birth registers. To account for the number of childbirths might change throughout the follow-up period, it was defined as a time-varying variable. We identified parity as a confounding factor due to studies suggesting a dual impact on the risk of certain cancers such as breast cancer: it may initially increase the risk of cancer following childbirth, but subsequently reduce the risk of cancer in the long term.[262,263]

Maternal Age (paper I and II)

Increasing maternal age is associated with an increased risk of cancer in both the child and the mother.[264] In paper I, we adjusted for maternal age in the analyses. In paper II, we used maternal age as the time scale acknowledging that individuals entering the study at an older age face a higher risk of cancer during the time of follow-up compared to individuals entering at a younger age.

Statistical Methods

Stata version 16 was used for paper I, and version 17 for paper II and III.[265,266]

For both paper I and II, a survival analysis was used to deal with the time-to-event nature of the study objective, assessing the risk of cancer after exposure to high-dose folic acid ($\geq$1 mg), in which the time from exposure to event differs from person to person. The term survival does not mean the survival of the participant, but that the

event of interest, cancer, has not yet occurred. Using a survival analysis offers the advantage of accounting for censoring, meaning handling of situations where individuals exit the study before the event of interest occurs, this being emigration, death, or no diagnosis of cancer until end of follow up (December 31st, 2017).

The survival analysis applied for both paper I and II was the Cox proportional hazards model.[267] The Cox model is especially useful when investigating the relationship between independent variables and the hazard rate, which is a representation of the risk of an event occurring at a specific time. As the Cox model does not assume any specific distribution for survival times, it is considered a semi-parametric model offering flexibility in adapting to a range of different types of data and distributions. A Cox model has in addition the capability of accommodating the effect of covariates that change status over time, allowing for a dynamic modelling of risk factors. For paper II, we updated the exposure status of high-dose folic acid to reflect that a participant's use of high-dose folic acid could shift from unexposed to exposed after the start of follow-up, based on the age at which the mother filled the first prescription for high-dose folic acid.

The Cox proportional hazards model supports the inclusion of adjustments for confounders. In each regression model, we applied certain as strata to account data heterogeneity: Source country was applied to address differences across country of origin (paper I and II), child sex to consider variations between the sexes (paper I), and year of delivery to accommodate for temporal changes throughout our study period (paper I and II).

To handle missingness in the data, we used multiple imputations by chained equations.[268,269] This is a technique used to impute (replace) missing values in a dataset with multiple sets of plausible values, thus creating complete datasets with the imputed values. For both paper I and II, 20 iterations were applied. These imputed datasets were then analyzed separately, with the results combined at the end to provide more accurate and robust estimates. We made imputations for missingness on maternal BMI (29% missingness in paper I and II), maternal smoking status (8% missingness in

paper I and II), and for maternal educational level (3% missingness in paper I and 4% in paper II).

Ethical Approvals and Considerations

Ethical approvals were obtained in accordance with the requirements of the three participating countries, ensuring compliance with each nation's data protection laws and the broader general data protection regulation (GDPR) framework. Central to the project's governance is the Data Protection Agreement, a multilateral agreement endorsed by the institutional representants involved in the SCAN-AED project, delineating the responsibilities and procedures concerning data management within the project. Data transfer agreements were established between Aarhus University Hospital and each institution exporting the data. We have done a Data Protection Impact Assessment (DPIA) ensuring a thorough evaluation of privacy risks associated with data processing activities. The DPIA was assessed by the Personal data protection officer at the University of Bergen and approved by the University of Bergen. Approval details include the Data Protection Agency and Aarhus University Hospital in Denmark (project number 618541), the Norwegian Data Inspectorate and the Regional Ethics Committee for Medical Research in Western Norway (approval ID 2017/1196), and the Regional Ethical Board at The Karolinska Institute in Sweden (approval ID 2017/1420-31).

As the data used for this PhD are purely register-based, leveraging data that already have been mandatorily registered, thus the need for an individually informed consent was waived by the approving committees by law (in Denmark). However, it is potentially possible to identify individuals in the registers due to the vast amount of information contained in the dataset consisting of detailed information from several nationwide registers. In the Nordic nations, each individual is assigned a unique personal identification number by the government upon birth or immigration. Prior to transmitting data from the data holders to the project, the personal identification numbers were pseudonymized by substituting them with project-specific identification numbers, and in Norway reference dates is further applied to secure anonymization of

the individuals. The linkage encryption key between the personal identification numbers and the project-specific identification numbers is securely maintained by the data holders within their respective countries and is not accessible to the people who have analyzed data within the project. To safeguard personal data within the project, all information has been securely stored on a server at Statistics Denmark, with access granted solely to a restricted group of researchers. It is strictly forbidden to combine information to identify individuals according to the data access regulations provided by Statistics Denmark. Exporting data related to individual persons or any files containing project-specific IDs is not permitted. We have refrained from exporting tables containing results with fewer than five individuals in a cell or graphs displaying less than five individuals within a group, in order to prevent the possibility of reverse identification.

Performing studies on whether use of high-dose folic acid in pregnancy increases the risk of cancer, a devastating disease, might concern and even further complicate the dialogue between prescribing clinician and the pregnant women. This aspect is discussed in further detail in the Clinical implications section at the end of the Discussion section, chapter 6.

Chapter 5

Summary of Results

Paper I – Cancer Risk in Children of Mothers With Epilepsy and High-Dose Folic Acid Use During Pregnancy

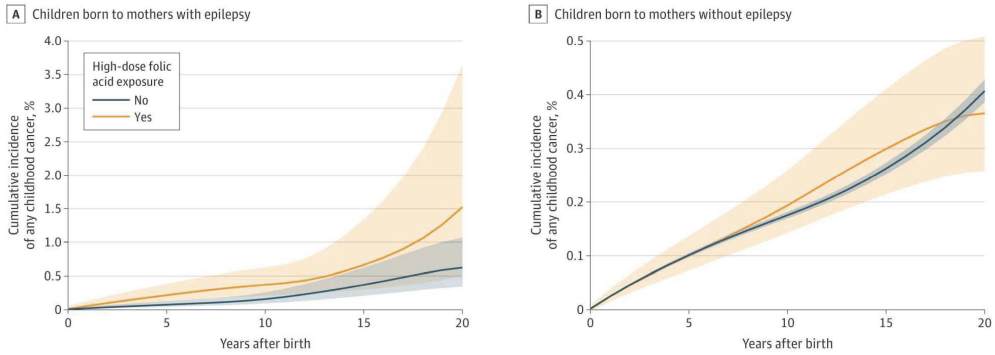
From a total of 3,379,171 children in Denmark (1997–2017), Norway (2005–2017), and Sweden (2006–2017), we identified 27,784 children born to mothers with epilepsy (0.8%) among whom 5,934 children (21.4%) were prenatally exposed to high-dose folic acid (≥1 mg daily). Among children in the general population born to mothers without epilepsy, only 1.4% were prenatally exposed to high-dose folic acid. Mothers with epilepsy using high-dose folic acid had a higher mean daily folic acid dose (4.3 mg) compared to those without epilepsy (2.9 mg). The median-follow-up time was 7.3 years (interquartile range (IQR) 3.5–10.9 years) for the entire cohort.

Children born to mothers with epilepsy who used high-dose folic acid had an incidence rate of childhood cancer of 42.5 cases per 100,000 person-years, compared to 18.4 cases per 100,000 person-years in children of mothers with epilepsy not exposed to high-dose folic acid (Figure 2). The absolute risk of cancer in children between birth and 20 years of age who were born to mothers with epilepsy treated with ASM during pregnancy was 0.6% (95% CI 0.3–1.1%), compared to 0.4% (95% CI 0.3%–0.5%) in children of mothers without epilepsy. Prenatal exposure to high-dose folic acid was associated with an aHR of 2.7 (95% CI 1.2–6.3) in children born to mothers with epilepsy compared to unexposed children of mothers with epilepsy. Leukemia was the most common cancer type in children born to mothers with epilepsy using high-dose folic acid, constituting 40% of all cancers in this group.

Children with an average prenatal exposure ≥4 mg folic acid daily had an aHR for cancer of 3.4 (95% CI 1.1–10.7), whereas those exposed to an average daily dose <4 mg had an aHR of 2.9 (95% CI 1.2–7.2). This difference was not statistically significant. The risk of cancer in children exposed to ASM, regardless of maternal epilepsy or not and high-dose folic acid prescription fill, yielded an aHR of 1.5 (95% CI 1.1–2.1) with no statistically significant differences in cancer risk between specific

ASM monotherapies or polytherapy. Maternal epilepsy alone was not associated with childhood cancer risk (aHR, 1.0, 95% CI 0.7–1.4).

Figure 2: Cumulative incidence of Childhood Cancer (Paper I)



Adapted from Vegrim et al.[270] Cumulative incidence of childhood cancer diagnosed between the ages of one and 20, born to mothers with or without prescription fill(s) for high-dose folic acid. Separate graphs for children to mothers with a diagnose of epilepsy (A) or no such diagnosis (B).

Paper II – High-Dose Folic Acid Use and Cancer Risk in People Who Have Given Birth: A Register-based Cohort Study

Counts presented for paper II are rounded as the manuscripts await final publication.

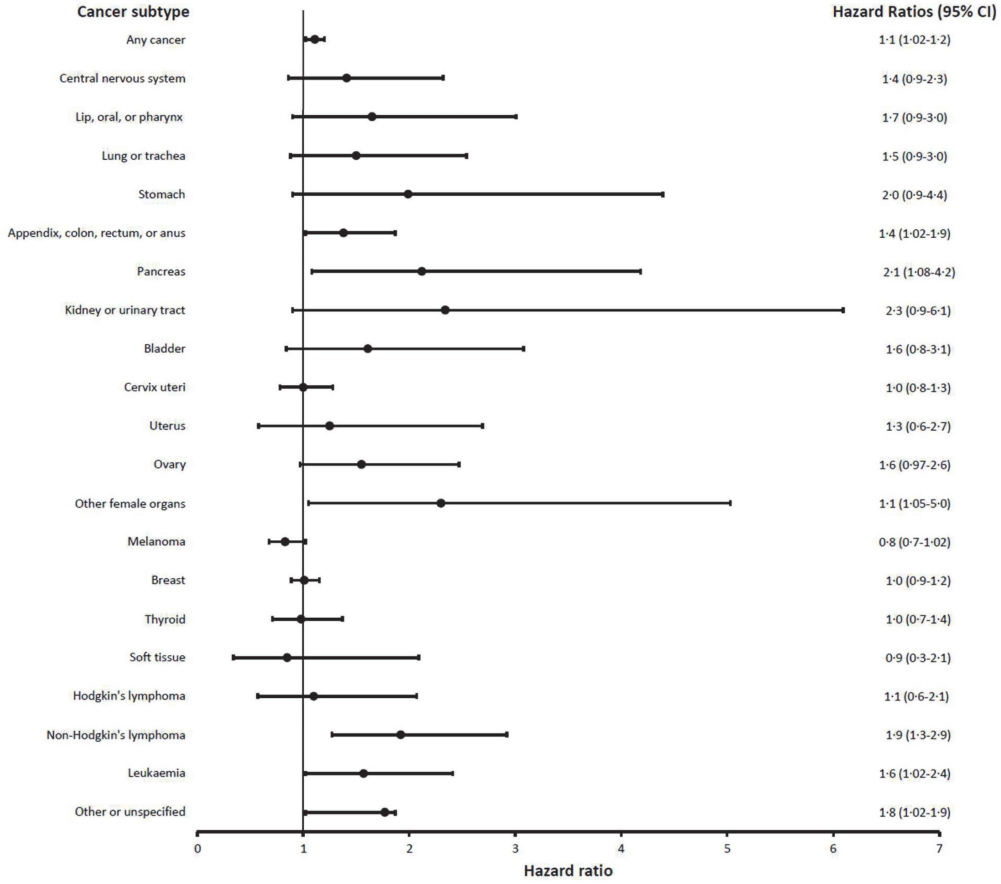
From a total of 1,465,790 women who gave birth in Denmark, Norway, and Sweden, we identified 64,490 (4.4%) women who had used high-dose folic acid during the time of follow-up. The median follow-up time was 5 years (IQR 2–8 years) for high-dose folic acid users and 7 years (IQR 4–11 years) for the entire cohort. We identified 760 cancer cases among the exposed group of women and 18,700 cases among the unexposed, corresponding to incidence rates of 208 and 164 cancer cases per 100,000 person-years, respectively. The most common cancer types in both groups were breast cancer (34.2% among exposed, 35.8% among unexposed), melanoma (13.2% among exposed, 19.6% among unexposed), and cervical cancer of the uterus (9.2% among exposed, 11.7% among unexposed).

We found that high-dose folic acid use was associated with an increased overall cancer risk (aHR 1.2, 95% CI 1.1–1.2). To lower the probability that the risk was due to doctors prescribing folic acid in response to symptoms that are later revealed to be caused by cancer, we subtracted six months of follow-up time from the analysis. This subtraction of follow-up time was also done to account for the unlikely case that cancer occurs immediately after medication exposure. This subtraction of six months of follow-up time slightly attenuated the association (aHR 1.1, 95% CI 1.0–1.2). High-dose folic acid exposure was associated with increased risk for stomach cancer (adjusted HR 2.2, 95% CI 1.1–4.7, p=0.04), cancer in the appendix, colon, rectum, or anus (adjusted HR 1.6, 95% CI 1.2–2.1, p=0.002), non-Hodgkin's lymphoma (adjusted HR 2.0, 95% CI 1.3–2.9, p=0.0009), and leukemia (adjusted HR 2.0, 95% CI 1.3–2.9, p=0.0006). The risk of non-Hodgkin's lymphoma was the only cancer subtype that remained significantly elevated after subtracting six months of follow-up time (adjusted HR 1.9, 1.3–2.9, Figure 3).

We did not observe any significant protective association between high-dose folic acid on cancer risk. No dose-response relationship emerged between cumulative folic acid dose and cancer risk. A secondary analysis stratified for mothers using ASM during

their first pregnancy, comparing those with and without use of high-dose folic acid, revealed an overall cancer risk with an adjusted HR of 1.2 (95% CI 0.9–1.6), hence not significantly different from the risk of folic acid supplementation observed in the general population.

Figure 3: Risk of cancer in women using high-dose folic acid after six months of follow-up subtracted (Paper II)



Forrest plot illustrating the risk of cancer comparing women who have given birth who have used high-dose folic acid, compared to unexposed women who have given birth. Six months of follow-up time has been subtracted as cancer is not likely to develop immediately after high-dose folic acid exposure, thus reducing the chance of reverse causation. Only the risk of non-Hodgkin's lymphoma remained significant after Bonferroni correction of p-values.

Paper III – Prescribing Patterns for Higher Dose Folic Acid In Pregnant Women with Epilepsy Treated with Antiseizure Medication

Counts presented for paper III are rounded as the manuscripts await final publication.

From a total of 2,748,880 pregnancies in Denmark, Norway, and Sweden that were registered between 2006–2017, we identified 8,700 pregnancies (0.3%) in mothers with ASM-treated epilepsy. Among these 4,720 pregnancies (54.3%) were supplemented with high-dose folic acid, while 3,980 pregnancies (45.7%) were not. The highest proportion of pregnancies in women with ASM-treated epilepsy supplemented with high-dose folic acid was observed in Sweden (74.2%, 2,940 out of 3,960 pregnancies), followed by Norway (41.4%, 920 out of 2,220 pregnancies), and Denmark (34.4%, 870 out of 2,530 pregnancies) (Figure 4). The proportion of pregnancies supplemented with high-dose folic acid in women with ASM-treated epilepsy remained stable in Sweden but was decreasing in Denmark and Norway from 2012 and until the end of follow-up.

The proportion of pregnancies supplemented with high-dose folic acid was equally high in all three countries when the woman was treated with carbamazepine or valproate. While the proportion of pregnancies supplemented with high-dose folic acid in Sweden was high regardless of type of ASM used, supplementation with high-dose folic acid in pregnancies in Denmark and Norway was lower if treated with lamotrigine, levetiracetam, oxcarbazepine, and topiramate monotherapy.

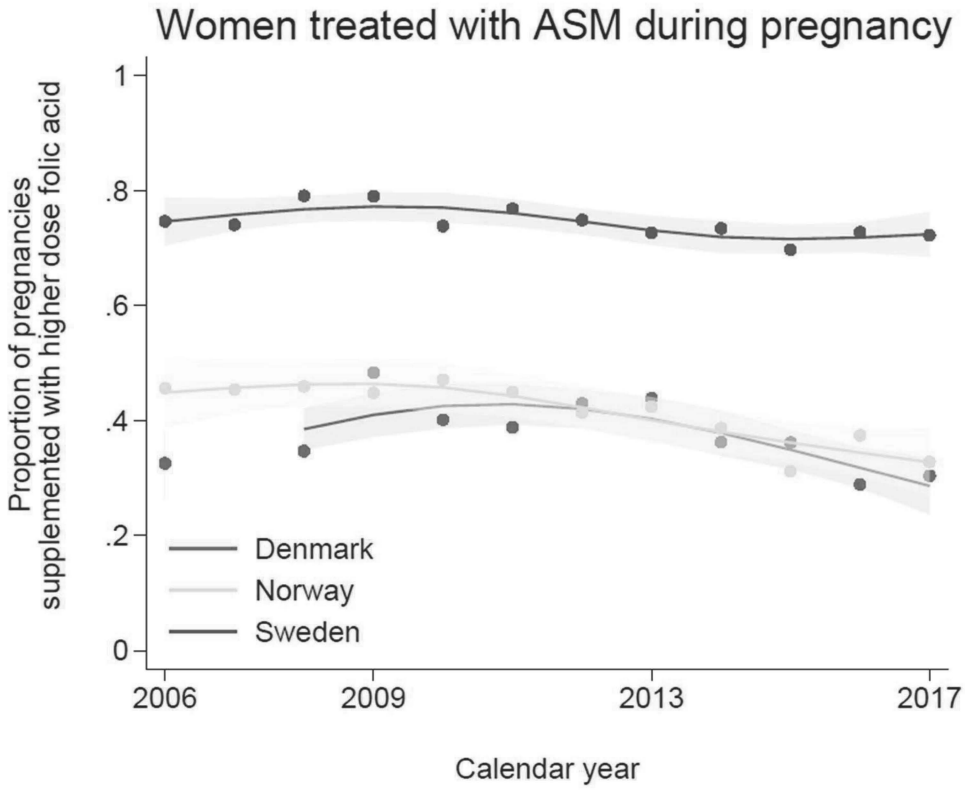
Regardless of concomitant high-dose folic acid supplementation, a higher proportion of pregnancies in individuals with ASM-treated epilepsy in Sweden were prescribed carbamazepine (22.0%) and valproate (12.2%) compared to Denmark (4.7% and 7.1%, respectively). Lamotrigine (55.7%) and levetiracetam (11.5%) were the most commonly prescribed ASMs during pregnancy for women with epilepsy, but less in Sweden (43.3% and 8.6%, respectively) than in Norway and Denmark.

We observed that 48.6% (n=2,220) pregnancies to mothers with ASM-treated epilepsy in Norway used over-the-counter folic acid supplements and filled no prescriptions for either 1 mg or 5 mg folic acid during pregnancy, while 10% did not use any folic acid

at all. The proportion of non-supplementation varied by ASM type, with the lowest rate (5%) among those treated with valproate during pregnancy.

Among pregnancies in women with ASM-treated epilepsy who were supplemented with high-dose folic acid and with a one-year lookback prior to the last menstrual period and until birth, 42% (2,100 out of 4,990 pregnancies) did not fill the first prescription for high-dose folic acid until after the pregnancy had started.

Figure 4: Proportion of pregnancies to women with ASM-treated epilepsy that were supplemented with high-dose folic acid during pregnancy (Paper III)



Logistic regression with restricted cubic splines were used to predict the proportion of pregnancies to women with ASM-treated epilepsy that were supplemented with high-dose folic acid during pregnancy with corresponding 95% CIs. Observed proportion have been plotted as a scatter plot.

Chapter 6

Discussion

In this thesis using an extensive, multinational dataset, we found robust associations between the use of high-dose folic acid (≥1 mg daily) in relation to pregnancy and increased risks of new cancers during the following years in children born to mothers with epilepsy and in women who have given birth in the general population who had used high-dose folic acid. The use of high-dose folic acid in pregnancy varied widely in women with ASM-treated epilepsy between Denmark, Norway and Sweden, countries which share similar demographic characteristics and health care systems. The studies conducted during this PhD are to the best of our knowledge the largest population-based studies hitherto to have investigated the association between the use of high-dose folic acid during pregnancy and risk of cancer using the relevant study population, i.e., women who have given birth.

To be able to evaluate the findings and conclusions of this thesis, it is important to contextualize the results and review strengths and weaknesses of the materials and methods used.[271] The current and potential implications associated with this work will be discussed at the end of this chapter.

Results in context

Use of Folic Acid During Pregnancy and Risk of Childhood Cancer

We identified a close to three-fold increased risk of cancer in children born to mothers with epilepsy who used high doses of folic acid (≥1 mg daily) during pregnancy. The risk of cancer was not attenuated when adjusting for important confounding factors such as concomitant use of ASM, or excluding several important mediators: diagnosis of congenital anomalies or chromosomal abnormalities,[255] maternal treatment with valproate or carbamazepine, maternal cancer, maternal tuberous sclerosis,[260,261] and maternal diabetes mellitus.[257,258] The association between high-dose folic acid and cancer could not be explained by maternal use of ASM alone as we did not observe any significantly increased risk of cancer in children to mothers with ASM-treated

epilepsy who did not take high-dose folic acid supplementation during pregnancy. Furthermore, a maternal diagnosis of epilepsy was not associated with the cancer risk.

