Maternal pregnancy folate status and association to language impairment in children of women with epilepsy after prenatal antiseizure medication exposure

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Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
2022

UNIVERSITY OF BERGEN

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Date of defense: 05.05.2022

Year: 2022

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Name: Elisabeth Synnøve Nilsen Husebye

Print: Skipnes Kommunikasjon / University of Bergen

Contents

Acknowledgements

Thank you to all the participating families in The Norwegian Mother, Father and Child Cohort Study (MoBa) for contributing data and biobank material, making this research project possible.

I also want to thank the Norwegian Epilepsy Foundation, Norwegian Chapter International League Against Epilepsy, Det alminnelige medisinske forskningsfond ved Universitetet i Bergen, Dr. Nils Henrichsen og hustru Anna Henrichsens legat, and Advokat Rolf Sandberg Reberg og Ellen Marie Rebergs Legat til Epilepsiforskning for research funding.

Thank you to my supervisors Marte Helene Bjørk (main supervisor) and Nils Erik Gilhus (co-supervisor) for great supervision and professional guidance throughout this project. I am positive that I could not have had better or more patient supervisors than both of you.

I also want to thank my co-authors Bettina Riedel, Anne Kjersti Daltveit, Olav Spigset, and Anne-Lise Bjørke-Monsen for great teamwork and invaluable help with the papers of this research project.

I am particularly happy to be part of the scientific environment of Bergen Epilepsy Research Group. To all my colleagues at the Department of Neurology at Haukeland University Hospital and the Rotunden office, thank you for support and for great conversations and discussions during the years. I also want to thank Håkon, Jenny, and Karine for the fruitful daily digital Teams meetings we have had during the past year.

To my wonderful husband Åsmund, thank you for your everyday support and encouragement. You are a great father to our children, Åsta Pauline and Eystein Salmund, that we welcomed in 2018 and 2019.

Thank you to my parents, Mona and Eystein Sverre Jr., for being such great and supportive parents. I am particularly thankful for all the professional discussions I have shared with my father. I also wish to thank my sisters, my elder sister Maria Helene and my younger sisters Ingrid Victoria, Birgitta Hildur Ida Ulrikke, Johanna Magdalena, Mona Kristina, and Emma Yael. Thank you for encouragement, sisterly support, and countless sister gatherings. You are my very best of friends.

Scientific environment

The work presented in this thesis was carried out at the Department of Neurology, Haukeland University Hospital, Bergen and the Department of Clinical Medicine, University of Bergen, and as a member of the research group Bergen Epilepsy Research Group (BERG).

BERG is an active research group based at the Department of Neurology at Haukeland University Hospital and Department of Clinical Medicine, University of Bergen led by Marte Helene Bjørk, associate professor and specialist in neurology. The group has 17 regular members including five PhD candidates and two post doctors/senior researchers. It consists of neurologists, a neuropsychologist, research nurses, a pharmacologist, epidemiologists, an obstetrician, neurophysiologists, a neuropediatric specialist, and a user representative. BERG works with registry-based epidemiological studies, electroencephalogram (EEG) research, and clinical epilepsy studies. The group collaborates extensively with other research environments locally, nationally, and internationally including Karolinska Institutet, Aarhus University and Aarhus University Hospital, University of Iceland, Finnish Institute for Health and Welfare (THL), Norwegian Institute of Public Health, Oslo University Hospital, Norwegian University of Science and Technology, and St. Olav University Hospital.

List of publications

I. Husebye ESN, Gilhus NE, Riedel B, Spigset O, Daltveit AK, Bjork MH. Verbal abilities in children of mothers with epilepsy: Association to maternal folate status. *Neurology* 2018;91:e811-e821.

II. Husebye ESN, Gilhus NE, Spigset O, Daltveit AK, Bjørk MH. Language impairment in children aged 5 and 8 years after antiepileptic drug exposure in utero - the Norwegian Mother and Child Cohort Study. *Eur J Neurol* 2020;27:667-675.

III. Husebye ESN, Riedel B, Bjørke-Monsen AL, Spigset O, Daltveit AK, Gilhus NE, et al. Vitamin B status and association with antiseizure medication in pregnant women with epilepsy. *Epilepsia* 2021;62:2968-2980.

Other publications not included in the degree of Philosophiae doctor (PhD)

- Husebye ESN, Wendel AWK, Gilhus NE, Riedel B, Bjørk MH. Plasma unmetabolized folic acid in pregnancy and risk of autistic traits and language impairment in antiseizure medication-exposed children of women with epilepsy. *Am J Clin Nutr* 2022. doi: 10.1093/ajcn/nqab436. Online ahead of print.
- Husebye ESN and Vederhus J, Eid K, Gilhus NE, Bjørk MH. Prevalence of self-reported emotional, physical, and sexual abuse and association with fear of childbirth in pregnant women with epilepsy - The Norwegian Mother, Father and Child Cohort Study. *Second revision submitted.*

- Alvestad S, Husebye ESN, Christensen J, Dreier JW, Sun Y, Igland J, Leinonen MK, Gissler M, Gilhus NE, Tomson T, Bjørk MH. Folic acid and risk of preterm birth, preeclampsia and fetal growth restriction among women with epilepsy: A prospective cohort study. *Second revision submitted in Neurology*
- Husebye ESN, Moen G, Storstein A. Memory loss during spinning. *Tidsskr Nor Laegeforen*. 2017 Oct 2;137(18).

List of abbreviations

5,10-MTHF	5,10-methylenetetrahydrofolate
AA	Anthranilic acid
AAN	American Academy of Neurology
ADHD	Attention-deficit/hyperactivity disorder
aOR	Adjusted odds ratio
apABG	Acetamidobenzoylglutamate
APR	The Australian Register of Antiepileptic Drugs
ASD	Autism Spectrum Disorder
ASM	Antiseizure medication
ASQ	The Ages and Stages Questionnaire
ATP	Adenosine triphosphate
B	Unstandardized beta
BMI	Body Mass Index
CI	95% Confidence interval
DHF	Dihydrofolate
DHFR	Dihydrofolate reductase
DNA	Deoxyribonucleic acid
EMA	European Medicines Agency
EURAP	The European and International Registry of Antiepileptic Drugs and Pregnancy
EUROCAT	The European Concerted Action on Congenital Anomalies and Twins
FVSD	Fetal Valproate Spectrum Disorder
HAA	3-hydroxyanthranilic acid
HK	3-hydroxykynurenine
HKr	HK ratio (3-hydroxykynurenine ratio)
hmTHF	4-alfa-hydroxy-5-methyltetrahydrofolate
ILAE	The International League Against Epilepsy
IQ	Intelligence quotient

KA	Kynurenic acid
KREP	The Kerala Registry of Epilepsy and Pregnancy
Language 20	The Twenty Statements about Language-Related Difficulties
LC-MS	Liquid chromatography-mass spectrometry
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LMNDG	The Liverpool and Manchester Neurodevelopmental Group
MBRN	The Medical Birth Registry of Norway
MCM	Major Congenital Malformation
MoBa	The Norwegian Mother, Father and Child Cohort Study
MONEAD	The Maternal Outcomes and Neurodevelopmental Effects of Antiepileptic Drugs Investigator group
MRI	Magnetic resonance imaging
MTHF	5-methyltetrahydrofolate
MTHFR	5-methyltetrahydrofolate reductase
NAAPR	The North American Antiepileptic Drug Pregnancy Registry
NEAD	The Neurodevelopmental Effects of Antiepileptic Drugs Group
NICE	The National Institute for Health and Care Excellence
NICU	The Neonatal Intensive Care Unit
p	P-value
pABG	Para-aminobenzoylglutamate
PCOS	Polycystic ovarian syndrome
PLP	Pyridoxal-5-phosphate
PRAC	Pharmacovigilance Risk Assessment Committee
r	Spearman's rho
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SAM	S-adenosylmethionine
SAH	S-adenosylhomocysteine
SD	Standard deviation
SANAD	Standard and New Antiepileptic Drugs
SGA	Small for gestational age

SLAS	The Speech and Language Assessment Scale
SNP	Single-nucleotide polymorphisms
SUDEP	Sudden unexpected death in epilepsy
THF	Tetrahydrofolate
UK	United Kingdom
UKEPR	The United Kingdom Epilepsy and Pregnancy Register
UKIEPR	The United Kingdom and Ireland Epilepsy and Pregnancy Register
UMFA	Unmetabolized folic acid
US	United States
XA	Xanthurenic acid

Abstract

Background: 2-8 out of 1000 pregnancies occur in women with epilepsy. Most women use antiseizure medication (ASM) during pregnancy to avoid potentially harmful epileptic seizures. Fetal exposure to ASM is associated with an increased risk of congenital malformations and adverse neurodevelopment. It is important to identify factors that modulate the risk of ASM-associated fetal harm. Folate is a B vitamin important for normal brain development and associated with favorable neurodevelopmental outcome in the children. Many ASM interact with folate metabolism causing reduced folate concentrations, in addition to the folate-lowering effect of the pregnancy itself.

Aims: The aims of this research project were to examine the risk of language impairment in children of women with epilepsy aged 5 and 8 years and associated risk factors, and to examine the association between maternal folate status during pregnancy, plasma ASM concentrations, and ASM-associated language impairment in children of women aged 1.5-8 years. The overall aim was to find ways to improve the outcome in children of women with epilepsy after prenatal ASM exposure.

Material and methods: The data source was The Norwegian Mother, Father and Child Cohort Study (MoBa). MoBa is a prospective, population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health and linked to the Medical Birth Registry of Norway (MBRN). Pregnant women from all over Norway were invited to participate during the years 1999-2008. 41% of the invited pregnancies consented to participate. Data on medical background, social background, epilepsy diagnosis, ASM use, vitamin supplement use, and language impairment in the children were collected from parental-reported questionnaires during the pregnancy and after birth when the child was at age 1.5, 3, 5 and 8 years. We measured plasma ASM concentrations and vitamin and metabolite concentrations in maternal samples from gestation week 17-19 and ASM concentrations in umbilical cord samples collected immediately after birth. Language impairment was examined

based on the following parental-reported screening instruments: The Ages and Stages Questionnaire (ASQ); a one-item question regarding expressive language delay; the Speech and Language Assessment Scale (SLAS); and the Norwegian instrument The Twenty Statements about Language-Related Difficulties (Language 20).

Results: The maternal epilepsy cohort in MoBa consisted of 346 ASM-exposed children of 297 women and 388 ASM-unexposed children of 323 women with epilepsy. The control group consisted of 113,674 children of 94,338 women without epilepsy. For ASM-exposed children, the adjusted odds ratio (aOR) for language impairment at age 5 years was 1.6, 95% confidence interval (CI) 1.1-2.5 compared to children of women without epilepsy. At age 8 years, the corresponding aOR for language impairment was 2.0, CI 1.4-3.0. Maternal use of periconceptional folic acid was associated with decreased risk of ASM-associated language impairment at ages 1.5, 3, 5, and 8 years. High maternal valproate concentrations correlated with poor language score at ages 1.5 (ASQ: Spearman's rho (r) = -0.50, n = 17, p-value (p) = 0.04) and 5 years (ASQ: r = -0.77, n = 9, p = 0.02; Language 20: r = 0.82, n = 9, p = 0.01). High maternal ASM concentrations correlated with high concentrations of unmetabolized folic acid (UMFA; n = 199, r = 0.22, p = 0.002), and with low concentrations of riboflavin (n = 188, r = -0.32, p <0.001) and metabolically active pyridoxine (PLP; n = 188, r = -0.19, p = 0.01). There was no association between ASM and plasma niacin status.

Conclusions and implications: Fetal ASM exposure in utero may have long term consequences for language outcome in children of women with epilepsy. Use of folic acid in the periconceptional period was associated with better language outcome in ASM-exposed children. Maternal ASM concentrations in the second trimester interacted with folate metabolism and non-folate B vitamins associated with folate function, and with language score. Folate may play a role in ASM-associated risk of language impairment. Periconceptional folic acid supplementation seems to improve the outcome in ASM-exposed children of women with epilepsy.

1. Introduction

1.1 Epilepsy – a heterogenous disease

Epilepsy is a heterogenous neurological disease with different etiologies and that affects 0.65% of adults worldwide.[1-3] It is characterized by an enduring predisposition to generate epileptic seizures and by neurobiological, cognitive, psychological, and social consequences related to this disease.[4]

An epileptic seizure is defined as a "transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain."[4] It is defined in clinical practice by; 1. The occurrence of two unprovoked epileptic seizures more than 24 hours apart, or 2. One unprovoked epileptic seizure in a person considered to have a high recurrence risk defined as >60% risk in a 10-year period, or 3. By the diagnosis of an epilepsy syndrome.[5] Factors that determine the seizure recurrence risk include abnormal electroencephalography (EEG) findings, an abnormal neurological examination, magnetic resonance imaging (MRI), and a second unprovoked epileptic seizure.[2,3] The International League Against Epilepsy (ILAE) presented an updated classification framework of epilepsy in 2017, introducing three levels of classification; A. Classification of seizure type according to the appearance of the seizure and which parts of the brain that is involved in the seizure (generalized onset, focal onset, and unknown onset), B. Classification of epilepsy type (generalized, focal, combined generalized and focal, and unknown epilepsy), and C. The diagnosis of an epilepsy syndrome.[6,7] The ILAE classification framework also includes six different etiology groups; structural, genetic, infectious, metabolic, immune, and unknown.[6] Correct classification in this framework is important to determine the epilepsy etiology, to initiate optimal treatment, and to assess the prognosis in all patients with epilepsy.[6,7]

Epilepsy is associated with cognitive, behavioral and psychiatric disorders such as autism spectrum disorders (ASD), attention deficit hyperactivity disorder (ADHD), anxiety and depression disorders, and poor academic performance.[8,9] People with

epilepsy also have an increased risk of injuries and accidents, such as burns, drowning, and traumatic brain injury.[3] The mortality risk is increased compared to people from the general population, mostly due to an increased risk of sudden unexpected death in epilepsy (SUDEP).[10] The SUDEP risk is approximately 1 per 1000 adults with active epilepsy per year.[11,12]

The incidence of epilepsy is similar among men and women.[1] However, management challenges and treatment guidelines differ between men and women, as female sex hormones, the menstrual cycle, and pregnancy can alter the activity of the epilepsy disease and interact with the treatment of epilepsy.[2,3,13-15] My research project focuses on language impairment in children of women with epilepsy after prenatal antiseizure medication (ASM) exposure and the association between ASM use and maternal folate status during pregnancy. Individual ASMs that will be highlighted include valproate, lamotrigine, carbamazepine, levetiracetam, topiramate, and oxcarbazepine, and treatment with two or more ASMs; ASM polytherapy. A brief overview of the management of women with epilepsy during pregnancy is important to understand why epilepsy treatment during pregnancy is necessary, complex, and represents a balance between the risk of epileptic seizures and the risk of ASM-associated adverse effects in the mother and the child.[13]

1.2 Women with epilepsy during pregnancy

2-8 of 1000 pregnancies occur in women with epilepsy in highly developed countries.[16-18] Most of these women have uncomplicated pregnancies and deliver healthy babies, but not all.[19] Management challenges in pregnant women with epilepsy include an increased risk of pregnancy complications, peripartum psychiatric disorders, and increased morbidity and mortality risks compared to women without epilepsy.[13,14] Treatment with some ASMs during pregnancy is associated with an increased risk of congenital malformations and adverse neurodevelopment in the child.[13,14] Uncontrolled epileptic seizures during pregnancy may harm the fetus, the mother, or both.[13] Appropriate management and counselling before conception, during pregnancy and in the postpartum period is essential for an optimal pregnancy

outcome for the mother and the child.[2,3,13,14] Most of the research involving pregnant women with epilepsy is based on observational data, as randomized controlled trials would in most cases be considered unethical.[20] Internationally used guidelines on the management of pregnant women with epilepsy have been published from The American Academy of Neurology (AAN) and the American Epilepsy Society, The National Institute for Health and Care Excellence (NICE) in the United Kingdom (UK), and the ILAE .[15,21-25] In 2021, the Medicine and Healthcare products Regulatory Agency in the UK published a report on the safety of ASM use during pregnancy.[26] In Norway, we use national guidelines developed from The Norwegian Medical Association.[27,28] No official, globally applicable treatment guideline exists, and many of the local guidelines used by different countries are outdated and lack important recommendations.[25] Although the knowledge regarding management of pregnant women with epilepsy has increased during the past decades, there is still much to be learned.[20,29] Mechanisms involved in ASM teratogenesis are largely unknown, although several hypothesizes exist, and data on the long-term outcome associated with prenatal ASM exposure are still scarce for most ASMs.[30,31]

1.2.1 The menstrual cycle

About one-third of women with epilepsy have increased frequency of epileptic seizures in relation to the menstrual cycle.[32,33] This cyclic seizure exacerbation across the menstrual cycle is termed catamenial epilepsy, and is most likely due to cyclic hormonal changes of female sex steroid hormones affecting neuronal excitability.[14,32] Catamenial epilepsy usually manifests as worsening of seizure frequency around menstruation, but also before ovulation or in the luteal phases of anovulatory cycles.[14,34] Women with epilepsy have increased risk of menstrual disturbances, either due to the epilepsy disease itself or associated ASM treatment, particularly valproate.[32,35,36] Examples of such menstrual disturbances include polycystic ovarian syndrome (PCOS), anovulatory cycles, hypothalamic amenorrhea, and premature menopause.[32,36]

1.2.2 Contraception

The choice of contraception in women with epilepsy can be challenging. Several types of ASM can interact with the female sex steroid hormones and the other way around, leading to either contraceptive failure or reduced efficacy of the ASM.[32,35,37,38] Use of enzyme-inducing ASMs such as carbamazepine, oxcarbazepine, phenytoin, topiramate, and phenobarbital decrease the levels of estrogen and progestins in oral contraceptives.[35] The combined oral contraceptive pill, dermal patch, or vaginal ring, or the progestogen-only pill or subcutaneous implant are not recommended to women using enzyme-inducing ASMs.[24,27,37] Contraceptives such as depot medroxyprogesterone injections with mechanical barrier methods, or intrauterine devices are possible options.[24,27,37] The estrogen compound of the combined oral contraceptive pill or other estrogen-based contraceptives may reduce the lamotrigine concentrations by up to 60%, and hence lead to breakthrough seizures unless the dose is adjusted.[35,38] Progestin-only contraceptives such as subcutaneous implant or hormone-releasing intrauterine devices should be preferred.[35,37]

Women with epilepsy should be educated about choice of contraception and the potential interactions with ASM treatment before they become sexually active, as unplanned pregnancies are unfortunate considering the teratogenic risks associated with ASM exposure in utero.[24,32,35,37] Up to 65% of pregnancies in women with epilepsy have been reported to be unplanned.[39,40] One study reported that the proportion of women with epilepsy with unplanned pregnancies was not higher than in women without epilepsy after adjustment for age, race, ethnicity, and socioeconomic status.[40] This indicates that demographic factors associated with epilepsy partly or fully explain the increased frequency of unintended pregnancies in epilepsy.[40,41]

1.2.3 Fertility

Birth rates are lower in women with epilepsy compared to the general population, but any association between infertility and epilepsy is not clear.[14,32,36,37] Lower birth rates could be explained by several factors such as lower marriage rates among women

with epilepsy, stigma associated with epilepsy, or active avoidance of pregnancy by the woman herself because of fear about the effects of epileptic seizures or ASMs on the pregnancy.[14,36,37,42] One prospective study in women who actively wanted to become pregnant found similar rates of pregnancy in women with epilepsy compared to women without epilepsy.[43] In this study, only participants with no known infertility cause were included, and most of the women with epilepsy used either lamotrigine or levetiracetam in monotherapy.[43] Another study found increased risk of infertility after phenobarbital use and ASM polytherapy use.[44]

1.2.4 Seizures during pregnancy

Most women with epilepsy have an unchanged seizure control during pregnancy.[14,45] Epileptic seizures worsen during the pregnancy for approximately 20-30% of women with epilepsy.[15,45] A few women experience a reduced seizure frequency.[45] Increased seizure frequency during pregnancy has been reported also among women with ASM-untreated epilepsy, indicating that the changes in female sex hormone concentrations during the pregnancy affect epileptogenesis.[45] Seizure-freedom during the last year prior to the pregnancy is the best predictor of seizure-freedom during the ensuing pregnancy.[45] Epileptic seizures during the year prior to the pregnancy, ASM polytherapy, ASM non-compliance, and pregnancy-associated pharmacokinetic changes causing lower ASM concentrations are all factors associated with seizures during pregnancy.[45] Seizure deterioration during the pregnancy is more common in women with focal compared to generalized epilepsy.[45,46] One study reported better seizure control during pregnancy in women with perimenstrual catamenial epilepsy.[47] One study with data from the European and International Registry of Antiepileptic Drugs and Pregnancy (EURAP) found that women using lamotrigine monotherapy were less likely to be seizure-free during pregnancy compared to women with monotherapy treatment with carbamazepine, phenobarbital, or valproate.[46] That study also reported that 21 of 3451 (0.6%) women with epilepsy had status epilepticus during the pregnancy.[46] Seizures during birth occurred in 2.6% of pregnancies exposed to lamotrigine and carbamazepine, in 1.9% of those exposed to phenobarbital, and in 1.4% of those exposed to valproate treatment.[46]

Fetal risks associated with seizures during pregnancy

The risk of fetal harm is mainly associated with generalized epileptic seizures during the pregnancy, although fetal distress has been reported after focal seizures with impaired awareness.[13,19,48] In generalized epileptic seizures, changes in electrolytes, blood pressure and oxygenation can cause changes in fetal heart rate and fetal asphyxia.[48] Studies have reported an increased risk of small for gestational age, low birth weight, preterm delivery, need for educational support, and low intelligence quotient (IQ) in children exposed to generalized seizures in utero.[49-52] However, findings have not been consistent,[53-56] and is generally difficult to examine as high seizure frequency during pregnancy may be associated with other factors such as poorly controlled maternal epilepsy, ASM polytherapy treatment, or low ASM compliance.[45] Perinatal death has been reported after status epilepticus in one study using EURAP data, but these two events were not directly associated in time.[46] In addition, any epileptic seizure with impaired awareness can lead to injuries or falls with possible abdominal trauma, leading to fetal and maternal harm.[13,19]

Maternal risks associated with seizures during pregnancy

Epileptic seizures during pregnancy can be associated with maternal risks.[13,19] Women with epilepsy have more than a five-fold increased mortality risk during pregnancy or in the postpartum period compared to women without epilepsy.[57-59] The estimated combined death rate during pregnancy and in the postpartum period in a study from the UK was 100 per 100,000 maternities in women with epilepsy compared to 11 per 100,000 maternities in women without epilepsy.[58] A Danish study reported 41.7 deaths per 100 000 pregnancies in women with epilepsy compared to 8.2 deaths per 100 000 pregnancies in women without epilepsy.[57] A study from the United States (US) reporting the risk of death during the delivery hospitalization period found 80 deaths per 100 000 pregnancies in women with epilepsy compared to 6 deaths per 100 000 pregnancies among women without epilepsy.[59] The majority of deaths were seizure-related, with SUDEP being the main cause.[58] In the EURAP

study reporting 21 cases of status epilepticus during pregnancy out of 3781 pregnancies with seizure data, no maternal deaths were reported.[46]

1.2.5 Treatment of epilepsy in pregnancy

To avoid epileptic seizures during pregnancy, most women with active epilepsy will need continuous treatment with ASM.[42] In people with epilepsy in general, the choice of medication is guided by type of seizure and epilepsy, and has largely been informed by the Standard and New Antiepileptic Drugs (SANAD) trials;[2] SANAD I and SANAD II.[60-63] Briefly, recommended first-line treatment of focal-onset seizures are often lamotrigine or levetiracetam in girls and women of childbearing potential.[24] In boys or women who are not of childbearing potential, lamotrigine or carbamazepine are possible options, alternatively oxcarbazepine.[24] The recommended first-line treatment of generalized-onset seizures are typically valproate in boys or women who are not of childbearing potential, and levetiracetam or lamotrigine in girls and women of childbearing potential.[24] In girls and women with epilepsy of childbearing potential, the choice of medication also depends on the risk of unplanned pregnancies, contraception use, and the teratogenic potential of the different ASMs.[64,65] Comorbid disorders and previous pregnancy complications may also play a role.[64]

The pregnancy registries

Treatment guidelines for epilepsy during pregnancy are largely informed by observational data from different independent pregnancy registries, such as the North American Antiepileptic Drug Pregnancy Registry (NAAPR), The United Kingdom and Ireland Epilepsy and Pregnancy Register (UKIEPR), and the EURAP registry which also includes data from the Australian Register of Antiepileptic Drugs in Pregnancy (APR) and the Kerala Registry of Epilepsy and Pregnancy (KREP).[13,20,64] Other sources of information include nationwide health registry data, pharmaceutical industry-sponsored pregnancy registries, and non-disease specific registries such as the European Concerted Action on Congenital Anomalies and Twins (EUROCAT).[20] The independent pregnancy registries were established to monitor pregnancy and

birth outcomes in women with epilepsy, with particular focus on ASM-associated, major congenital malformations (MCMs).[20,64] During the last decade, an increasing amount of data have emerged showing ASM-associated adverse neurodevelopment in children of women with epilepsy.[13,20] These data emerged as a consequence of multinational research teams working alongside and in extension of the pregnancy registries.[20] Examples of such teams are the US and UK Neurodevelopmental Effects of Antiepileptic Drugs (NEAD) group, the associated Maternal Outcomes and Neurodevelopmental Effects of Antiepileptic Drugs (MONEAD) Investigator group, and The Liverpool and Manchester Neurodevelopmental Group (LMNDG).[20]