Few studies have examined the association between prenatal exposure to high-dose folic acid (≥1 mg daily) and risk of childhood cancer. However, there have been conducted several studies assessing the risk of childhood cancer with similar study design associated with in utero exposure to 0.4-0.8 mg folic acid daily. This lower folic acid dose is recommended in the general population before and during pregnancy.[23,272-274] The daily doses used in our papers were up to 5 mg daily, that is up to 13 times higher than the regular 0.4 mg recommended for the general population. One systematic review and meta-analysis from 2019 on maternal intake of folic acid and risk of childhood brain- and spinal cord tumors showed a reduced risk of these cancer types (OR 0.77, 95% CI 0.67-0.88).[275] However, the authors did not assess risk associated with different doses of folic acid. They only included one case-control study with a daily folic acid dose >1 mg which assessed the risk of only one type of cancer, astrocytic glioma.[276] However, there was no significant increased or reduced risk for such gliomas. The meta-analysis and systematic review also included a population-based study using the medical birth register of Norway which reported no association between periconceptional use of folic acid and an increased risk of any childhood cancer or cancer subtypes.[23] However, the authors were not able to assess the risk associated with daily folic acid intake above 0.6 mg (both 0.4 mg folic acid and multivitamins containing 0.2 mg folic acid), and they relied on self-reported usage prone to reporting bias and omission bias. The group of "non-users" is a mixture of mothers not using folic acid and with missing information on folic acid use, which could cause bias in the association towards the null. A study on missingness on folic acid use based on data about self-reported folic acid use in the Medical Birth Register of Norway reported that 12% of all births had missing information on folic acid usage.[277] However, the risk of recall bias was low as the information of folic acid use was collected in the first trimester. Another systematic review and meta-analysis of case-control studies from 2019 found a protective effect of folic acid used in pregnancy on the risk of childhood acute lymphoblastic leukemia (OR 0.75, 95% CI 0.66-0.86), but no significant association for other types of cancer. However, they did

not assess the risk for specific doses of folic acid.[273] A protective association between supplementation with folic acid during pregnancy and childhood acute lymphoblastic leukemia was observed in a 2001 case-control study. The study lacked information on what folic acid doses that had been used and the length of folic acid treatment.[278]

A more recent population-based case-control study in Denmark investigating the risk of childhood cancer associated with maternal anemia found an elevated cancer risk in children born to the mothers who were supplemented with vitamin supplements containing folic acid (OR 4.03, 95% CI 1.91-8.50) compared to children to mothers with no such supplements in pregnancy.[279] However, they did not have access to information about folic acid supplementation higher than 0.4 mg daily. The results may be prone to confounding by indication since it is likely that the mothers with severe anemia are more likely to receive active treatment with vitamin supplements and in higher doses, with maternal anemia being reported to be associated with increased risk of cancer in their children.[280]

Use of Folic Acid During Pregnancy and Maternal Cancer Risk

We observed a 1.2-fold increased risk of cancer in mothers who had used high-dose folic acid and a 2.0-fold increased risk of non-Hodgkin's lymphoma, compared to mothers who had not used high-dose folic acid. The risk of non-Hodgkin's lymphoma withstood adjustment for confounding factors such as use of ASM and after subtracting six-months of follow-up time to reduce the chance of reverse causality and Bonferroni correction for multiple comparisons. However, our analyses on cumulative dose of high-dose folic acid did not reveal any dose-response association between high-dose folic acid use and risk of cancer. This study is the largest study to examine the risk of maternal cancer other than breast cancer associated with the use folic acid doses equal to or higher than 1 mg daily in premenopausal women.[233]

The results of previous studies on the risk of cancer related to folic acid use in pregnancy have been conflicting. A large meta-analysis and systematic review from 2013 on the effects of folic acid supplementation on cancer risk included approximately 50,000 participants from 16 randomized trials before 2011. The study

showed no increase or decrease in the incidence of overall and site-specific cancers during the first five years after treatment (relative risk 1.06, 95% CI 0.99-1.13).[25] The daily dose of folic acid ranged from 0.5 to 5 mg daily except for one study applying 40 m daily. Only four of the included studies utilizing doses less than 1 mg daily. Two thirds of the participants were men and the mean age at study entry was 64 years (SD 10 years). The lowest mean age at study entry in of the included studies for the meta-analysis was 52 years (SD 15 years) with 51% of the participants were males and the study was primarily designed to examine risks related to venous thrombosis.[281] The only study included in the meta-analysis with a majority of women had a mean age at study entry of 65 years (SD 7 years), which found no association between use of 1 mg folic acid daily and risk of colorectal adenoma.[282] Despite the meta-analysis commented on the use of folate in relation to pregnancy, none of the included studies assessed the risk of cancer due to folic acid use in pregnant populations. A Norwegian study which included participants with ischemic heart disease treated with B-vitamins from 1998 to 2005 found an 1.38-fold increased risk of cancer (95% CI 1.07–1.79) in the B-vitamin treated group, with the risk mainly driven by an increased incidence of lung cancers.[221] This study did not include doses of folic acid higher than 0.8 mg daily. The mean age at study entry was 62 years (SD 11 years) and only 23.5% of the study participants were women.

A common limitation in previous studies addressing the safety of folic acid use during pregnancy in terms of cancer risk has been that the risk has not been assessed in the relevant population of pregnant women. Furthermore, previous studies lack information on folic acid doses $\geq$1 mg daily. However, in 2004, a short report on the Aberdeen folic acid supplementation trial found doubled risk of breast cancer and five times higher mortality rate in young mothers who had used high-doses of folic acid during pregnancy.[283] This RCT study included 3,187 women who had been enrolled for antenatal care between 1966 and 1967 and who were randomly assigned to one of three groups: 0.2 mg of daily folic acid (466 individuals), 5 mg of daily folic acid (485 individuals), or a placebo (1,977 individuals). The women started their supplementation at less than 40 weeks of gestational age and continued until delivery. At the beginning of the trial, the participant had a mean age of 25-26 years of age (SD

5.2-5.6 years). However, the report did not report information about the exact risk estimates and did not describe precise group comparisons that were used. A follow-up study on the same cohort conducted in 2014 reported no evidence of an excess risk of morbidity or mortality in either the group receiving 5 mg of folic acid daily or the placebo group when compared with the low-dose group.[233]

A study with similar design including mothers identified in the Norwegian Medical Birth Registry from 1999 to 2010 consisting of 429,004 women (mean age 29 years of age) and 3781 cancer cases, found no association between the use of folic acid and risk of maternal cancer.[24] However, they reported an aHR of 1.09 (95% CI 1.01-1.17) for folic acid use during the first pregnancy, and this risk was only marginally attenuated in the fully adjusted model (aHR 1.08, 95% 1.00-1.18). There was no association between cancer and folic acid use in later pregnancies (two or more pregnancies (fully aHR 1.06 (95% CI 0.91-1.22)). They did not report the risk regarding any folic acid use regardless of the number of pregnancies per mother. They stated no association between folic acid use and risk of 13 different cancer subtypes, including non-Hodgkin's lymphoma (HR 1.34, 95% CI 0.81-2.23). However, they did find an increased hazard ratio for colorectal cancer in mothers who had used folic acid during two or more pregnancies in fully adjusted models (HR 1.96, 95% CI 1.10-3.50, p-trend 0.16). They had no information on folic acid dose, and the great majority of women probably used the regular dose of 0.4 mg daily. The definition of cancer outcome was only based on a single ICD registration of cancer in the Norwegian Cancer Registry, and they did not fortify their definition of cancer with information found in the ICD-O/3. Additionally, they adjusted for maternal age and did not use age as the time scale, meaning that they did not fully consider the increased risk of cancer with start of supplementation at a higher age.

The risk of colorectal cancer due to the use of folic acid has been of particular concern. In a 2007 review it was described how folic acid may promote carcinogenesis in preexisting neoplastic tissue which otherwise would not develop into cancer based on both human and animal studies.[284] However, clinical studies have been inconclusive. Meta-analyses and systematic reviews have reported both an increased risk and no

association between folic acid use and risk of colorectal cancer.[25,216] In our study, we identified an increased risk of cancer in the appendix, colon, rectum, or anus in the fully adjusted model (HR 1.6, 95% CI 1.2-2.1, p=0.002). However, this risk was not significant after Bonferroni correction for multiple comparison (p-value not less than 0.002), and the risk did not persist after subtracting six months of follow-up between the first prescription fill for high-dose folic acid and date of cancer diagnosis (HR 1.4, 95% CI 1.0-1.9).

High-Dose Folic Acid Use in Pregnancy in Women With ASM-Treated Epilepsy

We observed that the proportion of pregnancies in women who had ASM-treated epilepsy supplemented with high-dose folic acid varied between Denmark, Norway and Sweden, despite similar demographical properties and healthcare systems. Most of such pregnancies were supplemented with high-dose folic acid in Sweden, but in contrast, less than half received such supplementation in Norway and only one-third in Denmark. The proportion of pregnancies supplemented with high-dose folic acid was high in all countries for those ASMs that were specifically mentioned as an indication for high-dose folic acid supplementation in the guidelines in all three countries: carbamazepine and valproate. In Norway, we found that approximately half of the pregnancies among mothers with ASM-treated epilepsy opted for over-the-counter low-dose folic acid and did not fill prescriptions for higher doses (1 mg or 5 mg) during pregnancy. About 10% avoided folic acid supplementation entirely and it was least common to not use folic acid supplementation in women treated with valproate during their pregnancy. Among the pregnancies which were supplemented with high-dose folic acid, such supplementation was not initiated until after the pregnancy had started in 42% of the pregnancies.

Our findings align with the results in a recent UK study which reported that 54% (n=183) of the examined women on ASM treatment did not start supplementation with folic acid until after pregnancy had started.[285] Among those who were supplemented, they found no difference in the initiation of folic acid supplementation comparing mothers who were supplemented with $\geq$5 mg daily versus <5 mg daily. This study

reported that it was most common to use folic acid if treated with carbamazepine (65%). This is similar to our results where approximately 70% of those treated with valproate or carbamazepine were supplemented with high-dose folic acid during pregnancy. A lower rate of folic acid supplementation during pregnancy in women with epilepsy has been reported from low-income countries. In a study about reproductive health challenges in women with epilepsy in sub-Saharan Africa, only 25% of all women with epilepsy were supplemented with folic acid prior to conception.[286]

The observed patterns for high-dose folic acid supplementation in pregnancies to mothers with ASM-treated epilepsy aligned with the national guidelines applicable in Scandinavia during the included time period of our paper. The high proportion of pregnancies that received high-dose folic acid supplementation across all ASM therapies in Sweden reflects the guidelines that were applicable in the country during the time of registration, recommending 5 mg for all types of ASMs.[116,287,288] In Denmark and Norway, we observed a high proportion of pregnancies supplemented with high-dose folic acid for ASMs that were specifically mentioned in the national guidelines. However, the proportion that were supplemented if treated with other types of ASMs varied between the countries and during the time-span of registration.[111,114,115,289,290] Norwegian clinicians and patients had access to several clinical guidelines with slightly different wording as to how and when women with epilepsy should be supplemented with folic acid during pregnancy. We observed a decline in the proportion of pregnancies supplemented with high-dose folic acid in Denmark and Norway. This trend may align with the decreased use of valproate and carbamazepine during pregnancy - two of the ASMs specifically mentioned in the Danish and Norwegian clinical guidelines for high-dose folic acid supplementation – over the course of our study.[291] Although our SCAN-AED data were recorded until the end of 2017, it is worth noting that the Swedish guidelines changed from 5 mg for all ASMs to 0.4 mg regardless of treatment with ASM during pregnancy in 2019. The lack of evidence for the optimal folic acid dose has been stated in all Swedish guidelines, ultimately being the cause for the change from 5 mg daily to 0.4 mg daily during pregnancy if treated with an ASM. Guidelines on folic acid supplementation for

women treated with an ASM during pregnancy vary also internationally, reflecting the lacking evidence about the optimal dose and timing of folic acid and the need to balance potential beneficial and adverse effects (Table 1, page 30).

Methodological Considerations

In clinical epidemiology, it is crucial to implement rigorous methodology, to embrace diverse perspectives from different specialties, and to implement robust data validation techniques to effectively handle bias and avoid errors, thereby ensuring the integrity and accuracy of research findings that could impact public health decisions.[271] We have tried to follow these principles during the conduct of the studies in this thesis. However, we acknowledge that there are limitations that we were not able to account for. In this section, the methodological considerations will be discussed in further detail.

Data Sources

All three studies in this thesis were conducted using data from the SCAN-AED project, and derived from nationwide registers in Denmark, Norway, and Sweden. These registers are mandatory and collect information regarding several health aspects on an individual level, and then stored in specific health registers.[234] These high quality resources provide the possibility to perform large population-based studies with long and complete follow-up.[234,292] Although RCTs are considered the gold standard for study design in medical research, RCTs often face limitations such as a limited follow-up period, high resource costs, limited generalizability, and stringent participant selection.[293] Larger, specific cohorts that collect prospective data over extended periods and offer detailed clinical information, such as the Mother, Father, and Child study in Norway or The Neurodevelopmental Effects of Antiepileptic Drugs Study in the USA and UK, encounter several other challenges.[14,294,295] These challenges include potential information bias (like recall or reporting bias), limited generalizability and external validity due to self-selection of participants, and non-random loss to follow-up. The restricted focus of these selected cohorts may impact

the researchers' ability to adjust and stratify for relevant covariates, as well as influence the choice of relevant outcomes to investigate.

Register-based research using nationwide data has several advantages compared to controlled trials and specific cohorts. First, these large studies encompass entire populations, in our case, every pregnant woman and their child who are enrolled in the mandatory and nationwide medical birth registers in Denmark, Norway and Sweden. Pregnant women not included in the medical birth registers are women who experience early abortions before the 12th week of gestation in Norway and before the 22nd week of gestation in Denmark and Sweden. This enabled us to investigate associations between rare exposures and rare outcomes in a specific group of individuals. In our case, we were able to examine the use of high doses of folic acid during pregnancy and the risk of cancer in the mother and child. Second, conducting a trial specifically designed to evaluate the risk of cancer associated with pharmaceutical exposure in pregnant individuals would be considered ethically inappropriate. Third, cancer may occur several years after the medication exposure. Fourth, the use of nationwide registers limits the risk of selection bias, differential misclassification, and eliminates recall bias, as the data are prospectively and mandatorily collected, and with exposure and outcome recorded independently. Last, performing register-based studies are cost- and time-effective relative to clinical trials and specific cohorts as the data are more readily available. However, study designs that are typically applied to such register-based research, like the observational cohort study design applied for our paper I and II, cannot establish causal relationships between an exposure and an outcome like an RCT, but rather report study associations of relationships between exposures and outcomes. Depending on how observational cohort studies are designed, the directionality of the association can be assessed, thus be an indicator of the potentially true causal pathway.

The Nordic health registers are primarily designed for administrative purposes and not necessarily for clinical research, except for the medical birth registers and the national cancer registers.[296] Disadvantages of register-based research used in this PhD include different degree of validation of the registered information. However, the key variables

used for this thesis, pharmaceutical exposure of folic acid, occurrence of cancer, and a diagnosis of epilepsy, are deemed to have high validity in the registers used.[239,248,297,298]

Misclassification

Information found in the nationwide registers may in some instances contain incorrect registrations that can lead to misclassification of the exposure, the outcome, or other relevant variables.[236] Registration errors can be random or systematic, depending on the situation. For example, we observed in our data that registration of cervical cancer of the uterus in Sweden seemed to have been done administratively as a part of the national screening program for cervical cancer, while the same practice was not common in Norway. When we added precise information of tumor behavior, the incidence rate per 100,000 person years became similar in the two countries, as expected.

Misclassification is categorized into two types: differential and non-differential misclassification. In non-differential misclassification, the error is evenly distributed between the exposed and unexposed groups, whereas in differential misclassification, the error predominantly affects one direction.[271] While non-differential misclassification usually does not cause bias or bias towards the null, differential misclassification can lead to bias in both directions; both over- and underestimation of the actual outcome.

The main exposure in all three papers included for this thesis was prescription-based folic acid, consisting of doses of either 1 mg or 5 mg, and with information retrieved from the national prescription registers. The specific exposure definitions and windows used in each of the papers have been explained in detail in the Materials and Methods section. Women were regarded as exposed after they had filled at least one prescription of high-dose folic acid in relation to pregnancy. However, some individuals in the study population might have been misclassified as exposed if they never took the medication even though they retrieved the prescription from the

pharmacy. The comparison group was not truly unexposed to folic acid, only to high-dose folic acid, as it is generally recommended in all three countries to take low doses of folic acid (0.4 mg daily) during pregnancy. No prescription is needed for 0.4 mg tablets, and the purchase is not recorded in any registry. For women with epilepsy treated with ASM, it has been recommended to take 0.4 mg folic acid daily continuously during all their fertile years.[7] We cannot know if the woman did ingest the tablets that were dispensed or not, and we did not have data on serum concentrations of ASMs or for folic acid. Non-adherence is common in pregnant people due to fear of drugs being fetotoxic.[299] However, compliance has been shown to be higher (70-100%) in pregnant women that use drugs for chronic diseases such as epilepsy.[298] Furthermore, the indication for high-dose folic acid was to protect the child against fetotoxicity. The agreement between information on filled prescriptions recorded in the Swedish Prescribed Drug Register and antenatal use registered in the Swedish Medical Birth Register, was high for ASM, approximately 70%.[300] Studies examining the adherence to high-dose folic acid supplementation during pregnancy in women with epilepsy are scarce. In a Norwegian cross-sectional study using the MOBA data from 1999 to 2008, supplementation with folic acid was reported in 94% of the included pregnancies (n=208), with ASM concentrations being associated with vitamin B-status in blood: High serum concentrations with ASM correlated with high concentrations of UMFA and inactive folate metabolites.[98] In another cross-sectional study conducted in Saudi Arabia, an adherence rate of 64% to use of folic acid was observed in women with epilepsy during pregnancy.[301] The rate of adherence may have been influenced by sampling bias, the women included in the study receiving a more careful follow up than women with epilepsy in general.

Cancer was the main outcome in our first and second paper. Diagnosis of cancer has been shown to have a high validity in all the Nordic countries with a close to 100% completeness of incident solid malignancies in each of the cancer registers[248] To ensure that our outcome represented malignant cancer rather than less aggressive tumor forms (such as in situ, borderline, or benign tumors), we applied the ICD-O system, considering tumor behavior, location (topography), and structure (morphology). A malignancy was confirmed by an ICD-O value $\geq$3. Tumors that made

the transmission from borderline to malignant tumors were registered, and thus identified, in our analyses. It is unlikely that our cancers have been misclassified.

We defined maternal epilepsy as any single registration of either ICD-10 codes G40 (epilepsy) or G41 (status epilepticus) prior to delivery, or as any registration of maternal epilepsy in the medical birth registers, except for Denmark which do not register maternal epilepsy in the Danish Medical Birther Register. A 2009 study which evaluated the validity of maternal morbidities in the Medical Birth Registry of Norway, including maternal epilepsy, showed that a diagnosis of epilepsy had a sensitivity of 74%.[237] In this study, registration of maternal epilepsy in the Medical Birth Registry of Norway was compared to on whether an ASM prescription was reimbursed for epilepsy using the Norwegian Prescription Database as the reference standard. However, ASMs prescriptions might also be issued for women without a confirmed epilepsy diagnosis and the study did not capture mothers with untreated epilepsy. In Denmark, the diagnosis of epilepsy in the Danish Patient Register had a positive predictive value (i.e., the probability that the registration represents a true case of epilepsy) of 81% (95% CI 75–87%).[302] The positive predictive value would have increased even further if we had required two or more separate registration with a second diagnosis.[303] However, this could lead to loss of study participants who truly have epilepsy. The validity of diagnoses in the Swedish patient register is also considered to be high with a positive predictive value of any registered diagnosis in the register to be 85-98%.[250] However, the validity of epilepsy in the Swedish patient register was not mentioned specifically. While we did not require subsequent registrations of epilepsy in any of our studies, we required at least one filled prescription for ASM between date of LMP and delivery in paper III. The prevalence of maternal epilepsy in paper I of 0.8% reflects the expected amount of epilepsy in the general population from Denmark and Norway previously reported in other studies.[304-306] The limited number of maternal epilepsy cases that may still have been misclassified is likely to be due to non-differential misclassification, implying a bias towards the null.

Missingness

Missing data is common in register-based research, and if the missing data is related to the outcome of a study, it may cause bias.[307] Data can be missing in three ways: Missing completely at random, at random, or not at random.[308,309] Missing completely at random refers to the situation where the likelihood of missing data is entirely unrelated to both the observed and unobserved variables. Missing at random occurs when the probability of missing data is related to the observed variables, but not directly to the unobserved variables. In the situation of missing not at random, the probability of missing data is associated with the unobserved variables itself, which is challenging to address statistically. Missingness that is either completely at random or at random may reduce the precision and power of the analyses, whereas data missing not at random may introduce systematic bias. Often, missingness is a mixture of being completely at random, at random, and not at random. In paper I and II, the variables with a missingness $\geq$2.5% were maternal BMI (29%) which we deemed to be most likely missing not at random, maternal smoking status (8%) which was also viewed to be most likely missing not at random, and maternal educational level (3–4%), which can be both at random and not at random. In recent years with increasing demand on how to identify and deal with missing data to reduce potential bias, multivariate imputation by chained equations (MICE) has become a preferred technique in register-based research to handle missing data. This method is acknowledged to be flexible by being able to handle a wide range of different variable types.[268,269] Although there are no precise rules on when to make imputations for missing data, a rule of thumb has been to handle missingness if a variable has more than 5% missing.[269] However, we chose to make imputations for missingness for variables with more than 2.5% missingness since we had access to millions of observations, meaning that even a low percentage of missing observations can implicate thousands of missing observations. We observed that using MICE to handle missingness for maternal BMI, smoking status, and education, did not alter any of the estimates presented in either paper I or II. For covariates that are missing not at random, performing complete case analyses, meaning that we only keep observations with no missing values on relevant covariates, might not have given results that differed from using MICE.[309] But removing 29% of

our observations due to missing on maternal BMI would have yielded a crucial loss of statistical power, so performing a complete case analysis was not feasible when investigating the association combining a relatively rare exposure and cancer in children and young adults.

Confounding

Confounding is a crucial factor in medical research, also for observational studies which may cause bias by influencing variables that are independently related to both the exposure and the outcome, skewing the relationship if not handled appropriately.[310] It is important to ensure that studies are internally valid and that the association between exposure and outcome are reliably assessed. Although we have been able to control for many different confounders due to the access to an extensive dataset, there is always the possibility of unobserved and residual (imperfectly measured variables) confounding that we have not controlled for.[311]

There are several strategies on how to select the appropriate set of confounders.[312] In our paper, we used a technique called the directed-acyclic graphs (DAG) method. DAG is a preferred method in epidemiology to select covariates and a minimum adjustment set.[254,313] When choosing confounders to adjust for in an analysis, it is important to avoid overadjustment, as this may decrease the precision of estimates.[314]

The indication for high-dose folic acid before and during pregnancy is based on an already existing risk of health complications, not only for the child, but also for the mother.[82] The assessment of the indication for high-dose folic acid use includes factors such as maternal obesity, maternal smoking status, maternal diabetes mellitus, and the use of certain medications such as ASMs. All these factors are associated with an increased risk of adverse health outcomes including an increased risk of cancer. The potential links between these factors and cancer have been discussed in the Background and Materials and Methods sections. We added maternal age as a confounder in paper I, as increasing maternal age has been associated with an increased risk of cancer in their children, and can also increase the risk of maternal comorbidities contributing to the indication for high-dose folic treatment that we

otherwise were not able to identify and account for in our analyses.[264] In paper II, we applied maternal age as the time scale rather than the chronological time in years since the first prescription fill in relation to the first pregnancy. This was done as it is likely that a woman who became pregnant at age 40 at the start of follow-up will have a higher risk of cancer after five years of follow up due to increasing age, compared to a pregnant woman who was 18 years of age at start of the follow-up, as an example. Maternal educational level and number of hospital admissions prior to delivery were defined as potential confounders. Hospital admission was regarded as a proxy for having an illness increasing the indication for high-dose folic acid treatment. In paper I, we adjusted for major congenital anomalies, and in paper II, we specifically added maternal inflammatory bowel disease as a potential confounder as such disease is associated with an increased risk of cancer in the gastrointestinal tract.[259]

Although association between maternal use of high-dose folic acid before and during pregnancy in mothers with epilepsy and risk of cancer in their children was adjusted for concomitant use of ASM, we were not able to fully separate the effect of ASM and high-dose folic acid on the risk of childhood cancer due to the collinearity of the variables. This was because almost all mothers with epilepsy who were supplemented with high-dose folic acid during pregnancy were also treated with an ASM. Further, it is likely that the higher ASM dose that were applied during pregnancy, the more likely is it that the mothers would have been supplemented with high-dose folic acid during pregnancy, i.e., confounded by ASM dose used before and during pregnancy.

We did not have access to information on diagnoses registered by general practitioners which could provide information on important confounders such as folate insufficiency and vitamin B12 insufficiency. We also did not have information about surgical procedures including bariatric surgery which can lead to folate deficiency.[315] It is also likely that some of our covariates may be measured imperfectly as previously discussed for high-dose folic acid and ASM use, but also for other covariates including maternal smoking status and maternal BMI. Information on smoking habits during pregnancy and maternal obesity may be more likely to be not reported or reported in a

favorable way (i.e., smokers reporting “no” in the medical birth registers, weight rounded downwards rather than exact weight).

Statistical Methods and Variable Definitions

Assessing the risk of cancer after exposure to high doses of folic acid represents a research question eligible for time-to-event analyses, also known as survival analyses. In both paper I and II, we applied a Cox proportional hazards model, which is a semi-parametric method assuming that the risk of an occurring event in exposed individuals is proportional over time. It does not require any assumptions on a specific distribution of the baseline hazard function, i.e. that study participants may have different values on selected covariates at baseline, making the Cox model a realistic and adaptable approach to perform time-to-event analyses.[267] A Cox proportional hazards model is a preferred method in survival analyses, as it censors competing events such as death, emigration, or no event during the time of follow-up. It also allows independent variables to be time-varying. The option of defining folic acid as a time-varying exposure was applied in paper II, allowing us to examine how increasing doses of folic acid over time influenced the risk of cancer.

A Cox-proportional hazards model does not produce absolute or relative risks, but hazard ratios. A hazard ratio quantifies the relative likelihood of an event occurring at a given time for one group compared to another group. This differs from simple relative risk which would take the entire study period into account. However, “risk” has been used when referring to hazard ratios, as it serves communicative purposes in reporting being more easily accessible.

To calculate and report the average daily dose of prescription-based folic acid in a precise and meaningful way is challenging as one package can contain up to 1,000 pills (available in Sweden). Such a quantity can suffice for several years’ worth of continuous use. The use of folic acid in our study populations is likely to be restricted to supplementation in relation to pregnancy in paper I. In paper II, the use can for some women last longer due to a maternal comorbidity that served as part of the indication for high-dose folic acid supplementation during pregnancy. Additionally,

we were limited to calculate the dose based on the tablet dosage strengths of either 1 mg or 5 mg. These tablet strengths do not correspond well to Norwegian guidelines on folic acid supplementation for women treated with ASM, often recommending 4 mg daily (Table 1). We did not have access to specific instructions for use written by the prescribing clinician on the package or information about serum folate concentrations folate or red blood cell folate concentrations to assess precise high-dose folic acid intake and the true folic acid exposure in the mother and child.

In both paper I and paper II assessing cancer risk, we evaluated the likelihood of cancer diagnoses occurring shortly after high-dose folic acid exposure. Relevant for study I, a study has reported that incident preleukemic cells can occur prior to birth, supporting that cancers diagnosed immediately after birth are relevant for our study.[316] However, the same assumption cannot be made for cancer risk in the adult population. It is unlikely that cancer can develop immediately after exposure to high doses of folic acid. Therefore, we subtracted six months of follow-up after the first fill of high-dose folic acid in paper II, meaning that cancers diagnosed within these first six first months were not included in the estimation of the risk estimates. While subtraction of an even longer follow-up time might have been feasible, a six-month subtraction has been employed in other comparable observational studies assessing cancer risk after medication use.[317] Regarding the risk of non-Hodgkin's lymphoma in mothers who had used high-dose folic acid, which was consistently increased throughout all analyses, the median time from initial symptoms to diagnosis is typically less than six months.[318]

Several different time-windows for exposure of high-dose folic acid have been applied in papers I-III. In paper I, we defined a child to have been prenatally exposed to high-dose folic acid if the mother had filled a prescription for high-dose folic acid between 90 days prior to LMP and until the date of birth. Mothers treated with ASM in relation to pregnancy are generally recommended to start supplementation with high-dose folic acid early from when they wish to become pregnant and continue the treatment at least until the end of the first trimester. One single prescription fill is sufficient to cover this time period, as one filled prescription can contain up to 1,000 tablets. Additionally, the

half-life of whole-body folate typically last around 100 days in humans.[319] A shortening of the time-window to less than 90 days prior to LMP, would not only exclude mothers who started early to use high-dose folic acid before pregnancy, but also increase the contrast between those exposed and unexposed to high-dose folic acid by defining those who were truly exposed to high-dose folic acid as unexposed. This in turn could lead to even higher hazard ratios. In paper II we regarded the high-dose folic acid to be a time-varying exposure meaning that we accounted for the risk of cancer after subsequent prescription fills for high-dose folic acid during the time of follow-up. We also included women that filled the first prescription for high-dose folic within two years prior to LMP in their first pregnancy. This widening of exposure window was done to capture mothers who had unsuccessfully tried to become pregnant for a longer period, hence had used high-dose folic acid supplementation for a long time before conception. This was possible since we only included women who had their first pregnancy at the baseline of our study.

Generalizability

Epilepsy is a complex heterogeneous disease with a broad range of etiologies, phenotypes, and treatment approaches. This diversity extends to mothers with epilepsy, particularly those who require ASM treatment and who may possess genetic and non-genetic factors that inherently increase the risk of cancer compared to mothers without epilepsy. In paper I, we found an association between prenatal exposure to high-dose folic acid and an increased risk of cancer in children born to mothers with epilepsy. However, we did not have enough observations to report on the cancer risk in children to mothers with epilepsy who were not treated with ASM and were supplemented with high-dose folic acid during pregnancy. Therefore, the results may not be generalizable to children prenatally exposed to high doses of folic acid and born to mothers without epilepsy. In paper II, we found an association between the use of high-dose folic acid and an increased overall cancer risk, including the risk of non-Hodgkin's lymphoma, in the general population. Cancer risk may differ in subgroups within our study population with specific disorders which served as the indication for

high-dose folic acid use. In paper III, our observations regarding the proportion of pregnancies to women with epilepsy who used high-dose folic acid supplementation may not be generalizable to high-dose folic acid use during pregnancy in women with other indications than ASM-treated epilepsy.

As all our papers are population-based and derived from nationwide registers, the results should be generalizable to other populations of pregnant women with similar demographical properties and healthcare systems, but not necessarily to countries with more ethnically diverse backgrounds and more variable access to healthcare. Unlike most other countries in the world, the Nordic countries have not introduced mandatory folic acid fortification in foods. Hence, the results may not be transferable to countries with such fortification.

Clinical Implications and Considerations

The findings reported in this work provide important insights which should be considered when developing new clinical guidelines on folic acid supplementation. We have been the first to address the risk of cancer due to high-dose folic acid exposure in the relevant study population, pregnant women, and their children. We have also contributed with data regarding varying prescription patterns of high-dose folic acid between countries with similar populations and health care systems.

To ensure the safety of dietary supplements typically used during pregnancy is vital. As outlined in the Background section of this dissertation, previous research has primarily focused on assessing the potential benefits of high-dose folic acid supplementation in pregnancies that are at an increased risk of adverse health outcomes for the child. A lack of studies investigating potential risks associated with such supplementation may complicate the discussion on how these doses might elevate the risk of cancer in both the mother and child. Cancer, especially when it affects young people and children, is a profoundly devastating disease. To identify the optimal dose of folic acid for supplementation before and during pregnancy, research needs to delve in to potential adverse risks, as well as the potential benefits. The limited amount

of research investigating risks associated with high-dose folic acid supplementation for pregnant women may partly explain the observed variations in clinical guidelines, even across countries with similar characteristics.

Our results have been a topic of international public discussions, they have been broadcasted in news media, and they have spiked the debate about folic acid supplementation for pregnant women treated with ASM.[320-325] The increased risk of cancer due to prenatal exposure to high-dose folic acid in children born to mothers with epilepsy was one of the main topics discussed in several sessions during the American Epilepsy Society Meeting in Nashville, 2022, and scheduled as one of the main topics for epilepsy during the World Congress on Controversies in Neurology 2024.[326] In 2023, our work has been referenced in the updated clinical guidelines on folic acid supplementation during pregnancy in women with epilepsy in Switzerland, and they have lowered their recommendations from 4-5 mg daily to 1-3 mg daily.[122] In September 2023, The Guidelines Commission of the German Society of Neurology, in collaboration with the German Society of Epileptology, decided to lower the daily dose of folic acid during pregnancy for women with epilepsy from 5 mg folic acid daily to 0.4-0.8 mg daily, referencing our work.[121] Cancer as a risk that should be considered before high-dose folic acid supplementation in women with and without epilepsy is now included in UpToDate, which is an online, evidence-based and physician-authored clinical decision support resource assisting clinicians in making the right point-of-care decisions.[327,328]

Recommendations on high-dose folic acid supplementation in relation to pregnancy have not been adequately supported by evidence-based research, particularly in determining the appropriate daily dosage of folic acid and the necessary duration of supplementation. Clinical guidelines that are simple to adhere to might have played a role in the higher proportion of pregnancies in women with epilepsy that were supplemented with high-dose folic acid in Sweden compared to pregnancies in Denmark and Norway. As the volume of pregnancy-related information and guidelines continues to expand, both clinicians and patients face the challenge of navigating this overabundance of guidelines. Therefore, it is crucial to present recommendations in a

clear, straightforward, and cohesive manner, as well as make sure that the guidelines are regularly updated. There should not be competing guidelines with different recommendations relevant for the same group of individuals.

While our results should be considered in the development of new clinical guidelines on high-dose folic acid supplementation during pregnancy, a comprehensive evaluation of all available literature should be done acknowledging the numerous studies reporting benefits from high-dose folic acid use for children prenatally exposed to ASM. Treatment during pregnancy should always be done at an individual level balancing risks and benefits, and with precise information delivered in an accessible way to the pregnant woman ensuring an informed choice is made. Folic acid doses of 0.4 mg to 0.8 mg daily seem to be safe regarding cancer also when used long-term. However, in alignment our results regarding folic acid supplementation for women with epilepsy receiving ASM, we recommend limiting folic acid intake during pregnancy to less than 1–2 mg daily, and with the daily intake being at least 0.4 mg daily from actively planning for pregnancy and continuing until delivery.

Chapter 7

Future Directions

More studies are needed to understand the underlying etiologic mechanisms for the association between high-dose folic acid and risk of cancer, particularly in selected populations such as women diagnosed with epilepsy and exposed to varying doses of folic acid during pregnancy with and without the presence of ASM. This is needed to determine optimal folic acid dosage that balances the benefits and risks associated with folic acid supplementation in pregnant women, thereby defining safer and more clear clinical guidelines.

Several experimental studies have reported increased growth of tumor tissue from different organ systems if exposed to folic acid. There is a need for studies assessing the risk of tumor growth in women who have previously been exposed to high doses of folic acid to examine whether this induces or promotes tumor growth, using register-based data. This is especially important in pregnant women who have already been diagnosed with cancer, such as those treated with ASM for epilepsy caused by a glioma. An experimental study could investigate growth in cancer tissue in the presence of folic acid in different concentrations, similar to those appearing in vivo, and with and without the presence of different ASMs.

Studies assessing the epigenetic effects of folic acid on methylation of genes that could impact oncogenes and tumor suppressor genes are warranted. Such studies should include the impact of concomitant medications that are involved in methylation, such as valproate. Future studies should include the MTHFR gene variants to assess whether certain MTHFR polymorphisms are associated with an altered cancer risk if exposed to folic acid, and if such associations depend on the doses of folic acid.

We still lack knowledge about whether supplementation with high-dose folic acid reduces the risk of neural tube defects in children prenatally exposed to ASM. This has not been answered as previous studies have been limited by confounding by indication. A study combining data from the nationwide registers of all the Nordic countries should have sufficient power and quality to assess this association.

Chapter 8

Conclusions

By utilizing a comprehensive dataset that combines registry information from several nationwide and mandatory health registers from Denmark, Norway and Sweden, we found that filled prescriptions for high-dose folic acid (≥1 mg daily) was associated with an increased risk of cancer in children born to mothers with epilepsy. This association was also observed in women who have given birth in the general population. However, we observed no increased cancer risk in children born to mothers without epilepsy who were treated with ASM, and we lacked sufficient power to assess cancer risk in children of mothers with epilepsy who received high-dose folic acid during pregnancy but were not treated with ASM. We observed disparities in the use of high-dose folic acid in pregnancies to mothers with epilepsy treated with an ASM comparing the three Scandinavian countries, all with free universal healthcare and similar population characteristics.

Our findings should be considered when addressing risks and benefits of high-dose folic acid supplementation before and during pregnancy and when outlining new clinical guidelines for such folic acid supplementation. The results highlight the importance of considering adverse effects of high-dose folic acid supplementation in women with specific indications of use during pregnancy, such as ASM treatment. Future studies should focus on possible etiological mechanisms behind the risk of cancer due to folic acid exposure with and without the presence of ASMs, and to define the optimal level of folate and folate metabolites in women before and during pregnancy.

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Paper I, II and III

Paper I:

Vegrim HM, Dreier JW, Alvestad S, et al. "*Cancer Risk in Children of Mothers With Epilepsy and High-Dose Folic Acid Use During Pregnancy*". JAMA Neurol. 2022 Sep 26.

Paper II:

Vegrim HM, Dreier JW, Igland J, et al. "*High-Dose Folic Acid Use and Cancer Risk in Women Who Have Given Birth: A Register-based Cohort Study*". Submitted 2023 December 18th (under review).

Paper III:

Vegrim HM, Dreier JW, Igland J, et al. "*Prescribing Patterns for Higher Dose Folic Acid In Pregnant Women with Epilepsy Treated with Antiseizure Medication*". Submitted 2023 October 19th (under review).

Paper I

JAMA Neurology | Original Investigation

Cancer Risk in Children of Mothers With Epilepsy and High-Dose Folic Acid Use During Pregnancy

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IMPORTANCE Women with epilepsy are recommended high doses of folic acid before and during pregnancy owing to risk of congenital anomalies associated with antiseizure medications. Whether prenatal exposure to high-dose folic acid is associated with increases in the risk of childhood cancer is unknown.

OBJECTIVE To assess whether high-dose folic acid supplementation in mothers with epilepsy is associated with childhood cancer.

DESIGN, SETTING, AND PARTICIPANTS Observational cohort study conducted with nationwide registers in Denmark, Norway, and Sweden from 1997 to 2017. Analyses were performed during January 10, 2022, to January 31, 2022. Mother-child pairs were identified in medical birth registers and linked with information from patient, prescription, and cancer registers, as well as with sociodemographic information from statistical agencies, and were categorized by maternal diagnosis of epilepsy. The study population consisted of 3 379 171 children after exclusion of 126 711 children because of stillbirth or missing or erroneous values on important covariates.

EXPOSURES Maternal prescription fills for high-dose folic acid tablets (≥1 mg daily) between 90 days before pregnancy start and birth.

MAIN OUTCOMES AND MEASURES First onset of childhood cancer at younger than 20 years. Cox proportional hazards models were used to calculate adjusted hazard ratios with corresponding 95% CIs, adjusted for potential confounders. Cumulative incidence at aged 20 years was used as a measure of absolute risk.

RESULTS The median age at the end of follow-up in the study population of 3 379 171 children was 7.3 years (IQR, 3.5-10.9 years). Among the 27 784 children (51.4% male) born to mothers with epilepsy, 5934 (21.4%) were exposed to high-dose folic acid (mean dose, 4.3 mg), with 18 exposed cancer cases compared with 29 unexposed, producing an adjusted hazard ratio of 2.7 (95% CI, 1.2-6.3), absolute risk if exposed of 1.4% (95% CI, 0.5%-3.6%), and absolute risk if unexposed of 0.6% (95% CI, 0.3%-1.1%). In children of mothers without epilepsy, 46 646 (1.4%) were exposed to high-dose folic acid (mean dose, 2.9 mg), with 69 exposed and 4927 unexposed cancer cases and an adjusted hazard ratio of 1.1 (95% CI, 0.9-1.4; absolute risk, 0.4% [95% CI, 0.3%-0.5%]). There was no association between children born to mothers with epilepsy who were prenatally exposed to antiseizure medications, but not high-dose folic acid, and an increased risk of cancer (absolute risk, 0.6%; 95% CI, 0.2%-1.3%).

CONCLUSIONS AND RELEVANCE Prenatal exposure to high-dose folic acid was associated with increased risk of cancer in children of mothers with epilepsy.

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Multimedia

Supplemental content

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Women with epilepsy are often recommended high doses of folic acid (up to 5 mg daily) before and during pregnancy to reduce the risk of congenital anomalies associated with prenatal exposure to antiseizure medication (ASM).[1] Folate is an important vitamin for synthesis and repair of nucleic acids, and supplementation during pregnancy to women in general (0.4-0.8 mg of folic acid daily) has been shown to reduce the risk of neural tube defects in the child.[2] However, concern has been raised about a possible cancer risk with folic acid supplementation, possibly through altered DNA methylation.[3,4] High levels of folate may promote progression of neoplastic lesions and induce oxidative stress.[5,6] Folate deficiency could impair synthesis and repair of DNA and hence increase the risk of cancer.[7]

Cancer is the second most frequent cause of death in children in high-income countries, and the incidence is increasing.[8] There are few known risk factors apart from ionizing radiation, chemotherapy, and high maternal age.[9-15] Data on the association between prenatal exposure to folic acid and childhood cancer derive from studies of folic acid dose levels commonly used by mothers in the general population, but little is known regarding prenatal exposure to higher doses.[16-21] Because ASMs use can be an indication for high-dose folic acid supplementation in pregnancy, it is essential to consider their role in the potential association between childhood cancer and high-dose folic acid. In this study, we used nationwide register data from 3 Nordic countries to examine maternal prescription fill for high-dose folic acid (≥1 mg daily) during pregnancy and risk of cancer in the child, considering the potential role of maternal ASM use.

Key Points

Question Is prenatal exposure to high-dose folic acid (≥1 mg daily) associated with risk of cancer in children born to mothers with epilepsy?

Findings In this cohort study of 3 379 171 children, compared with no high-dose folic acid use in pregnancy for mothers with epilepsy, use among those with epilepsy was associated with an increased risk of cancer in their children.

Meaning Findings suggest that cancer risk in children should be considered in the risk-benefit analysis of folic acid supplementation for pregnant women with epilepsy.

Methods

Study Population

For this cohort study, we identified 3 505 882 singletons born in Denmark (1997-2017), Norway (2005-2017), and Sweden (2006-2017) from the national medical birth registers. These countries have personal identification numbers enabling individual linkage between national registers providing detailed health and socioeconomic information on all inhabitants (eAppendix in the Supplement).[22] Data were collected in the Nordic Register-Based Study of Antiepileptic Drugs in Pregnancy (SCAN-AED) collaboration project.[23]

The final study population consisted of 3 379 171 mother-child pairs, including 27 784 children of mothers with epilepsy. An overview of excluded pairs (n = 126 711 [3.7%]) is shown in **Figure 1**.

This study was approved by all relevant authorities in each country. Informed consent was not obtained (eTable 1 in the Supplement) because this was a purely register-based study with anonymized data from mandatory registers and no informed consent procedures. We used the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline. A complete and detailed overview for all the included covariates is available in eTable 12 in the Supplement.

High-Dose Folic Acid

We defined prenatal exposure to high-dose folic acid as at least 1 filled prescription of either 1 mg or 5 mg folic acid supplement (Anatomical Therapeutic Chemical code B03BB01)[24] between 90 days before the first day of the last menstrual period (hereinafter "pregnancy start") and birth.[1,25] The mean folic acid dose (in milligrams) was calculated according to the number of pills dispensed per day after pregnancy start and until birth, requiring at least 1 prescription.

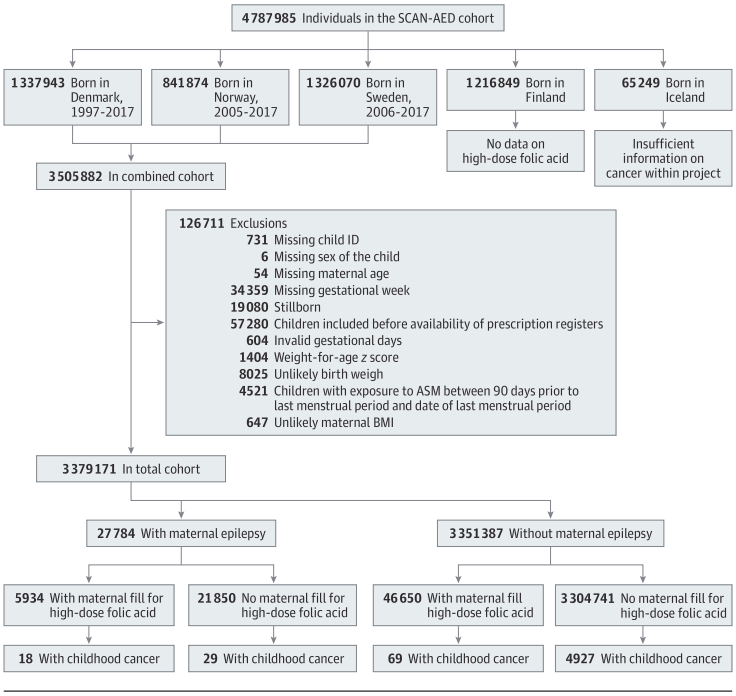
Childhood Cancer Diagnoses

We used the national cancer registers to identify cases of childhood cancer. These registers are considered close to complete in all 3 countries.[26] The *International Classification of Diseases for Oncology, Third Edition* contains information on cancer topography and morphology and was used to define cancers as malignant according to behavioral code value greater than or equal to 3.[27] Categorization of childhood cancers was according to the *International Classification of Childhood Cancer, Third Edition*.[28]

Maternal Epilepsy and ASM Exposure

Maternal epilepsy was defined as a recorded diagnosis of epilepsy any time before date of delivery, using *International Statistical Classification of Diseases and Related Health Problems, Tenth Edition (ICD-10)* codes G40 to G41.[29] We defined prenatal exposure to ASMs as at least 1 prescription fill from pregnancy start until birth, using Anatomical Therapeutic Chemical code N03, N05BA09, or S01EC01. To reduce misclassification of ASM exposure, we excluded mother-child pairs in which the mothers had an ASM prescription fill only during the 90 days before the date of the last menstrual period and the date of last menstrual period, but none during later the stages of pregnancy. We identified the most frequently used monotherapy ASMs during the pregnancies: carbamazepine, lamotrigine, levetiracetam, and valproate. Separate variables for these ASMs in combination with any other ASM were made. We classified the less frequently prescribed ASM monotherapies of topiramate, oxcarbazepine, clonazepam, and phenobarbital as "other monotherapy." ASM polytherapy exposure was defined as prescription fills of more than 1 type of ASM.