Current treatment guidelines

Lamotrigine and levetiracetam are often recommended as first-line ASMs during pregnancy, as both are associated with no or a low teratogenic risk.[3,19] ASMs with high teratogenic risks include valproate, phenobarbital, and topiramate, all of which should be avoided if possible in women with epilepsy with childbearing potential.[3] Valproate is associated with the highest teratogenic risk, but also represents a very effective treatment for idiopathic generalized epilepsy.[14,66] In 2014, the Pharmacovigilance Risk Assessment Committee (PRAC) of the European Medicines Agency (EMA) endorsed restrictions regarding valproate use in girls, women of childbearing potential, and pregnant women with epilepsy.[67] These restrictions were further strengthened in 2018, when EMA announced that valproate must not be used in pregnancy in women with epilepsy or in girls or women with epilepsy of childbearing age unless the conditions of an associated pregnancy prevention programme are met.[68] The teratogenic potential of several ASMs are dose-related, and hence the lowest possible ASM dose to achieve seizure control in pregnancy is always recommended.[13,37] In addition, the clinician should avoid ASM polytherapy as this is associated with increased teratogenic risk.[13,37]

Therapeutic drug monitoring during pregnancy

The absorption, distribution, metabolism, and excretion of different ASMs are affected by the physiological changes during pregnancy.[14,69,70] Such changes include

vomiting causing reduced ASM absorption; increased volume of distribution causing reduction in highly protein-bound ASMs such as valproate and phenytoin; increased renal blood flow that reduces the concentrations of ASMs such as levetiracetam, pregabalin, topiramate, and gabapentin; and induction of hepatic enzymes causing increased elimination and reduced concentrations of ASMs such as lamotrigine, valproate, and the active metabolite of oxcarbazepine.[14,69,70] Overall, the plasma concentrations of several ASM will decrease relative to the ASM dose, and hence the seizure-preventive effect becomes reduced during the pregnancy unless the dose is increased.[45] To avoid epileptic seizures, therapeutic drug monitoring is recommended: The clinician should establish a therapeutic ASM concentration range before pregnancy in each patient, and then monitor the ASM concentrations during the different trimesters of the pregnancy and after birth.[37,69,70] Treatment with lamotrigine, levetiracetam, and oxcarbazepine warrants an especially close therapeutic drug monitoring during pregnancy, at least monthly, as their concentrations can change markedly and in an unpredictable manner.[37,69,70] The concentration of carbamazepine does usually not change due to pregnancy.[13,69,70] After birth, the physiological changes will rapidly normalize, and if ASM dose adjustments were performed during pregnancy this could lead to side-effects or ASM toxicity.[70] Hence, the ASM dose should be gradually adjusted after birth under concomitant monitoring of ASM concentrations.[70]

1.2.6 Obstetrical complications

Having epilepsy is associated with a small but increased risk of obstetrical complications, both with and without ASM treatment during pregnancy.[16,54,71,72] In 2015, a systematic review and meta-analysis on epilepsy in pregnancy and reproductive outcomes reported increased risk of spontaneous miscarriage, antepartum and postpartum haemorrhage, caesarean section, preterm birth, induction of labour, and hypertensive disorders in women with epilepsy compared to women without epilepsy.[72] In a more recent study from the US, a higher risk of obstetrical complications including preeclampsia, preterm birth, stillbirth, and delivery by caesarean section was similarly found among women with epilepsy compared to

women without epilepsy.[59] Data from the NAAPR found an association between ASM use during pregnancy and risk of preterm birth.[52] It has been debated in the literature whether the increased obstetrical risk associated with epilepsy is due to pathological factors related to the epilepsy disease itself, epileptic seizures during pregnancy, use of ASM, unintended pregnancies, or a result of differences in socioeconomic status.[37,40,42,73] Nevertheless, the overall risk of obstetrical complications is increased.[15,37] In light of this, and of other factors discussed above such as the potential risk of seizures during birth and the importance of ASM dose adjustment postpartum, home births should be highly discouraged in women with epilepsy.[37] Women should be recommended to give birth in obstetrical units with facilities for maternal and neonatal resuscitation and epileptic seizure treatment.[24]

1.2.7 Management and counseling before, during, and after pregnancy

Detailed recommendations regarding management and counseling in women with epilepsy before, during and after pregnancy have been published.[14,15,37] Management should be shared between the specialist in neurology and the obstetrician.[24] Briefly, all women with epilepsy with childbearing potential should be routinely informed about fertility, choice of contraception, the importance of planning pregnancy, and associated adverse effects for different ASMs related to pregnancy.[13,37] They should also be reassured that most of the pregnancies in women with epilepsy are uneventful.[15] Women with ASM-treated epilepsy of childbearing age should take low-dose folic acid supplement routinely considering the incidence of unplanned pregnancies in this population.[13,37] Preconceptionally, ASM treatment should be optimized with low dose and a monotherapy regime if possible, and ASM concentrations measured as a baseline.[13,37] Any changes in the ASM treatment should be done in good time before pregnancy start in order to evaluate efficiency.[13,37] Recommended folic acid dose during the different trimesters of pregnancy varies in different guidelines[13] and will be discussed further in a section below. In the third trimester, a plan for childbirth and ASM dose adjustment postpartum should be established in cooperation with the patient, information should be given about breastfeeding, and practical safety advise given in case of epileptic seizures after

birth.[13,37,69] Women with epilepsy should be encouraged to breastfeed.[15,37,74] Maternal use of carbamazepine, lamotrigine, phenytoin, or valproate have not been associated with long-term developmental problems in breastfed infants, but studies on other ASMs are lacking.[74] Women with epilepsy have an increased risk of depression and anxiety during pregnancy and postpartum compared to women without epilepsy.[75-77] Hence, screening for depression and anxiety symptoms during pregnancy and postpartum should be performed routinely as part of the management of pregnant women with epilepsy.[13]

1.3 Outcomes in children of women with epilepsy after prenatal antiseizure medication exposure

Adverse outcomes in children of women with epilepsy after prenatal ASM exposure include fetal growth restriction, increased risk of MCMs which are structural abnormalities of surgical, medical, functional, or cosmetic importance, and adverse effects on neurodevelopment such as poor language abilities or increased risk of ASD.[30,78-82] Regarding individual ASMs, topiramate exposure has particularly been associated with fetal growth restriction, but also zonisamide and phenobarbital.[65] ASMs associated with increased risk of MCMs are valproate, phenobarbital, phenytoin, carbamazepine, and topiramate.[82] Valproate and possibly phenobarbital and phenytoin are associated with adverse effects on child neurodevelopment.[30,82] Exposure to lamotrigine, carbamazepine or levetiracetam have not been consistently associated with reduced neurodevelopmental outcomes.[30,65,83] However, current data available cannot rule out a possible increased risk as data on specific neurodevelopmental domains, ASM doses, follow-up data in children beyond early school-age, and data comparisons with non-exposed control children are lacking.[30,65,83] Data on topiramate, oxcarbazepine, and other newer ASMs are too limited for any conclusions regarding neurodevelopmental outcomes.[83]

1.3.1 Perinatal outcomes

Several studies have reported adverse perinatal outcomes such as low birth weight and being born small for gestational age (SGA) in infants of women with

epilepsy.[52,59,72] Data from the Medical Birth Registries of Sweden, Norway, Finland, and Denmark have in addition shown increased risk of stillbirth, neonatal infections, asphyxia-related complications, neonatal hypoglycemia, need for respiratory care, low Apgar score 5 minutes after birth, small head circumference, admission to a neonatal intensive care unit (NICU), and preterm birth.[16,71,84-88] Adverse perinatal outcomes have been associated with the epilepsy disease itself as well as with prenatal ASM exposure.[16,54,71,72,87] A study from EURAP reported increased risk of intrauterine death in women on ASM polytherapy treatment.[89] Other predictors of increased risk were parental MCMs and previous pregnancies ending in intrauterine death.[89] ASM monotherapy and ASM dose in monotherapy had no impact on the rate of spontaneous abortions and stillbirths.[89] The risk of SGA has been associated with use of clonazepam, carbamazepine, oxcarbazepine, valproate, phenobarbital, zonisamide, topiramate, and ASM polytherapy[52,84,87] but not with lamotrigine.[84,87] The prevalence of SGA was higher for topiramate doses >50 mg compared to doses below in a study from the NAAPR.[52] A consistent association between ASM exposure and fetal growth restriction seems to exist, although it varies between the individual ASMs.[13] The long-term consequences of fetal growth restriction due to epilepsy and ASM are unknown.[13]

1.3.2 Major congenital malformations

Prenatal ASM exposure is associated with an increased risk of neural tube defects, cardiac malformations, and facial clefts.[37] In a Cochrane review on ASM monotherapy during pregnancy and MCM outcomes in the child, valproate was associated with the highest risk of MCMs (prevalence 10.9%), whereas the risk of MCMs was lowest with no increased risk compared to the general population for levetiracetam (prevalence 1.8%), lamotrigine (prevalence 2.3%), and oxcarbazepine (prevalence 2.4%).[81] The risks of MCM were increased after exposure to topiramate (prevalence 4.3%), carbamazepine (prevalence 4.9%), phenytoin (prevalence 6.3%), and phenobarbital (prevalence 7.1%).[81] These prevalence estimates were comparable to the estimates reported from the NAAPR,[90] UKIEPR,[91] and EURAP[92] registries. A systematic review and network meta-analysis reported no increased risk of MCMs

after levetiracetam or lamotrigine exposure.[79] Dose-dependent increased risks of MCMs have been reported for valproate, phenobarbital, lamotrigine, phenytoin, and carbamazepine exposure.[79,81,90-92] Data on risk of MCMs were few for many ASMs such as levetiracetam, oxcarbazepine, zonisamide, primidone, and gabapentin.[79,81] Other factors that contribute to the risk of MCM during a particular pregnancy include previous history of a pregnancy with the same ASM resulting in a child with MCM, particularly if valproate, and parental history of MCM.[85,92-94] EURAP data found an approximately 27% decrease in the prevalence of MCMs in parallel with decreasing use of valproate and carbamazepine and increasing use of lamotrigine and levetiracetam during pregnancy.[95]

Specific major congenital malformations

Neural tube defects, which are severe malformations consisting of midline defects such as spina bifida, hypospadias, and brain malformations, are particularly associated with valproate,[37,81] but also with other ASMs such as carbamazepine.[96] An increased risk of hypospadias has been reported after valproate, gabapentin, primidone, and clonazepam exposure.[79] Cardiac malformations have been associated with prenatal exposure to gabapentin, valproate, phenobarbital, and ASM polyherapy.[79,81] Increased risk of facial clefts have been associated with valproate, ethosuximide, primidone, topiramate, phenobarbital, phenytoin, and carbamazepine, and ASM polytherapy.[79,81] Increased risk of other malformations such as club foot, inguinal hernia, genitourinary defects, limb and skeletal malformations and minor congenital malformations has also been reported after prenatal ASM exposure.[79,81,90-92,97]

1.3.3 Neurodevelopmental outcomes

Neurodevelopmental disorders are complex disorders characterized by early-onset deficits of variable severity in personal, social, academic, or occupational functioning.[98,99] The term include disorders such as ADHD, ASD, communication disorders, motor disorders, tic disorders, specific learning disorders, and intellectual disorders.[98,100] They have typically onset in childhood, have multi-factorial causes,

and the different disorders often overlap.[98] ADHD is characterized by age-inappropriate problems with inattention, disorganization, hyperactivity, and impulsivity.[98] Behavioral problems include disruptive, inattentive, aggressive, anxious, or socially awkward behavior, all of which have a negative impact on the daily life of the child.[98,99] ASD is characterized by social and communication difficulties with stereotyped or repetitive behaviors and interests.[98,99] Communication disorders include language disorders, which is defined as persistent difficulties with understanding and usage of spoken and written words and sentences.[101] Neurodevelopmental disorders are associated with poor academical achievement, social problems, and potentially a severe impact on daily life and functioning of the children and their families.[98,102]

Dose-dependent adverse effects on neurodevelopment have been consistently reported for valproate.[30] The prevalence of adverse neurodevelopment in children after prenatal valproate exposure has been estimated to be as high as 30-40% compared to children of women without epilepsy or ASM-unexposed children of women with epilepsy.[30,103] The occurrence of a pattern of major and minor congenital malformations, facial dysmorphic features, and impaired neurodevelopment in some children after prenatal valproate exposure have been termed Fetal Valproate Spectrum Disorder (FVSD).[104]

Verbal and non-verbal abilities and prenatal antiseizure medication exposure

Poorer verbal and non-verbal abilities have been reported in ASM-exposed children, particularly after valproate exposure.[51,53,55,56,105-110] The NEAD group has published several studies regarding neurodevelopmental outcomes in children of women with epilepsy aged 3 and 6 years old.[53,105,111-113] They performed a prospective, observational study with blinded cognitive assessment by trained neuropsychological examiners, where children exposed to either carbamazepine, lamotrigine, valproate, or phenytoin monotherapy treatment were compared.[53] Children exposed to valproate monotherapy had significantly lower verbal abilities at age 6 years compared to children exposed to lamotrigine, carbamazepine, or phenytoin.[53] High doses of

valproate were negatively associated with verbal and non-verbal abilities at age 6 years.[53] In the valproate and in the lamotrigine groups, the verbal abilities at age 6 years were significantly lower than the non-verbal abilities for both ASMs.[53] In a linked study from the LMNDG group that included comparison with children of women without epilepsy, children exposed to valproate had reduced verbal abilities at age 6 years compared to children of women without epilepsy.[51] In utero exposure to carbamazepine was also associated with reduced verbal abilities at age 6 years compared to control children.[51] In data from the NEAD group, carbamazepine was associated with reduced verbal abilities at age 3 years,[105] but not at age 6 years.[53] A study with data from the APR examining language skills in children aged 7 years found an increased risk of language impairment after valproate exposure in monotherapy or polytherapy, but not after exposure to carbamazepine, lamotrigine, or polytherapy without valproate.[56] High doses of valproate during the first trimester correlated with and predicted low language score, and also after adjustment for confounders.[56] Overall, 19% of the ASM-children met criteria for moderate or severe language delay, which was higher than the population rate of 6%.[56] In The Norwegian Mother, Father and Child Cohort Study (MoBa), the parents reported language impairment as impaired sentence skills in children aged 3 years after prenatal exposure to valproate and lamotrigine.[108] Maternal IQ was not adjusted for in the MoBa study, but adjustment for maternal education was applied.[108] Another study from the LMNDG with adjustment for maternal IQ included found reduced comprehensive and expressive language abilities in children aged 3 years after prenatal valproate exposure compared to children of women without epilepsy.[106] The language abilities of children exposed to levetiracetam did not differ significantly from the control children.[106] In children aged 5-9 years enrolled from The United Kingdom Epilepsy and Pregnancy Register (UKEPR) with prenatal exposure to levetiracetam, valproate, or topiramate, no reduction in verbal or non-verbal abilities were reported after levetiracetam or topiramate exposure.[55] Children exposed to valproate had dose-dependent reduced verbal abilities compared to children of women without epilepsy.[55]

Intellectual functioning and prenatal antiseizure medication exposure

Prenatal exposure to valproate have been associated with an increased risk of reduced IQ.[55,78,80,109] In a study from the NEAD group, children exposed to valproate in utero had significantly lower IQ (mean 97, 95% confidence interval (CI) 94-101) at age 6 years compared to children exposed to carbamazepine (mean 105, CI 102-108), lamotrigine (mean 108, CI 105-110), and phenytoin (mean 108, CI 104-112).[53] Independent predictors of the child's IQ at age 6 years were type of ASM, maternal IQ, periconceptional folic acid supplement use, standardized dose of ASM, and gestational age at birth.[53] Higher dose of valproate correlated with lower IQ at age 6 years, whereas no other ASMs had such dose-dependent effects.[53] The linked study from the LMNDG reported an adjusted mean reduction in IQ of 9.7 points (CI -4.9- -14.6) in children exposed to valproate doses >800 mg daily compared to children of women without epilepsy.[51] The IQ in children exposed to lamotrigine, carbamazepine, or ASM polytherapy combinations not including valproate was not reduced compared to the control children, but increased frequency of children with IQ<85 was reported for carbamazepine.[51] In another study from the NEAD group examining learning and memory functioning in children aged 6 years in comparison with a group of normally developed children age 6 years, children exposed to valproate and carbamazepine in monotherapy had significantly lower adjusted mean IQ compared to the control children.[113]

Autism spectrum disorder and prenatal antiseizure medication exposure

An increased risk of ASD has mainly been reported after valproate exposure in utero.[30] A Danish population-based, prospective cohort study in children with mean age 8.8 years at the end of follow-up reported an increased risk of ASD after prenatal valproate exposure compared to non-exposure, but not after exposure to carbamazepine, oxcarbazepine, lamotrigine, or clonazepam.[114] A Swedish population-based study also reported increased risk of ASD after prenatal valproate exposure.[115] In a Dutch study with EURAP data examining parental-reported behavioral problems in children of women with epilepsy exposed to valproate, carbamazepine, lamotrigine, or levetiracetam, a higher proportions of children aged 6-7 years had

parent-reported autistic behavior symptoms compared to Dutch population norms after prenatal lamotrigine or valproate exposure.[116] Prenatal exposure to valproate, oxcarbazepine, lamotrigine, or valproate and lamotrigine as polytherapy was associated with increased risk of developing autism in a systematic review and network meta-analysis.[80] These associations attenuated except for valproate monotherapy in the sensitivity analyses where women with epilepsy as treatment indication were analyzed separately and only high quality studies were included.[80]

Attention-deficit/hyperactivity disorder and prenatal antiseizure medication exposure
Valproate exposure in utero was associated with a small, but significantly increased risk of ADHD compared to unexposed children in two large Danish and Swedish population-based, prospective cohort studies.[115,117] The risk was not increased in children exposed to carbamazepine, clonazepam, oxcarbazepine, or lamotrigine.[115,117] In the Dutch study with EURAP data, a high proportion of children in all four ASM groups examined had parental-reported clear or frequent behavioral problems compared to Dutch population norms.[116]

Lower memory abilities and executive functions have been reported in children exposed to valproate compared to other ASMs.[53,109] In the study from the NEAD group examining learning and memory functioning in ASM-exposed children, this was assessed by comparing their performance on the Children's Memory Scale (CMS) with a sample of normally developing children aged 6 years from the standardization sample of the CMS.[113] Although the ASM group means were in the average range, valproate exposed children aged 6 years had significantly lower mean performance levels across all seven CMS indexes compared to the control children.[113] Children exposed to lamotrigine performed significantly below the control children on the attention/concentration index and learning index.[113] Children exposed to carbamazepine performed below the control group on the learning index, and children exposed to phenytoin performed below the control group on the learning and delayed recognition indexes.[113] Increasing valproate dose correlated significantly with lower performance on five of seven CMS indexes, whereas increasing dose of

carbamazepine correlated with lower performance on the verbal immediate index.[113] A study in children of women with epilepsy in Wales reported lower educational attainment at national tests at age 7 after prenatal exposure to valproate or ASM polytherapy.[118] Children exposed to carbamazepine, lamotrigine, or ASM-unexposed children of women with epilepsy did not differ from the control group of children of women without epilepsy.[118] In a similar study of children aged 12 years in Denmark, valproate-exposed children performed worse in the sixth-grade Danish language test and a mathematics test compared to ASM-unexposed children.[119] Children exposed to clonazepam performed worse in the sixth-grade Danish language test, and children exposed to oxcarbazepine or carbamazepine performed worse at the sixth-grade mathematics test compared to unexposed children.[119] Exposure to lamotrigine or phenobarbital was not associated with a reduced test performance.[119]

1.4 Antiseizure medication and association to folate biochemistry and one-carbon metabolism

The mechanisms that explain the ASM-associated increased risk of MCMs and adverse neurodevelopment are still largely unknown.[29,31] The folate metabolism has been hypothesized to be involved in the susceptibility to ASM-associated adverse effects.[29,120,121] This is mainly because low folate status during pregnancy is associated with increased risk of neural tube defects, and also with adverse neurodevelopmental outcomes.[122,123] Several ASMs interact with the maternal folate metabolism, causing low maternal folate concentration.[124] Women with epilepsy are consequently often recommended higher doses of folic acid supplement periconceptionally compared to women from the general population.[124,125]

1.4.1 Folate status during pregnancy and risk of adverse health effects

Folate and one carbon metabolism

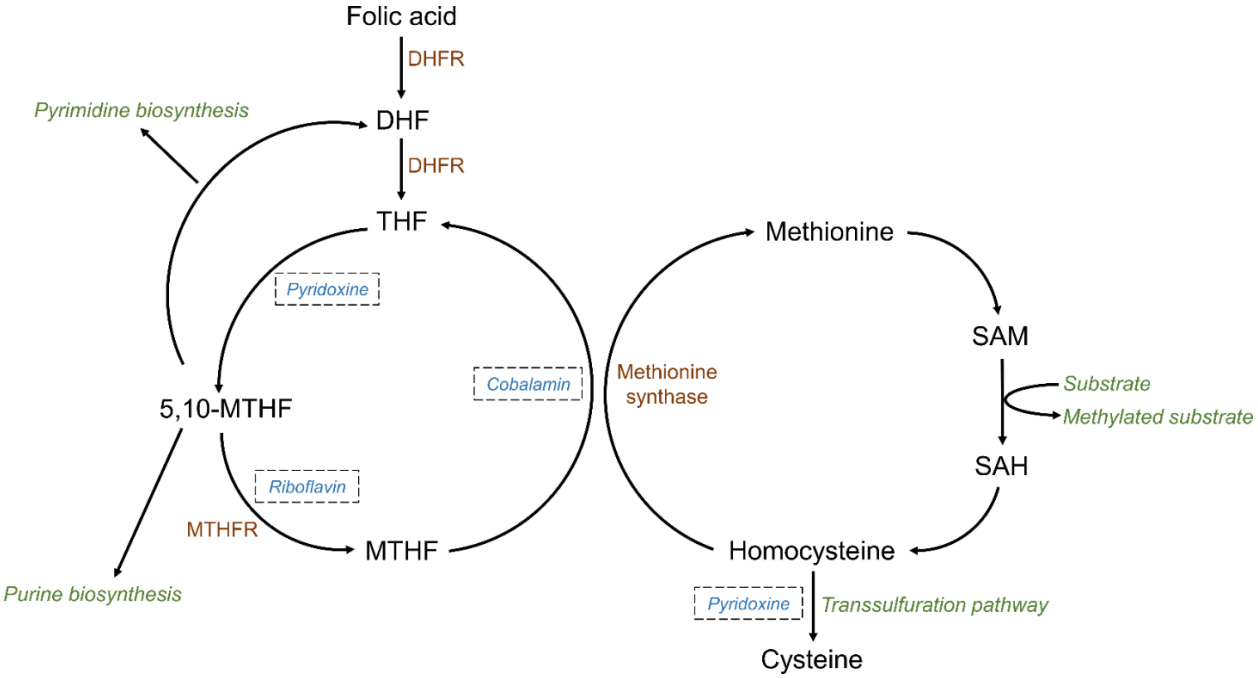
Folate (vitamin B9) is a B vitamin essential throughout life, but particularly important during the early stages of human development.[123,126,127] It functions as a family of cofactors that participate in one-carbon transfer reactions, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) biosynthesis, amino acid metabolism, and cellular

methylation reactions (one-carbon metabolism).[123,126,127] These pathways support cellular functions such as maintenance, growth, proliferation, differentiation, mitochondrial respiration, and epigenetic regulation that are crucial for normal brain development.[123,127-129]

The one-carbon metabolism consists of the folate cycle, the methionine cycle, and the transsulfuration pathway (Figure 1).[123,127] Briefly, folate is present naturally in a reduced form in many types of food, whereas folic acid is the synthetic, oxidized folate form typically present in supplements and fortified food.[126] After ingestion, folic acid will be transported into cells through the folate receptor or the intestinal proton-coupled transporter and reduced to dihydrofolate (DHF) and tetrahydrofolate (THF), this catalyzed by the enzyme dihydrofolate reductase (DHFR).[126] THF enters the one-carbon cycle and acquires a carbon unit from serine in a pyridoxine-dependent reaction to form 5,10-methylenetetrahydrofolate (5,10-MTHF).[127] 5,10-MTHF will either serve as a carbon unit donor in the synthesis of nucleic acids, or it will be reduced to 5-methyltetrahydrofolate (MTHF) in a riboflavin-dependent enzymatic reaction catalyzed by 5-methyltetrahydrofolate reductase (MTHFR).[127] The MTHFR reaction is the only source of MTHF, which is used by cobalamin-dependent methionine synthetase to convert homocysteine into methionine and to form THF.[127] Methionine is activated by adenosine triphosphate (ATP) to form S-adenosylmethionine (SAM), which acts as a universal methyl donor in the body.[127] SAM donates a methyl group to more than hundred different methyltransferases for a wide range of substrates such as DNA, proteins, neurotransmitters, membrane phospholipids, and hormones, and is thus essential for normal cell function.[127,130] It is degraded to S-adenosylhomocysteine (SAH) and reversibly hydrolyzed to homocysteine and adenosine.[123,130] Homocysteine can also be converted to cysteine through the pyridoxine-dependent transsulfuration pathway.[123] This pathway is essential for the synthesis of glutathione, which plays an important role in cellular antioxidant defence.[128] Folate catabolism is accounted for by the appearance of para-aminobenzoylglutamate (pABG) and acetamidobenzoylglutamate (apABG) in urine, but the cellular pathways for folate catabolism are still poorly understood.[129]

Figure 1.