Figure 1. Flowchart of the Study Population



Selection of study and control population between mothers with and without epilepsy who did and did not fill a prescription of high-dose folic acid. ASM indicates antiseizure medication; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); and SCAN-AED, Nordic Register-Based Study of Antiepileptic Drugs in Pregnancy.

Covariates

We applied covariates according to known risk factors for childhood cancer that could also be associated with high-dose folic acid supplementation.[10] Maternal covariates included age, educational level, smoking status, and body mass index (calculated as weight in kilograms divided by height in meters squared) recorded at the first prenatal appointment and previous birth of a child with a major congenital anomaly. Number of hospital admissions during the year before pregnancy start and until birth was used as a proxy for maternal comorbidity. We also included maternal diagnoses of diabetes (gestational diabetes or type 1 or 2 diabetes), tuberous sclerosis, and cancer before pregnancy.

Child covariates included any major congenital anomaly (*ICD-10* codes Q00-Q89) or chromosomal abnormality (*ICD-10* codes Q90-Q99), both recorded until 1 year after birth. Congenital anomalies are associated with cancer in childhood and adulthood.[30]

Statistical Analysis

Primary Analyses

We applied a Cox proportional hazards regression model to report hazard ratios (HRs) and corresponding 95% CIs with child age as the time scale. Statistical significance was determined via 95% CI. Children were followed up from birth until date of first cancer diagnosis or censored at the date of death, emigration, 20th birthday, or the end of follow-up (December 31, 2017), whichever came first. Birth year, source country, and sex of the child were applied as strata within each model. Assumption of proportional hazards was evaluated for all variables and models.

In the primary analysis, we examined prenatal exposure to high-dose folic acid and childhood cancer in children of mothers with epilepsy, with corresponding incidence rates per 100 000 person-years including a cumulative incidence at age 20 years as an absolute measure of risk. We adjusted for maternal age and educational level, ASM prescription fill, number of hospitalizations, smoking, body mass index, and previous birth of a child with a major congenital anomaly. We used multiple imputation by chained equations to handle missingness on maternal educational level (122 510 of 3 379 171; 3.6%), smoking (280 934; 8.3%), and body mass index (978 863; 29.0%), with 20 imputations.[31] Analyses were performed with Stata version 16 (StataCorp) from January 10, 2022, to January 31, 2022.

Secondary Analyses

We reran the primary analysis stratified by maternal ASM prescription fill during pregnancy to explore ASM as a variable and calculated the interaction effect between ASM and high-dose folic acid.

By constructing cumulative incidence curves separately for exposed and unexposed children of mothers with or with-

out epilepsy, we made a graph comparing the cumulative incidence with corresponding 95% CIs of cancer in children of mothers with or without epilepsy who were exposed or unexposed to high-dose folic acid. Death was treated as a competing event and age was used as the time scale. The graph line and corresponding CIs were smoothed owing to data protection legislation.

The primary analysis was repeated with end of follow-up at 10 years of age to investigate whether the risk was higher for the youngest children. We compared the frequency of the 3 most common cancer types according to prenatal exposure to high-dose folic acid and maternal epilepsy status and calculated the HRs. The increasing mean daily dose of filled prescriptions of folic acid after pregnancy start (categorized into >0 mg to <4 mg and >4 mg, and >0 mg to <3 mg and >3 mg, respectively) and cancer in the child were investigated compared with unexposed children.

Sensitivity Analyses

We investigated childhood cancer and prenatal exposure to maternal prescription fill for any ASM and for different ASM types and compared them with those of children of mothers not filling a prescription for any ASM. We calculated the adjusted HRs (aHRs) for childhood cancer by using a maternal diagnosis of epilepsy as the exposure and compared them with those of children of mothers without epilepsy. This calculation was performed regardless of folic acid prescription to have a sufficient number of exposed cancer cases.

Factors that could modify the risk of childhood cancer were separately excluded from the primary analysis in post hoc analyses: maternal cancer, tuberous sclerosis,[32] diabetes,[33,34] and major congenital anomalies or chromosomal abnormalities in children.[30] We also removed any maternal prescription fills for carbamazepine or valproate after pregnancy start and until birth because prenatal use of these ASMs leads to a higher risk of congenital anomalies.[35]

Results

Among the 3 379 171 children in the study, we identified 27 784 born to mothers with epilepsy (0.8%), with median age 7.3 years (IQR, 3.5-10.9 years) at the end of follow-up, 13 501 (48.6%) female, and 14 283 (51.4%) male. A total of 5934 children (21.4%) were exposed to high-dose folic acid. Among 3 351 387 children of mothers without epilepsy (99.2%), 46 646 (1.4%) were exposed to high-dose folic acid (**Table 1**). The median follow-up time in the entire cohort was 7.3 years (IQR, 3.5-10.9 years). The estimated mean daily folic acid dose for mothers with epilepsy was higher than that for mothers without epilepsy (4.3 mg [SD = 4.1] vs 2.9 mg [SD = 2.9]) (Table 1 and eTable 2 in the Supplement). Most mothers with epilepsy with prescription fills for folic acid also filled ASM prescriptions (5531 of 5934 [93.2%]). Among mothers with epilepsy who used ASM, 48.4% (5195 of 10 726) did not fill high-dose folic acid prescriptions (Table 1). The mean doses of lamotrigine, levetiracetam, carbamazepine, and valproate were highest among mothers with epilepsy prescription filling for high-dose folic acid (Table 1).

The incidence rate of cancer in children of mothers with epilepsy who filled prescriptions for high-dose folic acid was 42.5 (95% CI, 26.8-67.5) per 100 000 person-years (n = 18) compared with 18.4 (95% CI, 12.8-26.5) per 100 000 person-years (n = 29) in children of mothers with epilepsy who did not fill folic acid prescriptions. In children of mothers without epilepsy, the aHR of cancer in 69 children exposed to high-dose folic acid was 1.1 (95% CI, 0.9-1.4; absolute risk, 0.4% [95% CI, 0.3%-0.5%]) compared with 4927 children unexposed to high-dose folic acid (absolute risk, 0.4; 95% CI, 0.4-0.4) (**Table 2**). The absolute risk of cancer was 1.5% (95% CI, 0.5%-3.5%) (Table 2) in exposed children of mothers with epilepsy, with an aHR for cancer of 2.7 (95% CI, 1.2-6.3), compared with children of mothers with epilepsy who were unexposed to high-dose folic acid (absolute risk, 0.6%; 95% CI, 0.3%-1.1%).

Secondary Analyses

In children of mothers with epilepsy who filled prescriptions for ASM, a 3.0-fold (95% CI, 1.1-fold to 7.9-fold) increased risk of childhood cancer associated with exposure to high-dose folic acid was observed. Women with epilepsy who filled prescriptions for ASM but not for high-dose folic acid did not have an increased risk of cancer in their offspring (absolute risk, 0.6%; 95% CI, 0.2%-1.3%). There were insufficient data to estimate the cancer risk associated with high-dose folic acid use in children of mothers with epilepsy who had no filled prescriptions for ASM (**Table 3**). There was no statistically significant interaction between ASM and high-dose folic acid among children born to mothers with or without epilepsy (eTable 3 in the Supplement).

For children younger than 10 years born to mothers with epilepsy, the cumulative cancer incidence was higher in exposed children than unexposed ones (**Figure 2**), with an aHR of 3.2 (95% CI, 1.2-8.7) (eTable 4 in the Supplement). In all exposure groups, leukemia was the most common type of cancer, followed by lymphoma and central nervous system tumors (eTable 5 in the Supplement). The aHR for leukemia among children born to mothers with epilepsy who filled a prescription for high-dose folic acid was 7.3 (95% CI, 1.5-35.2) (eTable 6 in the Supplement). We did not have sufficient exposed cancer cases to report HRs for other cancer types.

Children of mothers with epilepsy with prenatal exposure to average prescribed daily dose greater than 4 mg folic acid had an aHR for cancer of 3.4 (95% CI, 1.1-10.7) compared with unexposed children of mothers with epilepsy, whereas the aHR was 2.9 (95% CI, 1.2-7.2) in children exposed to doses below 4 mg. There was no increased risk of cancer in children born to mothers without epilepsy within the same groups of estimated daily dose of folic acid (eTable 7 in the Supplement). When the same calculations based on average prescribed daily dose greater than 0 to less than 3 mg and at least 3 mg were performed, the results remained unchanged compared with the estimates with a 4-mg cutoff (eTable 8 in the Supplement).

Sensitivity Analyses

The aHR of cancer in children exposed to ASM was 1.5 (95% CI, 1.1-2.1) compared with that of children unexposed to any ASM regardless of maternal epilepsy and prescription fill for high-dose folic acid. These estimates were similar for

Table 1. Population Characteristics of 3 379 171 Children Born in Denmark, Norway, and Sweden Stratified on Maternal Epilepsy and Prenatal Exposure to High-Dose Folic Acid

Characteristic	No. (%)			
	No maternal epilepsy (3 351 387 [99.2%])		Maternal epilepsy (27 784 [0.8%])	
	No high-dose folic acid	High-dose folic acid	No high-dose folic acid	High-dose folic acid
Total[a]	3 304 741 (98.6)	46 646 (1.4)	21 850 (78.6)	5934 (21.4)
Countries[a]				
Denmark	1 272 265 (98.4)	8457 (0.7)	10 657 (0.8)	1654 (0.1)
Norway	759 367 (98.7)	4105 (0.5)	4940 (0.6)	1045 (0.1)
Sweden	1 273 109 (96.7)	34 084 (2.6)	6253 (0.5)	3235 (0.2)
Birth year[a]				
1997-2001	313 290 (98.1)	4278 (1.3)	1340 (0.4)	377 (0.1)
2002-2006	526 355 (98.3)	4977 (0.9)	3378 (0.6)	809 (0.2)
2007-2011	1 125 287 (97.8)	16 263 (1.4)	7510 (0.7)	2119 (0.2)
2012-2016	1 118 014 (97.6)	16 745 (1.5)	8013 (0.7)	2221 (0.2)
2017	221 795 (97.2)	4383 (1.9)	1609 (0.7)	408 (0.2)
Characteristics of the mother				
Maternal age, mean (SD), y	30.3 (5.1)	31.4 (5.5)	29.6 (5.3)	30.6 (5.0)
Maternal educational level[b]				
Compulsory	490 881 (14.9)	7931 (17.0)	5551 (25.4)	1105 (18.6)
Pre-university	1 452 762 (44.0)	22 429 (48.1)	9422 (43.1)	2838 (47.9)
College/university	792 322 (24.0)	8912 (19.1)	4314 (19.7)	1367 (23.1)
Postgraduate	448 353 (13.6)	5947 (12.7)	1996 (9.1)	531 (8.9)
Missing	120 423 (3.6)	1427 (3.1)	567 (2.6)	93 (1.6)
Smoking at the beginning of pregnancy[b]				
No	2 711 554 (82.1)	38 394 (82.3)	16 829 (77.0)	4890 (82.5)
Yes	318 094 (9.6)	4248 (9.1)	3612 (16.5)	616 (10.4)
Missing	275 093 (8.3)	4004 (8.6)	1409 (6.5)	428 (7.2)
BMI[b]				
Mean (SD)	24.6 (5.1)	25.5 (5.3)	25.0 (5.4)	25.2 (5.0)
<30	2 050 366 (62.0)	30 258 (64.9)	13 932 (63.8)	3877 (65.4)
≥30	292 140 (8.8)	6444 (13.8)	2559 (11.7)	732 (12.3)
Missing	962 235 (29.1)	9944 (21.3)	5359 (24.5)	1325 (22.3)
Maternal diabetes[b]				
Type 1 or 2 diabetes	30 144 (0.9)	1196 (2.6)	582 (2.7)	111 (1.9)
Gestational diabetes	77 705 (2.4)	1493 (3.2)	655 (3.0)	141 (2.4)
Maternal hospital admissions[b]				
0	3 020 239 (91.4)	38 035 (81.5)	19 154 (87.7)	5201 (87.6)
1	266 604 (8.1)	7473 (16.0)	2204 (10.1)	655 (11.0)
≥2	17 876 (0.5)	1138 (2.4)	492 (2.2)	78 (1.3)
Maternal medication				
Folic acid dose, mean (SD), mg	0	2.9 (2.9)	0	4.3 (4.1)
Any ASM[b]	6045 (0.2)	819 (1.8)	5195 (23.8)	5531 (93.2)
ASM polytherapy (yes/no)[c]	20 (0.3)	6 (0.7)	137 (2.6)	223 (4.0)
ASM monotherapy[c]				
Valproate	196 (3.2)	39 (4.8)	337 (6.5)	572 (10.3)
Lamotrigine	2655 (43.9)	451 (55.1)	2176 (41.9)	1931 (34.9)
Levetiracetam	16 (0.3)	6 (0.7)	490 (9.4)	348 (6.3)
Carbamazepine	201 (3.3)	47 (5.7)	529 (10.2)	1025 (18.5)
Topiramate	193 (3.2)	13 (1.6)	80 (1.5)	93 (1.7)
Oxcarbazepine	33 (0.5)	11 (1.3)	289 (5.6)	194 (3.5)
Phenobarbital	102 (1.7)	7 (0.8)	26 (0.5)	17 (0.3)
Clonazepam	374 (6.2)	19 (2.3)	166 (3.2)	49 (0.9)

(continued)

Table 1. Population Characteristics of 3 379 171 Children Born in Denmark, Norway, and Sweden Stratified on Maternal Epilepsy and Prenatal Exposure to High-Dose Folic Acid (continued)

Characteristic	No. (%)			
	No maternal epilepsy (3 351 387 [99.2%])		Maternal epilepsy (27 784 [0.8%])	
	No high-dose folic acid	High-dose folic acid	No high-dose folic acid	High-dose folic acid
Dose of different ASM before and during pregnancy, mean (SD), mg				
Valproate	79.6 (73.4)	119.2 (123.2)	151.5 (162.1)	223.0 (177.5)
Lamotrigine	164.4 (164.3)	246.0 (206.6)	301.3 (248.7)	450.9 (363.9)
Carbamazepine	85.3 (99.3)	143.8 (130.0)	225.5 (180.3)	284.5 (205.3)
Levetiracetam	52.8 (53.0)	173.1 (162.3)	312.2 (248.4)	433.8 (347.1)
Child characteristics[b]				
Male sex	1 697 735 (51.4)	23 620 (50.6)	11 261 (51.5)	3022 (50.9)
Female sex	1 607 006 (48.6)	23 026 (49.4)	10 589 (48.5)	2912 (49.1)
Gestational age, mean (SD), wk	39.3 (2.0)	38.9 (2.3)	39.0 (2.2)	39.0 (2.3)
Infant birth weight, mean (SD), g	3497.4 (590.8)	3397.1 (656.9)	3432.8 (621.6)	3428.8 (643.3)
Major congenital anomaly	165 024 (5.0)	2457 (5.3)	1339 (6.1)	422 (7.1)

Abbreviations: ASM, antiseizure medication; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared).

[a] Expressed as number (rowwise percentage) of women with or without high-dose folic acid exposure.

[b] Expressed as number (columnwise percentage) of women with or without high-dose folic acid exposure.

[c] Columnwise percentages among the total number of any ASM usage in each subgroup.

Table 2. Association Between Maternal Epilepsy, Filled Prescription of High-Dose Folic Acid, and Risk of Childhood Cancer in the Offspring[a]

Maternal epilepsy	High-dose folic acid	Live births	Childhood cancer cases	Incidence rate per 100 000 person-years (95% CI)	Cumulative incidence at 20 y (95% CI)[b]	Crude HR (95% CI)	aHR 1 (95% CI)[c]	aHR 2 (95% CI)[d]	aHR 3 (95% CI)[e]
Yes	Yes	5934	18	42.5 (26.8-67.5)	1.5 (0.5-3.6)	2.4 (1.3-4.5)	2.4 (1.3-4.4)	2.5 (1.1-5.8)	2.7 (1.2-6.3)
	No	21 850	29	18.4 (12.8-26.5)	0.6 (0.3-1.1)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
No	Yes	46 646	69	20.0 (15.8-25.4)	0.4 (0.3-0.5)	1.1 (0.9-1.4)	1.1 (0.9-1.4)	1.1 (0.9-1.4)	1.1 (0.9-1.4)
	No	3 304 741	4927	18.9 (18.4-19.5)	0.4 (0.4-0.4)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]

Abbreviations: aHR, adjusted hazard ratio; aHR 1, first model with basic adjustment; aHR 2, second model including adjustment for antiseizure medication prescription fill; aHR 3, fully adjusted model.

[a] Birth year, sex of the child and source country were applied as stratum for all models.

[b] The cumulative incidence at aged 10 years was 0.4 (95% CI, 0.2-0.6) for maternal epilepsy yes and high-dose folic acid yes; 0.2 (95% CI, 0.1-0.3) for maternal epilepsy yes and high-dose folic acid no; 0.2 (95% CI, 0.1-0.3) for maternal epilepsy no and high-dose folic acid yes; and 0.2 (95% CI, 0.2-0.2) for maternal epilepsy no and high dose folic acid no.

[c] Adjustment for maternal age and educational level.

[d] Including adjustment for antiseizure medication exposure.

[e] Including adjustment for maternal body mass index, prior births with major congenital anomalies, smoking during pregnancy, and number of hospitalizations.

children of mothers with or without epilepsy (aHR, 1.6 [95% CI, 0.9-2.8] vs 1.4 [95% CI, 0.8-2.6]). There were no risk differences between specified ASM monotherapies or polytherapy because all point estimates overlapped the 95% CI of the overall association between ASM and cancer in children of mothers with or without epilepsy (eTable 9 in the Supplement).

Maternal epilepsy was not associated with childhood cancer (aHR, 1.0; 95% CI, 0.7-1.4) (eTable 10 in the Supplement). In the primary analysis, removal of mothers with cancer before pregnancy (n = 454), tuberous sclerosis (n = 23), or diabetes (n = 1292, including gestational diabetes) did not change the findings between high-dose folic acid and cancer in the child. Excluding mothers with epilepsy with any prescription for carbamazepine or valproate (n = 3407) only slightly attenuated the aHR (aHR, 2.4; 95% CI, 0.9-6.5). Excluding children with major congenital anomalies (n = 1761) or chromosomal abnormalities (n = 68) who were born to mothers with epilepsy did not change the risk estimates (eTable 11 in the Supplement).

Discussion

In this multinational population-based study of more than 3 million pregnancies, we identified a 2.7-fold increased risk of cancer in children of mothers with epilepsy who immediately before or during pregnancy filled a prescription for high-dose folic acid (≥1 mg daily) compared with children of mothers with epilepsy who did not fill such a prescription. The estimates were consistent after adjustment for potential confounders, were unchanged after removal of children with other possible risk factors for childhood cancer, and could not readily be explained by maternal use of ASM. However, because ASM was used by almost the entire cohort of women with epilepsy filling prescriptions for high-dose folic acid (5531 of 5934 [93.2%]) (Table 1), it was not possible to completely rule out that maternal use of ASM in pregnancy or other characteristics inherent in mothers with epilepsy who used high-dose folic acid could partly or fully explain the finding. Pathologies of inherent diseases other than

Table 3. Association Between Maternal Epilepsy, Maternal Prescription Fill for Antiseizure Medication (ASM), High-Dose Folic Acid, and Risk of Childhood Cancer in the Offspring[a]

Maternal epilepsy	Maternal ASM	High-dose folic acid	Live births	Incidence rate per 100 000 person-years (95% CI)	Cumulative incidence at 20 y (95% CI)[b]	Crude HR (95% CI)	aHR 1 (95% CI)[c]	aHR 2 (95% CI)[d]
Yes	Yes	Yes	5531	43.0 (26.8-69.2)	1.4 (0.5-3.1)	2.9 (1.1-7.5)	2.9 (1.1-7.7)	3.0 (1.1-7.9)
		No	5195	19.1 (9.5-38.1)	0.6 (0.2-0.13)	1 [Reference]	1 [Reference]	1 [Reference]
	No	Yes	403	NA	NA	NA	NA	NA
		No	16 655	18.2 (11.8-27.9)	0.6 (0.3-1.1)	1 [Reference]	1 [Reference]	1 [Reference]
No	Yes	Yes	819	NA	NA	NA	NA	NA
		No	6045	23.0 (11.5-46.0)	0.4 (0.1-1.0)	1 [Reference]	1 [Reference]	1 [Reference]
	No	Yes	45 827	19.7 (15.4-25.1)	0.4 (0.3-0.5)	1.1 (0.8-1.4)	1.1 (0.8-1.4)	1.1 (0.8-1.4)
		No	3 298 696	18.9 (18.4-19.5)	0.4 (0.4-0.4)	1 [Reference]	1 [Reference]	1 [Reference]

Abbreviations: aHR, adjusted hazard ratio; aHR 1, first model with basic adjustment; aHR 2, second model including adjustment for antiseizure medication prescription fill; NA, not available.

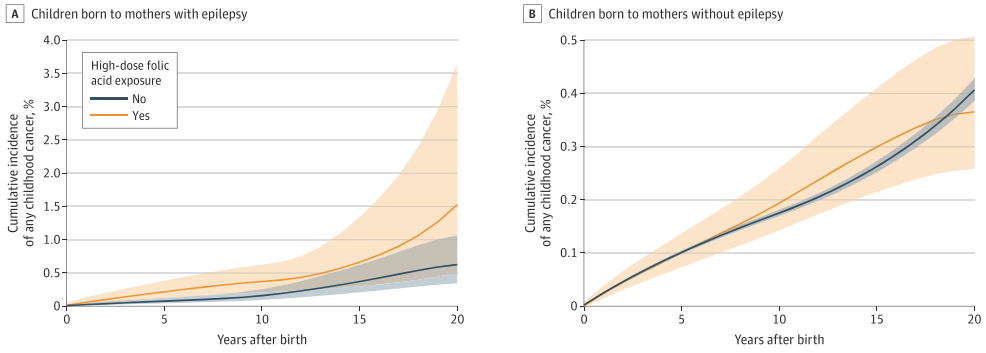
[a] Birth year, sex of the child and source country were applied as stratum for all models.