Overview of B vitamins in one-carbon metabolism.



Adapted from McNulty et al.[127] DHF, dihydrofolate; THF, tetrahydrofolate; DHFR, dihydrofolate reductase; 5,10-MTHF, 5,10-methylenetetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; MTHF, 5-methyltetrahydrofolate; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.

Folate interacts closely with riboflavin (vitamin B2), pyridoxine (vitamin B6), and cobalamin (vitamin B12) in one-carbon metabolism (Figure 1), and normal folate function is thus dependent on the concentrations of these vitamins as well.[127] Single-nucleotide polymorphisms (SNPs) in genes related to one-carbon metabolism are relatively common in the general population and may cause disturbances in vitamin B absorption, transport, cellular uptake, and intracellular interconversion resulting in changes in enzymatic activity and increased demand of folate.[123,131] One of the most studied SNPs is the alanine-to-valine substitution in the gene encoding MTHFR (MTHFR 677C→T), which results in a thermolabile enzyme with a 50% reduction in enzyme activity in homozygous persons and low folate concentrations.[131,132] This

polymorphism has been associated with increased risk of neural tube defects and ASD.[131]

When folic acid is excessive, unmetabolized folic acid (UMFA) can accumulate in plasma.[126] UMFA is presumed to accumulate in biological fluids when the catalytic capacity of DHFR has been saturated, and is associated with the dose, frequency, and timing of ingested folic acid.[126] Both low folate status and excess folic acid intake and/or elevated folate status has been associated with increased risk of adverse health effects, such as cancer, mortality, and changes in offspring neurodevelopment.[126,133-135] However, data have been conflicting and the physiological effects of folic acid supplementation and/or excess folate are still largely unknown.[126]

Folate status during pregnancy and associated adverse effects

There is an increased demand of folic acid during pregnancy due to rapid growth of the fetus, placenta, and maternal tissue.[136] Folate deficiency during pregnancy has been associated with increased risk of anemia in the mother, neutral tube defects and other MCMs, fetal growth restriction, low birth weight, preterm delivery, and neonatal folate deficiency.[136] Periconceptional folic acid supplementation prevents the occurrence of neural tube defects.[122,127] More recently, periconceptional folic acid supplementation has also been associated with a decreased risk of adverse neurodevelopment in the children, such as ASD and language impairment, and with improved cognitive performance.[123,127,137-139] However, excess folate during pregnancy has been an increasing concern,[126,133,135] as high doses of folic acid during pregnancy and high UMFA concentrations in cord blood have been associated with adverse neurodevelopment such as increased risk of ASD,[140,141] and with impaired psychomotor development in children aged 1 year.[142]

The exact mechanisms by which folate deficiency causes increased risk of neural tube defects or impaired neurodevelopment are still largely unknown.[128,143] Folate deficiency are hypothesized to influence fetal outcome by three different mechanisms: alteration of nucleotide biosynthesis, accumulation of toxic levels of

homocysteine, and alterations of methylation reactions.[127,131,135] Homocysteine acts as a pro-oxidative agent through generation of reactive oxygen species (ROS).[144] Oxidative stress represents an alteration in the balance of pro-oxidant and antioxidant in favor of ROS overload leading to cellular damage.[144] A recent meta-analysis reported that folic acid supplementation improved markers within the antioxidative defense system such as the concentration of glutathione, possibly through a homocysteine-lowering mechanism.[144] DNA methylation represents an epigenetic mechanism for gene regulation; folate deficiency leading to decreased supply of methyl donors via SAM may lead to aberrant gene expression.[127,131] The importance of folate in DNA biosynthesis and DNA methylation has been shown in several studies: Folate deficiency has been associated with global DNA hypomethylation and brain cell alterations such as brain cell apoptosis in mice.[135,145,146] Recently, another study in mice showed that both folate deficiency and excess folate were associated with altered cortical neurogenesis, neurodevelopmental changes, and disruption of the folate metabolism in the offspring.[147] Particularly, during the growth spurt phase of pregnancy at gestational weeks 24-42 there is a rapid structural and synaptic development in the brain making it particularly vulnerable to inadequate nutrition, with folate being hypothesized to play an important role.[123,127]

1.4.2 Folate status during pregnancy and interaction with antiseizure medication

Folate and non-folate B vitamins and chronic antiseizure medication use

The interaction between epilepsy, ASM and the folate metabolism has mostly been examined in non-pregnant epilepsy populations.[124,148-152] Non-pregnant patients with epilepsy on chronic ASM therapy more often have elevated homocysteine concentrations because of low folate concentration than people from the general population.[124,153] This is because several ASMs such as carbamazepine, phenytoin, phenobarbital, and primidone increase folate catabolism which in turn impedes the metabolism of homocysteine to methionine.[124,154,155] Also, ASMs can interfere with the absorption of folic acid.[153,154] Low folate concentrations and high homocysteine concentrations have also been reported after chronic use of valproate, topiramate, gabapentin, oxcarbazepine, and lamotrigine, but data have been conflicting for

lamotrigine and oxcarbazepine.[150,152,154,156,157] Low folate concentrations have not been associated with levetiracetam, but increased homocysteine concentrations after chronic levetiracetam use have been reported in one study.[124,157]

Influence on non-folate vitamin B concentrations has been reported during chronic ASM use in non-pregnant epilepsy populations. Chronic use of topiramate, carbamazepine, phenobarbital, pregabalin, primidone, and phenytoin has been associated with low cobalamin concentrations, but data have been inconsistent.[148,150] Low concentrations of riboflavin compared to controls were reported in patients with epilepsy using carbamazepine, primidone, phenobarbital, or phenytoin.[149] Chronic ASM use, particularly of enzyme-inducing ASMs, has also been associated with low pyridoxine status, in some, but not in all studies.[148,149,158,159] In addition, chronic ASM use has been linked to low concentrations of thiamine (vitamin B1), biotin, and other vitamins such as carnitine, vitamin K, and vitamin D.[153] This will not be discussed further in this thesis.

Studies regarding vitamin B concentrations in pregnant women with epilepsy are scarce. One study from 1987 reported an association between low blood folate concentrations and high concentrations of phenobarbital and phenytoin in plasma, and also an association between low blood folate and ASM polytherapy during pregnancy in women with epilepsy.[160] Another study found low concentrations of MTHF and THF in pregnant women with epilepsy using lamotrigine, whereas no association between the folate metabolism and levetiracetam was found.[161]

Folate, prenatal antiseizure medication exposure, and major congenital malformations

In contrast to the studies from the general population, periconceptional folic acid supplementation has not been convincingly shown to reduce the risk of MCMs in pregnant women with epilepsy.[91,92,97,162,163] On the contrary, the risk of MCMs has tended to be higher in the group of women receiving periconceptional folic acid supplement. [91,92,162] An explanation for this could be that this group taking folic acid

had an increased risk of MCMs due to their ASM use.[92] In two studies,[91,162] the number of neural tube defects was numerically lower, but not statistically significantly different, in pregnancies with both valproate and periconceptional folic acid supplement use compared to pregnancies with valproate but no folic acid supplementation. However, such an association was not seen in a study with data from EURAP.[92] One study in women with ASM-treated epilepsy in pregnancy reported a higher risk of congenital malformations in mothers who carried the MTHFR 677TT mutation compared to those carrying the MTHFR 677CC wildtype.[164] This association was less pronounced in another similar study.[165] One Austrian study of 388 pregnancies of women with epilepsy enrolled in EURAP found a protective effect of periconceptional folic acid supplementation on risk of spontaneous abortion,[166] but this was not reproduced in a larger EURAP study.[89] Serum folate and red cell folate concentrations were significantly lower prior to pregnancy in women who had a spontaneous abortion or a child with MCM than in women with a healthy pregnancy outcome.[160]

Folate, prenatal antiseizure medication exposure, and adverse neurodevelopment

Studies from the NEAD group found that periconceptional folic acid supplement use was associated with better neurodevelopment in ASM-exposed children of women with epilepsy.[53,112] At age 6 years, mean IQ for children exposed to periconceptional folate supplement was 108 (CI 106–111) compared with 101 (CI 98–104) for supplement unexposed children.[53] This association was also present in subanalyses of children exposed to lamotrigine.[53] Data on folic acid supplement use was collected retrospectively from maternal interviews.[53,112] A study from the Norwegian MoBa study found a 5 to 8 times increased risk of having a child with autistic traits if the mother reported no use of periconceptional folic acid supplement compared to supplement use.[167] Similar findings have not been reported in other studies.[51,109,168] Most of the studies on this topic were not designed to examine associations to periconceptional folic acid exposure, and hence did not include prospective data or information on folic acid intake periconceptionally and during the different trimesters.[53,92]

Folic acid supplement dose during pregnancy in women with epilepsy

In the study from the NEAD group, the positive association between periconceptional folic acid supplement and higher IQ in the children was dose dependent: IQ was higher in children exposed to periconceptional folic acid doses of 0.4-1 mg daily, in those exposed to folic acid doses >1.0-4.0 mg daily, and in those exposed to doses >4.0 mg daily compared to the IQ of children with no supplement, respectively.[53] The group exposed to doses between 0 and 0.4 mg included only 6 children, hence the majority of the supplemented cohort used more than 0.4 mg.[53] Few other studies have compared different doses of folic acid during pregnancy and outcome in children of women with epilepsy. One study from the APR found no dose relationship between folic acid intake and risk of MCMs in pregnant women with ASM-treated epilepsy.[169] In the MoBa study examining autistic traits in children of women with epilepsy, the maternal plasma folate concentration in gestation week 18 was inversely associated with autistic traits at age 3 years.[167] Higher doses of folic acid supplement preconceptionally and during all three trimesters were associated with less autistic traits at age 3 years.[167] Few studies have examined the safety of high-dose (≥4 mg daily) folic acid supplementation during pregnancy in women with ASM-treated epilepsy.[13,125] We used data from MoBa to examine whether high pregnancy concentrations of UMFA in women with ASM-treated epilepsy were associated with increased risk of autistic traits or language impairment in their children at ages 1.5-8 years old, and found no such association.[170]

In Norway, women with ASM-treated epilepsy planning pregnancy have been previously recommended 1-5 mg folic acid daily in the periconceptional period depending on the ASM prescribed, but current guidelines now recommend 4-5 mg folic acid periconceptionally and 0.4 mg daily in the second and third trimester.[27] No formal recommendations exist regarding non-folate B vitamins in Norway, but women with epilepsy are often encouraged to use multivitamins during the pregnancy. The optimal dose of folic acid supplement that should be recommended to women with epilepsy before and during pregnancy is not known.[13,121,124,171,172]

Globally, guidelines differ regarding folic acid doses recommended to women with epilepsy: The AAN and Epilepsy Foundation recommend at least 0.4 mg daily and up to 4 mg daily, the NICE guidelines recommend 5 mg folic acid daily before any possible pregnancy, the ILAE guidelines recommend at least 0.4 mg daily.[13-15,24,37] In a global survey among ILAE chapters regarding use of guidelines for the management of women with epilepsy in pregnancy, 52 of 57 responders had guidelines that included folate recommendations.[25] Most of the guidelines recommended ≥4 mg folic acid daily, but doses ranged from 0.4 mg to ≥4 mg daily.[25] Due to the high prevalence of unplanned pregnancies in women with epilepsy, many guidelines including the Norwegian guidelines, the ILAE guidelines, and the AAN recommend use of at least 0.4 mg folic acid daily in all women with ASM-treated epilepsy that may become pregnant regardless of pregnancy plans.[13,15,23,27,37]

1.4.3 Mechanisms of antiseizure medication teratogenesis and the role of folate

ASMs are neuroactive compounds that reduce seizure incidence in patients with epilepsy by different and complex biochemical mechanisms that result in decreased pathological hyperexcitability of the cerebral cortex.[29,30,173] Folate and folate derivatives have convulsive properties, and it was previously hypothesised that the antiepileptic actions of ASMs were related to an ASM-induced antifolate action.[120] However, this hypothesis waned as new and effective ASMs such as lamotrigine were marketed with little or no antifolate activity, and with studies reporting that folic acid treatment did not exacerbate the epilepsy.[120] In addition to folate deficiency, other hypotheses for ASM-associated adverse effects include brain cell apoptosis, cell proliferation alterations, synaptic changes, neuronal suppression, and reactive intermediates such as ROS.[29,174] In recent years, additional hypotheses have emerged: ASM-induced altered expression of placental transporters, ASM-induced modification of gene expression through histone deacetylase inhibition, and ASM-induced interference with endogenous bioelectric mechanisms guiding embryonic development.[173,175,176]

Several studies have examined the role of folate in ASM teratogenesis.[120,121] Valproate in particular has been associated with impaired folate absorption and metabolism, accumulation of homocysteine, impaired DNA methylation, inhibition of folate receptors, and with lowered brain folate concentrations.[121,130,152,177-179] One study examined fetal DNA methylation in nine umbilical cord blood samples from women with ASM-treated epilepsy and nine such samples from women without epilepsy.[180] There was a significant difference in genome-wide methylation with exposure to ASM.[180] However, the women with epilepsy used high-dose folic acid supplement and the study could not separate the effects from such supplementation from the effects of ASM use.[180] Another study reported decreased global DNA methylation in umbilical cord blood samples and placenta tissue after prenatal exposure mainly to lamotrigine.[181] A study of mice treated with lamotrigine reported additional benefit on epilepsy, mood and memory when adding folic acid supplement,[182] and another study in pregnant mice found a protective effect of folic acid supplement on lamotrigine-induced offspring anomalies.[183] An in vitro study of human embryonic stem cells exposed to carbamazepine, gabapentin, lamotrigine, levetiracetam, or topiramate alone or in combination with folic acid found that all ASMs were associated with DNA damage, particularly levetiracetam and topiramate, and folic acid decreased this DNA damage.[184] Folic acid reduced valproate-induced structural brain defects and neurotoxicity in zebrafish embryo.[185] ASMs, particularly valproate, may adversely affect fetal growth and development through altered expression of several placental carriers, including folate carriers.[176,186-188] In human placenta, valproate altered the mRNA levels of major carriers for folic acid, glucose, choline, thyroid hormones, and serotonin.[186] Valproate at the same time reduced placental folate concentrations by 25-35% and enhanced placental histone acetylation.[186]

Animal studies and one study in humans have indicated ASM-induced brain morphology alterations such as brain cell apoptosis, cell proliferation alterations, and synaptic changes in the developing brain.[29,31,121,174,177,189] The exact mechanisms of how these alterations can cause ASM-induced MCMs or adverse neurodevelopment

are not understood.[29,31] Valproate-induced apoptosis in the embryonic cerebral wall, increased number of neocortical neurons, and increased expression of cell cycle regulatory proteins in neural progenitor cells have been reported in rodents after valproate exposure.[31] Carbamazepine exposure has been associated with decreased numbers of neurons in the hippocampus and the neocortex of mice.[31] Rat studies have reported impaired neuronal migration after lamotrigine exposure.[31] Animal studies have not reported structural abnormalities in the brain after levetiracetam exposure, but such studies are few.[31] Both phenobarbital and phenytoin have been associated with ASM-induced apoptosis in the immature animal brain.[29] A MRI study of the brain in adults with prenatal ASM exposure compared to unexposed controls found lower cell numbers in the basal ganglia and the hypothalamus in the ASM exposed adults, most pronounced in the left hemisphere.[190]

Considering that all ASMs affect neuronal transmission and voltage gradients in cells, and through different mechanisms, a new hypothesis was suggested in 2014: ASM-induced alterations in the voltage gradients in cells could be a mechanism of ASM teratogenesis.[173] Such gradients function as cues to guide cell division, apoptosis, cell positioning, orientation, and differentiation. Alterations in such bioelectrically gradients and related neurotransmitter-regulated mechanisms could impair developmental events.[173] Another hypothesis is that valproate as a potent inhibitor of histone deacetylase activates gene transcription and consequently alters gene expression resulting in teratogenicity and cell toxicity.[191,192] Yet another hypothesis involves ASM-induced oxidative stress.[29,153,193] ASMs may reduce antioxidant capacity, leading to increased production of free radicals and lower levels of endogenous antioxidants.[153] This might contribute to ASM-associated cognitive impairment. However, data for several ASMs regarding oxidative stress have been conflicting.[193]

Although several hypotheses for mechanisms of ASM teratogenesis have been suggested, there is currently no strong evidence for a single mechanism.[29,174] It is not clear whether the ASM-induced low folate concentrations are responsible for the

ASM-induced MCMs.[120,173] Several ASMs interact with folate metabolism, but it is unknown why prenatal valproate exposure is associated with the greatest risk of teratogenic effects.[120,121] Considering that ASM exposure has been associated with a limited number of specific MCMs, it has been proposed that mechanisms of ASM teratogenesis are unlikely to be explained by a global effect on cells and cell division.[173] Furthermore, it has been suggested that the ASM-associated increased risks of MCMs and adverse neurodevelopment involve different mechanisms at different stages of the pregnancy.[29] Nevertheless, it is intriguing that many of the hypotheses regarding mechanisms of ASM-induced teratogenesis and adverse neurodevelopment can be linked to folate and one-carbon metabolism.

2. Aims

The overall aim of this work was to improve the outcome in children of women with epilepsy after prenatal ASM exposure. We wanted to identify factors that reduce the risk of ASM-associated adverse neurodevelopment in children of women with epilepsy. We also wanted to examine whether ASM use and ASM concentrations are associated with maternal folate status in pregnant women with epilepsy. Our specific aims were to:

1. Examine language impairment in children of women with epilepsy aged 5 and 8 years and associated risk factors (Paper II)
2. Examine the association between ASM concentrations during pregnancy and language impairment in children of women with epilepsy aged 1.5-8 years (Papers I and II)
3. Examine the association between maternal folic acid supplement use, plasma folate concentrations, and ASM-associated language impairment in children of women with epilepsy aged 1.5-8 years (Papers I and II)
4. Examine the association between various ASM concentrations and maternal folate metabolites in pregnant women with epilepsy (Paper III)
5. Examine the association between various ASM concentrations and maternal non-folate B vitamins in pregnant women with epilepsy (Paper III)

3. Material and methods

3.1 The Norwegian Mother, Father and Child Cohort Study

The data source for this research project was pregnant women and their children enrolled in MoBa. MoBa is a prospective, population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health and linked to the Medical Birth Registry of Norway (MBRN), a national health registry containing information about all births in Norway.[194] The main aim of MoBa is to detect causes of diseases among parents and children through estimation of exposure-outcome associations.[194,195] The recruitment period was 1999-2008.[194] During this period, invitations were sent to women in 277,702 pregnancies.[194] The overall participation rate was 41%.[194,195] In total, the MoBa cohort includes more than 114,000 children, 95,000 mothers, and 75,000 fathers.[194]

3.1.1 Cohort characteristics

All pregnant women in Norway were eligible for participation, but a restriction was that the information material and questionnaires were in Norwegian only.[194,195] The postal invitation to participate was sent in gestation week 15 together with an appointment for routine ultrasound that occurs in gestational weeks 17-19 for all pregnant women in Norway.[194,195] Women not responding to the postal invitation could consent to participate during the routine ultrasound appointment.[195] Recruitment began in the county of Hordaland in Western Norway in 1999, and gradually expanded during the coming years until it became nationwide in 2005, when 50 of 52 hospitals with maternity units in Norway recruited study participants.[194,195] Fathers were invited to participate from year 2000.[194,196] A woman can participate in MoBa with more than one pregnancy. Participants can at any time withdraw from the study by not receiving more questionnaires, and also be deleted from the study with deletion of data and biological material.[194,195] MoBa data can upon application be linked to many of the health registries in Norway.[194]

3.1.2 Data collection

Mothers in MoBa responded to three questionnaires during pregnancy.[194] The first questionnaire (Q1) was sent together with the postal invitation to participate in gestation week 15 or filled out at the routine ultrasound appointment during gestational weeks 17-19.[194,195] The second questionnaire was filled out around gestation week 22 (Q2), and the third questionnaire in gestation week 30 (Q3).[194,195] Q1 and Q3 contained questions regarding social background, medical background, as well as detailed questions on previous and present health problems and exposures, including vitamin supplement use and ASM use. Q2 was a food frequency questionnaire, and data from this questionnaire was not included in this thesis.[194] After the child was born, questionnaires were sent out at age 6 months (Q4), 1.5 years (Q5), 3 years (Q6), 5 years (Q5Y), and 8 years (Q8Y). Q4-Q6 contained questions on child development, health of the mother and the child, and lifestyle exposures.[194] Q5Y and Q8Y contained questions regarding neurocognitive development.[194]

Biological samples including blood, DNA, RNA, and urine have been collected from all or subgroups of parents and children in MoBa and stored in a biobank, details are described elsewhere.[197] As a general rule, no laboratory results will be provided to the participants.[197] Recent years, MoBa Genetics has emerged as a research infrastructure aiming to genotype all participants in MoBa.[194] Blood samples from the mother were collected twice; once during the routine ultrasound appointment and once after birth.[197] An umbilical cord blood sample was also collected immediately after birth.[197]

MoBa data files released for research have been updated regularly during the years as more questionnaire data has become available. My research project was based on MoBa version 8 (95,267 pregnant women) and 10 (95,229 pregnant women).

3.2 Study population and study design

The study population was based on MoBa version 8 in paper I and MoBa version 10 in papers II and III. In paper I, the study population was all children of women with

and without epilepsy in MoBa and with available information on folic acid supplement use or folate concentrations during pregnancy. In paper II, the study population was all children of women with and without epilepsy in MoBa. In paper III, the study population was all pregnancies of women with ASM-treated epilepsy in MoBa and with available plasma vitamin B concentrations. Papers I and II were cohort studies, whereas paper III was a cross-sectional study.

3.3 Variables

3.3.1 Epilepsy diagnosis and antiseizure medication

Self-reported information on epilepsy diagnosis[4] was collected from the MoBa questionnaires and from MBRN where this information was filled in by the midwife or primary care physician. If the diagnosis of epilepsy was reported in the MBRN only and with no report of ASM use, either in MBRN or in MoBa, it was considered uncertain, and these women were excluded. Women with self-reported epilepsy were further divided into two groups: ASM-treated and ASM-untreated epilepsy. Data on ASM use during pregnancy was collected from Q1, Q3, and the MBRN.

The maternal epilepsy diagnosis in MoBa has been validated in previous MoBa data file versions by three different methods; 1. A retrospective validation questionnaire sent to all women with epilepsy in MoBa version 7 (n = 604, 50% responded) with questions regarding type of epilepsy, folic acid dose in pregnancy, and epileptic seizures during pregnancy, 2. Analysis of ASM concentrations in maternal plasma samples from gestation week 17-19 (n = 226) and in umbilical cord blood right after birth (n = 196) in singleton pregnancies of women with ASM-treated epilepsy with available biological samples from the MoBa biobank, and 3. A medical record examination for women with epilepsy residing in Western Norway based on MoBa version 5 (n = 78, 51% consented).[108,198] The validity was good, the diagnosis of epilepsy as reported in MoBa was confirmed by 98% of the women that responded to the retrospective validation questionnaire.[198] Characteristics of women with epilepsy in MoBa were similar to the Norwegian women with epilepsy included in EURAP.[198] The reported ASMs were detected in 93% of the biological samples.[198] According to

the medical record examination, 21 of 40 women that consented were treated with ASM during pregnancy, and type of ASM was in 100% agreement with the patient-reported ASM in MoBa.[108] In women with ASM-untreated epilepsy (n = 19), the epilepsy diagnosis could not be verified for two women due to lack of data.[108,198] Generally the women in the ASM-untreated group had inactive epilepsy, as only one of these 19 women had experienced epileptic seizures within two years prior to the pregnancy.[108]

3.3.2 Plasma antiseizure medication concentrations

We have previously analysed the concentrations of valproate, lamotrigine, carbamazepine, levetiracetam, topiramate, and the oxcarbazepine monohydroxyderivative metabolite in maternal plasma samples from gestation week 17-19, and in umbilical cord plasma immediately after birth.[198] The analyses of lamotrigine, carbamazepine, levetiracetam, topiramate, and the oxcarbazepine monohydroxyderivative metabolite were performed in 100 µl plasma with liquid chromatography-mass spectrometry (LC-MS) methods.[198] The analysis of valproate was performed by a commercial kit using a Cobas Integra 400 plus system (Roche Diagnostics, Rotkreuz, Switzerland).[198] All analyses were performed at the Department of Clinical Pharmacology, St. Olav University Hospital, Trondheim, Norway.[198] The limits of quantification were 0.5 µmol/L for lamotrigine, 2.5 µmol/L for carbamazepine, 5 µmol/L for levetiracetam, 1 µmol/L for topiramate, 2.5 µmol/L for the oxcarbazepine monohydroxyderivative metabolite, and 25 µmol/L for valproate.[198] We calculated standardized ASM concentrations by normalizing the plasma concentrations to the concentration range observed for that drug in our data according to the formula: *100 × (observed concentration − minimum concentration measured for that drug) / concentration range measured for that drug*.[105] For each child, we calculated the mean standardized ASM concentration based on the maternal and umbilical cord ASM concentration (Papers I and II) or based on the maternal ASM concentration only (Paper III). If only one of them was present, this was used (Papers I and II). For ASM polytherapy, the standardized ASM concentration for

each ASM was added together. A high standardized ASM concentration reflected high plasma concentrations of ASM.