[b] The cumulative incidence at age 10 years was 0.4 (95% CI, 0.2-0.6) for maternal epilepsy yes, maternal ASM yes, and high-dose folic acid yes; 0.1 (95% CI, 0.1-0.3) for maternal epilepsy yes, maternal ASM yes, and high-dose folic acid no; 0.2 (95% CI, 0.1-0.3) for maternal epilepsy yes, maternal ASM no, and maternal high-dose folic acid no; 0.2 (95% CI, 0.1-0.6) for maternal epilepsy no, maternal ASM yes, and maternal high-dose folic acid no; 0.2 (95% CI, 0.1-0.3) for maternal epilepsy no, maternal ASM no, and maternal high-dose folic acid yes; and 0.2 (95% CI, 0.2-0.2) for maternal epilepsy no, maternal ASM no, and maternal high-dose folic acid no.

[c] Adjustment for maternal age and educational level.

[d] Including adjustment for maternal body mass index, prior births with major congenital anomalies, smoking during pregnancy, and number of hospitalizations.

Figure 2. Cumulative Incidence of Childhood Cancer



Cumulative incidence of first onset of childhood cancer recorded from birth until 20 years of age with or without maternal prescription fill for high-dose folic acid for mothers with or without a diagnosis of epilepsy. The graph lines were smoothed owing to the Danish Data Protection Act to prevent identification of individuals.

those we were able to control for might still be associated with the cancer risk.

The increased risk associated with high-dose folic acid was also found when stratified for ASM use. However, we found no association between maternal prescription fills for any specific ASM and childhood cancer. Removing mothers with any prescription fills for carbamazepine and valproate was not associated with the point estimate. Hence, these 2 ASMs were not important effect modifiers for the cancer association. Maternal epilepsy was not associated with an increased risk of cancer among the study children. The most frequent childhood cancer types in children among mothers with epilepsy who filled prescriptions for high-dose folic acid did not differ from the distribution in the general population.[8]

We did not find any increased risk of cancer in exposed children of mothers without epilepsy. A previous population-based study from the Norwegian Birth Register did not find an increased risk of cancer in children in the general population after maternal use of folic acid in doses up to 0.6 mg daily during pregnancy.[36] Ecologic studies in the US and Canada described a reduction of childhood cancer incidence after implementation of folic acid fortification in foods, but with estimated folic acid doses below 0.4 mg daily.[20,21,37] Other studies have shown a reduced risk of childhood central nervous system tumors[17] and acute lymphoblastic leukemia,[16,18,19] based on self-reported folic acid usage after doses of approximately 0.4 mg daily, and no certain supplementation greater than 0.8 mg.

In mothers who filled prescriptions for high-dose folic acid, the mean calculated daily dose after pregnancy start and until birth was higher among those with epilepsy (4.3 mg) than without it (2.9 mg). Although an elevated risk of cancer was observed in children born to mothers with epilepsy who were exposed to increasing mean doses of folic acid, the differences between those with more than 4 mg daily compared with less than 4 mg daily were not significant. The mean calculated ASM doses were higher among mothers with epilepsy with a filled prescription for high-dose folic acid compared with those with epilepsy with no prescription, which may imply that an increasing dose of ASM plays a role in carcinogenesis among children born to mothers with epilepsy, possibly through an interaction between ASM and folic acid in high doses. However, we did not identify a statistically significant interaction between ASM and high-dose folic acid, but the statistical power of this analysis was limited.

Strengths and Limitations

A major strength of this study is the use of population-based information from mandatory and nationwide health and administrative registers, providing a sufficient sample size to examine associations between rare combinations of exposures and outcomes. The exposure information was based on filled prescriptions, eliminating recall bias. The likelihood of misclassification of cancers is negligible owing to the high validity of the data from the national cancer registers.[26]

There are some limitations to this study. A filled prescription of high-dose folic acid does not guarantee that mothers took the medication every day.[38] This affects the calculation for the mean daily folic acid dose. An increasing mean daily dose can also depict the length of supplementation rather than the daily dose taken. We did not have information on serum levels of folic acid to assess maternal intake, any over-the-counter supplements, and dietary intake. Despite the large study sample, we did not have enough exposed childhood cancers to report estimates for children of mothers with epilepsy who filled prescriptions for high-dose folic acid but not for ASM or risk estimates for cancer subtypes except for leukemia.

Conclusions

In this cohort study, an association was found between increased risk of cancer among children of mothers with epilepsy and maternal use of high-dose folic acid. In contrast, prenatal exposure to high-dose folic acid was not associated with an increased risk of childhood cancer in children born to mothers without epilepsy.

Results of this study should be considered when the risks and benefits of folic acid supplements for women with epilepsy are discussed and before decisions about optimal dose recommendations are made. Because of the combined use of ASM and folic acid in high doses in mothers with epilepsy, future studies should investigate possible etiologic mechanisms between folic acid and ASM exposure in pregnancy and the risk of cancer.

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Author Contributions: Dr Vegrim had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
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Supplementary Online Content

Vegrim HM, Dreier JW, Alvestad S, et al. Cancer risk in children of mothers with epilepsy and high-dose folic acid use during pregnancy. *JAMA Neurol*. Published online September 26, 2022. doi:10.1001/jamaneurol.2022.2977

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix. Description of Data Sources

Data from several nationwide, mandatory health registers were collected to perform this study. Below is a simple flowchart showing which and how the different registers were used.

Medical birth registers (MBR)

MBR contains information on all births in mothers including national identification number, age, smoking, maternal parity, pregnancy and delivery diagnoses and interventions, offspring anomalies, birth outcome, gestational age, weight at birth, Apgar score. It is mandatory for health care providers to report to this register, and information is collected from medical records from the prenatal, delivery and neonatal care. The Medical Birth Register of Norway was founded in 1967, and the Danish and Swedish ones in 1973.

In Norway, the use of folic acid supplement (any dose) pre-conception and/or during pregnancy is recorded in the birth register.

Prescription registers (PDR)

PDR contains data on dispensed prescriptions from pharmacies. This includes ATC-codes, drug strength, total defined daily dose dispensed, package size, and dispensing date. FDR does not contain information about over-the-counter medicines and medicines used during in-patient hospital stays. The Danish Registry of Medicinal Product Statistics contains information from 1995 and onwards, the Norwegian Prescription Database from 2004 and the Swedish Prescribed Drug Register from July 2005. The Norwegian and Danish Prescription Databases also contain information about the indication for reimbursement.

National patient registers (NPR)

NPR contains information about diagnosis according to ICD-10 codes. The Norwegian Patient Registry contains information on all persons treated at hospitals as well in private clinics if they are reimbursed by the public health system. This information has been linked to the personal, national identification numbers since 2008. The Danish National Hospital Register contains information from hospital stays from 1977. ICD codes for psychiatric treatment are available from 1969 from the Psychiatric Central Research Register. From 1995, also data from somatic and psychiatric outpatient contacts are available. The Swedish National patient register includes all in-patient care in Sweden from 1987. The outpatient visits including psychiatric care from both private and public caregivers were included from 2001.

National statistical agencies (NSA)

Each of the Nordic countries have national statistical agencies containing information on socioeconomic and demographic data on all inhabitants. These data contain yearly information on household income, education, occupation and demographical data on emigration and death. Most of the variables are digitized and follow international standards (ISCO-88 for occupation, ISCED2011 for education). Hence harmonization between the registers was feasible.

Cancer registers (CR)

The Danish CR was established in 1941, in Norway in 1951 and in Sweden in 1958. It is mandatory for healthcare-providers to report information on diagnosis, treatment, and cause of death to the national cancer-registers.

All the CRs contain information about age, sex, time of diagnosis, site and type of tumor, stage, or extent of the cancer, in addition to several other variables, this for the entire study-period. CR uses an international standard (ICD-O-3) which contains precise information on morphology and topography.

eTable 1. Ethical Approvals

Ethical approvals were necessary to retrieve and manage data from the national social and health registers. This included approvals from the Data Protection Agency in Denmark; the Norwegian Inspectorate and the Regional Ethics Committee for Medical Research in Western Norway; and the Regional Ethical Board at Karolinska Institutet in Sweden. Below is an overview of approvals.

Ethical approvals from included countries

Country	Ethical approval	Data protection approvals	Data transfer agreement	Data protection agreement
Denmark	Not required for register-based research	Data protection agency	N/A	✓
Norway	✓	Data protection impact assessment	✓	✓
Sweden	✓	Local Data protection Officer	✓	✓

Abbreviations: N/A: Not applicable.
All register holders must approve of the project. Necessary approvals can change over time.
All data was exported to Denmark; thus, a data transfer agreement was not necessary.

eTable 2. Mean Dose Delivered of High-Dose Folic Acid Among Different Maternal Comorbidities

Maternal characteristics or comorbidities	Mean dose in mg (SD)
General population	3.1 (3.1)
General population without maternal epilepsy	2.9 (2.9)
Maternal epilepsy	4.3 (4.1)
Maternal use of ASM	4.3 (4.1)
Maternal use of ASM without epilepsy	3.1 (3.2)
Maternal use of VPA in combination with any ASM	4.4 (4.5)
Maternal use of LTG in combination with any ASM	4.1 (3.8)
Maternal diabetes mellitus	2.9 (3.1)
Maternal smoking during the start of pregnancy	2.6 (2.8)
Maternal BMI $\geq$30 kg/m^2	3.4 (3.5)

Abbreviations: ASM: Antiseizure medication; BMI: Body Mass Index; LTG: Lamotrigine; SD: Standard deviation; VPA: Valproic acid
The mean dose delivered of high dose folic acid in mg was calculated along with corresponding standard deviation for different maternal characteristics, comorbidities and comedications.

eTable 3. Interaction Between Antiseizure Medication and High-Dose Folic Acid in Mothers With and Without Epilepsy

Test for interaction between antiseizure medication and high dose folic acid on the risk of cancer in the offspring was performed in children to mothers with and without epilepsy. Level of significance was set to $p<0.05$. Test for interaction was based on the fully adjusted model as presented in Table 2 by adding an interaction term to the analysis.

Test for interaction between antiseizure medication (ASM) and high dose folic acid on cancer risk in children to mothers with and without epilepsy

Test for interaction	p-value
Children to mothers with epilepsy	0.76
Children to mothers without epilepsy	0.48

Abbreviations: ASM: Antiseizure medication.

eTable 4. Risk of Childhood Cancer During the First 10 Years of Life in Children Born to Mothers With and Without Epilepsy Filling Prescriptions for High-Dose Folic Acid

The risk of childhood cancer during the first ten first years of follow-up after birth was investigated for children born to mothers with and without diagnosis of epilepsy, filling prescription for high dose folic acid before and during pregnancy. The risk of cancer was 3.2 [95% CI 1.2-8.7] in children during the first ten years of follow-up if they were born to mothers with epilepsy filling prescription of high dose folic acid, compared to mothers with epilepsy that did not have such a prescription.

Association between maternal epilepsy, filled prescription of high dose folic acid, and risk of childhood cancer in the offspring up to age 10 years

Maternal epilepsy	High dose folic acid	Live births	Incidence rate per 100 000 person-years, 95% CI	Crude HR, 95% CI	aHR1[a], 95% CI	aHR2[b], 95% CI	aHR3[c], 95% CI
Yes	Yes	4462	40.5 (24.4-67.2)	2.9 (1.4-6.0)	2.8 (1.3-5.7)	3.1 (1.1-8.4)	3.2 (1.2-8.7)
	No	15 750	14.6 (9.4-22.6)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
No	Yes	34 264	20.0 (15.4-25.9)	1.1 (0.9-1.5)	1.1 (0.9-1.5)	1.1 (0.9-1.4)	1.1 (0.9-1.4)
	No	2 270 632	18.2 (17.7-18.8)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]

a) aHR1: Adjustment for maternal age and education
b) aHR2: Including adjustment for antiseizure medication exposure.
c) aHR3: Including adjustment for maternal BMI, prior births with congenital anomalies, smoking during pregnancy, number of hospitalizations.
Birth year, sex of the child and source country were applied as stratum for all models.

eTable 5. High-Dose Folic Acid and Type of Childhood Cancer

Occurrence (number and percentage) of the three most ICCC3 subgroups of cancer among children born to mothers with and without epilepsy filling for high dose folic acid. The three most common types of childhood cancers were also the most common types of cancer in children of mothers with epilepsy filling for high dose folic acid.

Frequency of cancer cases in different childhood cancer subgroups and fill for high dose folic acid

ICCC3 subgroups	No epilepsy		Epilepsy	
	No high dose folic acid	High dose folic acid	No high dose folic acid	High dose folic acid
Group I: Leukemia	1620 (30%)	28 (40%)	8 (30%)	8 (40%)

Group II & III: Lymphoma and CNS tumors	1007 (20%)	14 (20%)	7 (30%)	4 (<25%)
Other childhood cancer subtypes	2300	27	15	6

Abbreviations: ICCC3: International Classification of Childhood Cancer 3rd edition.

eTable 6. Risk of Leukemia in Children Born to Mothers With and Without Epilepsy Filling Prescriptions for High-Dose Folic Acid

We calculated the HR for the most common childhood cancer type, leukemia, in children to mothers with or without epilepsy filling for high dose folic acid (1mg or 5mg folic acid). There was not sufficient no. of exposed cancer cases to report HRs for other cancer subtypes.

Association between fill for high dose folic acid by mothers with and without epilepsy and risk of leukemia in the offspring

Maternal epilepsy	High dose folic acid	Live births	Cancer cases	Incidence rate per 100 000 person-years, 95% CI	Crude HR, 95% CI	aHR1[a], 95% CI	aHR2[b], 95% CI	aHR3[c], 95% CI
Yes	Yes	4462	8	18.9 (9.5-37.8)	4.2 (1.5-12.3)	4.2 (1.4-12.2)	6.3 (1.3-30.6)	7.3 (1.5-35.2)
	No	15 750	8	5.1 (2.5-10.2)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
No	Yes	34 264	28	8.1 (5.5-11.8)	1.3 (0.9-1.9)	1.3 (0.9-1.9)	1.3 (0.9-1.9)	1.3 (0.9-1.9)
	No	2 270 632	1620	6.3 (6.0-6.6)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]

a) aHR1: Adjustment for maternal age and education
b) aHR2: Including adjustment for antiseizure medication exposure.
c) aHR3: Including adjustment for maternal BMI, prior births with congenital anomalies, smoking during pregnancy, number of hospitalizations.
Birth year, sex of the child and source country were applied as stratum for all models.

eTable 7. Association Between Cancer in the Child and Average Delivered Dose of Folic Acid in Mothers With and Without Epilepsy

The average delivered dose of folic acid was calculated based on prescription fills for 1 mg and 5 mg of folic acid, filled between 90 days prior to the date of last menstrual period and until birth. This was categorized into >0 mg to <4 mg daily and ≥4 mg daily. These categories were examined for mothers with and without epilepsy, comparing both groups with mothers that had no fill for high dose folic acid. We were unable to make more categories due to few cancer cases among the exposed children.

Association between average daily dose of folic acid and risk of cancer in the offspring to mothers with and without epilepsy								
Maternal epilepsy	**Average daily dose of folic acid**	**Live births**	**Cancer cases**	**Incidence rate per 100 000 person-years, 95% CI**	**Crude HR, 95% CI**	**aHR1, 95% CI[a]**	**aHR2, 95% CI[b]**	**aHR3, 95% CI[c]**
Yes	>0 mg to <4 mg	3389	11	44.0 (24.3-79.4)	2.5 (1.2-5.1)	2.4 (1.2-4.9)	2.8 (1.1-6.8)	2.9 (1.2-7.2)
	No folic acid	21 850	29	18.4 (12.8-26.5)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
	≥4 mg	2545	7	40.5 (19.3-85.0)	2.9 (1.1-7.4)	2.8 (1.1-7.1)	3.1 (1.0-9.4)	3.4 (1.1-10.7)
	No folic acid	21 850	29	18.4 (12.8-26.5)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
No	>0 mg to <4 mg	35 307	54	20.7 (15.8-27.1)	1.1 (0.9-1.5)	1.1 (0.9-1.5)	1.1 (0.9-1.5)	1.1 (0.9-1.5)
	No folic acid	3 304 741	4874	19.0 (18.5-19.5)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
	≥4 mg	11 339	14	17.8 (10.5-30.1)	1.0 (0.6-1.7)	1.0 (0.6-1.6)	1.0 (0.6-1.6)	1.0 (0.6-1.6)
	No folic acid	3 304 741	4874	19.0 (18.5-19.5)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]

a) aHR1: Adjustment for maternal age and education
b) aHR2: Including adjustment for ASM exposure.
c) aHR3: Including adjustment for maternal BMI, prior births with congenital anomalies, smoking during pregnancy, number of hospitalizations.
Birth year, sex of the child and source country were applied as stratum for all models.

eTable 8. Association Between Cancer in the Child and Average Delivered Dose of Folic Acid in Mothers With and Without Epilepsy, Based on Estimated Mean Dose of Folic Acid

The estimated mean dose in mothers with and without epilepsy were 4.3mg and 2.9mg, respectively. Therefore, we reran the analysis as presented in eTable 7, but here comparing use of mean daily prescribed dose of folic acid >0 to <3mg and ≥3mg. The numbers have for this table been rounded to closest ten due to close similarity with eTable 7 with the possibility to calculate difference with less than 5 exposed cancer cases.

Association between average daily dose of folic acid and risk of cancer in the offspring to mothers with and without epilepsy, based on estimated mean dose folic acid.								
Maternal epilepsy	**Average daily dose of folic acid**	**Live births**	**Cancer cases**	**Incidence rate per 100 000 person-years, 95% CI**	**Crude HR, 95% CI**	**aHR1, 95% CI[a]**	**aHR2, 95% CI[b]**	**aHR3, 95% CI[c]**

Yes	>0 mg to <3 mg	3070	10	39.7 (20.7-76.3)	2.2 (1.0-4.7)	2.1 (1.0-4.6)	2.5 (1.0-6.6)	2.6 (1.0-6.9)
	No folic acid	21 850	30	18.4 (12.8-26.5)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
	≥3 mg	2860	10	45.8 (23.8-88.0)	2.9 (1.3-6.7)	2.8 (1.2-6.4)	3.0 (1.1-8.3)	3.3 (1.2-9.4)
	No folic acid	21 850	30	18.4 (12.8-26.5)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
No	>0 mg to <3 mg	33 660	50	20.8 (15.8-27.4)	1.1 (0.9-1.5)	1.1 (0.9-1.5)	1.1 (0.9-1.5)	1.1 (0.9-1.5)
	No folic acid	3 304 740	4870	18.9 (18.4-19.5)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
	≥3 mg	12 990	20	17.9 (10.9-29.2)	1.0 (0.6-1.6)	1.0 (0.6-1.6)	1.0 (0.6-1.6)	1.0 (0.6-1.6)
	N/A	3 304 740	4870	18.9 (18.4-19.5)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]

a) aHR1: Adjustment for maternal age and education
b) aHR2: Including adjustment for ASM exposure.
c) aHR3: Including adjustment for maternal BMI, prior births with congenital anomalies, smoking during pregnancy, number of hospitalizations.
Birth year, sex of the child and source country were applied as stratum for all models.
Counts have been rounded to closest ten due to close similarity to counts presented in eTable 7.

eTable 9. Prescription Fill for Antiseizure Medication and Risk of Childhood Cancer

The risk of childhood cancers with maternal fills for ASM was examined. This was performed regardless of high dose folic acid use or not to be able to obtain a sufficient number of exposed cancer cases to report risk estimates.

Hazard rates (HR) of cancer in offspring of women with prescription fills for antiseizure-medication (ASM) regardless of high dose folic acid prescriptions, stratified for maternal epilepsy and types and combinations of antiseizure medications.

Type of ASM	Filled prescription of ASM	Incidence rates per 100 000 person years	Crude HR, 95% CI	aHR1*, 95% CI	aHR2**, 95% CI
Any ASM	Yes	29.00 (20.82-30.49)	1.47 (1.04-2.06)	1.46 (1.04-2.06)	1.49 (1.07-2.09)
	No	18.93 (18.41-19.47)	1 [Reference]	1 [Reference]	1 [Reference]
	Yes	30.69 (20.73-45.41)	1.54 (0.84-2.78)	1.48 (0.82-2.69)	1.56 (0.86-2.83)

Hazard rates (HR) of cancer in offspring of women with prescription fills for antiseizure-medication (ASM) regardless of high dose folic acid prescriptions, stratified for maternal epilepsy and types and combinations of antiseizure medications.

Type of ASM	Filled prescription of ASM	Incidence rates per 100 000 person years	Crude HR, 95% CI	aHR1*, 95% CI	aHR2**, 95% CI
Any ASM within mothers with epilepsy	No	18.58 (12.24-28.22)	1 [Reference]	1 [Reference]	1 [Reference]
Any ASM within mothers without epilepsy	Yes	25.49 (13.71-47.37)	1.36 (0.73-2.52)	1.34 (0.72-2.50)	1.42 (0.76-2.64)
	No	18.93 (18.41-19.47)	1 [Reference]	1 [Reference]	1 [Reference]
≥ 2 prescriptions of any ASM	Yes	28.11 (19.28-40.99)	1.39 (0.94-2.05)	1.39 (0.94-2.05)	1.40 (0.95-2.07)
	No	18.95 (18.42-19.48)	1 [Reference]	1 [Reference]	1 [Reference]
Any polytherapy	Yes	N/A[a]	N/A[a]	N/A[a]	N/A[a]
	No	18.49 (17.16-19.93)	1 [Reference]	1 [Reference]	1 [Reference]
Any monotherapy	Yes	26.89 (18.02-40.12)	1.36 (0.99-2.06)	1.35 (0.89-2.05)	1.36 (0.90-2.07)
	No	18.95 (18.43-19.49)	1 [Reference]	1 [Reference]	1 [Reference]
Valproate in combination with any ASM	Yes	35.79 (16.08-79.67)	2.04 (0.91-4.54)	2.04 (0.92-4.54)	2.13 (0.96-4.74)
	No	18.97 (18.45-19.50)	1 [Reference]	1 [Reference]	1 [Reference]
Levetiracetam in combination with any ASM	Yes	N/A[a]	N/A[a]	N/A[a]	N/A[a]
	No	18.97 (18.45-19.50)	1 [Reference]	1 [Reference]	1 [Reference]

Hazard rates (HR) of cancer in offspring of women with prescription fills for antiseizure-medication (ASM) regardless of high dose folic acid prescriptions, stratified for maternal epilepsy and types and combinations of antiseizure medications.