3.3.3 Vitamin supplement use

Information on vitamin supplement use was collected from Q1 and Q3. The women reported on use of folic acid, riboflavin, pyridoxine, and niacin (vitamin B3) supplement in different gestation week intervals before and during pregnancy, with week 0 starting with the first day of the last menstrual period. In Q1, the women reported use of supplement in the following weekly time intervals: -4 to 0, 0-4, 5-8, 9-12, and 13+. In Q3, the weekly time intervals were: 13-16, 17-20, 21-24, 25-28, and 29+. In each questionnaire, they also reported on the frequency of supplement intake by ticking one of the following response options: daily, 4-6 times a week, or 1-3 times a week. We defined periconceptional supplement use as any use of folic acid supplement during gestational weeks -4 to 12 (Papers I and II). As maternal plasma samples were collected in the second trimester, which has been shown to be associated with folic acid supplement use during both the first and second trimester,[199,200] we additionally defined pregnancies with any supplement use during gestational weeks -4 to 20 as supplemented pregnancies (Paper III).

Folic acid supplement dose

In the retrospective validation questionnaire, the women reported folic acid supplement dose during the following gestation week intervals: -4 to 0, 0-12, 13-24, and 25-40. They were asked to tick one of the following dose options for each time interval: 0.4 mg, 1 or 2 mg, and ≥4 mg.

3.3.4 Plasma vitamin and metabolite concentrations

We analysed plasma concentrations of vitamins and metabolites related to folate, riboflavin, pyridoxine, and niacin status at Bevital Laboratory, Bergen (www.bevital.no). We analysed 227 maternal plasma samples from gestation week 17-19 from singleton pregnancies of women with ASM-treated epilepsy and with

available maternal sample in the MoBa biobank. The analyses were performed by using liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods.[201-203]

Plasma folate status

Plasma folate status was examined by analysis of the biologically active MTHF metabolite, the MTHF-derived 4-alfa-hydroxy-5-methyltetrahydrofolate (hmTHF) metabolite, UMFA, and the inactive metabolites pABG and apABG.[201] The limits of quantification were 0.13 nmol/L, 0.40 nmol/L, 0.53 nmol/L, 0.17 nmol/L, and 0.27 nmol/L, respectively.[201] Plasma UMFA values below the limit of quantification were reported as 0 nmol/L.[201] MTHF is unstable in samples kept in room temperature but can be recovered as hmTHF.[201] Hence, we defined the metabolically active folate concentration as the sum of MTHF and hmTHF ("folate").[201,202] We calculated the ratio between active and inactive folate metabolites *(MTHF + hmTHF / pABG + apABG*) as a measurement of folate catabolism. We also calculated the ratio between UMFA and folate (*UMFA / MTHF + hmTHF*) to examine the UMFA concentrations in relation to the metabolically active folate.

Plasma riboflavin, pyridoxine, and niacin status

Riboflavin status was examined by analysis of the maternal plasma riboflavin concentration, the limit of detection was 0.2 nmol/L.[203] Plasma pyridoxine status was examined by analysis of the metabolically active pyridoxine metabolite, pyridoxal-5-phosphate (PLP), and the metabolites 3-hydroxykynurenine (HK), kynurenic acid (KA), xanthurenic acid (XA), anthranilic acid (AA), and 3-hydroxyanthranilic acid (HAA).[203,204] The limits of detection were 0.2 nmol/L, 2 nmol/L, 0.4 nmol/L, 0.5 nmol/L, 0.7 nmol/L, and 2 nmol/L, respectively.[203] The functional marker of pyridoxine status, HK ratio (HKr), was calculated based on the formula *HK / KA + XA + AA + HAA*, as described in detail elsewhere.[204] High HKr indicates low pyridoxine status.[204] To examine plasma niacin status, we analyzed the plasma concentration of nicotinamide, [203] the limit of detection was 20 nmol/L.[205]

3.3.5 Language impairment

Children aged 1.5 and 3 years (Paper I)

Language impairment ("language delay" in Paper I) in children aged 1.5 and 3 years was assessed by two different screening instruments in Q5 and Q6: The communication scale of The Ages and Stages Questionnaires (ASQ) ("global language delay" in Paper I), and a one-item question regarding expressive language skills ("expressive language delay" in Paper I). The ASQ is a validated, diagnostic tool to identify language developmental delay.[206,207] The communication scale consists of three items at age 1.5 years and six items at age 3 years (Paper I, Supplemental data). Each item was scored 10 points ("yes"), 5 points ("sometimes"), and 0 points ("no"), with a high score indicating no language impairment (continuous variable).[207] We defined a dichotomous variable of language impairment as a score of 1.5 standard deviations (SD) or more below the mean score for the total MoBa study population.[207] In the one-item question on expressive language skills (Paper I, Supplemental data),[208] the parents were asked to describe how their child talks at age 3 years by ticking an option from 1 ("not yet talking") to 6 ("talking in long and complicated sentences"). Children who talked in 2- to 3-word sentences or less (option 4 or below) were defined as having expressive language impairment (dichotomous variable).[138,208] Maximum score was 6 points, with a high score indicating no language impairment (continuous variable).[138]

Children aged 5 and 8 years (Paper II)

Language impairment in children aged 5 and 8 years was based on the following screening instruments from Q5Y (Paper II, Supplemental data): The communication scale of ASQ, The Speech and Language Assessment Scale (SLAS), the Norwegian instrument The Twenty Statements about Language-Related Difficulties (Language 20), and on the semantic subscale of Language 20 from Q8Y. In Q5Y, the communication scale of ASQ consisted of seven items, six items from the age 5 years version, and one item from the age 4 years version of ASQ. Maximum score reflecting no language impairment was 70 points (continuous variable). The cut-off for language impairment was defined as already described for age 1.5 and 3 years.

The SLAS is a validated, 14-item screening instrument used to identify children with language disorders.[209] The instrument consisted of 13 items in Q5Y, where each item was scored from 0 ("very much lower") to 5 ("very much higher").[209] A score of 3 ("typical for age") or more reflected no language impairment.[209] We calculated the mean SLAS score for each child, SLAS composite scale mean score, with a maximum score of 5 reflecting no language impairment (continuous variable).[209] The Language 20 instrument consists of 20 items, and is a validated screening instrument designed to identify children with language impairment.[210] It consists of three subscales: semantic (8 items), receptive (6 items), and expressive (6 items). Each item was scored from 1 ("doesn't fit the child, absolutely wrong") to 5 ("fits well with the child, absolutely right").[210] A low score reflected no language impairment (continuous variable).[210] Maximum score was 100 points at age 5 years and 40 points at age 8 years. Cut-off for language impairment was a score of 31% or more of maximum possible score.[210] We defined the child as having language impairment at age 5 years if the child scored according to our criteria for impairment in any of the three instruments in Q5Y (dichotomous variable). At age 8 years, language impairment was defined if the child scored according to our criteria for impairment of Language 20 (dichotomous variable).

3.3.6 Other variables

We collected relevant variables from the MoBa questionnaires and the MBRN:[138,199,200,211,212] maternal age; maternal low education (≤9 years of schooling); parity (previous pregnancies >21 gestation weeks (Papers I and II) or >12 gestation weeks (Paper III)); unplanned pregnancy; low household income (<400,000 NOK annually (Papers I and II) or <60% of the national median in the child's birth year (Paper III)); non-cohabiting mother; prepregnancy body mass index (BMI); smoking (any) during pregnancy; alcohol consumption during the first trimester (≥1 time per month); report of anxiety or depression symptoms in pregnancy (mean score >1.75 on the Hopkins Symptom Checklist[213] in gestational weeks 17-19); offspring sex; twin or triplet child; Apgar score 5 minutes after birth; gestational age (calculated from routine ultrasound measurements in gestational weeks 17-19, if not available the first

day of the last menstrual period was used); maternal report of familial language delay (a sibling, parent, grandparent, aunt, uncle or cousin who was a late talker or had difficulties with reading, writing or pronunciation, Q5Y), maternal report of seldom/never helping their child read during a typical week at age 5 years (Q5Y), or never reading for their child at age 8 years (Q8Y); and any epileptic or any tonic-clonic seizures during pregnancy (data from the retrospective validation questionnaire).

3.4 Statistical analysis

All analyses were performed by using the IBM SPSS software version 24 (Papers I and II), and version 25 (Paper III).

3.4.1 Main statistical methods

We used non-parametrical statistical methods to compare the groups due to violation of the assumption of normal distribution and due to relatively small numbers of children or pregnancies in many of the analyses. We compared dichotomous variables with Chi-square test for independence or Fisher's exact test when appropriate. Continuous variables were compared with Mann-Whitney U Test (comparison between two groups) or Kruskal-Wallis Test (comparison between three or more groups, Paper III). In paper III, we adjusted for multiple testing by multiplying the observed p-value by the number of comparisons made (Dunn-Bonferroni post hoc method). The relationship between variables was examined in correlation analyses by calculating the non-parametric Spearman rank order correlation (rho) coefficient. We examined exposure-outcome associations with logistic regression models adjusted for relevant confounders. Interaction analyses were performed with multiple regression analysis with an interaction term depending on the interaction examined. When examining language impairment in paper I and II, each age group was examined as independent observations. Two-sided p-values <0.05 or effect estimates where the CI did not include 1 were considered statistically significant.

3.4.2 Main comparisons

The main comparisons in paper I and II were between ASM-exposed children of women with epilepsy and ASM-unexposed children of women with epilepsy, respectively, and a control group of children of women without epilepsy. In both papers, the analyses were stratified for periconceptional folic acid supplement use. Hence, groups with similar folic acid supplementation status were compared. This stratification was the main comparison in paper I, in paper II this represented a subanalysis. In paper II, we examined language impairment in ASM-exposed children compared to control children with and without stratification for individual ASMs and ASM polytherapy. We also examined risk factors associated with language impairment at age 5 or 8 years in each of the two epilepsy groups and in the control group. In paper III, the main comparisons were between different ASM groups, as no control group of pregnancies of women without epilepsy and with plasma vitamin concentrations was available. We analysed vitamin B concentrations stratified for ASM group and with and without stratification for supplement use.

3.4.3 Missing data

In MoBa version 8 or 10, we had no information about MoBa participants that previously had been deleted from the cohort. We provided flowchart of included and excluded participants in papers I-III. In paper II, we provided a more detailed description and comparison of Q5Y and Q8Y responders and non-responders, respectively, within each of the two epilepsy groups and the control group. Furthermore, there were missing answers in some of the questions in the questionnaires among the responders. We imputed missing answers to the questions regarding language impairment with the estimation-maximation procedure in SPSS (Papers I and II). Imputation was performed if 1 of 6 answers (ASQ Q6), ≤2 of 7 answers (ASQ Q5Y), ≤3 of 13 answers (SLAS Q5Y), ≤4 of 20 answers (Language 20 Q5Y) and ≤2 of 8 answers (Language 20 Q8Y), respectively, were missing. Children or pregnancies with missing data on main exposure or outcome variables were excluded from the main analyses, and this was described in the table footnotes and flowcharts of papers I-III.

3.5 Ethics

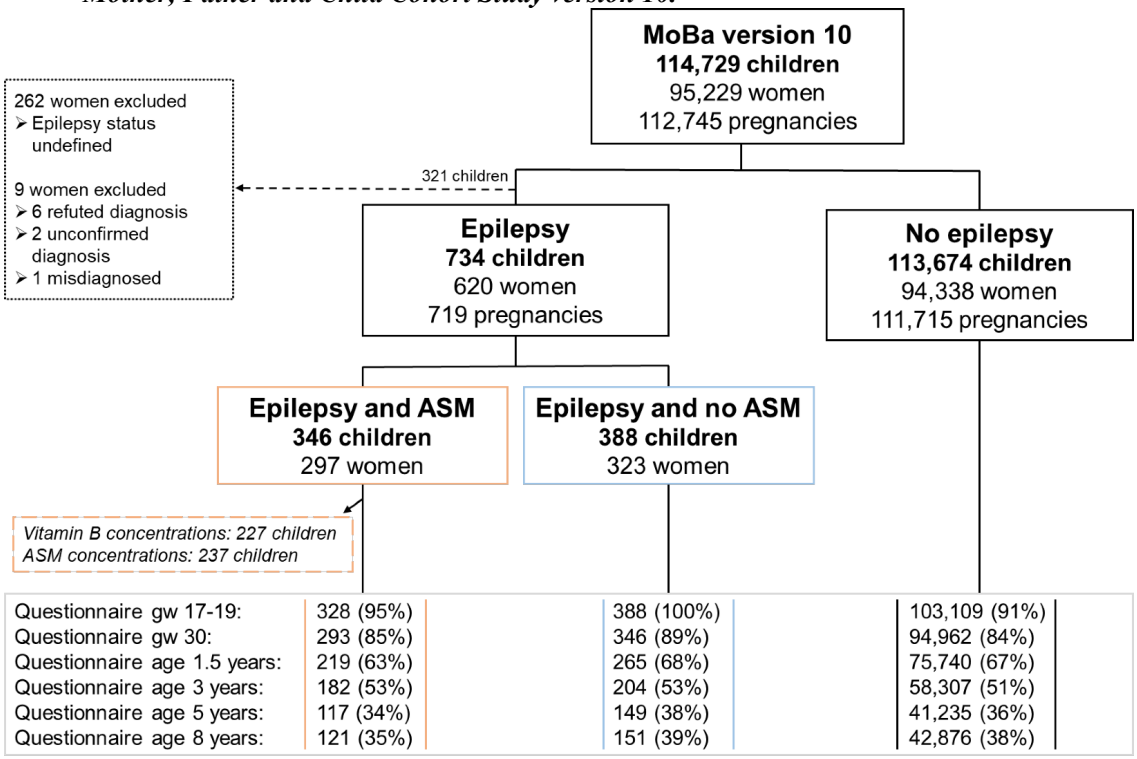
This research project has been approved by The Regional Committee for Medical Research Ethics with reference number 2011/1616. The MoBa cohort is regulated by the Norwegian Health Registry Act. The establishment of MoBa and initial data collection were based on a license from The Norwegian Data Protection Agency and approval from The Regional Committee for Medical Research Ethics.[196] All parents participating in MoBa have given written informed consent to participate. Children are included based on consent from the mother.[196] All children in MoBa will be informed about the study at age 15 years and will need to consent to further data storage at age 18 years.[196] The MoBa data files released for research contained anonymized data which was collected from all over Norway, and the laboratory results are unknown to the participants. Examples of ethical issues in this project are parents consenting and giving information about their child on behalf of the child, and risk of violating personal data privacy with data that can be identified, such as reporting rare combinations of ASM polytherapy. However, participation was voluntarily and based on informed, written consent. Randomized controlled trials to examine adverse neurodevelopment in children prenatally exposed to different ASMs would be unethical. Hence, the maternal epilepsy cohort in MoBa represents a unique opportunity to perform observational studies as the number of pregnant women with epilepsy with and without ASM treatment are relatively high, and data on several exposures and outcomes was prospectively collected during and after pregnancy. We thus believe that the ethical issues above are minor in comparison to the potential beneficial effects this research project may have for pregnant women with epilepsy and their children in the future. Nevertheless, during the years of this research project, my attention to personal data privacy was increased with consequently gradual avoidance of reporting too small numbers in the tables and supplemental data sets.

4. Summary of results

MoBa version 10 consists of 95,229 women, 112,745 pregnancies, and 114,729 children (Figure 2). The epilepsy cohort consisted of 620 women with epilepsy, 719 pregnancies, and 734 children (Figure 2). A total of 346 children were exposed to ASM in utero, whereas 388 children were not. ASM monotherapy exposure, most commonly lamotrigine (n = 112), carbamazepine (n = 72), and valproate (n = 40), was reported in 280 children, and ASM polytherapy exposure or unspecified ASM exposure (n = <5) were reported in 66 children. The response rates for the different MoBa questionnaires are shown in Figure 2.

Figure 2.

Overview of children of women with and without epilepsy in The Norwegian Mother, Father and Child Cohort Study version 10.



ASM, antiseizure medication; gw, gestation week; MoBa, The Norwegian Mother, Father and Child Cohort Study.

In the group of ASM-exposed children, 227 children had available plasma vitamin B concentrations, and 237 children had plasma ASM concentrations from maternal samples and/or umbilical cord blood samples (Figure 2). A total of 140 ASM-exposed and 159 ASM-unexposed children had data from the retrospective validation questionnaire. Seizure data was available for 161 ASM-exposed and 178 ASM-unexposed children of women with epilepsy in version 10. Exposure to any epileptic seizure during pregnancy or birth was reported in 42 ASM-exposed children and 13 ASM-unexposed children. Corresponding numbers for exposure to tonic-clonic seizures were 20 and 5 children, respectively. Only a total of two women reported 3 or more tonic-clonic seizures during pregnancy or birth in version 10.

Children with response to Q5Y differed from children that were non-responders (Paper II). ASM-exposed children that did not respond to Q5Y had mothers who were significantly younger, less often used periconceptional folic acid supplementation, and more often belonged to a low-income household, reported anxiety and/or depression symptoms during pregnancy, smoked during pregnancy, consumed alcohol during pregnancy, and more often were non-cohabiting compared to ASM-exposed children that responded to Q5Y. These differences were less pronounced between responders and non-responders of Q8Y; non-responders had significantly younger mothers and mothers who more often reported anxiety and/or depression symptoms during pregnancy than responders of Q8Y (Paper II). Similar differences between responders and non-responders were also found in ASM-unexposed children and in children of women without epilepsy at age 5 and 8 years (Paper II).

4.1 Language impairment and associated risk factors (Paper II)

Among the ASM-exposed children, 35 of 117 (30%) children had language impairment at age 5 years compared to 9011 of 41,194 (22%) children of women without epilepsy (Paper II). The adjusted odds ratio (aOR) of language impairment was 1.6, CI 1.1-2.5. At age 8 years, 38 of 120 (32%) ASM-exposed children had language impairment compared to 8250 of 42,550 (19%) children of women without epilepsy (paper II). The aOR for language impairment was 2.0, CI 1.4-3.0. When

individual ASMs were examined, children exposed to carbamazepine monotherapy had increased risk of language impairment compared to children of women without epilepsy (n = 10 of 23 (43%), aOR 3.8, CI 1.6-9.0) at age 8 years, but not at age 5 years (n = 6 of 17 (35%), aOR 1.9, CI 0.6-5.6). There was no increased risk of language impairment in ASM-unexposed children of women with epilepsy compared to children of women without epilepsy at age 5 (39 of 149 (26%) children, aOR 1.2, CI 0.8-1.8) or age 8 years (35 of 150 (23%) children, aOR 1.2, CI 0.8-1.8).

We identified several risk factors associated with language impairment at age 5 and/or 8 years (Paper II) in ASM-exposed children of women with epilepsy: male offspring, no periconceptional folic acid supplement use, smoking during pregnancy, and language impairment in the same child at ages 1.5 or 3 years. In ASM-unexposed children of women with epilepsy, one or more epileptic seizures during pregnancy and language impairment at age 1.5 years were associated with increased risk of language impairment at age 5 and/or 8 years. No periconceptional folic acid supplementation was not associated with increased risk of language impairment in this group, nor in children of women without epilepsy (Paper II).

4.2 Antiseizure medication concentrations during pregnancy and association to language impairment (Papers I and II)

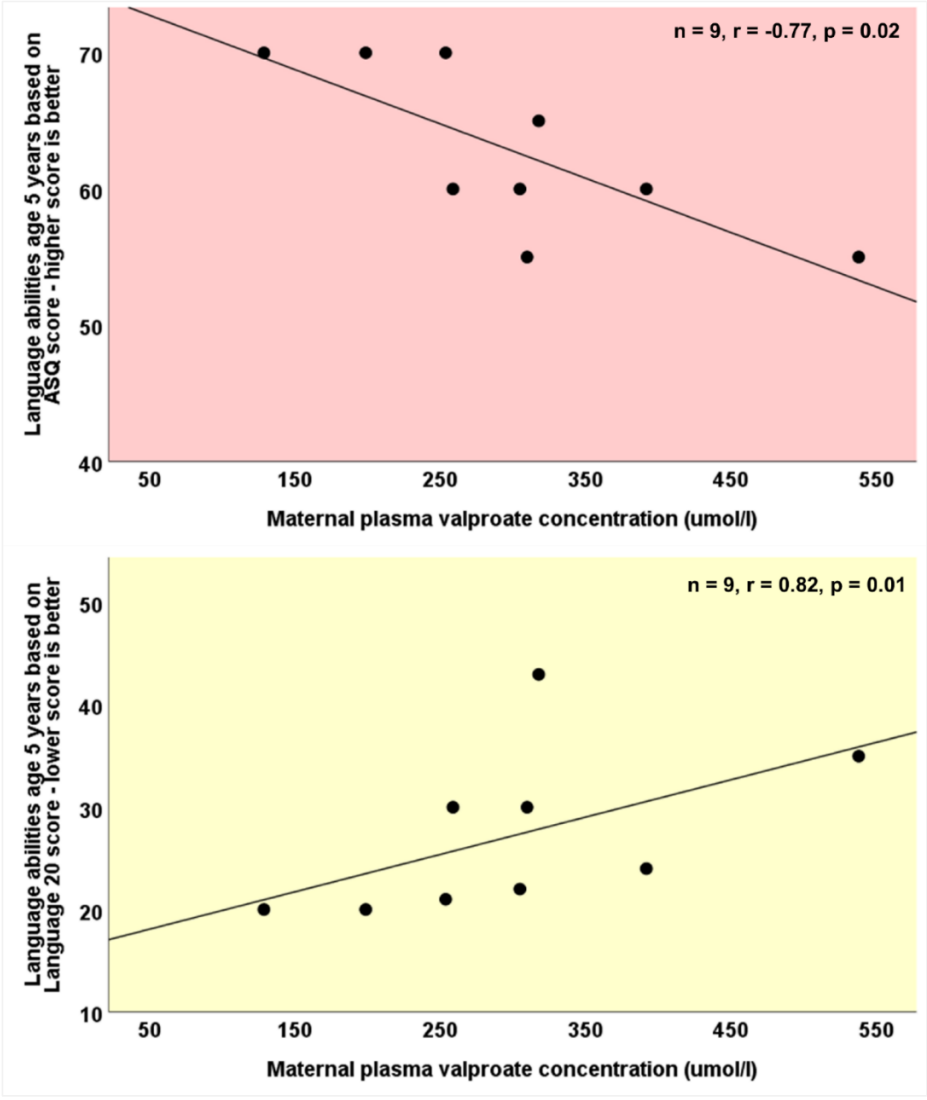
In paper I, high maternal valproate concentrations correlated strongly with low ASQ score and hence language impairment at age 1.5 years (n = 17, Spearman's rho (r) = -0.50, p-value (p) = 0.04). We found no correlation between maternal ASM concentrations and language score in children aged 3 years. In paper II, high maternal valproate concentrations correlated with low ASQ score and hence language impairment (n = 9, r = -0.77, p = 0.02), and also with high Language 20 score indicating language impairment (n = 9, r = 0.82, p = 0.01) at age 5 years (Figure 3).

Children exposed to high maternal carbamazepine concentrations tended to have low Language 20 scores (no language impairment) at age 5 years (n = 19, r = -0.47, p = 0.04), but outliers in the scatter plot made this result difficult to interpret (Paper II).

We found no correlation between any ASM concentrations and language score at age 8 years (Paper II).

Figure 3.

Correlation between maternal valproate concentrations in gestational weeks 17-19 and the child's language score at age 5 years (Paper II).



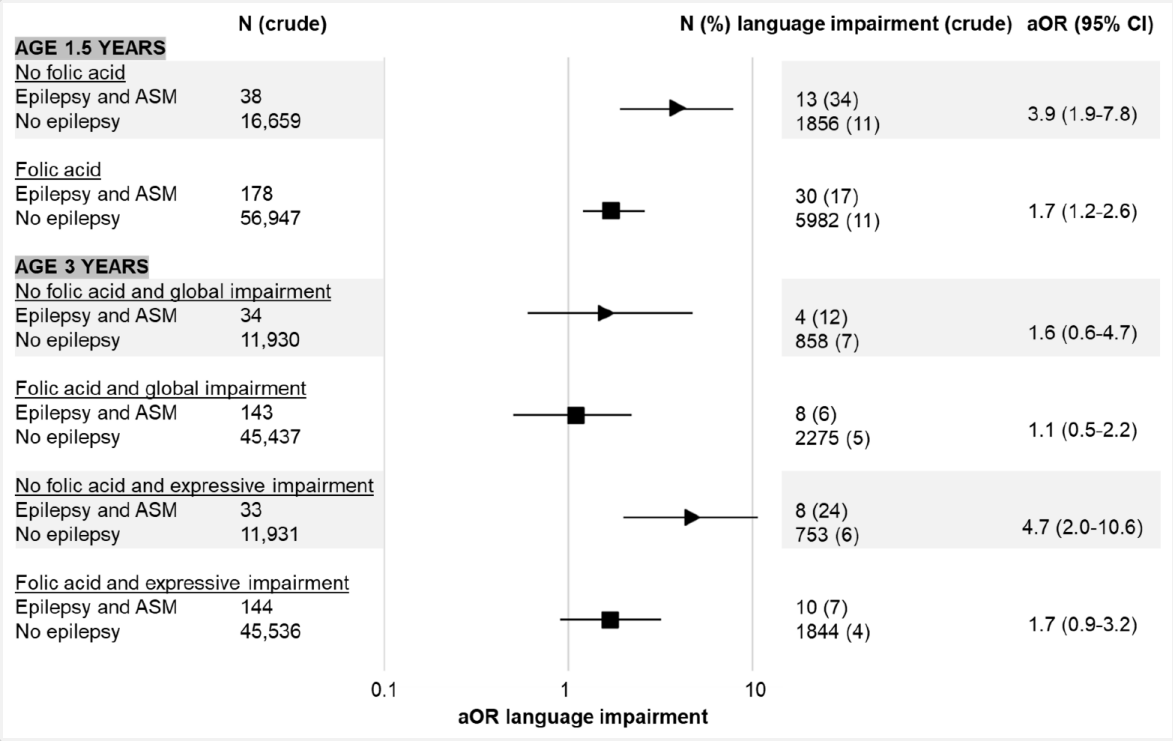
ASQ, The Ages and Stages Questionnaire; Language 20, The Twenty Statements about Language-Related Difficulties; r, Spearman's rho; p, p-value.

4.3 Maternal pregnancy folate and association to language impairment in children of women with epilepsy (Papers I and II)

In ASM-exposed children of women with epilepsy with no periconceptional folic acid supplementation, the risks of language impairment at age 1.5 years and of expressive language impairment at age 3 years were increased compared to children of women without epilepsy with no periconceptional folic acid supplementation. The aORs were 3.9, CI 1.9-7.8 and 4.7, CI 2.0-10.6, respectively (Figure 4 and Paper I).

Figure 4.

Adjusted odds ratios of language impairment in antiseizure medication-exposed children of women with epilepsy compared to children of women without epilepsy at ages 1.5 and 3 years stratified for periconceptional folic acid supplement use.

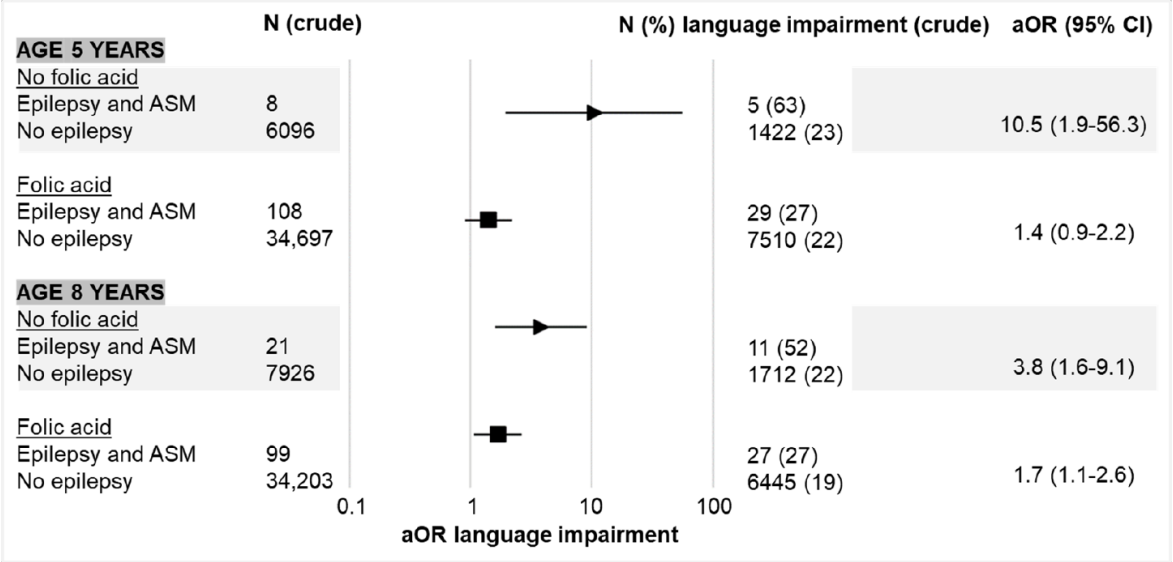


ASM, antiseizure medication; aOR, adjusted odds ratio; CI, 95% confidence interval. Language impairment at age 1.5 years according to the Ages and Stages Questionnaires (ASQ). Global language impairment at age 3 years according the ASQ, expressive language impairment age 3 years according to the one-item question regarding expressive language skills (Paper I).

When the mother reported use of periconceptional folic acid supplementation, the risk of language impairment at both ages were lower, and no longer statistically different from the children in the control group at age 3 years. The aORs were 1.7, CI 1.2-2.6 and 1.7, CI 0.9-3.2, respectively (Figure 4 and Paper I). Similar to the findings at age 1.5 and 3 years, we also found an association between periconceptional folic acid supplement use and decreased risk of language impairment in ASM-exposed children of women with epilepsy at age 5 and 8 years (Figure 5 and Paper II).

Figure 5.

Adjusted odds ratios of language impairment in antiseizure medication-exposed children of women with epilepsy compared to children of women without epilepsy at ages 5 and 8 years stratified for periconceptional folic acid supplement use.



ASM, antiseizure medication; aOR, adjusted odds ratio; CI, confidence interval. Language impairment at age 5 years according to the Ages and Stages Questionnaires (ASQ), the Speech and Language Assessment Scale (SLAS) or the Twenty Statements about Language-related Difficulties (language 20), and at age 8 years according to the Language 20 (Paper II).

Without any periconceptional folic acid supplementation, there was an increased risk of language impairment in ASM-exposed children compared to control children at

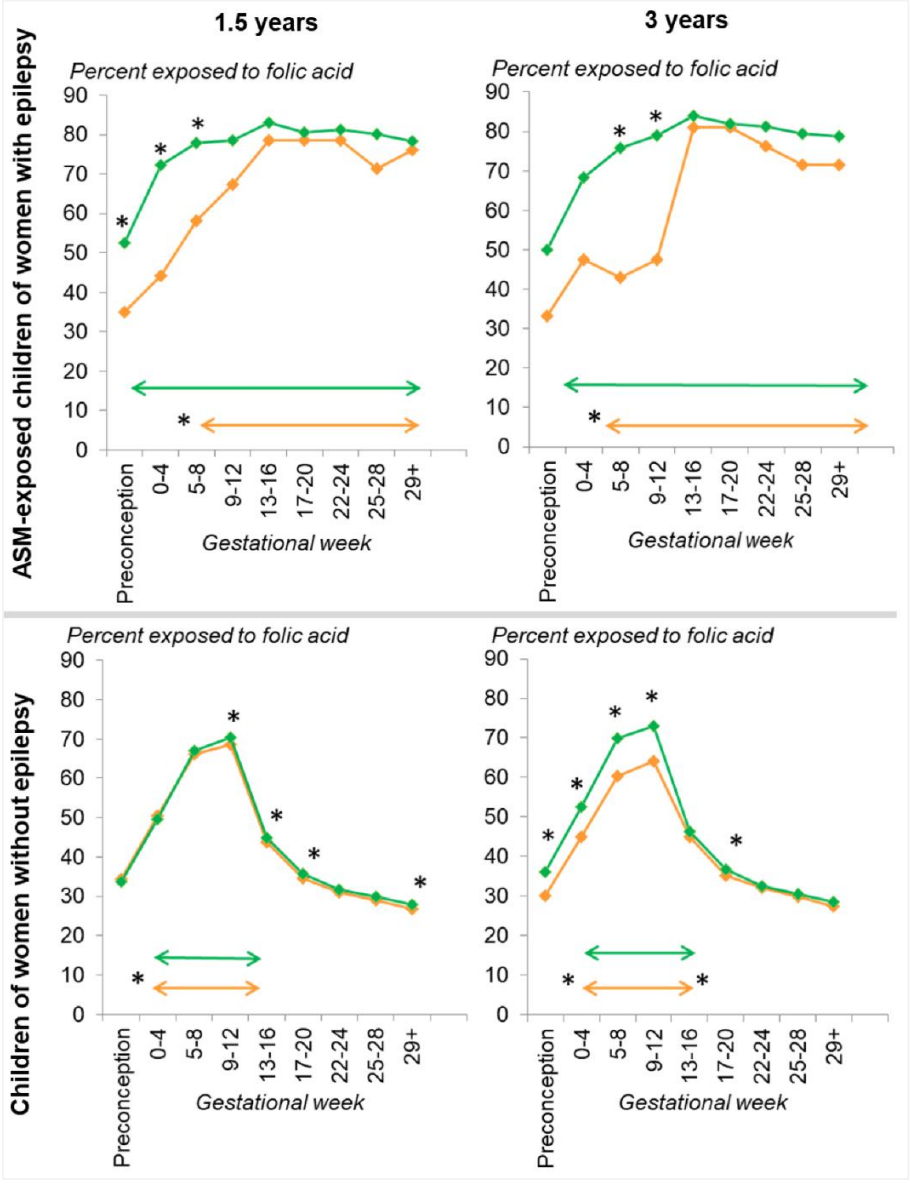
age 5 and 8 years, the aORs being 10.5, CI 1.9-56.3 and 3.8, CI 1.6-9.1, respectively (Figure 5). When the mother reported periconceptional folic acid supplementation, the risk of language impairment in ASM-exposed children were lower at both ages and no longer statistically different from the control children at age 5 years (aOR 1.4, CI 0.9-2.2 and 1.7, CI 1.1-2.6, respectively; Figure 5). The were no significant differences in risk of language impairment between ASM-unexposed children of women with epilepsy and children in the control group stratified for periconceptional folic acid supplementation at ages 1.5 and 3 years (Paper I) and at ages 5 and 8 years (Paper II).

In the adjusted multiple regression analysis, we found that the interaction between prenatal ASM exposure and no periconceptional folic acid supplementation had a synergistic negative association with language impairment at ages 1.5 and 3 years (Paper I; ASQ score: $p = 0.04$ age 1.5 years, $p < 0.001$ age 3 years; expressive language score: $p = 0.01$ age 3 years), and at age 5 years (Paper II; ASQ score: $p = 0.01$ age 5 years). We found no correlation between maternal folate concentrations during gestational weeks 17-19 and language score at ages 1.5 and 3 years (Paper I). At age 5 years (Paper II), high maternal folate concentrations during pregnancy correlated with low Language 20 score (unstandardized beta (B) -0.07, CI -0.13 - -0.005, $p = 0.034$) and hence less language impairment in the unadjusted linear regression model, but not in the adjusted model (B -0.07, CI -0.13-0.002, $p = 0.057$). There was no correlation between maternal folate concentrations and language score at age 8 years (Paper II).

In paper I, mothers of ASM-exposed children with language impairment at ages 1.5 and 3 years started their folic acid supplement use later than mothers of ASM-exposed children with no language impairment (median start age 1.5 years: gestation week 6.5 versus 3 weeks before conception, p 0.01; median start age 3 years: gestation week 4.3 versus 3 weeks before conception, p 0.05; Figure 6).

Figure 6.

The proportion of children (percent) exposed to maternal folic acid supplementation at different time intervals during pregnancy and association to language impairment (orange lines) and no language impairment (green lines) at age 1.5 and 3 years (Paper I).



ASM, antiseizure medication. Arrows illustrate median start and stop of maternal supplement use. Statistically significant differences are marked with an asterisk.

The proportion of ASM-exposed children exposed to folic acid early in pregnancy was higher in children with no language impairment at ages 1.5 and 3 years compared to children with language impairment (Figure 6). There was no such difference during the last part of the pregnancy (Figure 6). In ASM-unexposed children of women with epilepsy, the median start of folic acid supplement use and the proportion exposed to folic acid supplement in pregnancy did not differ between children with and without language impairment (Paper I).

4.4 Plasma antiseizure medication concentrations and association to vitamin B status during pregnancy (Paper III)

We included 227 singleton pregnancies of 203 women with ASM-treated epilepsy with available plasma samples from gestational weeks 17-19 from MoBa version 10. Two women contributed with three pregnancies and 20 women with two pregnancies. We divided the pregnancies into six ASM monotherapy groups, in which the reported ASM was detected in plasma; valproate (n = 24), lamotrigine (n = 65), carbamazepine (n = 48), levetiracetam (n = 11), topiramate (n = 8), and oxcarbazepine (n = 5), and ASM polytherapy (n = 40), where at least one of the reported ASMs was detected in plasma. Pregnancies where none of the reported ASMs were detected in plasma were categorized into a low-adherence group (n = 26) with suspected low ASM-compliance. During the period from gestational weeks -4 to 20, the women reported any supplement use in 221 pregnancies: any folic acid supplement in 208 pregnancies (94%), any riboflavin supplement in 72 (33%), any pyridoxine supplement in 77 (35%), and any niacin supplement in 45 (20%) pregnancies.

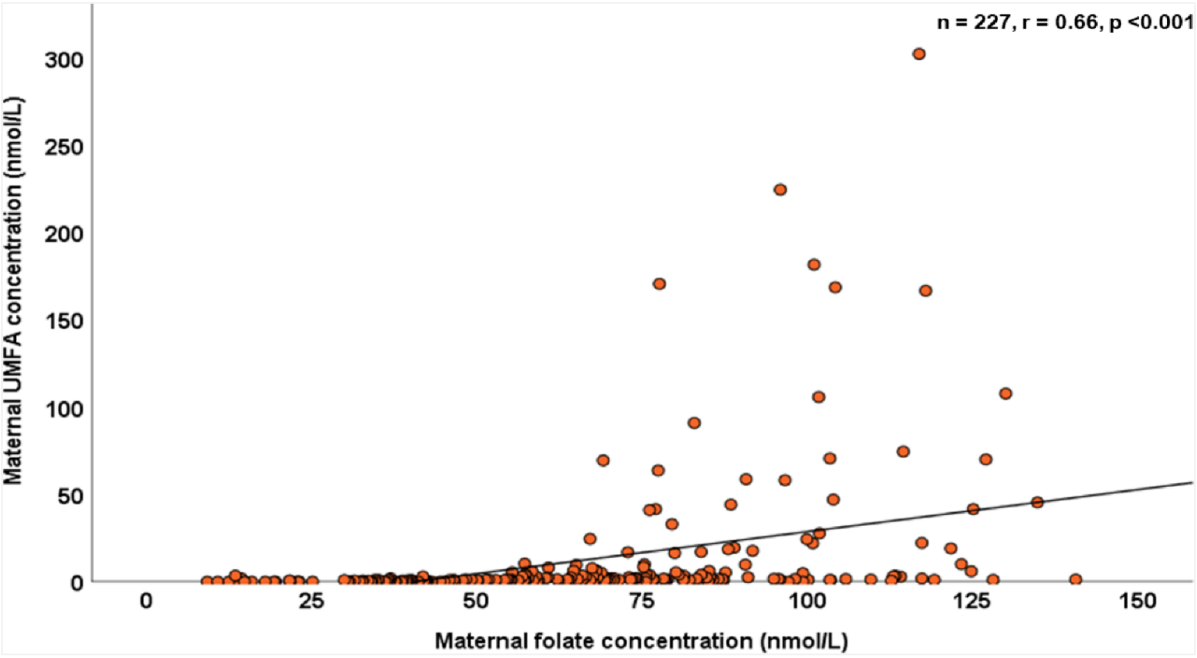
High standardized maternal plasma ASM concentrations correlated with high concentrations of UMFA (n = 199, r = 0.22, p = 0.002) and inactive folate metabolites (pABG: n = 199, r = 0.23, p = 0.001, apABG: n = 199, r = 0.25, p <0.001), and with low concentrations of riboflavin (n = 188, r = -0.32, p <0.001). Furthermore, high standardized ASM concentrations correlated with low metabolically active pyridoxine (PLP; n = 188, r = -0.19, p = 0.01) and with high

value of the marker of low pyridoxine status (HKr; n = 188, r = 0.15, p = 0.04). There was no association between ASM and plasma niacin status. High maternal valproate concentrations correlated with high concentrations of apABG (n = 24, r = 0.51, p = 0.01), and high topiramate concentrations with high UMFA concentrations (n = 8, r = 0.83, p = 0.01) in the respective monotherapy groups. High lamotrigine concentrations correlated with low riboflavin concentrations (n = 65, r = -0.27, p = 0.03). High maternal valproate concentrations correlated with a high value of the marker of low pyridoxine status (HKr; n = 24, r = 0.43, p = 0.04).

In the overall study population in paper III, high folate concentrations correlated strongly with high UMFA concentrations (Figure 7).

Figure 7.

Correlation between maternal folate concentrations and unmetabolized folic acid concentrations in gestational weeks 17-19.



UMFA, unmetabolized folic acid; r, Spearman's rho; p, p-value.

Maternal plasma folate concentrations were significantly lower in the low-adherence group compared to the other ASM groups except for oxcarbazepine, and lower in pregnancies with carbamazepine monotherapy compared to ASM polytherapy pregnancies (Table 1). The values of HKr were higher in the valproate group compared to all the other ASM groups (median value 0.4 compared to median values 0.2-0.3, all p-values <0.05). The concentrations of PLP and riboflavin did not differ between individual ASM groups.

Table 1.

Maternal plasma folate status in gestational weeks 17-19 stratified for antiseizure medication use.

Plasma folate status (nmol/L); median (range)								
	Valproate n = 24	**Lamotrigine n = 65**	**Carbama-zepine n = 48**	**Levetir-acetam n = 11**	**Topiramate n = 8**	**Oxcarba-zepine n = 5**	**Polytherapy n = 40**	**Low-adherence group[1] n = 26**
Folate[2]	**80.0 (84.7)**	**65.9 (119.0)**	**65.5 (103.8)**[b]	**73.9 (107.1)**	**66.9 (86.4)**	67.1 (68.4)	**74.2 (112.1)**[b]	**38.2 (96.7)**[a]
pABG	**1.6 (19.5)**	1.1 (20.7)[b]	**1.3 (29.5)**	0.9 (17.4)	1.2 (9.2)	1.0 (5.3)	**1.5 (24.8)**[b]	**0.8 (9.7)**[a]
apABG	**0.9 (2.4)**	0.8 (6.7)[b]	**0.9 (2.6)**	**0.6 (1.1)**[a]	**1.2 (1.3)**	0.6 (2.0)	**1.1 (2.4)**[b,c]	0.8 (2.2)[c]
UMFA	**2.7 (303.0)**	**1.1 (169.0)**	**1.5 (182.0)**	0.8 (108.0)	1.9 (41.6)	0.8 (8.4)	**1.6 (167.0)**	**0.6 (63.8)**[a]

mTHF, 5-methyltetrahydrofolate; hmTHF, 4-alfa-hydroxy-5-methyltetrahydrofolate; pABG, para-aminobenzoylglutamate; apABG, acetamidobenzoylglutamate; UMFA, unmetabolized folic acid; ASM, antiseizure medication. All ASM groups are compared with each other by using Kruskal-Wallis Test. Groups that differ significantly (p-value <0.05) from the group marked with an [a] are marked with bold text, additional groups that significantly differ from each other are both marked with [b] and than with [c]. Significant differences after Bonferroni correction for multiple tests are underlined.

[1] Consists of pregnancies where the mother reported ASM use, but no ASM was detected in plasma samples [2] Based on sum of mTHF and hmTHF

5. Discussion

5.1 Methodological considerations

The research field of epidemiology includes several methodological challenges.[214] In the following section, I will discuss methodological considerations in this research project. These are important for the following discussion and interpretation of my results.

5.1.1 Data source – The Norwegian Mother, Father and Child Cohort Study

We used data from the MoBa cohort because of the large number of pregnant women with and without epilepsy enrolled in this cohort, available biobank material, the linkage to the mandatory MBRN, the large amount of prospectively collected data on exposures and outcomes, and the long follow-up time. Large cohort studies are important for identifying risk factors for disease and to make "new discoveries" within a research field.[215] The large sample size, a prospective design, and information on a range of outcomes and exposures are classical strengths of large cohort studies.[216,217] The limitations typically include lack of detailed clinical information on the participants, unbalanced characteristics of exposed and unexposed participants due to non-randomization, possible loss to follow-up, and that the included participants may differ from the general population in unmeasurable ways.[216,217] MoBa differs from other large cohort studies as the self-reported questionnaires collected extensive clinical information about the participants, their medication and supplement use during different time periods of the pregnancy including questions on compliance, and there is available biobank material. Nevertheless, in contrast to cohort studies dedicated to women with epilepsy, such as the studies by the NEAD group, MoBa do not contain detailed prospective information on epilepsy type, epileptic seizures, or dose of ASM. Also, there was no blinded cognitive assessment of the children. However, a much larger number of children of women with and without epilepsy were assessed in MoBa, and the maternal epilepsy cohort in MoBa has been validated.[198] The parental-reported questionnaires contained numerous questions regarding language impairment based

on different, validated screening instruments, and the data were collected at different ages. Furthermore, the information reported in MoBa was independent of the medical follow-up during and after pregnancy and would not in itself lead to any advantage or disadvantage for the participant. MoBa is population-based in the sense that a segment of the pregnant population in Norway was invited without regard to exposure status.[217] Women that smoke, non-cohabiting women, women with two or more previous pregnancies, and women under age 25 years were underrepresented in MoBa compared to the general population in Norway, whereas multivitamin and folic acid supplement users were overrepresented.[218] However, the prevalence of epilepsy in MoBa was similar to the prevalence in the general population.[218] The self-reported data from the periconceptional period of the pregnancy was retrospective, as women answered the first questionnaire during gestation weeks 15-19, but given before the pregnancy outcomes were known.[194] In summary, MoBa was considered a good data source for the conduction of our research project. The sources of error and validity of our findings based on this data source will be discussed in more detail below.

5.1.2 Study design

The cohort study design of papers I and II with prospective collection of data on language impairment in exposed and unexposed children during the years after birth, was appropriate for the aims of this research project. Prospectively collected data on folic acid supplement use during the periconceptional period, prospective data on folic acid supplement dose with exact timing on intake, data on maternal IQ, less children lost to follow-up, and available maternal folate and ASM concentrations from the periconceptional period would have been useful to further improve the methodology of the studies. The cross-sectional design of paper III was a result of the exposure and outcomes being measured at the same time point in MoBa.[219] Prospective and multiple maternal blood sampling in unexposed and exposed pregnancies, more detailed information on vitamin supplement dose and frequency of intake during the different trimesters, and a higher number of pregnancies to increase statistical power would have improved the study methodology.

5.1.3 Sources of error and validity

Errors in epidemiology can be random or systematic, both detract from accuracy.[220] Sources of systematic errors are mainly selection bias, information bias, and confounding.[220-222] In epidemiology, bias is defined as: "Deviation of results or inferences from the truth, or processes lead to such deviation. Any trend in the collection, analysis, interpretation, publication, or review of data that can lead to conclusions that are systematically different from the truth".[221] Random errors are often defined as errors that happen by chance or random variation.[220] It can be measured as the variance of an estimation process, which is inversely related to the statistical precision of the process.[220] Precision tells the closeness of the measurements to each other: when the variance of an estimate is reduced, the precision is improved.[220] However, a high precision is of no value if the measurements are systematically wrong or measure something else than what was intended. This is called the validity of a measurement: the degree to which it measures what it is supposed to measure.[223] The internal validity expresses whether our results are true for the source population that the study is based on.[221] Selection bias, information bias, or confounding are all threats to the internal validity of a study.[224] To what extent the inferences we make in the study can be generalized to another population or future patients represents the external validity of a study.[221]

Random errors and precision

Random errors are typically associated with the process of selecting the specific study participants, termed sampling.[220] The random variation of MoBa participants is called sampling variation and reflects a source of random error in this research project. Furthermore, samples from ASM-treated pregnancies and umbilical cord blood were randomly selected for measurement of vitamin B and ASM concentrations based on previous MoBa data file versions and available samples in the MoBa data biobank. This random selection could be a source of random error. Other sources of random errors are random errors that occur during the laboratory analyses, or analyses including small groups of children where random variation may influence the results.[220] This is reflected by the wide CIs in some of the estimates involving groups

with few children or pregnancies in papers I-III. However, as random errors can be reduced and the precision increased by increasing the study population,[220] we examined ASM-exposed children or pregnancies both as one group and according to their specific ASM. Children exposed to valproate, carbamazepine, or lamotrigine represented the largest ASM groups in our material and the number of children in each of these groups was relatively high.