Type of ASM	Filled prescription of ASM	Incidence rates per 100 000 person years	Crude HR, 95% CI	aHR1*, 95% CI	aHR2**, 95% CI
Lamotrigine monotherapy	Yes	29.19 (16.58-51.40)	1.44 (0.79-2.63)	1.43 (0.79-2.61)	1.47 (0.89-2.43)
	No	18.96 (18.44-19.50)	1 [Reference]	1 [Reference]	1 [Reference]
Lamotrigine in combination with any ASM	Yes	32.23 (20.03-51.84)	1.47 (0.89-2.42)	1.47 (0.89-2.42)	1.47 (0.89-2.42)
	No	18.95 (18.43-19.49)	1 [Reference]	1 [Reference]	1 [Reference]
Carbamazepine monotherapy	Yes	37.81 (16.99-84.17)	2.07 (0.93-4.64)	2.05 (0.92-4.59)	2.17 (0.97-4.86)
	No	18.97 (17.1-19.9)	1 [Reference]	1 [Reference]	1 [Reference]
Carbamazepine in combination with any ASM	Yes	30.04 (13.50-66.87)	1.69 (0.76-3.76)	1.67 (0.75-3.72)	1.77 (0.79-3.94)
	No	18.97 (18.45-19.51)	1 [Reference]	1 [Reference]	1 [Reference]
Other monotherapy[b]	Yes	N/A[a]	N/A[a]	N/A[a]	N/A[a]
	No	18.98 (18.46-19.52)	1 [Reference]	1 [Reference]	1 [Reference]

Abbreviations: aHR: adjusted hazard ratio; ASM: antiseizure medication; HR: hazard ratio
* aHR1: Adjustment for sex, maternal age and education
** aHR2: Including adjustment for maternal BMI, prior births with congenital anomalies, smoking during pregnancy, number of hospitalizations
Birth year, sex of the child and source country were applied as stratum for all models.
Analyses were performed without stratification on filled prescription for high dose folic acid to have sufficient number of exposed cancer cases to report HRs.

Superscript
[a] Not sufficient no. of exposed cancer cases to be reported.
[b] Other ASM: Topiramate, Clonazepam, Oxcarbazepine, Phenobarbital

eTable 10. Effect of Maternal Epilepsy on Risk of Childhood Cancer

We stratified children born to women with and without epilepsy with no further stratification on whether they were exposed to high dose folic acid or anti-seizure medication, to examine any effect of maternal epilepsy. Three adjustment models were performed as described in the statistical analysis. We did not observe any association between maternal epilepsy and risk of childhood cancer in the offspring (aHR=1.0, 95% CI 0.7-1.4).

Association between maternal epilepsy and risk of childhood cancer in the offspring							
Maternal epilepsy	Live births	Childhood cancer cases	Incidence rate per 100 000 person-years, 95% CI	Crude HR, 95% CI	aHR1, 95% CI[a]	aHR2, 95% CI[b]	aHR3, 95% CI[c]
Yes	27 784	48	23.5 (17.7-31.3)	1.2 (0.9-1.6)	1.2 (0.9-1.6)	1.0 (0.7-1.5)	1.0 (0.7-1.4)
No	3 351 387	4996	18.9 (18.4-19.5)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]

Abbreviations: aHR: adjusted hazard ratio; HR: hazard ratio
a) aHR1: Adjustment for maternal age and education
b) aHR2: Including adjustment for antiseizure medication exposure.
c) aHR3: Including adjustment for maternal BMI, prior births with congenital anomalies, smoking during pregnancy, number of hospitalizations.
Birth year, sex of the child and source country were applied as stratum for all models.

eTable 11. Restrictions

For the main analysis in Table 2, showing risk of cancer in children born to mothers with epilepsy and with and without high dose folic acid exposure, we reran the analysis with separate restrictions on covariates that might have influenced the association. This included maternal cancer before pregnancy, maternal tuberous sclerosis, maternal diabetes mellitus one year before birth. Maternal fill for valproate or carbamazepine included both monotherapy and combination with other ASMs. We also included separate exclusions on children with major congenital anomaly and chromosomal abnormality.

Restriction by main analysis in Table 2: Association between maternal epilepsy, high dose folic acid and childhood cancer

Restrictions	No. excluded from the analysis	Crude HR, 95% CI	aHR, 95% CI*
Maternal cancer before pregnancy	454	2.5 (1.3-4.6)	2.7 (1.2-6.3)
Maternal diagnosis of tuberous sclerosis	23	2.4 (1.3-4.5)	2.8 (1.2-6.3)
Maternal diabetes mellitus	1292	2.2 (1.2-4.1)	2.6 (1.1-5.9)
Maternal prescription fills for CBZ or VPA	3407	2.1 (0.9-4.5)	2.4 (0.9-6.5)
Major congenital anomaly in the child	1761	2.5 (1.3-5.0)	2.3 (1.0-5.7)
Chromosomal abnormalities in the child	68	2.6 (1.4-5.0)	2.7 (1.2-6.3)

Abbreviations: aHR: adjusted hazard ratio; ASM: antiseizure medication; CBZ: Carbamazepine; HR: hazard ratio; VPA: Valproate.
* aHR: Adjusting for maternal age- and education, maternal BMI, prior births with congenital anomalies, smoking during pregnancy, number of hospitalizations.

Birth year, sex of the child and source country were applied as stratum for all models.

eTable 12. Definition of Included Covariates

Variable definitions

This table provide a complete overview with definitions on covariates and their sources that have been used in the paper "*High Dose Folic Acid during Pregnancy in Mothers with and without Epilepsy and Cancer Risk in their Children: A Scandinavian Register-based Cohort study*" by Vegrim et al.

The lay-out of the table is based on the S2 table from: "Relation of in-utero exposure to antiepileptic drugs to pregnancy duration and size at birth" by Margulis AV, Hernandez-Diaz H, McElrath T, Rothman KJ, Plana E, Almqvist C, D´Onofrio BM, Oberg AS.[1]

Contents

Abbreviations

ATC: Anatomical therapeutic chemical; CR: Cancer registers; BMI: Body mass index; ICCC3: International Classification of Childhood Cancer 3rd edition; ICD: International Classification of Diseases; ICD-O/3: International Classification of Diseases for Oncology, 3rd edition; LMP: Last menstrual period; MBR: Medical birth registers; NPR: National patient registers; NSA: National statistical agencies; N/A; Not applicable; PDR: Prescription registers.

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Maternal definitions								
First day of last menstrual period (LMP).	N/A	Date of first day in last menstrual period. Estimated subtracting the date of birth from gestational age at birth.	MBR					
Maternal age.	At delivery.	Continuous.	MBR					
Maternal education.	Year of delivery.	Categorical: 0: Compulsory. 1: Pre-university. 2: College/University. 3: Post-graduate. 4: Missing information on education.	MBR					
Maternal country of birth.	N/A	Mother born in the country of delivery or not. 1) Source country. 2) All other countries.	NSA, MBR					
Calendar year of delivery.	Year of delivery	Continuous.	MBR					
Parity.	N/A	Number of previous deliveries: 0, 1 or ≥2.	MBR					
Child sex.	At delivery.	Categorical: Female or male.	MBR					

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Child birthweight.	At delivery.	Continuous.	MBR					
Child gestational age.	At delivery	Continuous.	MBR					
BMI.	At start of pregnancy.	Categorical: As recorded in MBR or calculated from maternal weight and height at first antenatal visit (kg/m^2). - Non-obese <35 kg/m^2. - Obese ≥35 kg/m^2. - Missing.	MBR					
Smoking.	Start of pregnancy.	Categorical: Y/N/Missing.	MBR	Checkbox MBR				
Previous congenital anomaly.	N/A	Categorial: Y/N. Computed as summarized previous births with congenital anomalies.	MBR					
Prior birth to child with major congenital anomaly.	Full lookback.	Categorical: Y/N. Definition of major congenital malformation in accordance with EUROCAT definition[2].	NPR, MBR			Q00-Q99		

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Child definitions								
Child sex.	At delivery.	Categorical: Female or male.	MBR					
Child birthweight.	At delivery.	Continuous.	MBR					
Child gestational age.	At delivery	Continuous.	MBR					
Chromosomal abnormality.	Any time.	Categorical: Y/N. Composite variable: Any of the codes for chromosomal abnormality.	NPR			Q90-Q99		
Congenital major anomaly	Any time.	Categorical: Y/N. Composite variable: Any of the codes for major congenital anomalies. Definition of major congenital malformation in accordance with EUROCAT definition[2].	NPR			Q00-Q89		
Definition of pregnancy complications								
Gestational diabetes.	LMP-90 to delivery.		MBR, NPR	Checkbox MBR		O24.4 O24.9		

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Gestational hypertension.	LMP-90 to delivery.	Any code for gestational hypertension, preeclampsia, HELLP and/or eclampsia.	MBR, NPR	Checkbox MBR		O11, O13-16		
Medical exposure definitions								
Any high dose folic acid.	LMP-90 to delivery.	Categorical: Y/N. Any filled prescriptions of either 1mg or 5mg. Separate variables for filled prescriptions of 1mg or 5mg.	PDR		B03BB01			
Folic acid average dose.	LMP-90 to delivery.	Average daily dose of folic acid supplements calculated from the total amount of folic acid supplements dispensed, divided by the number within defined time frame. Separate variables based on 1mg or 5mg only.	PDR		B03BB01 B03BB51			
Folic acid cumulative dose.	LMP-90 to delivery.	Cumulative folic acid dose based on total no. of pills dispensed during the defined time frame. Separate variables based on 1mg or 5mg only.	PDR		B03BB01 B03BB51			
Any antiseizure medication.	LMP-90 to delivery.	Categorical: Y/N. Any monotherapy or polytherapy	PDR		N03 N05BA09 S01EC01			

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Valproate monotherapy.	LMP-90 to delivery.	Categorical: ≥1 prescriptions of valproate and no other type of ASM: Y/N.	PDR		N03AG01			
Lamotrigine monotherapy.	LMP-90 to delivery.	Categorical: ≥1 prescriptions of lamotrigine and no other type of ASM: Y/N.	PDR		N03AX09			
Levetiracetam monotherapy.	LMP-90 to delivery.	Categorical: ≥1 prescriptions of levetiracetam and no other type of ASM: Y/N.	PDR		N03AX14			
Carbamazepine monotherapy.	LMP-90 to delivery.	Categorical: ≥1 prescriptions of carbamazepine and no other type of ASM: Y/N.	PDR		N03AF01			
Topiramate monotherapy.	LMP-90 to delivery.	Categorical: ≥1 prescriptions: Y/N.	PDR		N03AX11			
Clonazepam monotherapy.	LMP-90 to delivery.	Categorical: ≥1 prescriptions: Y/N.	PDR		N03AE01			
Oxcarbazepine monotherapy.	LMP-90 to delivery.	Categorical: ≥1 prescriptions: Y/N.	PDR		N03AF02			
Phenobarbital monotherapy.	LMP-90 to delivery.	Categorical: ≥1 prescriptions: Y/N.	PDR		N03AB02			

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Phenytoin monotherapy.	LMP-90 to delivery.	Categorical: ≥1 prescriptions: Y/N.	PDR		N03AB02			
Maternal somatic comorbidities								
Epilepsy.	Full lookback.	Categorical: Y/N	NPR, MBR, PDR	Check-box MBR		G40-41		
Diabetes mellitus.	LMP-365 to delivery.	Categorical: Y/N	NPR, MBR	Checkbox MBR		E10-14		
Tuberous sclerosis.	Full lookback.	Categorical: Y/N	NPR			Q85.1		
Number of hospitalizations.	LMP-365 to delivery.	Categorical: 0, 1, ≥2.	NPR					
Maternal cancer.	Any time until delivery.	Categorical: Y/N.	CR			C00-97	ICD-O/3 behavioral code of 3 except for CNS cancers.	Group based on ICD-10 C-chapters.

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Childhood cancer definition according to the ICCC3								
I. Leukemias, myeloproliferative diseases and myelodysplastic diseases.								
Lymphoid leukemias.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9820, 9823, 9826, 9827, 9831-9837, 9940, 9948	C000-C809
Acute myeloid leukemias.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9840, 9861, 9866, 9867, 9870-9874, 9891, 9895-9897, 9910, 9920, 9931	C000-C809
I. Leukemias, myeloproliferative diseases and myelodyasplastic diseases: - *Chronic myeloproliferative diseases.*	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9863, 9875, 9876, 9950, 9960-9964	C000-C809
I. Leukemias, myeloproliferative diseases and myelodyasplastic diseases: - *Myelodysplastic syndrome and other myeloproliferative diseases.*	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9945, 9946, 9975, 9980, 9982-9987, 9989	C000-C809

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
I. Leukemias, myeloproliferative diseases and myelodyasplastic diseases: - *Unspecified and other specified leukemias.*	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9800, 9801, 9805, 9860, 9930	C000-C809
II. Lymphomas and reticuloendothelial neoplasms.								
Hodgkin lymphomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9650-9655, 9659, 9661-9665, 9667	C000-C809
Non-Hodgkin lymphomas (except Burkitt lymphoma).	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9591, 9670, 9671, 9673, 9675, 9678-9680, 9684, 9689-9691, 9695, 9698-9702, 9705, 9708, 9709, 9714, 9716-9719, 9727-9729, 9731-9734, 9760-9762, 9764-9769, 9970	C000-C809
Burkitt lymphoma.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9687	C000-C809
Miscellaneous lymphoreticular neoplasms.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9740-9742, 9750, 9754-9758	C000-C809

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Unspecified lymphomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9590, 9596	C000-C809
III. CNS and miscellaneous intracranial and intraspinal neoplasms.								
Ependymomas and choroid plexus tumor.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9383, 9390-9394	C000-C809
Astrocytomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9380 9384, 9400-9411, 9420, 9421-9424, 9440-9442	C723 C000-C809
Intracranial and intraspinal embryonal tumors.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9470-9474, 9480, 9508 9501-9504	C000-C809 C700-C729
Other gliomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9380 9381, 9382, 9430, 9444, 9450, 9451, 9460	C700-C722, C724-C729, C751, C753 C000-C809
Other specified intracranial and intraspinal neoplasms.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8270-8281, 8300, 9350-9352, 9360-9362, 9412, 9413, 9492, 9493, 9505-9507, 9530-9539, 9582	C000-C809

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Unspecified intracranial and intraspinal neoplasm.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8000-8005	C700-C729, C751-C753
IV. Neuroblastoma and other peripheral nervous cell tumors.								
Neuroblastoma and ganglioneuroblastoma.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9490, 9500	C000-C809
Other peripheral nervous cell tumors.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8680-8683, 8690-8693, 8700, 9520-9523 9501-9504	C000-C809 C000-C699, C739-C768, C809
V. Retinoblastoma.								
Retinoblastoma.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9510-9514	C000-C809
VI. Renal tumors.								

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Nephroblastoma and other nonepithelial renal tumors.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8959, 8960, 8964-8967 8963, 9364	C000-C809 C649
Renal carcinomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8010-8041, 8050-8075, 8082, 8120-8122, 8130-8141, 8143, 8155, 8190-8201, 8210, 8211, 8221-8231, 8240, 8241, 8244-8246, 8260-8263, 8290, 8310, 8320, 8323, 8401, 8430, 8440, 8480-8490, 8504, 8510, 8550, 8560-8576 8311, 8312, 8316-8319, 8361	C649 C000-C809
Unspecified malignant renal tumors.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				C8000-8005	C649
VII. Hepatic tumors.								
Hepatoblastoma.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8970	C000-C809

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Hepatic carcinomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8010-8041, 8050-8075, 8082, 8120-8122, 8140, 8141, 8143, 8155, 8190-8201, 8210, 8211, 8230, 8231, 8240, 8241, 8244-8246, 8260-8264, 8310, 8320, 8323, 8401, 8430, 8440, 8480-8490, 8504, 8510, 8550, 8560-8576 8160-8180	C220, C221 C000-C809
Unspecified malignant hepatic tumors.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8000-8005	C220, C221
VIII. Malignant bone tumors.								
Osteosarcomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9180-9187, 9191-9195, 9200	C400-C419, C760-C768, C809
Chondrosarcomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9210, 9220, 9240 9221, 9230, 9241-9243	C400-C419, C760-C768, C809 C000-C809
Ewing tumor and related sarcomas of bone.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9260 9363-9365	C400-C419, C760-C768, C809 C400-C419

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Other specified malignant bone tumors.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8810, 8811, 8823, 8830 8812, 9250, 9261, 9262, 9270-9275, 9280-9282, 9290, 9300-9302, 9310-9312, 9320-9322, 9330, 9340-9342, 9370-9372	C400-C419 C000-C809
Unspecified malignant bone tumors.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8000-8005, 8800, 8801, 8803-8805	C400-C419
IX. Soft tissue and other extraosseous sarcomas.								
Rhabdomyosarcomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8900-8905, 8910, 8912, 8920, 8991	C000-C809
Fibrosarcomas, peripheral nerve sheath tumors and other fibrous neoplasms.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8810, 8811, 8813-8815, 8821, 8823, 8834-8835 8820, 8822, 8824-8827, 9150, 9160, 9491, 9540-9571, 9580	C000-C399, C440-C768, C809 C000-C809
Kaposi sarcoma.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9140	C000-C809

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Other specified soft tissue sarcomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8587, 8710-8713, 8806, 8831-8833, 8836, 8840-8842, 8850-8858, 8860-8862, 8870, 8880, 8881, 8890-8898, 8921, 8982, 8990, 9040-9044, 9120-9125, 9130-9133, 9135, 9136, 9141, 9142, 9161, 9170-9175, 9231, 9251, 9252, 9373, 9581 8830 8963 9180, 9210, 9220, 9240 9260 9364 9365	C000-C809 C000-C399, C440-C768, C809 C000-C639, C659-C699, C739-C768, C809 C490-C499 C000-C399, C470-C759 C000-C399, C470-C639, C659-C699, C739-C768, C809 C000-C399, C470-C639, C659-C768, C809
Unspecified soft tissue sarcomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8800-8805	C000-C399, C440-C768, C809
X. Germ cell tumors, trophoblastic tumors and neoplasms of gonads.								

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Intracranial and intraspinal germ cell tumors.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9060-9065, 9070-9072, 9080-9085, 9100, 9101	C700-C729, C751-C753
Malignant extracranial and extragonadal germ cell tumors.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9060-9065, 9070-9072, 9080-9085, 9100-9105	C000-C559, C570-C619, C630-C699, C739-C750, C754-C768, C809
Malignant gonadal germ cell tumors.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				9060-9065, 9070-9073, 9080-9085, 9090, 9091, 9100, 9101	C569, C620-C629
Gonadal carcinomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8010-8041, 8050-8075, 8082, 8120-8122, 8130-8141, 8143, 8190-8201, 8210, 8211, 8221-8241, 8244-8246, 8260-8263, 8290, 8310, 8313, 8320, 8323, 8380-8384, 8430, 8440, 8480-8490, 8504, 8510, 8550, 8560-8573, 9000, 9014, 9015 8441-8444, 8450, 8451, 8460-8473	C569, C620-C629 C000-C809
Other and unspecified malignant gonadal tumors.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8590-8671 8000-8005	C000-C809 C569, C620-C629
XI. Other malignant epithelial neoplasms and malignant melanomas.								

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	Time frame	Functional form of Variable and Comments	Register	Non-ICD or ATC-Coded Variables	ATC	ICD-10	ICD-O/3 Morphology	ICD-O/3 Topography
Adrenocortical carcinomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8370-8375	C000-C809
Thyroid carcinomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8010-8041, 8050-8075, 8082, 8120-8122, 8130-8141, 8190, 8200, 8201, 8211, 8230, 8231, 8244-8246, 8260-8263, 8290, 8310, 8320, 8323, 8430, 8440, 8480, 8481, 8510, 8560-8573 8330-8337, 8340-8347, 8350	C739 C000-C809
Nasopharyngeal carcinomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8010-8041, 8050-8075, 8082, 8083, 8120-8122, 8130-8141, 8190, 8200, 8201, 8211, 8230, 8231, 8244-8246, 8260-8263, 8290, 8310, 8320, 8323, 8430, 8440, 8480, 8481, 8500-8576	C110-C119
Malignant melanomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8720-8780, 8790	C000-C809
Skin carcinomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8010-8041, 8050-8075, 8078, 8082, 8090-8110, 8140, 8143, 8147, 8190, 8200, 8240, 8246, 8247, 8260, 8310, 8320, 8323, 8390-8420, 8430, 8480, 8542, 8560, 8570-8573, 8940, 8941	C440-C449

eTable 12 Definition of included covariates								
					Codes			
Cohort characteristic	**Time frame**	**Functional form of Variable and Comments**	**Register**	**Non-ICD or ATC-Coded Variables**	**ATC**	**ICD-10**	**ICD-O/3 Morphology**	**ICD-O/3 Topography**
Other and unspecified carcinomas.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8010-8084, 8120-8157, 8190-8264, 8290, 8310, 8313-8315, 8320-8325, 8360, 8380-8384, 8430-8440, 8452-8454, 8480-8586, 8588-8589, 8940, 8941, 8983, 9000, 9010-9016, 9020, 9030	C000-C109, C129-C218, C239-C399, C480-C488, C500-C559, C570-C619, C630-C639, C659-C729, C750-C768, C809
XII. Other and unspecified malignant neoplasms.								
Other specified malignant tumors.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8930-8936, 8950, 8951, 8971-8981, 9050-9055, 9110 9363	C000-C809 C000-C399, C470-C759
Other unspecified malignant tumors.	Before age 20.	Categorical: Y/N. Composite variables: Any combination of specified ICD-O/3 morphology and topography codes.	CR				8000-8005	C000-C218, C239-C399, C420-C559, C570-C619, C630-C639, C659-C699, C739-C750, C754-C809

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Paper III

Prescribing Patterns for Higher Dose Folic Acid In Pregnant Women with Epilepsy Treated with Antiseizure Medication

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Ethical approvals

This study was approved by the Danish Data Protection Agency, the Norwegian Inspectorate and the Regional Ethics Committees of Norway and Sweden.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Data availability statement

In accordance with the Danish Data Protection Act, the SCAN AED data set are maintained at Statistics Denmark and cannot be exported or be made available outside of Statistics Denmark. However, the primary sources of data for the SCAN-AED project, derived from nationwide registers in the Nordic countries, may be obtained through the appropriate national authorities upon application.

Patient consent statement

Since this study solely relies on mandatory and anonymized data obtained from registries, there was no requirement for individual patient consent. The need for informed consent has been exempted by the relevant ethical and data protection authorities, or it is legally waived in all the countries included in this research.

Abstract

Objective To characterise the use of higher doses of folic acid (≥1 mg daily) in relation to pregnancy in Denmark, Norway, and Sweden in women with epilepsy treated with antiseizure medication (ASM).

Methods In this observational study, we used data from national medical birth, patient, and prescription registers in Denmark, Norway and Sweden to retrospectively identify pregnancies in women with epilepsy treated with ASM from 2006 to 2017. The proportion of higher dose folic acid supplementation in pregnancies among women receiving ASM for epilepsy was calculated according to country of origin, time periods and type of ASM. Logistic regression with restricted cubic splines was used to model country-specific time trends.

Results Among a total of 2,748,880 pregnancies, we identified 8,700 (0.3%) pregnancies after restricting the population to women with ASM-treated epilepsy. A prescription for higher dose folic acid was filled in 4,720 (54.3%) of these pregnancies. The proportion supplemented with higher dose folic acid was highest in Sweden (74.2%) and lower in Norway (41.4%) and Denmark (30.7%). Furthermore, we observed a decreasing trend of higher dose folic acid use in Denmark and Norway from year 2012 to 2017. Among those who used higher dose folic acid, 42% did not start preconception supplementation with higher dose folic acid.