Selection bias

Differences between participants and non-participants at study enrolment or between participants that continue to participate compared to those who are lost to follow-up may introduce selection bias.[224,225] Selection bias happens when the estimate of disease occurrence or etiologic effect in the study population differs systematically from the estimate that would have been obtained if data from the entire source population had been available.[224,225]

In MoBa, the participants were enrolled without knowing whether their child would have language impairment or not, and this reduces any selection bias at baseline.[224,225] However, the MoBa participants differed from those who did not participate, affecting the external validity of our results, but not necessary the internal validity as we calculated exposure-outcome associations adjusted for these differences such as smoking, maternal age, and socioeconomic factors.[218,225] In the invitation from MoBa the women were informed about the general aim and design of MoBa, potential outcomes, and about the data collection and the MoBa biobank.[226] If this information affected their decision to participate, this would introduce a type of selection bias termed self-selection bias.[224] Generally, healthy women are more likely to participate in research.[225] This could lead to women with epilepsy refusing to participate, but the opposite could also be true: MoBa aimed to examine risk of disease in the child, women with ASM-treated epilepsy might have known or feared that ASM or the epilepsy could harm the child and hence have a stronger personal wish to participate in MoBa. As the prevalence of women with epilepsy in MoBa was similar to the

prevalence of all women given birth in Norway, self-selection bias is less likely to have affected our internal comparisons.[218]

Loss to follow-up increased with increasing follow-up time in MoBa, and this may be a source of selection bias that can affect our internal comparisons.[225,227] If the reason for a participant being lost to follow-up is associated with the exposure and the outcome, this may cause selection bias.[224,225] In our research project, the percentages of non-responders and hence loss to follow-up were calculated compared to the total numbers of participants included in MoBa (Figure 2 in the Results section). The response rates in MoBa were higher if calculated based on the participants who responded compared with those who received the questionnaire.[227] The response rates at the different ages were similar between children of women with ASM-treated epilepsy, children of women with ASM-untreated epilepsy, and children of women without epilepsy. Furthermore, many parents in MoBa did not answer all the questionnaires from child ages 1.5-8 years, and the Q5Y was initiated after 20% of the children were too old to contribute with this questionnaire.[227] Low birth weight, familial history of language impairment, low maternal education, male offspring, and social disadvantage are associated with risk of language impairment, but most of the variance of language abilities during the preschool years are still unexplained.[101,211] Hence, language impairment would be difficult to predict in non-responders. The non-responders of Q5Y and Q8Y generally had a higher percentage of characteristics associated with lower socioeconomic status compared to the responders in all the groups examined. The percentages of children with language impairment measured in previous questionnaires were typically higher among non-responders compared to responders in Q5Y, but this was less pronounced in Q8Y. The percentage of children with ASM polytherapy and ASM monotherapy exposure during pregnancy were similar between non-responders and responders for both ages. The distribution of maternal epilepsy among the responders did not change during the eight years of follow-up in MoBa.[227] Overall, our analyses indicated that children with normal language may be overrepresented among the responders rather than the opposite in the three groups examined. Based on this and the similar response rates during

follow-up within the three groups examined, we believe that the potential selection bias due to loss to follow-up would bias our results towards the null.

Information bias

Measurement of exposures, outcomes, confounders, and other variables are subject to measurement error.[223] If such errors influence the exposure-outcome associations examined in a study, this is termed information bias.[223] Measurement errors that depend on the values of another variable is termed differential, whereas if errors do not depend on the values of other variables it is termed nondifferential.[223]

Nondifferential information bias

The questions in the questionnaires may be subject to measurement error if the questions do not really correspond to the underlying conceptual construct, this is called construct validity.[223] In example, in the question regarding familial history of language, reading and writing problems in Q5Y, the parent was instructed to answer yes if such problems were present in any of the child's biological siblings, parents, grandparents, uncles, aunts, or cousins. This variable was not associated with risk of language impairment in children of women with epilepsy, contrary to previous studies,[101,211] but an association was found in the much larger group of children of women without epilepsy. If this question had included the more clinically relevant first grade relatives only, this would have improved the construct validity and possibly changed the estimated associations. This is an example of a nondifferential information bias as the error do not depend on other variables including the outcome and hence affected the exposed and the unexposed groups of children equally.[223] Such bias typically leads to less precise estimates that are biased towards the null value.[223]

Another source of nondifferential bias are the questions from language screening instruments that we used to define variables of language impairment. Although we used validated cut-offs from the screening instruments to determine criteria for language impairment,[206-210] the construct validity of the parental-reported language questions as presented in the MoBa questionnaires have not been examined. Hence,

we cannot exclude that some children were misclassified as having language impairment or the other way around. However, any measurement error in these variables would affect the exposed and unexposed children equally and hence most likely bias our results towards the null.

Other sources of nondifferential measurement errors are errors that occur during the collection, management or transformation of data, or recall errors in the self-reported questionnaires.[223] In example, women that responded to Q1 might not recall correctly when they started folic acid supplement use in the periconceptional period or refuse to report alcohol consumption or smoking during pregnancy. Another example is the self-reported diagnosis of epilepsy in MoBa. Some women with epilepsy that resolved in childhood may choose to not report this, or women may have been wrongly diagnosed with epilepsy and this would lead to misclassification errors. However, the epilepsy diagnosis have previously been validated in MoBa, and women with an uncertain diagnosis or that refuted the diagnosis during this validation have been excluded.[198] In summary and considering that the data collection in MoBa were based on self-reported questionnaires, these measurement errors would likely affect the unexposed and exposed children in our research project equally and hence lead to less precise estimates and bias towards the null.

Measurement errors during management, handling, and transport of laboratory analyses are another source of nondifferential information bias. We only had biobank data available for pregnant women with ASM-treated epilepsy. All the samples were delivered for analysis by the MoBa biobank concomitantly and analysed as one batch. The within-day coefficients of variation of the plasma ASM analyses were acceptable and indicated adequate precision.[198]

Differential information bias

Differential information bias can lead to bias in any direction.[223] Women with ASM-treated epilepsy might for example have reported the language abilities of their children more precisely than women without ASM exposure because of fear that such

exposure would cause fetal harm. Another possible source of differential information bias are the questions regarding folic acid supplementation: Women with epilepsy are often prescribed high-dose folic acid supplementation and are aware of the increased risk of ASM-associated congenital malformations, and thus might have answered these questions more carefully than women without epilepsy. Information regarding ASM-associated language impairment was not commonly known during the enrolment period of MoBa, nor were any association between folic acid supplementation and language impairment. In addition, the data on language impairment was collected prospectively several months and years after the exposure data for all the participants, and numerous of exposures and outcomes were included in the MoBa questionnaires. Based on this, we believe that such bias is unlikely to have a large impact on our results, although we acknowledge this as a limitation of our research project.

Missing data

In addition to questionnaire non-responders, missing data was present among the responders as incomplete questionnaires with missing answers on one or more variables such as smoking, alcohol consumption, BMI, and some of the questions regarding language impairment. Missing data may be a source of information bias, particularly if it is related to the outcome of the study.[222] We imputed missing answers regarding language impairment if the number of missing answers was less than 29% (depending on the score), except for age 1.5 years where no missing answers were accepted (only three items). We considered the missing items as missing completely at random, for example that the participant randomly forgot to respond to one of seven language items. Language development in young children vary considerably, and delay may be early and then resolve, or early without resolving, or start later in the preschool years.[101] We chose a conservative approach regarding imputation of missing values, as we did not find it reasonable to impute language scores across the different ages. During the years, the strategies for identifying, handling and reducing bias due to missing data, including multiple imputation or inverse-probability weighted methods, have gained increased

attention.[228,229] Consequently, we provided a more detailed report of missing values in the tables of paper III compared to in papers I and II. We did not estimate or correct for any bias due to missing data in other variables such as confounders, mainly because we did not hypothesize that this would have a large impact on our internal comparisons as missing data were few within each of the three groups. We examined the missing data due to loss to follow-up with descriptive analyses only, but a recent study from MoBa suggests to consider using imputation or weighting methods to account for the loss to follow-up in MoBa.[227]

Confounding

Confounding occurs when the association between an exposure and an outcome is partly or fully explained by other factors that is associated with the outcome than the effect of the exposure.[230] A confounder must be a risk factor for the outcome, associated with the exposure, and not a results of the exposure or of the disease.[230] Confounding is a source of systematic error that can be reduced by adjusting the data by the potential confounder or eliminated by stratifying the data by the potential confounder.[230] Low folate status may be a result of ASM exposure and hence could not be a confounder. We hypothesized that low folate status during pregnancy was a mediator in the causal pathway between prenatal ASM exposure and language impairment. We stratified the analyses for periconceptional folic acid supplementation to examine whether periconceptional folic acid supplementation was an effect modifier, and to reduce bias due to confounding as periconceptional folic acid supplement users may differ from non-users.[199] The different strata of folic acid supplementation had different effect estimates, indicating that the ASM-associated risk of language impairment differed depending on whether the mother reported periconceptional folic acid supplementation, and hence we concluded that folic acid was an effect modifier.[231] We carefully considered variables to adjust for, as adjusting for variables that are not confounders may also introduce bias, termed overadjustment bias.[230] We did not adjust for unplanned pregnancies, as such pregnancies could be a result of ASM use and are more likely to have low maternal folate status as prior folic acid supplementation is unlikely. We adjusted for confounders such as

socioeconomic status including low maternal education, maternal age, parity, gestational age, smoking during pregnancy, and alcohol use during pregnancy. There were generally only minor differences between the CIs based on crude estimates and the CIs based on adjusted estimates (Papers I and II). This could be explained by genetic factors and unidentified risk factors explaining most of the variance regarding language abilities during the preschool years.[101,211] We adjusted for familial history of language impairment based on the Q5Y variable but did not have data on parental IQ. However, low maternal education was adjusted for. In paper III, the study population was less heterogenous being only pregnancies of women with ASM-treated epilepsy, and none of the covariates included were considered to fill criteria for being a confounder.

Women with ASM-treated epilepsy are likely to have a more severe disease than women with ASM-untreated epilepsy. If the indication for treatment, such as epileptic seizures, could explain the association between ASM and language impairment, and not the ASM exposure, this is termed confounding by indication.[232] We only had retrospective seizure data for a subsample of pregnancies, and these variables were thus not meaningful to include into the regression analyses. Nevertheless, the two epilepsy groups are likely to differ in severity of and type of epilepsy disease, and we cannot exclude that some of the ASM-associated language impairment could be related to the epilepsy disease itself, for example due to genetic causes or harmful effects of seizures on the fetus. Furthermore, although we had data on numerous possible confounders, there could still be unmeasured confounding present. Unmeasured confounding are confounding not dealt with adequately due to poor statistical modeling or misclassification of the confounder.[230]

Choice of statistical methods

We used mainly non-parametric methods, which do not make assumptions about the underlying population distribution and generally have less stringent assumptions than parametric methods.[233] However, such methods are less powerful than the corresponding parametric alternative and may fail to detect differences between

groups that exist.[233] The two main reasons for why we chose non-parametric methods were that the number of children or pregnancies in several analyses were few, and that the assumption of an underlying normal distribution of scores was violated. Language scores, many of the ASM concentrations, and the non-folate vitamin B concentrations had skewed distributions. In the main analyses the sample size was relatively large, and a parametric method approach would probably been possible for several of the analyses.[234]

Most of the children included did not have siblings in MoBa. However, some women with epilepsy contributed with more than one pregnancy. We did not account for the correlation between siblings in our analyses by using robust logistic regression estimations, as this was not possible in SPSS. This is a limitation of the study, as such analyses likely would influence the confidence intervals of the estimate, but the estimates are usually unchanged. We chose not to exclude siblings as this would reduce statistical power in the analyses. We adjusted for parity and environmental factors at age 5 and 8 years such as maternal report of seldom or never reading to their child or helping your child read letters or sounds. In paper III, twenty mothers contributed with two pregnancies and three mothers with three pregnancies. As these numbers were few in comparison to the total study population included, it is unlikely that it would have a major impact on the results although genetic factors that may influence vitamin B concentrations would be identical in pregnancies of the same mother.

Internal and external validity

We acknowledge that there are several methodological considerations in this research project, including several potential sources of random and systematic errors. We have relatively large groups of ASM-exposed and ASM-unexposed children of women with epilepsy and a control group of children of women without epilepsy from a population-based cohort. We have data on multiple exposures and outcomes and have adjusted for a range of possible confounders. Data on language impairment was prospectively collected and based on validated screening instruments. The response

rates during follow-up were similar between the three groups. None of the laboratory results were known to the participants. Our findings were consistent through the three different papers. In summary, based on the methodological considerations above we believe that the sources of random and systematic errors mainly caused less precise estimates and would bias our results towards the null. We thus believe that the internal validity of our exposure-outcomes associations is high.

We believe our findings can be generalized to the general population of pregnant women with epilepsy in Norway, as the prevalence of pregnant women with epilepsy in MoBa was similar to the prevalence among all women giving birth in Norway.[218] However, women with epilepsy in MoBa may differ from pregnant women with epilepsy that chose not to participate in MoBa, because the questionnaires were in Norwegian only[194] or for other reasons, or that they were not invited. We adjusted for several of the baseline characteristics that were different between participants in MoBa and pregnant women in the general population of Norway.[218] Such differences are nevertheless unlikely to impact the external validity of the exposure-outcome associations calculated in our research project.[218] Our findings based on pregnant women with epilepsy are not necessarily generalizable to pregnant women without epilepsy using ASM during pregnancy, as these women may differ from women with epilepsy regarding risk of adverse neurodevelopment in their children. Antenatal care is free in Norway and available to all women. Our findings may not be generalizable to countries where the antenatal follow-up differs between women with epilepsy. There is no mandatory folic acid food fortification in Norway, and our findings may not be generalizable to countries with different folic acid fortification guidelines.

5.2 Discussion of the results

5.2.1 Prenatal antiseizure medication exposure and risk of language impairment

We found an almost doubled increase in the odds of language impairment at age 5 years and a doubled increase in the odds of impairment at age 8 years in ASM-exposed children of women with epilepsy compared to control children. Increased risk of language impairment was reported in children of women with epilepsy in

MoBa compared to children of women without epilepsy at age 3 years,[108] and in children aged 3-4 years exposed to valproate enrolled in the UKEPR.[106] In the studies from the NEAD group, verbal abilities were poorer than non-verbal abilities for all ASM monotherapy exposures at age 3 years, and in the valproate and lamotrigine groups at age 6 years.[53,105] Other studies in children aged 6-13 years reported impaired verbal abilities after valproate,[51,55,235] carbamazepine,[51] or any prenatal ASM exposure[107,110] compared to children of women without epilepsy, or in valproate-exposed children compared to children exposed to carbamazepine or lamotrigine,[56] or children of women with ASM-untreated epilepsy.[109] Our method differed from these studies as we used dichotomous variables for language impairment as the main outcome variables, and the language abilities were evaluated by parents and not formally assessed by a neuropsychologist. We also used children of women with ASM-treated epilepsy that were treated in hospitals all over Norway, and by different specialists. The screening instruments that we used were specifically designed to detect language impairment, and not part of an assessment of cognitive function in general. The percentage of children with language impairment at age 5 and 8 years in our results (30% and 32%, respectively) are comparable to a study from APR assessing language disorder or delay specifically.[56] This study found mild, moderate or severe language delay in 28 of 102 (27%) ASM-exposed children aged 7 years.[56] We thus found an increased risk of language impairment in ASM-exposed children of epilepsy that was consistent at both age 5 and 8 years, and supported by previous findings from MoBa and from other studies despite different methodology.[51,53,55,56,105-110,235]

Regarding exposure to individual ASMs, we used ASM concentrations from gestational weeks 17-19 and umbilical cord plasma as a proxy of fetal ASM exposure in utero. We found a striking correlation between higher maternal valproate concentrations during the second trimester of the pregnancy and poor language score at ages 1.5 and 5 years. Valproate have particularly been associated with a dose-dependent increased risk of language impairment.[53,55,56,235] In the study from APR, first trimester valproate dose correlated with low language scores and significantly

predicted language scores in children aged 7 years.[56] When we examined individual ASM groups and risk of language impairment, the individual groups contained few children at both age 5 and 8 years. However, the prevalence of language impairment in valproate-exposed children at age 5 and 8 years (36% and 31%, respectively) were highly comparable with the study from APR where 7 of 23 valproate-exposed children (30%) had mild, moderate, or severe language delay.[56]

We reported an increased risk of language impairment in children exposed to carbamazepine monotherapy at age 8 years. Plasma concentrations of carbamazepine did not correlate with language impairment. Prenatal exposure to carbamazepine has been associated with reduced verbal abilities,[51,105,113] but other studies reported no such association.[53,110,235] In a population-based Danish study, children exposed to carbamazepine scored worse than unexposed control on the sixth-grade mathematics test.[119] In the APR study, 6 of 34 (18%) of carbamazepine-exposed children filled criteria for any language delay, although the mean language score did not differ significantly from the expected mean language score.[56] Children with MCMs or an epilepsy diagnosis were excluded from their analyses.[56] An increased frequency of IQ<85 compared to children of women without epilepsy was reported in carbamazepine-exposed children in the study from the LMNDG group.[51] Carbamazepine exposure in utero have been associated with increased risk of MCMs and dysmorphic features.[30] We did not find an association between carbamazepine and language impairment at age 5 years, but the number of children exposed to carbamazepine monotherapy was lower at this age. Findings have not consistently shown a linkage between prenatal carbamazepine exposure and neurodevelopmental impairment, but it has been suggested that neurodevelopmental difficulties may differ between subpopulation of carbamazepine-exposed children, such as those with dysmorphic features or MCMs.[30] Overall, our findings highlight valproate and carbamazepine as individual ASMs associated with language impairment in children of women with epilepsy aged 5 and 8 years. These findings are important, and emphasize, in line with several previous studies, the need for more studies regarding specific impairment in children exposed to other ASMs than valproate.[30,83]

The risk factors associated with language impairment in ASM-exposed children aged 5 or 8 years such as male offspring, no report of maternal periconceptional folic acid supplementation, smoking during pregnancy, and previous language impairment are in line with risk factors described in studies of children from the general population.[101,123,138,211] In ASM-exposed children, maternal IQ and socioeconomic status have been reported as predictors of language delay or lower verbal abilities,[55,56,109] and also maternal lack of periconceptional folic acid supplementation and maternal age.[53,105] In our findings, none of the ASM-exposed children with language impairment at age 5 or 8 years had mothers that reported low education. Epileptic seizures during pregnancy were retrospectively collected, and not associated with an increased risk of language impairment in ASM-exposed children. Epileptic seizures were associated with increased risk of language impairment in ASM-unexposed children of women with epilepsy, but this association was not adjusted for other possible confounders and based on small numbers. Generalized epileptic seizures during pregnancy have been associated with low verbal IQ[49] and increased risk of educational support[51] in two studies, but association to language delay or cognitive abilities were not reported in several other studies.[53,55,56]

Language disorders represent one of the most common neurodevelopmental disorders, and may have long-term consequences for educational attainment, employment, social life, and mental health.[102] Studies have shown that children with early language impairment that later resolved with scores within age-appropriate levels by school-age, still experience problems with specific language tasks during the following years compared with children with no early impairment.[211] Parents are good evaluators of the language abilities of their children.[207,209] Based on our findings, clinicians treating ASM-exposed children of women with epilepsy should be aware of association between prenatal ASM exposure and risk of language impairment and intervene early if parental concern or symptoms of language impairment.

5.2.2 Antiseizure medication-associated language impairment and the role of folate

We found a protective effect of periconceptional folic acid supplementation on risk of language impairment in ASM-exposed children of women with epilepsy aged 1.5, 3, 5, and 8 years. Our findings were in line with two studies from the NEAD group reporting higher mean verbal index score at age 3 years and higher mean IQ at age 6 years in ASM-exposed children of women with epilepsy who were exposed to periconceptional folic acid compared to children not exposed to periconceptional folic acid.[53,105] In MoBa, a protective effect of periconceptional folic acid supplementation on risk of autistic traits at age 1.5 and 3 years had already been shown for ASM-exposed children of women with epilepsy.[167] Our findings further indicated that use of folic acid supplementation could be a factor that reduced the risk of ASM-associated language impairment. We initially reported this association in children aged 1.5 and 3 years in paper I. Despite the number of children lost to follow-up in MoBa at age 5 and 8 years, we found this association to be highly significant also at age 5 and 8 years in paper II. The NEAD group further examined the role of periconceptional folic acid supplementation and found that use of periconceptional folic acid was associated with better neurodevelopmental scores across a range of cognitive variables in ASM-exposed children.[112] Another study from the LMNDG group linked to the NEAD study did not find any association between preconceptual folate and mean IQ in ASM-exposed children aged 6 years.[51] This study only noted folic acid supplement before conception, the NEAD study defined a mother as a periconceptional folate user if the reported taking folic acid one month or more before conception.[112] A study from KREP collected folic acid information prospectively in women with ASM-treated epilepsy, and found no association between folic acid during pregnancy and intellectual or language outcome in children aged 6 years, but only 71 children were examined.[107] In MoBa, intake of folic acid supplement in the periconceptional period was reported in time intervals of four weeks, and the frequency of intake was high. Being a folic acid user during the periconceptional period have been associated with higher maternal education, being a non-smoker, better socioeconomic status, better plasma vitamin B status, and a higher intake of other micronutrients in MoBa.[199] We have adjusted for several possible

confounders in our analyses. Any potential unmeasured confounding could nevertheless be present. However, it is very unlikely that this is the only explanation of our findings for several reasons; the studies from the NEAD group report similar results with inclusion of adjustment for maternal IQ, and chronic use of several different ASMs have been consistently reported to be associated with low folate status in studies of non-pregnant patients with epilepsy.[124,150] Also, we stratified the analyses for periconceptional folic acid status, this would reduce unmeasured confounding in our comparisons. If the protective effect of folic acid were not related to the ASM exposure, but to unmeasured confounding related to the epilepsy diagnosis, we should have found a similar association or tendency in the estimates in the ASM-untreated epilepsy group, but this was not the case. Furthermore, several studies from the general population have reported a positive association between high maternal folate status during pregnancy and better neurodevelopmental outcomes in the children.[123,127,137-139] Chronic ASM use prior to pregnancy in combination with lack of periconceptional folic acid supplementation could have resulted in a poor folate status in women with ASM-treated epilepsy. We thus believe that our findings highlighting folic acid supplementation as a factor that may reduce the ASM-associated risk of language impairment to be consistent throughout two papers and of clinical importance.

We examined at what time during the pregnancy folic acid supplementation was important. In paper I, folic acid supplementation during the periconceptional period was the critical period associated with reduced language impairment. We did not find an association between maternal folate concentrations during the second trimester and language scores at ages 1.5-8 years. Our findings are in line with several studies from the general population reporting better neurodevelopmental outcomes if exposure to folic acid supplement use compared to no exposure during the periconceptional period.[123,137,138] This has also been shown in ASM-exposed children, both in MoBa and in the studies from the NEAD group. However, folate is important for fetal brain development throughout the pregnancy, and it has been hypothesized that folate status during the growth spurt of the pregnancy is particularly important.[127] In a

randomized controlled trial among pregnant women from the general population, women were randomized to receive either continued folic acid supplementation or placebo during the second or third trimester.[236] At age 7 years, children of the folic acid treated mothers scored significantly higher on word reasoning compared to children of mothers that received placebo.[236] Our findings indicate that optimal maternal folate status in the periconceptional period seems to be important to reduce the risk of ASM-associated language impairment.

As we had found an association between ASM concentrations and language score at age 1.5 and 5 years, we wanted to examine further whether maternal folate concentrations were associated with ASM concentrations later during the pregnancy. Based on our findings from paper I and II, we hypothesized that folate and the one-carbon metabolism could be involved in the mechanisms behind the association between periconceptional folic acid supplementation and language impairment. The folate concentrations in MoBa were measured in the second trimester and do not reflect self-reported supplementation before gestation week 8.[200] In paper III, we found that almost all the included women with ASM-treated epilepsy during pregnancy reported supplement use by gestation week 20, in line with the clinical guidelines in Norway. This could explain why we did not find an association between folate concentrations and language score in the second trimester in papers I and II: by the time the maternal folate concentration was analysed, many women with a previous low folate status might have improved their status through supplementation. High concentrations of ASM correlated with high concentrations of inactive folate metabolites and hence increased folate catabolism, particularly in pregnancies with valproate or carbamazepine monotherapy, or ASM polytherapy. Folate catabolism may be influenced by the pregnancy itself and by extensive folic acid supplementation.[123,126,155] The widespread use of folic acid supplementation in the study population of paper III makes our findings difficult to interpret. Nevertheless, an interaction between individual ASMs and folate pathways could be possible based on the differences in folate metabolites between individual ASMs.

Supplementation of non-folate vitamins were less frequent in the pregnancies of women with ASM-treated epilepsy in paper III. Both riboflavin and pyridoxine are important co-factors for folate in one-carbon metabolism, and optimal status of both are crucial for optimal folate functioning.[127] Niacin is involved in neuronal development and survival.[237] We found that high ASM concentrations were associated with low riboflavin and low pyridoxine status, but not with niacin status. This finding was striking, as niacin was the only non-folate B vitamin examined which is not involved in the one-carbon metabolism. Low riboflavin status have been reported in non-pregnant patients with ASM-treated epilepsy, particularly enzyme-inducing ASMs,[149] whereas low pyridoxine status have been associated with several ASMs.[124,149] Regarding individual ASMs, lamotrigine was associated with low riboflavin status and valproate with low pyridoxine status. Both these ASMs have been associated with low folate concentrations and antifolate properties.[120,152] Changes in one-carbon metabolism with altered pathways involving folate, purine, and amino acid metabolism was associated with lamotrigine use in a study of pregnant women with epilepsy.[161] In the study from the NEAD group, the mean IQ was significantly higher in children exposed to lamotrigine monotherapy if the mother reported periconceptional folic acid supplementation compared to no such report.[53] In summary, we found an association between ASM concentrations, particularly valproate and lamotrigine, and low pyridoxine and riboflavin status, which are folate co-factors in one-carbon metabolism.[127] Taken together, our findings indicate that ASM exposure both early and later in pregnancy is associated with language impairment, and that maternal folate status may be of importance in both trimesters.

Finally, we used the retrospective data available on folic acid dose to examine whether the dose of folic acid was important regarding risk of language impairment. We did not find an association between language impairment and folic acid doses above ≥1 mg compared to doses of 0.4 mg in paper I. As these data were limited to a few of the children of women with epilepsy, we assessed the concentration of UMFA in paper III, as a proxy for excessive folic acid intake. We found a strong correlation

between high maternal folate and high UMFA concentrations in gestational weeks 17-19. This association is in line with studies of pregnant women from the general population; the UMFA concentrations increase when folic acid supplementation is excessive.[126] High UMFA concentrations is not solely related to the intake of folic acid, studies in both pregnant and non-pregnant women from the general population have suggested that there are mechanisms by which the body adapts to high folic acid intake to limit UMFA exposure.[238,239] This could be an explanation of the wide range of UMFA concentrations within individual ASM groups, even if most of the women included reported folic acid supplementation use by gestation week 20. Assessing UMFA in women with epilepsy is important as the optimal dose of folic acid to recommend to these women is not known,[13,125] nor is the safety of excess folate during pregnancy.[126] Studies in mice have found that both low and excessive folate during pregnancy are associated with adverse development in the offspring.[147,240,241] Altered methylation pattern in the embryo have been associated with low and high folate concentrations during pregnancy in animal and human studies.[135,145] High UMFA cord blood concentrations have been associated with increased risk of ASD in some population groups in the US.[141] High doses of folic acid have also been associated with increased risk of ASD or other adverse neurodevelopment in children from the general population.[140,142] We have recently examined whether high UMFA concentrations during pregnancy were associated with risk of language impairment or autistic traits in ASM-exposed children in MoBa but found no such association.[170] The mean IQ was higher in ASM-exposed children from the NEAD study if exposure to high-dose (≥4 mg) periconceptional folic acid supplementation compared to no folic acid exposure.[53] A similar association was also reported in the previous MoBa study as risk of autistic traits decreased with higher doses of folic acid during the pregnancy.[167] As several ASMs interact with maternal folate metabolism,[124] studies from the general population cannot be automatically generalized to women with ASM-treated epilepsy.

Based on our overall findings, the dose of folic acid to recommend to women with ASM-treated epilepsy should probably differ depending on the ASM treatment.

Prenatal exposure to valproate and carbamazepine were associated with risk of language impairment, and the risk differed depending on maternal report of periconceptional folic acid supplementation for lamotrigine-exposed children. The concentrations of all these ASMs were associated with plasma folate, riboflavin, or pyridoxine status. Valproate, lamotrigine, and carbamazepine have previously been linked to the folate metabolism.[121,124,152,161,184,242] Folate and the one-carbon metabolism is fundamental for normal brain development in utero, and for brain health throughout life.[127,135] It is intriguing that several of the mechanisms suggested to be associated with ASM-induced teratogenesis and adverse neurodevelopment, such as brain cell changes, oxidative stress, ASM-induced changes in placental transport, and altered gene expression and DNA methylation can be linked to folate and one-carbon metabolism.[29,120,121,186] However, as some women with ASM-treated epilepsy during pregnancy have very high concentrations of UMFA indicating excessive folate, our findings also point to a closer monitoring of folate status before and during pregnancy in women with epilepsy.

5.2.3 Strengths and limitations

Strengths of this research project include population-based, prospectively collected data on a large number of ASM-exposed and ASM-unexposed children of women with epilepsy, and a control group of children of women without epilepsy. The maternal epilepsy cohort has been validated and biobank data on a large subgroup of pregnancies of ASM-treated women were available. We adjusted for a range of possible confounders. Limitations include retrospectively collected data on folic acid supplementation periconceptionally, few children in some of the individual ASM groups, and no formal assessment of the language abilities of the children or parental IQ. The loss to follow-up in MoBa increased with increasing age, we do not know the language abilities of children of non-responders, and whether this influenced the decision for continued participation in MoBa. Women with ASM-treated epilepsy are likely to have a more severe epilepsy than women with ASM-untreated epilepsy, and we cannot exclude that non-ASM factors influence our results. Also, women using ASM during pregnancy may have reported their child's language abilities more

vigilantly than women of the control group. Maternal plasma samples were only measured once during pregnancy, and not in the periconceptional period. Genetic factors influence vitamin concentrations; we did not have access to such data. We did not adjust for multiple pregnancies in the same mother. The correlation coefficients between ASM concentrations and plasma vitamin and metabolite concentrations were relatively low in some of the analyses, which illustrates that factors other than ASM treatment also interfere with vitamin concentrations during pregnancy. The UMFA concentrations depend on the time gap between intake of folic acid and sample collection,[126] this was unknown in MoBa. We presented standardized maternal ASM concentrations in addition to individual ASM concentrations. By using relative plasma concentrations, the standardized ASM concentrations adjusted for differences in pharmacokinetics between the ASMs. The majority of the ASMs that were merged have been associated with folate status,[124,150,152] and although pharmacological mechanisms may differ between the ASMs, their mechanisms of action related to folate could share common pathways.

6. Conclusions

We found that prenatal ASM exposure was associated with increased risk of language impairment in children of women with epilepsy aged 5 and 8 years compared to children of women without epilepsy, and hence that prenatal ASM exposure can have long-term consequences for the child. Periconceptional folic acid supplementation was identified as a factor that reduced this risk, both in young children aged 1.5 and 3 years, and in children aged 5 and 8 years. Maternal valproate concentrations and prenatal exposure to carbamazepine was associated with language impairment. Other associated risk factors were male offspring, smoking during pregnancy, and previous language impairment at ages 1.5 or 3 years. Regarding timing of folic acid supplementation, the periconceptional period was important to reduce the risk of language impairment. In addition, the second trimester seemed to be of importance. High ASM concentrations during the second trimester were associated with folate metabolism and with low status of the non-folate B vitamins riboflavin and pyridoxine, both essential for normal folate function.[127] High valproate concentrations during the second trimester also correlated with low language scores.

Our findings support the recommendation that folic acid supplementation should be offered to all girls and women of childbearing age using ASMs that might become pregnant regardless of pregnancy plans.[13,37] Furthermore, our findings highlight other non-folate B vitamins necessary to ensure optimal folate status that may interact with ASM use during pregnancy: pyridoxine and riboflavin. The optimal folic acid dose to recommend to women with epilepsy have not been elucidated in this thesis, but our findings taken together suggest that the dose may differ depending on the ASM treatment, and that folate status should be monitored during pregnancy to avoid excess folate in women with epilepsy using ASM.

7. Implications and future directions

Our findings reporting long-term adverse consequences of prenatal ASM exposure on language abilities in children of women with epilepsy should be further examined in larger studies with inclusion of screening instruments or assessment specifically created to detect language impairment and language disorders. Subgroups of children exposed to specific ASMs should also be assessed, in example children exposed to carbamazepine with dysmorphic features. Population-based studies with data on educational attainment are of importance, in addition to prospective cohort studies with long follow-up and with assessment both from parents, the children themselves, and a formal, blinded assessment by a neuropsychologist. The impact of potential difficulties on the daily life and functioning of the child should be assessed.

More studies are needed to determine the optimal folic acid dose to recommend to women with epilepsy. This should include prospective studies with multiple sampling of plasma folate concentrations, ASM concentrations, different doses of folic acid, and with data on pregnancy outcome and neurodevelopment scores in the children. Other study designs could also be of importance, such as randomized controlled trials with different folic acid doses as there is currently very different recommendations for folic acid supplement internationally. I am currently leading a new research project based on the maternal epilepsy cohort in MoBa with inclusion of maternal genotyped data from MoBa Genetics. Our aim is to examine whether the maternal genetic risk of low folate concentrations influence the role of folate on ASM-associated autistic traits and language impairment in children of women with epilepsy. In addition to folic acid, studies should be conducted regarding other vitamins associated with folate during pregnancy, such as riboflavin or pyridoxine. The study design could be a prospective, observational design with comparison of folic acid only and folic acid plus multivitamin supplementation with outcomes related to the pregnancy or child neurodevelopment.

References

1. Fiest KM, Sauro KM, Wiebe S, Patten SB, Kwon CS, Dykeman J, et al. Prevalence and incidence of epilepsy: A systematic review and meta-analysis of international studies. *Neurology* 2017;88:296-303.
2. Smith PEM. Initial Management of Seizure in Adults. *N Engl J Med* 2021;385:251-263.
3. Milligan TA. Epilepsy: A Clinical Overview. *Am J Med* 2021;134:840-847.
4. Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* 2005;46:470-472.
5. Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, et al. ILAE official report: a practical clinical definition of epilepsy. *Epilepsia* 2014;55:475-482.
6. Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. *Epilepsia* 2017;58:512-521.
7. Fisher RS, Cross JH, D'Souza C, French JA, Haut SR, Higurashi N, et al. Instruction manual for the ILAE 2017 operational classification of seizure types. *Epilepsia* 2017;58:531-542.
8. Kanner AM, Helmstaedter C, Sadat-Hossieny Z, Meador K. Cognitive disorders in epilepsy I: Clinical experience, real-world evidence and recommendations. *Seizure* 2020;83:216-222.
9. Berg AT. Epilepsy, cognition, and behavior: The clinical picture. *Epilepsia* 2011;52 Suppl 1:7-12.
10. Kløvgaard M, Lynge TH, Tsiropoulos I, Uldall PV, Banner J, Winkel BG, et al. Epilepsy-Related Mortality in Children and Young Adults in Denmark: A Nationwide Cohort Study. *Neurology* 2022;98:e213-e224.
11. Kløvgaard M, Lynge TH, Tsiropoulos I, Uldall PV, Banner J, Winkel BG, et al. Sudden unexpected death in epilepsy in persons younger than 50 years: A retrospective nationwide cohort study in Denmark. *Epilepsia* 2021.
12. Einarsdottir AB, Sveinsson O, Olafsson E. Sudden unexpected death in epilepsy. A nationwide population-based study. *Epilepsia* 2019.
13. Tomson T, Battino D, Bromley R, Kochen S, Meador K, Pennell P, et al. Management of epilepsy in pregnancy: a report from the International League Against Epilepsy Task Force on Women and Pregnancy. *Epileptic Disord* 2019;21:497-517.
14. Stephen LJ, Harden C, Tomson T, Brodie MJ. Management of epilepsy in women. *Lancet Neurol* 2019.
15. Li Y, Meador KJ. Epilepsy and Pregnancy. *Continuum (Minneap Minn)* 2022;28:34-54.
16. Borthen I, Eide M, Daltveit A, Gilhus N. Delivery outcome of women with epilepsy:a population-based cohort study. *BJOG: An International Journal of Obstetrics & Gynaecology* 2010;117:1537-1543.

17. Katz O, Levy A, Wiznitzer A, Sheiner E. Pregnancy and perinatal outcome in epileptic women: a population-based study. *J Matern Fetal Neonatal Med* 2006;19:21-25.
18. Olafsson E, Hallgrimsson JT, Hauser WA, Ludvigsson P, Gudmundsson G. Pregnancies of women with epilepsy: a population-based study in Iceland. *Epilepsia* 1998;39:887-892.
19. Kinney MO, Morrow J. Epilepsy in pregnancy. *BMJ* 2016;353:i2880.
20. Kinney MO, Craig JJ. Pregnancy and epilepsy; meeting the challenges over the last 25 years: The rise of the pregnancy registries. *Seizure* 2017;44:162-168.
21. Harden CL, Hopp J, Ting TY, Pennell PB, French JA, Hauser WA, et al. Practice parameter update: management issues for women with epilepsy--focus on pregnancy (an evidence-based review): obstetrical complications and change in seizure frequency: report of the Quality Standards Subcommittee and Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology and American Epilepsy Society. *Neurology* 2009;73:126-132.
22. Harden CL, Meador KJ, Pennell PB, Hauser WA, Gronseth GS, French JA, et al. Practice parameter update: management issues for women with epilepsy--focus on pregnancy (an evidence-based review): teratogenesis and perinatal outcomes: report of the Quality Standards Subcommittee and Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology and American Epilepsy Society. *Neurology* 2009;73:133-141.
23. Harden CL, Pennell PB, Koppel BS, Hovinga CA, Gidal B, Meador KJ, et al. Practice parameter update: management issues for women with epilepsy--focus on pregnancy (an evidence-based review): vitamin K, folic acid, blood levels, and breastfeeding: report of the Quality Standards Subcommittee and Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology and American Epilepsy Society. *Neurology* 2009;73:142-149.
24. NICE Guidance. Epilepsies: Diagnosis and Management - Clinical guideline. Available at https://www.nice.org.uk/guidance/cg137.
25. Tomson T, Battino D, Bromley R, Kochen S, Meador KJ, Pennell PB, et al. Global Survey of Guidelines for the Management of Epilepsy in Pregnancy: A report from the International League Against Epilepsy Task Force on Women and Pregnancy. *Epilepsia Open* 2020;5:366-370.
26. The Medicines and Healthcare products Regulatory Agency. Antiepileptic drugs: review of safety of use during pregnancy 2021.
27. Den norske legeforening. Retningslinjer for behandling av kvinner med epilepsi 2018. Available at https://www.legeforeningen.no/contentassets/f3dde659c8e147e59b47eda62d02a621/retningslinjer-for-behandling-av-kvinner-med-epilepsi-2018.pdf .
28. NEL - Nevrologiske prosedyrer. Epilepsi og kvinner. Available at https://nevrologi.legehandboka.no/handboken/sykdommer/epilepsi/sykdommer-og-symptomer/epilepsi-og-kvinner/#fagmedarbeidere.
29. Kellogg M, Meador KJ. Neurodevelopmental Effects of Antiepileptic Drugs. *Neurochem Res* 2017;42:2065-2070.

30. Bromley RL, Bluett-Duncan M. Neurodevelopment following exposure to antiseizure medications in utero: a review. *Curr Neuropharmacol* 2021.
31. Fujimura K, Mitsuhashi T, Takahashi T. Adverse effects of prenatal and early postnatal exposure to antiepileptic drugs: Validation from clinical and basic researches. *Brain Dev* 2017;39:635-643.
32. Stefanidou M, Montouris G. Reproductive and Sexual Health Concerns in Transition-Age Adolescents and Young Adults With Epilepsy. *Semin Pediatr Neurol* 2020;36:100855.
33. Herzog AG, Fowler KM, Sperling MR, Massaro JM. Distribution of seizures across the menstrual cycle in women with epilepsy. *Epilepsia* 2015;56:e58-62.
34. Herzog AG. Catamenial epilepsy: Update on prevalence, pathophysiology and treatment from the findings of the NIH Progesterone Treatment Trial. *Seizure* 2015;28:18-25.
35. Reimers A. Contraception for women with epilepsy: counseling, choices, and concerns. *Open Access J Contracept* 2016;7:69-76.
36. Markoula S, Siarava E, Keramida A, Chatzistefanidis D, Zikopoulos A, Kyritsis AP, et al. Reproductive health in patients with epilepsy. *Epilepsy Behav* 2020;113:107563.
37. Harden C, Lu C. Epilepsy in Pregnancy. *Neurol Clin* 2019;37:53-62.
38. Reimers A, Brodtkorb E, Sabers A. Interactions between hormonal contraception and antiepileptic drugs: Clinical and mechanistic considerations. *Seizure* 2015;28:66-70.
39. Herzog AG, MacEachern DB, Mandle HB, Cahill KE, Fowler KM, Davis AR, et al. Folic acid use by women with epilepsy: Findings of the Epilepsy Birth Control Registry. *Epilepsy Behav* 2017;72:156-160.
40. Johnson EL, Burke AE, Wang A, Pennell PB. Unintended pregnancy, prenatal care, newborn outcomes, and breastfeeding in women with epilepsy. *Neurology* 2018;91:e1031-e1039.
41. Herzog AG, Mandle HB, Cahill KE, Fowler KM, Hauser WA. Predictors of unintended pregnancy in women with epilepsy. *Neurology* 2017;88:728-733.
42. Gerard EE, Meador KJ. Managing Epilepsy in Women. *Continuum (Minneap Minn)* 2016;22:204-226.
43. Pennell PB, French JA, Harden CL, Davis A, Bagiella E, Andreopoulos E, et al. Fertility and Birth Outcomes in Women With Epilepsy Seeking Pregnancy. *JAMA Neurol* 2018;75:962-969.
44. Sukumaran SC, Sarma PS, Thomas SV. Polytherapy increases the risk of infertility in women with epilepsy. *Neurology* 2010;75:1351-1355.
45. Eadie MJ. Pregnancy and the Control of Epileptic Seizures: A Review. *Neurol Ther* 2021.
46. Battino D, Tomson T, Bonizzoni E, Craig J, Lindhout D, Sabers A, et al. Seizure control and treatment changes in pregnancy: observations from the EURAP epilepsy pregnancy registry. *Epilepsia* 2013;54:1621-1627.
47. Cagnetti C, Lattanzi S, Foschi N, Provinciali L, Silvestrini M. Seizure course during pregnancy in catamenial epilepsy. *Neurology* 2014;83:339-344.
48. Sveberg L, Svalheim S, Tauboll E. The impact of seizures on pregnancy and delivery. *Seizure* 2015;28:35-38.

49. Adab N, Kini U, Vinten J, Ayres J, Baker G, Clayton-Smith J, et al. The longer term outcome of children born to mothers with epilepsy. *J Neurol Neurosurg Psychiatry* 2004;75:1575-1583.
50. Chen YH, Chiou HY, Lin HC, Lin HL. Affect of seizures during gestation on pregnancy outcomes in women with epilepsy. *Arch Neurol* 2009;66:979-984.
51. Baker GA, Bromley RL, Briggs M, Cheyne CP, Cohen MJ, Garcia-Finana M, et al. IQ at 6 years after in utero exposure to antiepileptic drugs: a controlled cohort study. *Neurology* 2015;84:382-390.
52. Hernandez-Diaz S, McElrath TF, Pennell PB, Hauser WA, Yerby M, Holmes LB. Fetal growth and premature delivery in pregnant women on antiepileptic drugs. *Ann Neurol* 2017;82:457-465.
53. Meador KJ, Baker GA, Browning N, Cohen MJ, Bromley RL, Clayton-Smith J, et al. Fetal antiepileptic drug exposure and cognitive outcomes at age 6 years (NEAD study): a prospective observational study. *The Lancet Neurology* 2013;12:244-252.
54. Borthen I, Eide MG, Daltveit AK, Gilhus NE. Obstetric outcome in women with epilepsy: a hospital-based, retrospective study. *Bjog* 2011;118:956-965.
55. Bromley RL, Calderbank R, Cheyne CP, Rooney C, Trayner P, Clayton-Smith J, et al. Cognition in school-age children exposed to levetiracetam, topiramate, or sodium valproate. *Neurology* 2016;87:1943-1953.
56. Nadebaum C, Anderson VA, Vajda F, Reutens DC, Barton S, Wood AG. Language skills of school-aged children prenatally exposed to antiepileptic drugs. *Neurology* 2011;76:719-726.
57. Christensen J, Vestergaard C, Hammer Bech B. Maternal death in women with epilepsy: Smaller scope studies. *Neurology* 2018;91:e1716-e1720.
58. Edey S, Moran N, Nashef L. SUDEP and epilepsy-related mortality in pregnancy. *Epilepsia* 2014;55:e72-74.
59. MacDonald SC, Bateman BT, McElrath TF, Hernández-Díaz S. Mortality and Morbidity During Delivery Hospitalization Among Pregnant Women With Epilepsy in the United States. *JAMA Neurol* 2015;72:981-988.
60. Marson AG, Appleton R, Baker GA, Chadwick DW, Doughty J, Eaton B, et al. A randomised controlled trial examining the longer-term outcomes of standard versus new antiepileptic drugs. The SANAD trial. *Health Technol Assess* 2007;11:iii-iv, ix-x, 1-134.
61. Marson AG, Al-Kharusi AM, Alwaidh M, Appleton R, Baker GA, Chadwick DW, et al. The SANAD study of effectiveness of valproate, lamotrigine, or topiramate for generalised and unclassifiable epilepsy: an unblinded randomised controlled trial. *Lancet* 2007;369:1016-1026.
62. Marson A, Burnside G, Appleton R, Smith D, Leach JP, Sills G, et al. The SANAD II study of the effectiveness and cost-effectiveness of levetiracetam, zonisamide, or lamotrigine for newly diagnosed focal epilepsy: an open-label, non-inferiority, multicentre, phase 4, randomised controlled trial. *Lancet* 2021;397:1363-1374.
63. Marson A, Burnside G, Appleton R, Smith D, Leach JP, Sills G, et al. The SANAD II study of the effectiveness and cost-effectiveness of valproate versus levetiracetam for newly diagnosed generalised and unclassifiable

epilepsy: an open-label, non-inferiority, multicentre, phase 4, randomised controlled trial. *Lancet* 2021;397:1375-1386.
64. Spiegel R, Merius H. Principles of Epilepsy Management for Women in Their Reproductive Years. *Front Neurol* 2020;11:322.
65. Agency MaHpR. Antiepileptic drugs in pregnancy: updated advice following comprehensive safety review 2021.
66. Toledo M, Mostacci B, Bosak M, Jedrzejzak J, Thomas RH, Salas-Puig J, et al. Expert opinion: use of valproate in girls and women of childbearing potential with epilepsy: recommendations and alternatives based on a review of the literature and clinical experience-a European perspective. *J Neurol* 2021;268:2735-2748.
67. European Medicines Agency. CMDh agrees to strengthen warnings on the use of valproate medicines in women and girls. Available at https://www.ema.europa.eu/en/medicines/human/referrals/valproate-related-substances#key-facts-section.
68. European Medicines Agency. PRAC recommends new measures to avoid valproate exposure in pregnancy. Available at https://www.ema.europa.eu/en/news/prac-recommends-new-measures-avoid-valproate-exposure-pregnancy.
69. Whelehan A, Delanty N. Therapeutic strategies for treating epilepsy during pregnancy. *Expert Opin Pharmacother* 2019;20:323-332.
70. Arfman IJ, Wammes-van der Heijden EA, Ter Horst PGJ, Lambrechts DA, Wegner I, Touw DJ. Therapeutic Drug Monitoring of Antiepileptic Drugs in Women with Epilepsy Before, During, and After Pregnancy. *Clin Pharmacokinet* 2020;59:427-445.
71. Razaz N, Tomson T, Wikstrom AK, Cnattingius S. Association Between Pregnancy and Perinatal Outcomes Among Women With Epilepsy. *JAMA Neurol* 2017;74:983-991.
72. Viale L, Allotey J, Cheong-See F, Arroyo-Manzano D, McCorry D, Bagary M, et al. Epilepsy in pregnancy and reproductive outcomes: a systematic review and meta-analysis. *The Lancet* 2015;386:1845-1852.
73. Borthen I. Obstetrical complications in women with epilepsy. *Seizure* 2015;28:32-34.
74. Anderson PO. Antiepileptic Drugs During Breastfeeding. *Breastfeed Med* 2020;15:2-4.
75. Galanti M, Newport DJ, Pennell PB, Titchner D, Newman M, Knight BT, et al. Postpartum depression in women with epilepsy: influence of antiepileptic drugs in a prospective study. *Epilepsy Behav* 2009;16:426-430.
76. Turner K, Piazzini A, Franza A, Fumarola C, Chifari R, Marconi AM, et al. Postpartum depression in women with epilepsy versus women without epilepsy. *Epilepsy Behav* 2006;9:293-297.
77. Bjork MH, Veiby G, Reiter SC, Berle JO, Daltveit AK, Spigset O, et al. Depression and anxiety in women with epilepsy during pregnancy and after delivery: a prospective population-based cohort study on frequency, risk factors, medication, and prognosis. *Epilepsia* 2015;56:28-39.