Significance Supplementation with higher dose folic acid occurred in approximately half of pregnancies in women with ASM-treated epilepsy, with many not starting supplementation until after becoming pregnant. Considerable variability was observed in the use of higher dose folic acid across the countries, despite similar population characteristics and healthcare systems. Future guidelines should be simplified with clear recommendations developed in a collaborative manner by relevant specialists including neurologists, obstetricians, pediatricians, and public health specialists to enhance real-world applicability.

Key points

- Only half of the pregnancies in women treated with an antiseizure medication (ASM) for their epilepsy were supplemented with higher doses of folic acid (≥1 mg daily).
- Whereas most pregnancies in women with ASM-treated epilepsy were supplemented with higher dose folic acid in Sweden (74.2%), a lower proportion received such supplementation in Norway (41.4%) and Denmark (30.7%)
- Among pregnancies in women with ASM-treated epilepsy supplemented with higher dose folic acid, supplementation was not initiated in more than 40% of the pregnancies until after becoming pregnant.

Introduction

Epilepsy is a common neurological disease affecting millions of women of childbearing age (1, 2). Women with epilepsy planning to become pregnant face various challenges, including balancing the benefits and adverse effects of antiseizure medication (ASM) and folic acid supplements during pregnancy (3). Clinical guidelines widely recommend using higher dose folic acid (≥1 mg daily) in conjunction with ASM treatment in relation to pregnancy as ASM is associated with teratogenic risks and lowered folate concentrations in blood (4-6). Despite strong evidence demonstrating the protective effects of folic acid supplementation against neural tube defects in the general population (7-9), similar evidence is lacking regarding protectiveness against congenital anomalies in women with epilepsy on ASM treatment during pregnancy. Nevertheless, association studies addressing health outcomes in children exposed to ASM prenatally have reported other benefits from higher dose folic acid use, such as a lowered risk of autistic traits (10), better intelligence quotient outcomes (except for valproate) (11) and a lowered risk of language impairment in some (12) but not all studies (13, 14). Conversely, use of higher dose folic acid in pregnancy has been associated with an increased risk of childhood cancer in children born to women with epilepsy (15).

Current clinical guidelines for pregnant women with ASM-treated epilepsy pose a challenge to both patients and clinicians as the optimal timing and dosage of folic acid supplementation before and during pregnancy for achieving potential benefits remain uncertain. Current recommendations vary widely from 0.4 mg to 5 mg of daily supplementation, with inconsistent guidance on whether to maintain or adjust the doses throughout pregnancy (16-28) (eTable 1, Supplementary material). Varying guidelines for pregnant women with ASM-treated epilepsy exist in Denmark, Norway and Sweden, despite their similar demographics and healthcare systems.

To better understand recent practices and pinpoint areas for improving folic acid supplementation guidelines for women with epilepsy during their pregnancy, we conducted a study in Denmark, Norway, and Sweden. Our objective was to explore and describe whether the use of higher doses of folic acid resonated with the national clinical guidelines, if the use differed across these different ASM therapies used during pregnancy and compare the use between the included countries.

Material and Methods

Study population

First, we retrospectively identified the pregnancies in the national medical birth registers. We were able to link information in the medical birth registers with information on filled prescriptions in the national prescription registers by using the unique personal identification number assigned to each citizen at birth or immigration in each of the three participating countries. Our data source was the SCAN-AED study, a Nordic multi-registry study with full data coverage from all countries and registers from year 2006 to 2017 (www.scanaed.org) (29, 30). The registers and variables used are described in further detail in the supplementary materials and eTable 2. The total number of pregnancies identified was 2,748,880 (Figure 1). We restricted the study population to pregnancies by women with epilepsy corresponding to the 10th version of International Statistical Classification of Diseases and Related Health Problems (ICD-10) code G40 or G41 registered in patient records at any time before the date of delivery and where these individuals also had at least one ASM prescription fill between the date of the last menstrual period and the date of delivery.

To accurately determine the time elapsed between the last menstrual period and the date of delivery in relation to the date of filling of higher dose folic acid and ASM prescriptions, we excluded women with improbable pregnancy durations of fewer than 154 days or more than 314 days, and those with missing pregnancy length (Figure 1, n=20, 0.2% of all pregnant women with ASM-treated epilepsy).

Higher dose folic acid

We defined higher dose folic acid use as having filled at least one prescription for folic acid tablets with a dosage strength of either 1 mg or 5 mg, which are the two available prescription-based dose strengths. These prescriptions were filled between 90 days before the last menstrual period and the date of delivery. The medications were identified in the prescription registers by using the Anatomical Therapeutic Chemical (ATC) code of B03BB01. One drug dispensing may cover a period exceeding 90 days as a prescription for higher dose folic acid typically contains between 250 and 1,000 pills per package.

Antiseizure medication

We defined use of ASM as filled prescriptions assigned with either of the ATC codes S01EC01, N05BA09 or N03 that were filled between the last menstrual period and the date of delivery. Use of ASM was categorised into any ASM use and stratified in to ASM polytherapy or ASM monotherapy. ASM monotherapy was defined as prescription fills for no more than one type of ASM between the

last menstrual period and the date of delivery. We focused on the eight most frequently used monotherapies in our population (31). Other monotherapies were pooled into a single category coined "other ASM monotherapy". ASM polytherapy was defined as use of at least two different types of ASM between the last menstrual period and the date of delivery.

Statistical analyses

We calculated the proportion of pregnancies in women with ASM-treated epilepsy who used higher dose folic acid in each country separately and combined. To illustrate changes in the proportion of higher dose folic acid use, we applied logistic regression with restricted cubic splines with calendar year as an independent variable and stratified data on country. This method allowed for a flexible and non-linear representation of the relationship between year of delivery, country and the proportion of pregnancies supplemented with higher dose folic acid with corresponding 95% confidence intervals (CIs). Changes in the proportion of higher dose folic acid use were similarly illustrated stratified for any ASM use, and then stratified for ASM monotherapy and ASM polytherapy. To enhance our understanding of higher dose folic acid supplementation in relation to differences in ASM treatments between the countries, we calculated the proportion of pregnancies receiving any ASM use, and then for ASM monotherapy and ASM polytherapy, regardless of concomitant higher dose folic acid use.

We estimated the use of over-the-counter folic acid (typically 0.4 mg or 0.8 mg daily) in pregnancies with ASM-treated epilepsy in Norway. This calculation was done by subtracting the number of pregnant women with filled prescriptions for 1 mg and/or 5 mg folic acid from all pregnant women self-reporting use of any folic acid before or during pregnancy and recorded in the Medical Birth Registry of Norway. Information of self-reported usage of folic acid in relation to pregnancy was not available for pregnancies in Denmark or Sweden.

The timing of the initial higher dose folic acid use in relation to pregnancy was assessed by producing country-specific histograms, allowing for a one-year lookback prior to the last menstrual period.

Stata version 17 was used for all analyses (32).

Results

From a total of 2,748,880 pregnancies in the general population in Denmark, Norway and Sweden, we identified 8,700 pregnancies (0.3%) in women with ASM-treated epilepsy (Figure 1). Among these, 4,720 (54.3%) pregnancies were supplemented with higher dose folic acid, whereas 3,980 pregnancies (45.7%) were not (Table 1).

Higher dose folic acid supplementation by country and ASM type

The proportion of pregnancies supplemented with higher dose folic acid was highest in Sweden with 74.2% (n=2,940 out of 3,960 pregnancies) compared with 41.4% in Norway (n=920 out of 2,220 pregnancies) and 34.4% in Denmark (n=870 out of 2,530 pregnancies) (Table 2). Whereas the proportion of pregnancies supplemented with higher dose folic acid did not change during the time of follow-up in Sweden, we observed a decreasing trend in Denmark and Norway from 2012 onwards by using logistic regression with cubic splines as illustrated in Figure 2 (point estimates of proportions provided in eTable 3 in the Supplementary material).

The proportion of pregnancies in women with epilepsy using higher dose folic acid and being treated with carbamazepine or valproate monotherapy was equally high in all countries (Figure 3). For lamotrigine, levetiracetam, oxcarbazepine and topiramate monotherapy, fewer pregnancies in Denmark and Norway were supplemented with higher dose folic acid than in Sweden. In the composite group "other ASM monotherapies", less than 60% of pregnancies were supplemented with higher dose folic acid from 2012 and onwards in all countries (Figure 3).

Regardless of higher dose folic acid use, we observed that a higher proportion used carbamazepine and valproate in relation to pregnancy in Sweden (22.0% and 12.2%, respectively) than in Norway (13.1% and 9.0%, respectively) and in Denmark (4.7% and 7.1%, respectively) (eTable 4, Supplementary material). Overall, women with ASM-treated epilepsy most frequently used lamotrigine (55.7%) and levetiracetam (11.5%) during pregnancy, although the proportions were lower in Sweden (43.3% and 8.6%, respectively) (eTable 4, Supplementary material).

Use of over-the-counter folic acid during pregnancy

Out of 2,220 pregnancies to mothers with ASM-treated epilepsy in Norway, we found that 1,080 (48.6%) reported folic acid use but did not fill prescriptions for higher doses of folic acid, suggesting that these mothers likely supplemented with lower doses of folic acid, which are available over-the-counter (eTable 5, Supplementary material). Only 220 (10.0%) were registered with no use of any folic acid supplementation before or during pregnancy and with no recorded prescription fills for

higher doses of folic acid. Across different types of ASMs used in relation to pregnancy, the percentage of cases with no folic acid supplementation varied between 5% to 14%. It was least common to not use any folic acid supplementation in mothers treated with valproate (5%) (eTable 6, Supplementary material).

Timing of first use of higher dose folic acid prescription in relation to pregnancy

By extending the lookback from 90 days to one year prior to the last menstrual period, a total of 4,990 pregnancies were identified and included when we assessed the timing for the first use of higher doses of folic acid in relation to pregnancy. We observed that 42% (2,100 out of 4,990 pregnancies) of pregnancies in women with ASM-treated epilepsy were not supplementation with higher dose folic acid until after the pregnancy had started (Figure 4, eTable 7 Supplementary material). Periconceptional higher dose folic acid supplementation was most common in Sweden (60%) and Denmark (60%), and least common in Norway (51%).

Discussion

In this large, multinational study describing supplementation of higher dose folic acid in relation to pregnancy in women with ASM-treated epilepsy, we observed that only half of the pregnancies were supplemented with higher dose folic acid. Among those who used higher dose folic acid, more than a third did not start using it before becoming pregnant.

We observed that it was most common to supplement pregnancies with higher dose folic acid in Sweden, regardless of the type of ASM used. The proportion of pregnancies in Sweden supplemented with higher dose folic acid remained stable from 2006 to 2017. This aligns with the guidelines in effect in Sweden during the study period. Swedish health authorities issued clear recommendations for 5 mg of folic acid daily for persons using valproate and carbamazepine, starting in 1997 and, subsequently, recommending 5 mg daily during pregnancy for any type of ASM used from 2011 to 2019 (23, 24). Whereas we were unable to assess folic supplementation practices for women treated with ASM in relation to pregnancy after 2017, it is worth noting that in 2019 the Swedish health authorities changed the recommendations to 0.4 mg folic acid daily regardless of whether the person is being treated with an ASM or not (25). The Swedish health authorities have consistently emphasised the absence of conclusive evidence regarding the optimal dose and timing of folic acid to achieve potential benefits from higher dose folic acid in the various clinical guidelines.

In contrast to pregnancies in Sweden, less than half of pregnancies in women with ASM-treated epilepsy were supplemented with higher dose folic acid in Norway (41.4%) and Denmark (34.4%). In both countries, we observed a decreasing proportion of pregnancies supplemented with higher dose folic acid in relation to pregnancy from 2012 to 2017. Plausible explanation for declining use of higher dose folic acid is reduced usage of valproate and carbamazepine, two ASMs for which higher doses of folic acid particularly were recommended during the study period (33, 34). Also, it is possible that clinicians have been increasingly aware that the evidence does not support higher dose folic acid being protective against neural tube defects associated with ASM and have changed their prescribing practice already before the national guidelines changed (35). However, most pregnancies involving valproate monotherapy, carbamazepine monotherapy or ASM polytherapy, were supplemented with higher dose folic acid. This observation aligns well with the recommendations in two of three current clinical guidelines in Norway, which recommend higher dose folic acid for pregnant women with epilepsy on carbamazepine, valproate or ASMs of "unknown teratogenic potential" (18, 21, 22). Additionally, the Danish clinical guideline for folic acid supplementation also recommends a daily 5 mg dosage for older types of ASM, including valproate and carbamazepine (16).

One explanation for the higher proportion of pregnancies consistently receiving higher dose folic acid supplementation may be the simplicity of Swedish guidelines, which did not distinguish between specific ASM types. Furthermore, unlike Norway, Sweden has only one available set of clinical

recommendations for folic acid supplementation for those treated with ASM during pregnancy, and unlike Denmark, the recommendations have been updated more frequently. For valproate and carbamazepine, both of which had higher dose folic acid supplementation recommendations in Denmark (at least valproate) and Norway, we noticed that the proportion of pregnancies supplemented with higher dose folic acid was more similar to what we observed in Sweden for these two ASM types. For other ASM therapies in Denmark and Norway, we observed more variability in the proportion of pregnancies receiving higher dose folic acid supplementation during pregnancy.

We observed that among pregnancies to women with ASM-treated epilepsy in Norway, the use of over-the-counter folic acid who did not fill prescriptions for higher doses of folic acid included approximately half of all pregnancies. No use of any folic acid before or during pregnancy occurred in only 10% of all the included pregnancies in Norway. No folic acid use was lest common in women who were treated with valproate during pregnancy. In contrast, only 27% were supplemented with folic acid doses <1 mg daily in a recent US study including 302 pregnant women with epilepsy enrolled between 2012 to 2016 studying behavioural outcomes and neurodevelopmental disorders in children born by women with epilepsy (36). Higher doses of folic acid ($\geq$1 mg daily) was used in 60% whereas 11% did not use any folic acid at all during pregnancy. Nearly all were treated with an ASM during their pregnancy and the study population was recruited from specialist healthcare centres and was not population-based.

In our study, we observed that more than 40% of the pregnancies in women with ASM-treated epilepsy were not started on preconception higher dose folic acid supplementation. Moreover, preconceptional supplementation of higher dose folic acid was more common in Sweden than in Denmark and Norway. This finding is in line with results on neurodevelopment of children born to women with epilepsy conducted in the UK showing that only 46% initiated folic supplements prior to conception, in which 70% of the population used $\geq$5 mg folic acid daily (14). The late initiation of folic acid supplementation underscores the need for enhanced pre-pregnancy planning protocols for women of childbearing age with epilepsy, given that pregnancies in this population are commonly unplanned (37).

This study has some limitations. The results may not be generalisable to other regions or countries with different clinical guidelines. We did not have detailed information on actual consumption of higher dose folic acid and ASM or biological levels reflecting the use of these two medications and therefore mainly relied on information about filled prescriptions as the best available surrogate (38). We did not have information on self-reported use of folic acid in Denmark or Sweden, which for Norway could be used to estimate the use of over-the-counter low dose folic acid. Since a folic acid prescription may contain as much as 1,000 pills per package and we did not have exact information concerning prescribed daily dose, we were unable to report the exact daily average exposure for folic

acid. Although it could be of interest to compare the timing of the first folic acid prescription in the first pregnancy to that of subsequent pregnancies, this comparison was not feasible due to limited observations in several key time intervals during pregnancy when stratifying for the first, second, and third pregnancy. We lacked exact information regarding the absence of data on higher dose folic acid use in Denmark for the year of 2007, but this finding is in line with publicly available data on Danish data use and is unlikely to impact the study results (39). The results may not be generalizable to pregnancies with other indications for higher dose folic acid supplementation than ASM.

Conclusions

We observed that only half of pregnancies in women with ASM-treated epilepsy received higher dose folic acid supplementation. Overall, Sweden had a higher rate of such supplementation than Denmark and Norway. Nevertheless, higher dose folic acid supplementation was common in all countries for individuals on valproate monotherapy or carbamazepine monotherapy. For other types of ASM therapies, we found considerable variations in higher dose folic acid usage between the countries. Among those who used higher dose folic acid supplementation during pregnancy, more than 40% did not begin until after pregnancy onset.

Our results emphasize a need for improved practice patterns to better reflect clinical guidelines. Future guidelines should be simplified and with clear recommendations, and they should be developed in cooperation between neurologists, obstetricians, pediatricians, and public health specialist. This should contribute to a better translation into real world practice.

Tables and figures

Table 1: Population characteristics in pregnancies in women receiving antiseizure medication for epilepsy, with and without higher dose folic acid supplementation.

Characteristics	Number of pregnancies in women with ASM-treated epilepsy	
	Higher dose folic acid	No higher dose folic acid
Total, (%)[a]	4,720 (54.3)	3,980 (45.7)
Country, N (%)[a]		
Denmark	870 (34.4)	1,660 (65.6)
Norway	920 (41.4)	1,300 (58.6)
Sweden	2,940 (74.2)	1,020 (25.8)
Year of delivery, N (%)[b]		
2006-2009	1,450 (30.7)	1,260 (31.7)
2010-2013	1,690 (35.8)	1,260 (31.7)
2014-2017	1,580 (33.5)	1,460 (36.7)
Antiseizure medication (ASM)		
Any ASM monotherapy, N (%)[b]	4,720 (100)	3,980 (100)
Carbamazepine, N (%)[b]	880 (18.6)	390 (9.8)
Levetiracetam, N (%)[b]	430 (9.1)	520 (13.1)
Lamotrigine, N (%)[b]	2,010 (42.6)	2,100 (52.8)
Oxcarbazepine, N (%)[b]	160 (3.4)	150 (3.8)
Topiramate, N (%)[b]	160 (3.4)	130 (3.3)
Valproate, N (%)[b]	610 (12.9)	250 (6.3)
Other monotherapy*, N (%)[b]	280 (5.9)	360 (9.0)
ASM polytherapy**, N (%)[b]	190 (4.0)	90 (2.3)

Use of higher dose folic acid was defined as prescription fills of a drug associated with an Anatomical Therapeutic Chemical (ATC) code of B03BB01 with dose strengths or 1 mg or 5 mg in the 90 days leading up to the last menstrual period and until the date of delivery.

Antiseizure medication was defined as prescription fills of drugs associated with ATC code S01EC01, N05BA09, or N03 between the last menstrual period and date of delivery.

[a] Row-wise percentage.
[b] Column-wise percentage.
* Other antiseizure monotherapy reimbursed corresponding to ATC code S01EC01, N05BA09, or N03.
** ASM polytherapy was defined as prescription of at least two different ASMs.

Table 2: Use of higher dose folic acid prescriptions during pregnancy in women with epilepsy treated with antiseizure medication, by country.

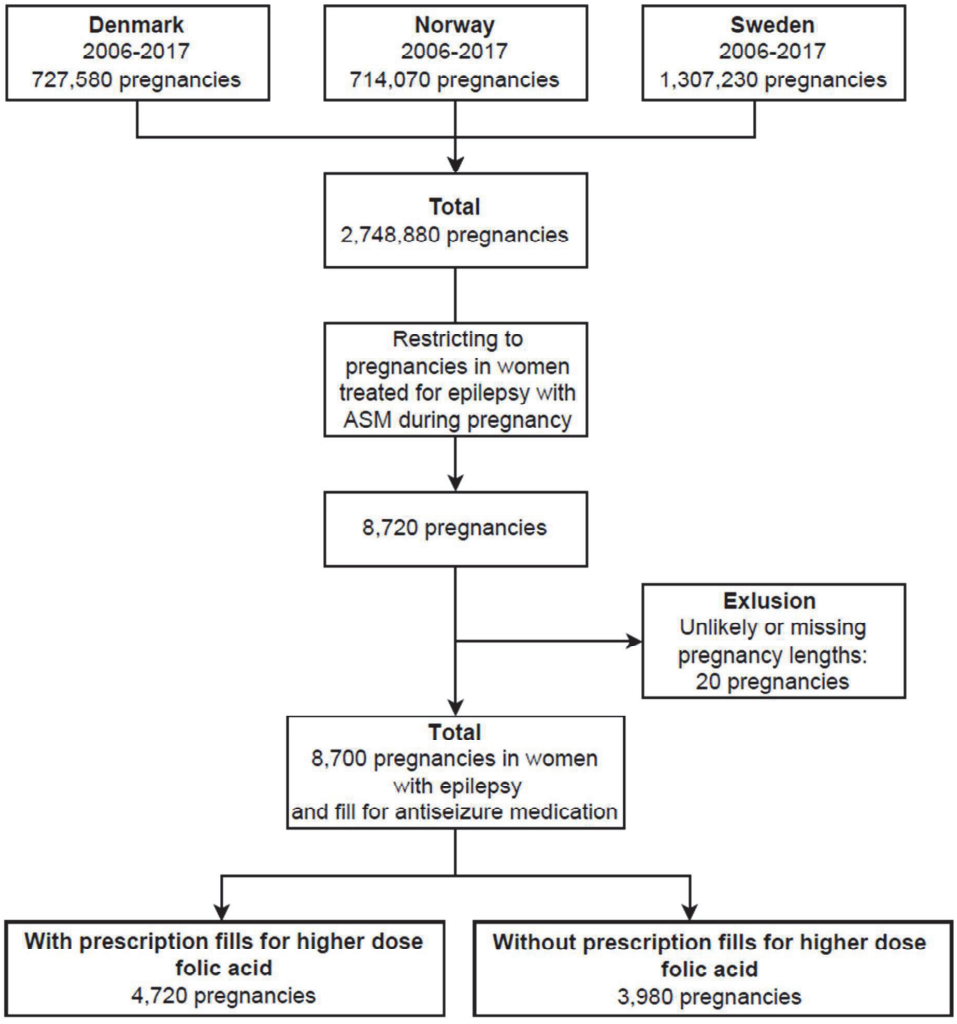
Higher dose folic acid characteristics	Countries			
	Denmark	Norway	Sweden	Combined
Total no. of pregnancies (%[a])	2,530 (29.1)	2,220 (25.5)	3,960 (45.5)	8,700 (100)
Any higher dose folic acid (%[b])	870 (34.3)	920 (41.4)	2,940 (74.2)	4,720 (54.3)
Total no. who used 5 mg folic acid, (%[c])	830 (95.4)	20 (2.2)	2,720 (92.5)	3,570 (75.6)
Total no. who used 1 mg folic acid, (%[c])	30 (3.4)	890 (96.7)	150 (5.1)	1070 (22.7)
Total no. who used 1 mg and 5 mg folic acid, (%[c])	10 (1.1)	10 (1.1)	70 (2.4)	90 (1.9)

Use of higher dose folic acid was defined as prescription fills of a drug associated with an Anatomical Therapeutic Chemical (ATC) code of B03BB01 with dose strengths or 1 mg or 5 mg in the 90 days leading up to the last menstrual period and until date of delivery.

Antiseizure medication was defined as prescription fills of drugs associated with ATC code S01EC01, N05BA09 or N03 between the last menstrual period and date of delivery.

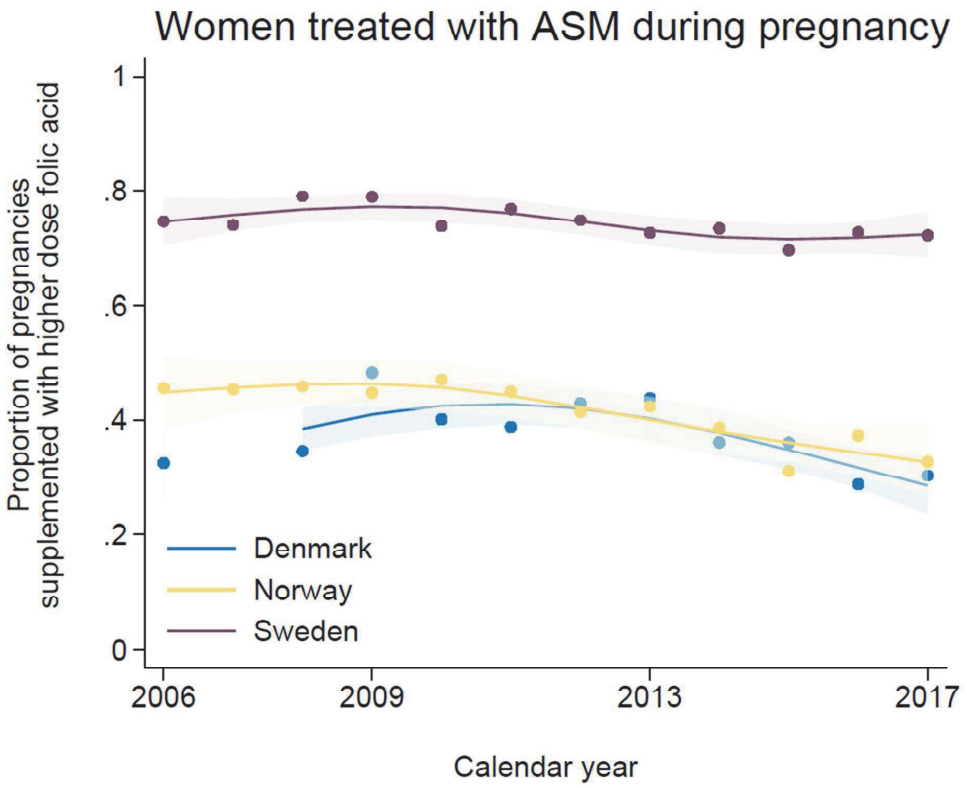
[a] Percentage of recorded pregnancies in women with epilepsy and fill for antiseizure medication.
[b] Column-wise percentage of total pregnancies per country or of all countries combined (total).
[c] Column-wise percentage of total amount of folic acid prescription fills.

Figure 1: Flow chart showing study population selection.



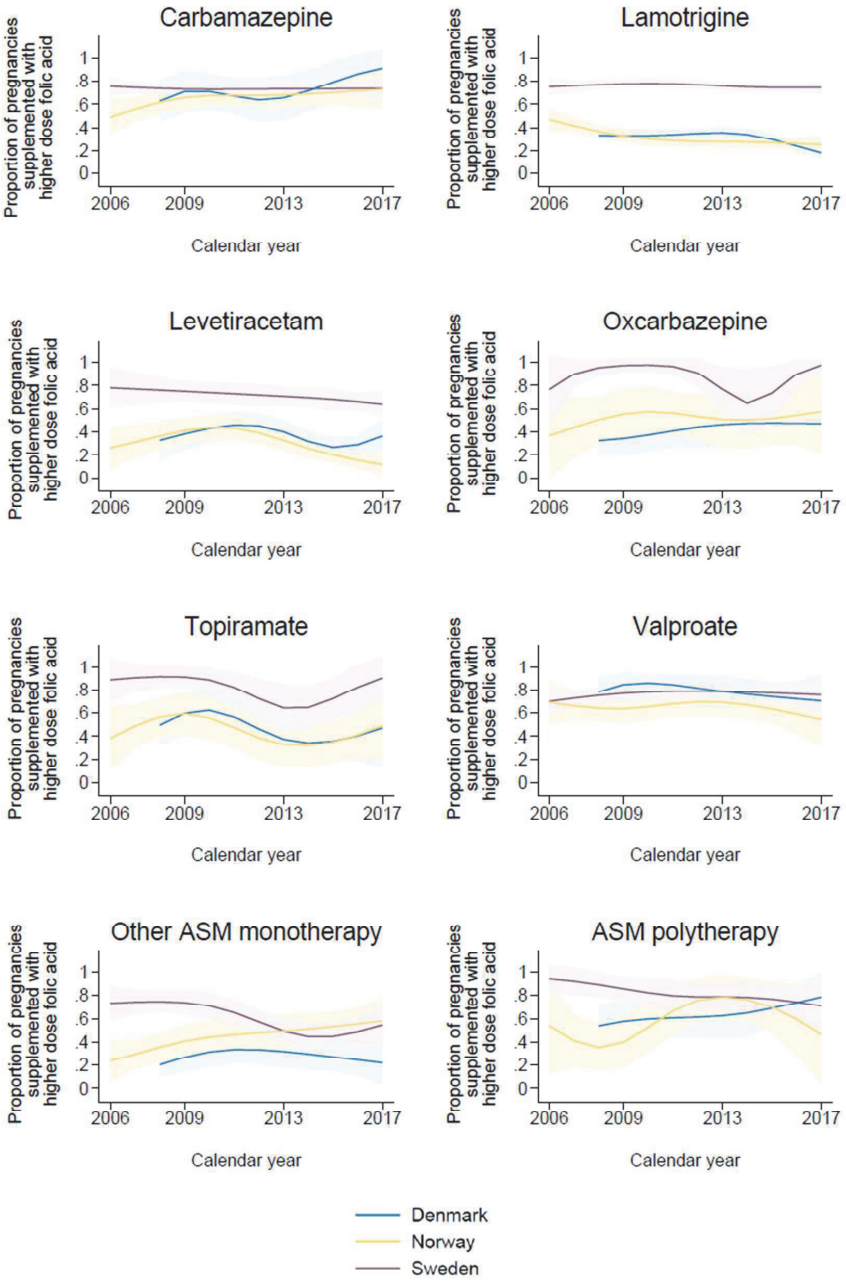
Flow chart of pregnancies in Denmark, Norway and Sweden from 2006-2017 and identification of women diagnosed with epilepsy and fill for antiseizure medication, grouped into those with and without filled prescriptions for higher dose folic acid.

Figure 2: Proportion of pregnancies in women with epilepsy on antiseizure medication using higher dose folic acid during pregnancy.



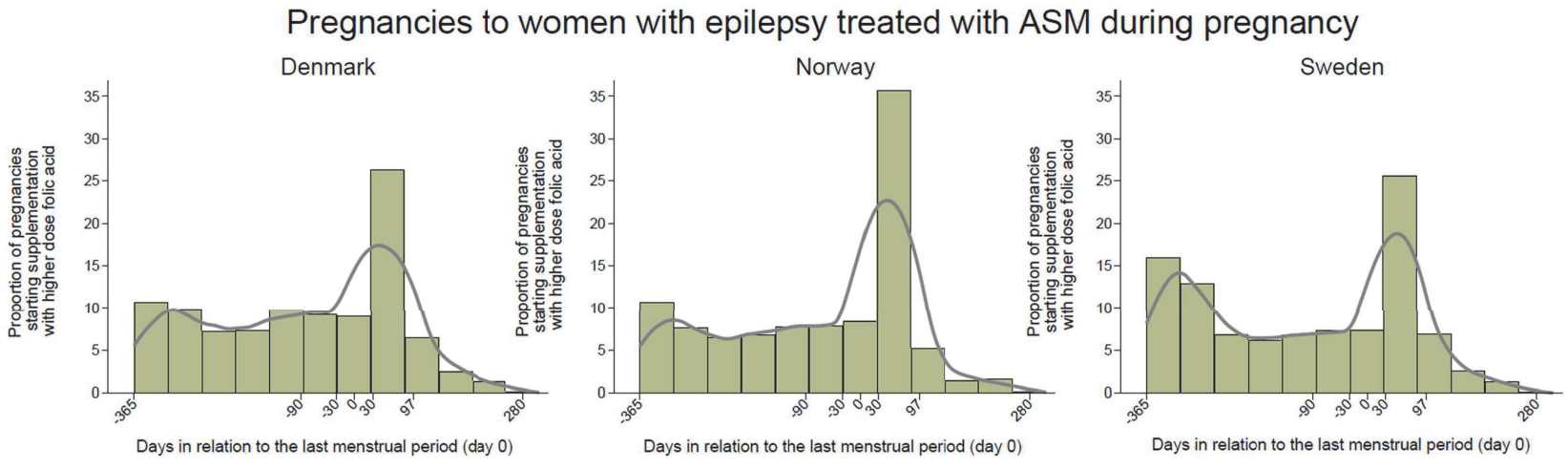
Trends in proportion of pregnancies in women on antiseizure medication for epilepsy filling a prescription for higher dose folic acid (≥1 mg). Lines with corresponding confidence intervals were produced using restricted cubic splines with observed proportions added as scatterplot. Splines were not added for Denmark in 2007 due to missing information on higher dose folic acid prescriptions fills. Specific point estimates with corresponding CIs are available in eTable 2 in the Supplement.

Figure 3: Higher dose folic acid utilisation during pregnancy among women across different antiseizure medication therapies.



Abbreviations: ASM: Antiseizure medication
Graphs showing trends in the proportion of pregnancies in women with ASM-treated epilepsy supplemented with higher dose folic acid (≥1 mg). Lines with corresponding confidence intervals were produced using restricted cubic splines.

Figure 4: Timing of the first usage of higher dose folic acid in relation to start of pregnancy.



Abbreviations: ASM: Antiseizure medication; LMP: Last menstrual period.

Individual histograms from each of the included countries showing the timing of higher dose folic acid (≥1 mg) prescription fills in relation LMP for pregnancies in women diagnosed with epilepsy and treated with ASM. In this analysis, we allowed for one year lookback prior to the last menstrual period. Grey lines illustrate the kernel density plot of the histograms. Bar graphs in each histogram are equal to a proportion of 1.0.

Days in relation to the last menstrual period (pregnancy start) reflecting one year prior to pregnancy start (-365 days), three months prior to pregnancy start in which supplementation is recommended to be initiated (-90), the time immediately before and after pregnancy start (-30 and 30 days), end of the first trimester (79 days) and mean pregnancy length (280 days).

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Supplemental material

This document contains supplemental information and tables relevant to "Prescribing Patterns for Higher Dose Folic Acid In Pregnant Women with Epilepsy Treated with Antiseizure Medication" by Vegrim HM et al.

Contents

Description of data sources

Medical birth registers

The national medical birth registers are mandatory health registers containing detailed data on all births such as parental identification number, age, parity, smoking status, pregnancy and delivery diagnoses, interventions, birth outcomes, selected maternal comorbidities including epilepsy and more. The registers collect information from medical records of prenatal, delivery and neonatal care, and were established in Norway in 1967 and in Denmark and Sweden in 1973. In Norway, the use of folic acid supplementation is also recorded in the register, both pre-conceptionally and during pregnancy.

Patient registers

The national patient registers store medical and administrative information from specialist care including International Classification of Diseases, 10th version (ICD-10) diagnoses. In Norway, the registry holds information on all individuals treated at hospitals as from 2008. Private clinic information is available if the treatment is reimbursed by the public healthcare system. In Denmark, the national hospital register contains information on hospital stays as from 1977, with ICD codes for psychiatric

treatment being available as from 1969. Data from outpatient contacts have been recorded since 1995. The Swedish national patient register includes all hospital care in Sweden as from 1987. Outpatient visits, including psychiatric care from both private and public caregivers, have been included as from 2001.

Prescription registers

The national prescription registers contain information about dispensed medications from pharmacies, including ATC codes, drug strength, total defined daily dose, package size and dispensing date. No information is available on over-the-counter medicines or medications used during in-patient hospital stays. The Danish Registry of Medicinal Product Statistics holds information as from 1995, the Norwegian Prescription Database as from 2004 and the Swedish Prescribed Drug Register as from July 2005. The Norwegian and Danish Prescription Databases also hold information on the reimbursement indication or diagnosis.

Ethical approvals

To access and handle data from the Nordic national social and health registers, ethical approvals were required from the Data Protection Agency in Denmark, the Norwegian Inspectorate and the Regional Ethics Committee for Medical Research in Western Norway, and from the Regional Ethical Board at the Karolinska Institute in Sweden.

eTables

eTable 1: Scandinavian recommendations on folic acid supplementation before and during pregnancy in parents with ASM-treated epilepsy

Country	Source of recommendation	Year published	Summary of recommendation
Denmark	Danish Health Authorities[1]	2005 (Recommendations valid since 1998)	0.4 mg folic acid daily is recommended for everyone planning pregnancy. If treated with phenobarbital, phenytoin, ethosuximide, carbamazepine, valproate, clonazepam, or clobazam, dose of 5 mg daily should be considered.
	Danish Neurological Society[2]	2022	5 mg daily if treated with valproate and for enzyme inducing ASM during first trimester. 0.4 mg daily is recommended for everyone planning pregnancy.
Norway	The Norwegian Society of Gynecology and Obstetrics[3,4]	1998	4 mg folic acid daily is recommended if treated with valproate or carbamazepine before pregnancy and throughout first trimester, and 0.4 mg daily after first trimester.
		2014	4 mg folic acid is recommended if treated with an enzyme inducing ASM during pregnancy, and 0.4 mg after the first trimester.
		2020	1-4 mg folic acid is recommended if treated with an ASM in relation to pregnancy, depending on the type of ASM (not further specified). After the first trimester, a dose of 0.4 mg daily is recommended. All fertile women of gestational capacity should be on a continuous supplementation of 0.4 folic acid daily regardless of pregnancy.
	The Norwegian Medical association: Consensus report on treatment of women with epilepsy[5,6]	2006	Documentation on the recommendation is no longer readily available. [7]
		2011	4 mg folic acid is recommended immediately before and throughout the first trimester especially if treated with valproate, but also if treated with carbamazepine of ASMs with unknown teratogenicity. Thereafter 0.4 mg folic acid daily until delivery.
		2018	4-5 mg folic acid daily is recommended for those treated with any ASM from the date contraception is discontinued and throughout the first trimester, thereafter 0.4 mg folic acid until delivery.
	The National Centre for Epilepsy[8]	2022	4 mg folic acid before and during first trimester is recommended if treated with valproate, and for ASM types in which teratogenicity is not fully known. A dose of 0.4 mg after the first trimester.
Sweden	The Swedish Medical Products Agency[9-11]	1997	0.4 mg folic acid is recommended to everyone planning to get pregnant, throughout the first trimester. A higher dose of 4-5 mg daily should be considered if treated with valproate or carbamazepine.

		2011	5 mg folic acid should be considered if treated with any ASM, but the individual should be informed that any protective effects of higher dose folic acid is lacking.
		2019	0.4 mg folic acid is recommended for everyone before and during pregnancy, regardless of concurrent treatment of ASM.

eTable 2 Definition of variables

Variable	Time frame	Description	Register	ATC	ICD-10
Last menstrual period (LMP	N/A	Date equal from the distance from date of delivery subtracted from gestational length in days	MBR		
Age of birthing parent	N/A	Continuous	MBR		
Country	N/A	Categorical, Denmark, Norway, Sweden	MBR		
Education	Year of birth	Ordinal: Compulsory, Preuniversity, Bachelor, Master/PhD	NSI		
Smoking	Start of pregnancy	Categorical: Y/N.	MBR		
BMI	Start of pregnancy	Ordinal: <30 kg/m^2 ≥30 kg/m^2	MBR		
Epilepsy	Any time before birth	Categorical: Y/N.	NPR MBR		G40, G41
ASM	Redeemed between LMP and delivery	Categorical: Y/N	NPR	S01EC01, N05BA09, or N03	
Higher dose folic acid	Redeemed between LMP-90 and delivery	Categorial: Y/N, 1 mg, 5 mg, both 1 and 5 mg.	NPR	B03BB01	

Table legends
Abbreviations: ATC: Anatomical Therapeutic Chemical code; ASM: Antiseizure medication; BMI: Body Mass Index. ICD-10: International Classification of Diseases, 10th revision; LMP: Last menstrual period; NPR: National patient registers; NSI: National statistical institutes; MBR: Medical birth registers; PDR: Prescribed drug registers; Y/N: Yes or no.

eTable 3: Estimates corresponding to Figure 2				
Year	**Point estimate, proportion**	**95% confidence intervals**	**Scatter**	**Number of birthing parents**
Denmark				
1997	0.29	0.24 - 0.34	0.27	50
1998	0.32	0.28 - 0.36	0.30	60
1999	0.35	0.32 - 0.38	0.40	80
2000	0.38	0.34 - 0.42	0.41	80
2001	0.40	0.36 - 0.44	0.39	80
2002	0.41	0.38 - 0.45	0.39	70
2003	0.42	0.39 - 0.45	0.37	70
2004	0.42	0.38 - 0.45	0.42	90
2005	0.41	0.37 - 0.45	0.55	110
2006	0.41	0.37 - 0.45	0.33	70
2008	0.41	0.37 - 0.44	0.35	70
2009	0.41	0.37 - 0.45	0.48	100
2010	0.41	0.37 - 0.45	0.40	90
2011	0.41	0.38 - 0.45	0.39	80
2012	0.42	0.38 - 0.45	0.43	90
2013	0.41	0.37 - 0.45	0.44	90
2014	0.38	0.35 - 0.42	0.36	80
2015	0.35	0.32 - 0.39	0.36	70
2016	0.32	0.28 - 0.35	0.29	70
2017	0.28	0.23 - 0.33	0.30	60
Norway				
2005	0.44	0.37 - 0.50	0.44	70
2006	0.45	0.40 - 0.49	0.46	70
2007	0.46	0.42 - 0.49	0.45	70
2008	0.46	0.42 - 0.50	0.46	90
2009	0.46	0.42 - 0.50	0.45	90
2010	0.45	0.42 - 0.49	0.47	90
2011	0.44	0.41 - 0.47	0.45	80
2012	0.42	0.38 - 0.45	0.41	90
2013	0.40	0.36 - 0.44	0.42	80
2014	0.38	0.35 - 0.42	0.39	70
2015	0.36	0.33 - 0.40	0.31	60
2016	0.34	0.30 - 0.38	0.37	70
2017	0.33	0.27 - 0.38	0.33	60
Sweden				
2006	0.75	0.70 - 0.79	0.75	210
2007	0.76	0.73 - 0.79	0.74	210
2008	0.77	0.74 - 0.79	0.79	220
2009	0.77	0.75 - 0.80	0.79	250
2010	0.77	0.74 - 0.80	0.74	250
2011	0.76	0.74 - 0.78	0.77	270
2012	0.75	0.72 - 0.77	0.75	260
2013	0.73	0.70 - 0.76	0.73	250
2014	0.72	0.69 - 0.75	0.73	260
2015	0.72	0.69 - 0.74	0.70	250
2016	0.72	0.69 - 0.74	0.73	270
2017	0.72	0.68 - 0.76	0.72	250
Table legends In Denmark, estimates for 2007 are not reported due to missing information about prescription fills using ATC code B03BB01.				

eTable 4: Number of pregnancies in women with epilepsy treated with ASM in Scandinavia

Types of ASM	Denmark	Norway	Sweden
Any ASM	2,530 (100)	2,220 (100)	3,950 (100)
Carbamazepine	120 (4.7)	290 (13.1)	870 (22.0)
Levetiracetam	290 (11.5)	330 (14.9)	340 (8.6)
Lamotrigine	1,410 (55.7)	1,000 (45.0)	1,710 (43.3)
Oxcarbazepine	160 (6.3)	70 (3.2)	70 (1.8)
Topiramate	100 (4.0)	100 (4.5)	90 (2.3)
Valproate	180 (7.1)	200 (9.0)	480 (12.2)
Other ASM monotherapy	200 (7.9)	180 (8.1)	260 (6.6)
ASM polytherapy	90 (3.6)	60 (2.7)	130 (3.3)

Table legends
Abbreviations: ASM: Antiseizure medication.
Antiseizure medication was defined as prescription fills of drugs associated with ATC code of S01EC01, N05BA09, or N03 between the last menstrual period and date of delivery.

eTable 5: Estimated use of over-the-counter folic acid in Norway before or during pregnancy in women with ASM-treated epilepsy

Any folic acid (yes/no)	Higher dose folic acid		Total
	No	Yes	
No	220 (10.0)	120 (5.4)	340 (15.3)
Yes	1,080 (48.6)	800 (36.0)	1880 (84.7)
Total	1,300 (58.6)	920 (41.4)	2220 (100)

Table legends
Use of over-the-counter folic acid was calculated by subtracting the number in which a fill for higher dose folic acid (1 mg or 5 mg) were filled at least once from 90 days prior to the last menstrual period and until date of delivery from the total number of pregnant women reporting "any" use of folic acid before or during pregnancy (yes/no) in the Medical Birth Register of Norway.

eTable 6: Estimated use of over-the-counter folic acid in Norway before or during pregnancy in women with ASM-treated epilepsy by different ASM types

Type of ASM	Use of any folic acid (yes/no)	
	Yes	No (and no other folic acid use)
Carbamazepine monotherapy	70	30
Levetiracetam monotherapy	190	40
Lamotrigine monotherapy	570	110
Oxcarbazepine monotherapy	30	10
Topiramate monotherapy	50	10
Valproate monotherapy	60	10
Other ASM monotherapy	80	20
ASM polytherapy	N/A	N/A

Table legends
Use of over-the-counter folic acid was calculated by subtracting the number in which a fill for higher dose folic acid (1 mg or 5 mg) were filled at least once from 90 days prior to the last menstrual period and until date of delivery from the total number of pregnant women reporting the use of any folic acid before or during pregnancy (yes/no) in the Medical Birth Register of Norway. There was not a sufficient number of exposed cases to report numbers for over-the-counter use of folic acid and no recorded use of folic acid for pregnant women treated with ASM polytherapy.

eTable 7: Timing for when the first prescription for higher dose folic acid was filled in relation to pregnancy in women with ASM-treated epilepsy who were supplemented with higher dose folic acid (corresponding to Figure 4 in the manuscript)		
Country	**Before pregnancy start**	**After pregnancy start**
Denmark, n (%)[a]	560 (60%)	380 (40%)
Norway, n (%)[a]	530 (51%)	500 (49%)
Sweden, n (%)[a]	1,800 (60%)	1,210 (40%)
All countries, n (%)[a]	2,890 (58%)	2,100 (42%).
Table legends [a]: Percentage of total n per row. Abbreviations: ASM: Antiseizure medication. Antiseizure medication was defined as prescription fills of drugs associated with ATC code of S01EC01, N05BA09, or N03 between the last menstrual period and date of delivery. Pregnancy start is defined as the date of the last menstrual period.		

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