78. Bromley R, Weston J, Adab N, Greenhalgh J, Sanniti A, McKay AJ, et al. Treatment for epilepsy in pregnancy: neurodevelopmental outcomes in the child. *Cochrane Database Syst Rev* 2014:CD010236.
79. Veroniki AA, Cogo E, Rios P, Straus SE, Finkelstein Y, Kealey R, et al. Comparative safety of anti-epileptic drugs during pregnancy: a systematic review and network meta-analysis of congenital malformations and prenatal outcomes. *BMC Med* 2017;15:95.
80. Veroniki AA, Rios P, Cogo E, Straus SE, Finkelstein Y, Kealey R, et al. Comparative safety of antiepileptic drugs for neurological development in children exposed during pregnancy and breast feeding: a systematic review and network meta-analysis. *BMJ Open* 2017;7:e017248.
81. Weston J, Bromley R, Jackson CF, Adab N, Clayton-Smith J, Greenhalgh J, et al. Monotherapy treatment of epilepsy in pregnancy: congenital malformation outcomes in the child. *Cochrane Database Syst Rev* 2016;11:Cd010224.
82. Tomson T, Battino D, Perucca E. Teratogenicity of antiepileptic drugs. *Curr Opin Neurol* 2019;32:246-252.
83. Knight R, Wittkowski A, Bromley RL. Neurodevelopmental outcomes in children exposed to newer antiseizure medications: A systematic review. *Epilepsia* 2021;62:1765-1779.
84. Veiby G, Daltveit AK, Engelsen BA, Gilhus NE. Pregnancy, delivery, and outcome for the child in maternal epilepsy. *Epilepsia* 2009;50:2130-2139.
85. Veiby G, Daltveit AK, Engelsen BA, Gilhus NE. Fetal growth restriction and birth defects with newer and older antiepileptic drugs during pregnancy. *J Neurol* 2014;261:579-588.
86. Artama M, Braumann J, Raitanen J, Uotila J, Gissler M, Isojarvi J, et al. Women treated for epilepsy during pregnancy: outcomes from a nationwide population-based cohort study. *Acta Obstet Gynecol Scand* 2017;96:812-820.
87. Kilic D, Pedersen H, Kjaersgaard MI, Parner ET, Vestergaard M, Sorensen MJ, et al. Birth outcomes after prenatal exposure to antiepileptic drugs--a population-based study. *Epilepsia* 2014;55:1714-1721.
88. Borthen I, Eide MG, Veiby G, Daltveit AK, Gilhus NE. Complications during pregnancy in women with epilepsy: population-based cohort study. *Bjog* 2009;116:1736-1742.
89. Tomson T, Battino D, Bonizzoni E, Craig JJ, Lindhout D, Perucca E, et al. Antiepileptic drugs and intrauterine death: A prospective observational study from EURAP. *Neurology* 2015;85:580-588.
90. Hernandez-Diaz S, Smith CR, Shen A, Mittendorf R, Hauser WA, Yerby M, et al. Comparative safety of antiepileptic drugs during pregnancy. *Neurology* 2012;78:1692-1699.
91. Campbell E, Kennedy F, Russell A, Smithson WH, Parsons L, Morrison PJ, et al. Malformation risks of antiepileptic drug monotherapies in pregnancy: updated results from the UK and Ireland Epilepsy and Pregnancy Registers. *J Neurol Neurosurg Psychiatry* 2014;85:1029-1034.
92. Tomson T, Battino D, Bonizzoni E, Craig J, Lindhout D, Perucca E, et al. Comparative risk of major congenital malformations with eight different

antiepileptic drugs: a prospective cohort study of the EURAP registry. *Lancet Neurol* 2018;17:530-538.
93. Vajda FJ, O'Brien TJ, Lander CM, Graham J, Roten A, Eadie MJ. Teratogenesis in repeated pregnancies in antiepileptic drug-treated women. *Epilepsia* 2013;54:181-186.
94. Campbell E, Devenney E, Morrow J, Russell A, Smithson WH, Parsons L, et al. Recurrence risk of congenital malformations in infants exposed to antiepileptic drugs in utero. *Epilepsia* 2013;54:165-171.
95. Tomson T, Battino D, Bonizzoni E, Craig J, Lindhout D, Perucca E, et al. Declining malformation rates with changed antiepileptic drug prescribing: An observational study. *Neurology* 2019;93:e831-e840.
96. Hernandez-Diaz S, Werler MM, Walker AM, Mitchell AA. Neural tube defects in relation to use of folic acid antagonists during pregnancy. *Am J Epidemiol* 2001;153:961-968.
97. Hernandez-Diaz S, Werler MM, Walker AM, Mitchell AA. Folic acid antagonists during pregnancy and the risk of birth defects. *N Engl J Med* 2000;343:1608-1614.
98. Thapar A, Cooper M, Rutter M. Neurodevelopmental disorders. *Lancet Psychiatry* 2017;4:339-346.
99. Doernberg E, Hollander E. Neurodevelopmental Disorders (ASD and ADHD): DSM-5, ICD-10, and ICD-11. *CNS Spectr* 2016;21:295-299.
100. Posar A, Visconti P. Neurodevelopmental Disorders between Past and Future. *J Pediatr Neurosci* 2017;12:301-302.
101. Reilly S, McKean C, Morgan A, Wake M. Identifying and managing common childhood language and speech impairments. *Bmj* 2015;350:h2318.
102. O'Hare A, Bremner L. Management of developmental speech and language disorders: Part 1. *Arch Dis Child* 2016;101:272-277.
103. Wieck A, Jones S. Dangers of valproate in pregnancy. *Bmj* 2018;361:k1609.
104. Clayton-Smith J, Bromley R, Dean J, Journel H, Odent S, Wood A, et al. Diagnosis and management of individuals with Fetal Valproate Spectrum Disorder; a consensus statement from the European Reference Network for Congenital Malformations and Intellectual Disability. *Orphanet J Rare Dis* 2019;14:180.
105. Meador KJ, Baker GA, Browning N, Cohen MJ, Clayton-Smith J, Kalayjian LA, et al. Foetal antiepileptic drug exposure and verbal versus non-verbal abilities at three years of age. *Brain* 2011;134:396-404.
106. Shallcross R, Bromley RL, Cheyne CP, Garcia-Finana M, Irwin B, Morrow J, et al. In utero exposure to levetiracetam vs valproate: development and language at 3 years of age. *Neurology* 2014;82:213-221.
107. Thomas SV, Sukumaran S, Lukose N, George A, Sarma PS. Intellectual and language functions in children of mothers with epilepsy. *Epilepsia* 2007;48:2234-2240.
108. Veiby G, Daltveit AK, Schjolberg S, Stoltenberg C, Oyen AS, Vollset SE, et al. Exposure to antiepileptic drugs in utero and child development: a prospective population-based study. *Epilepsia* 2013;54:1462-1472.

109. Huber-Mollema Y, van Iterson L, Oort FJ, Lindhout D, Rodenburg R. Neurocognition after prenatal levetiracetam, lamotrigine, carbamazepine or valproate exposure. *J Neurol* 2020;267:1724-1736.
110. Unnikrishnan G, Jacob NS, Salim S, Jose M, Salini RA, Pavithran V, et al. Enduring language deficits in children of women with epilepsy and the potential role of intrauterine exposure to antiepileptic drugs. *Epilepsia* 2020;61:2442-2451.
111. Meador KJ, Baker GA, Browning N, Clayton-Smith J, Combs-Cantrell DT, Cohen M, et al. Cognitive function at 3 years of age after fetal exposure to antiepileptic drugs. *N Engl J Med* 2009;360:1597-1605.
112. Meador KJ, Pennell PB, May RC, Brown CA, Baker G, Bromley R, et al. Effects of periconceptional folate on cognition in children of women with epilepsy: NEAD study. *Neurology* 2020;94:e729-e740.
113. Cohen MJ, Meador KJ, May R, Loblein H, Conrad T, Baker GA, et al. Fetal antiepileptic drug exposure and learning and memory functioning at 6years of age: The NEAD prospective observational study. *Epilepsy Behav* 2019;92:154-164.
114. Christensen J, Gronborg TK, Sorensen MJ, Schendel D, Parner ET, Pedersen LH, et al. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. *Jama* 2013;309:1696-1703.
115. Wiggs KK, Rickert ME, Sujan AC, Quinn PD, Larsson H, Lichtenstein P, et al. Antiseizure medication use during pregnancy and risk of ASD and ADHD in children. *Neurology* 2020;95:e3232-e3240.
116. Huber-Mollema Y, Oort FJ, Lindhout D, Rodenburg R. Behavioral problems in children of mothers with epilepsy prenatally exposed to valproate, carbamazepine, lamotrigine, or levetiracetam monotherapy. *Epilepsia* 2019;60:1069-1082.
117. Christensen J, Pedersen LH, Sun Y, Dreier JW, Brikell I, Dalsgaard S. Association of Prenatal Exposure to Valproate and Other Antiepileptic Drugs With Risk for Attention-Deficit/Hyperactivity Disorder in Offspring. *JAMA Netw Open* 2019;2:e186606.
118. Lacey AS, Pickrell WO, Thomas RH, Kerr MP, White CP, Rees MI. Educational attainment of children born to mothers with epilepsy. *J Neurol Neurosurg Psychiatry* 2018;89:736-740.
119. Elkjaer LS, Bech BH, Sun Y, Laursen TM, Christensen J. Association Between Prenatal Valproate Exposure and Performance on Standardized Language and Mathematics Tests in School-aged Children. *JAMA Neurol* 2018.
120. Reynolds EH. Antiepileptic drugs, folate and one carbon metabolism revisited. *Epilepsy Behav* 2020;112:107336.
121. Reynolds EH, Green R. Valproate and folate: Congenital and developmental risks. *Epilepsy Behav* 2020;108:107068.
122. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992;327:1832-1835.

123. McGarel C, Pentieva K, Strain JJ, McNulty H. Emerging roles for folate and related B-vitamins in brain health across the lifecycle. *Proc Nutr Soc* 2015;74:46-55.
124. Belcastro V, Striano P. Antiepileptic drugs, hyperhomocysteinemia and B-vitamins supplementation in patients with epilepsy. *Epilepsy Res* 2012;102:1-7.
125. Asadi-Pooya AA. High dose folic acid supplementation in women with epilepsy: are we sure it is safe? *Seizure* 2015;27:51-53.
126. Maruvada P, Stover PJ, Mason JB, Bailey RL, Davis CD, Field MS, et al. Knowledge gaps in understanding the metabolic and clinical effects of excess folates/folic acid: a summary, and perspectives, from an NIH workshop. *Am J Clin Nutr* 2020;112:1390-1403.
127. McNulty H, Ward M, Hoey L, Hughes CF, Pentieva K. Addressing optimal folate and related B-vitamin status through the lifecycle: health impacts and challenges. *Proc Nutr Soc* 2019:1-14.
128. Au KS, Findley TO, Northrup H. Finding the genetic mechanisms of folate deficiency and neural tube defects-Leaving no stone unturned. *Am J Med Genet A* 2017;173:3042-3057.
129. Zheng Y, Cantley LC. Toward a better understanding of folate metabolism in health and disease. *J Exp Med* 2019;216:253-266.
130. Semmler A, Frisch C, Smith D, Blom H, Linnebank M. The ratio of S-adenosylmethione and S-adenosyl-homocysteine is increased in the brains of newborn rats in a model of valproic acid teratogenicity. *Toxicology* 2012;293:132-133.
131. Zeisel SH. Importance of methyl donors during reproduction. *Am J Clin Nutr* 2009;89:673S-677S.
132. Bueno O, Molloy AM, Fernandez-Ballart JD, García-Minguillán CJ, Ceruelo S, Ríos L, et al. Common Polymorphisms That Affect Folate Transport or Metabolism Modify the Effect of the MTHFR 677C > T Polymorphism on Folate Status. *J Nutr* 2016;146:1-8.
133. Murray LK, Smith MJ, Jadavji NM. Maternal oversupplementation with folic acid and its impact on neurodevelopment of offspring. *Nutr Rev* 2018;76:708-721.
134. Liu M, Zhang Z, Zhou C, Li Q, He P, Zhang Y, et al. Relationship of several serum folate forms with the risk of mortality: A prospective cohort study. *Clin Nutr* 2021.
135. Liu HY, Liu SM, Zhang YZ. Maternal Folic Acid Supplementation Mediates Offspring Health via DNA Methylation. *Reprod Sci* 2020;27:963-976.
136. Bailey LB, Stover PJ, McNulty H, Fenech MF, Gregory JF, 3rd, Mills JL, et al. Biomarkers of Nutrition for Development-Folate Review. *J Nutr* 2015;145:1636S-1680S.
137. Liu X, Zou M, Sun C, Wu L, Chen WX. Prenatal Folic Acid Supplements and Offspring's Autism Spectrum Disorder: A Meta-analysis and Meta-regression. *J Autism Dev Disord* 2021.

138. Roth C, Magnus P, Schjolberg S, Stoltenberg C, Suren P, McKeague IW, et al. Folic acid supplements in pregnancy and severe language delay in children. *Jama* 2011;306:1566-1573.
139. Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, et al. Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. *Jama* 2013;309:570-577.
140. Raghavan R, Riley AW, Volk H, Caruso D, Hironaka L, Sices L, et al. Maternal Multivitamin Intake, Plasma Folate and Vitamin B(12) Levels and Autism Spectrum Disorder Risk in Offspring. *Paediatr Perinat Epidemiol* 2018;32:100-111.
141. Raghavan R, Selhub J, Paul L, Ji Y, Wang G, Hong X, et al. A prospective birth cohort study on cord blood folate subtypes and risk of autism spectrum disorder. *Am J Clin Nutr* 2020.
142. Valera-Gran D, Garcia de la Hera M, Navarrete-Munoz EM, Fernandez-Somoano A, Tardon A, Julvez J, et al. Folic acid supplements during pregnancy and child psychomotor development after the first year of life. *JAMA Pediatr* 2014;168:e142611.
143. Rubini E, Baijens IMM, Horánszky A, Schoenmakers S, Sinclair KD, Zana M, et al. Maternal One-Carbon Metabolism during the Periconceptional Period and Human Foetal Brain Growth: A Systematic Review. *Genes (Basel)* 2021;12.
144. Asbaghi O, Ghanavati M, Ashtary-Larky D, Bagheri R, Rezaei Kelishadi M, Nazarian B, et al. Effects of Folic Acid Supplementation on Oxidative Stress Markers: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Antioxidants (Basel)* 2021;10.
145. Lintas C. Linking genetics to epigenetics: The role of folate and folate-related pathways in neurodevelopmental disorders. *Clin Genet* 2019;95:241-252.
146. Irvine N, England-Mason G, Field CJ, Dewey D, Aghajafari F. Prenatal Folate and Choline Levels and Brain and Cognitive Development in Children: A Critical Narrative Review. *Nutrients* 2022;14.
147. Harlan De Crescenzo A, Panoutsopoulos AA, Tat L, Schaaf Z, Racherla S, Henderson L, et al. Deficient or Excess Folic Acid Supply During Pregnancy Alter Cortical Neurodevelopment in Mouse Offspring. *Cereb Cortex* 2021;31:635-649.
148. Apeland T, Mansoor MA, Pentieva K, McNulty H, Seljeflot I, Strandjord RE. The effect of B-vitamins on hyperhomocysteinemia in patients on antiepileptic drugs. *Epilepsy Res* 2002;51:237-247.
149. Apeland T, Mansoor MA, Pentieva K, McNulty H, Strandjord RE. Fasting and post-methionine loading concentrations of homocysteine, vitamin B2, and vitamin B6 in patients on antiepileptic drugs. *Clin Chem* 2003;49:1005-1008.
150. Linnebank M, Moskau S, Semmler A, Widman G, Stoffel-Wagner B, Weller M, et al. Antiepileptic drugs interact with folate and vitamin B12 serum levels. *Ann Neurol* 2011;69:352-359.
151. Ni G, Qin J, Fang Z, Chen Y, Chen Z, Zhou J, et al. Increased homocysteine levels in valproate-treated patients with epilepsy: a meta-analysis. *BMJ Open* 2014;4:e004936.

152. Ni G, Qin J, Li H, Chen Z, Zhou Y, Fang Z, et al. Effects of antiepileptic drug monotherapy on one-carbon metabolism and DNA methylation in patients with epilepsy. *PLoS One* 2015;10:e0125656.
153. Safahani M, Aligholi H, Asadi-Pooya AA. Management of antiepileptic drug-induced nutrition-related adverse effects. *Neurol Sci* 2020;41:3491-3502.
154. Rezaei S, Shab-Bidar S, Abdulahi Abdurahman A, Djafarian K. Oxcarbazepine administration and the serum levels of homocysteine, vitamin B12 and folate in epileptic patients: A systematic review and meta-analysis. *Seizure* 2017;45:87-94.
155. Suh JR, Herbig AK, Stover PJ. New perspectives on folate catabolism. *Annu Rev Nutr* 2001;21:255-282.
156. Belcastro V, Striano P, Gorgone G, Costa C, Ciampa C, Caccamo D, et al. Hyperhomocysteinemia in epileptic patients on new antiepileptic drugs. *Epilepsia* 2010;51:274-279.
157. Kim DW, Lee SY, Shon YM, Kim JH. Effects of new antiepileptic drugs on circulatory markers for vascular risk in patients with newly diagnosed epilepsy. *Epilepsia* 2013;54:e146-149.
158. Linnebank M, Moskau S, Semmler A, Widman G, Weller M, Kallweit U, et al. Antiepileptic drugs and vitamin B6 plasma levels in adult patients. *Epilepsy Res* 2012;101:182-184.
159. Mintzer S, Skidmore CT, Sperling MR. B-vitamin deficiency in patients treated with antiepileptic drugs. *Epilepsy Behav* 2012;24:341-344.
160. Dansky LV, Andermann E, Rosenblatt D, Sherwin AL, Andermann F. Anticonvulsants, folate levels, and pregnancy outcome: a prospective study. *Ann Neurol* 1987;21:176-182.
161. Walker DI, Perry-Walker K, Finnell RH, Pennell KD, Tran V, May RC, et al. Metabolome-wide association study of anti-epileptic drug treatment during pregnancy. *Toxicol Appl Pharmacol* 2019;363:122-130.
162. Morrow JI, Hunt SJ, Russell AJ, Smithson WH, Parsons L, Robertson I, et al. Folic acid use and major congenital malformations in offspring of women with epilepsy: a prospective study from the UK Epilepsy and Pregnancy Register. *J Neurol Neurosurg Psychiatry* 2009;80:506-511.
163. Kjaer D, Horvath-Puho E, Christensen J, Vestergaard M, Czeizel AE, Sorensen HT, et al. Antiepileptic drug use, folic acid supplementation, and congenital abnormalities: a population-based case-control study. *Bjog* 2008;115:98-103.
164. Dean J, Robertson Z, Reid V, Wang QD, Hailey H, Moore S, et al. Fetal anticonvulsant syndromes and polymorphisms in MTHFR, MTR, and MTRR. *Am J Med Genet A* 2007;143A:2303-2311.
165. Kini U, Lee R, Jones A, Smith S, Ramsden S, Fryer A, et al. Influence of the MTHFR genotype on the rate of malformations following exposure to antiepileptic drugs in utero. *Eur J Med Genet* 2007;50:411-420.
166. Pittschieler S, Brezinka C, Jahn B, Trinka E, Unterberger I, Dobesberger J, et al. Spontaneous abortion and the prophylactic effect of folic acid supplementation in epileptic women undergoing antiepileptic therapy. *J Neurol* 2008;255:1926-1931.

167. Bjork M, Riedel B, Spigset O, Veiby G, Kolstad E, Daltveit AK, et al. Association of Folic Acid Supplementation During Pregnancy With the Risk of Autistic Traits in Children Exposed to Antiepileptic Drugs In Utero. *JAMA Neurol* 2018;75:160-168.
168. Kasradze S, Gogatishvili N, Lomidze G, Ediberidze T, Lazariashvili M, Khomeriki K, et al. Cognitive functions in children exposed to antiepileptic drugs in utero - Study in Georgia. *Epilepsy Behav* 2017;66:105-112.
169. Vajda FJE, O'Brien TJ, Graham JE, Hitchcock AA, Perucca P, Lander CM, et al. Folic acid dose, valproate, and fetal malformations. *Epilepsy Behav* 2021;114:107569.
170. Husebye ESN, Wendel AWK, Gilhus NE, Riedel B, Bjørk MH. Plasma unmetabolized folic acid in pregnancy and risk of autistic traits and language impairment in antiseizure medication-exposed children of women with epilepsy. *Am J Clin Nutr* 2022.
171. Asadi-Pooya AA, Simani L. Clinical trials of vitamin-mineral supplementations in people with epilepsy: A systematic review. *Clin Nutr* 2020.
172. Sadat-Hossieny Z, Robalino CP, Pennell PB, Cohen MJ, Loring DW, May RC, et al. Folate fortification of food: Insufficient for women with epilepsy. *Epilepsy Behav* 2021;117:107688.
173. Hernandez-Diaz S, Levin M. Alteration of bioelectrically-controlled processes in the embryo: a teratogenic mechanism for anticonvulsants. *Reprod Toxicol* 2014;47:111-114.
174. Ikonomidou C, Turski L. Antiepileptic drugs and brain development. *Epilepsy Res* 2010;88:11-22.
175. Ornoy A, Becker M, Weinstein-Fudim L, Ergaz Z. S-Adenosine Methionine (SAMe) and Valproic Acid (VPA) as Epigenetic Modulators: Special Emphasis on their Interactions Affecting Nervous Tissue during Pregnancy. *Int J Mol Sci* 2020;21.
176. Tetro N, Hamed R, Berman E, Eyal S. Effects of antiseizure medications on placental cells: Focus on heterodimeric placental carriers. *Epilepsy Res* 2021;174:106664.
177. Semmler A, Frisch C, Bleul C, Smith D, Bigler L, Prost JC, et al. Intrauterine valproate exposure is associated with alterations in hippocampal cell numbers and folate metabolism in a rat model of valproate teratogenicity. *Seizure* 2017;46:7-12.
178. Fathe K, Palacios A, Finnell RH. Brief report novel mechanism for valproate-induced teratogenicity. *Birth Defects Res A Clin Mol Teratol* 2014;100:592-597.
179. Hsieh CL, Wang HE, Tsai WJ, Peng CC, Peng RY. Multiple point action mechanism of valproic acid-teratogenicity alleviated by folic acid, vitamin C, and N-acetylcysteine in chicken embryo model. *Toxicology* 2012;291:32-42.
180. Emes RD, Clifford H, Haworth KE, Farrell WE, Fryer AA, Carroll WD, et al. Antiepileptic drugs and the fetal epigenome. *Epilepsia* 2013;54:e16-19.
181. Smith AK, Conneely KN, Newport DJ, Kilaru V, Schroeder JW, Pennell PB, et al. Prenatal antiepileptic exposure associates with neonatal DNA methylation differences. *Epigenetics* 2012;7:458-463.

182. Ali A, Pillai KK, Pal SN. Effects of folic acid and lamotrigine therapy in some rodent models of epilepsy and behaviour. *J Pharm Pharmacol* 2003;55:387-391.
183. Abdulrazzaq YM, Shafiullah M, Kochyil J, Padmanabhan R, Bastaki SMA. Ameliorative effects of supplemental folinic acid on Lamotrigine-induced fetal malformations in the mouse. *Mol Cell Biochem* 2018.
184. Kardoost M, Hajizadeh-Saffar E, Ghorbanian MT, Ghezelayagh Z, Pooshang Bagheri K, Behdani M, et al. Genotoxicity assessment of antiepileptic drugs (AEDs) in human embryonic stem cells. *Epilepsy Res* 2019;158:106232.
185. Muhsen M, Youngs J, Riu A, Gustafsson J, Kondamadugu VS, Garyfalidis E, et al. Folic acid supplementation rescues valproic acid-induced developmental neurotoxicity and behavioral alterations in zebrafish embryos. *Epilepsia* 2021.
186. Rubinchik-Stern M, Shmuel M, Bar J, Kovo M, Eyal S. Adverse placental effects of valproic acid: Studies in perfused human placentas. *Epilepsia* 2018;59:993-1003.
187. Rubinchik-Stern M, Shmuel M, Eyal S. Antiepileptic drugs alter the expression of placental carriers: An in vitro study in a human placental cell line. *Epilepsia* 2015;56:1023-1032.
188. Tetro N, Imbar T, Wohl D, Eisenberg I, Yagel S, Shmuel M, et al. The effects of valproic acid on early pregnancy human placentas: Pilot ex vivo analysis in cultured placental villi. *Epilepsia* 2019;60:e47-e51.
189. Takeda K, Watanabe T, Oyabu K, Tsukamoto S, Oba Y, Nakano T, et al. Valproic acid-exposed astrocytes impair inhibitory synapse formation and function. *Sci Rep* 2021;11:23.
190. Ikonomidou C, Scheer I, Wilhelm T, Juengling FD, Titze K, Stover B, et al. Brain morphology alterations in the basal ganglia and the hypothalamus following prenatal exposure to antiepileptic drugs. *Eur J Paediatr Neurol* 2007;11:297-301.
191. Phiel CJ, Zhang F, Huang EY, Guenther MG, Lazar MA, Klein PS. Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. *J Biol Chem* 2001;276:36734-36741.
192. Gurvich N, Berman MG, Wittner BS, Gentleman RC, Klein PS, Green JB. Association of valproate-induced teratogenesis with histone deacetylase inhibition in vivo. *Faseb j* 2005;19:1166-1168.
193. Naziroglu M, Yurekli VA. Effects of antiepileptic drugs on antioxidant and oxidant molecular pathways: focus on trace elements. *Cell Mol Neurobiol* 2013;33:589-599.
194. Magnus P, Birke C, Vejrup K, Haugan A, Alsaker E, Daltveit AK, et al. Cohort Profile Update: The Norwegian Mother and Child Cohort Study (MoBa). *Int J Epidemiol* 2016;45:382-388.
195. Magnus P, Irgens LM, Haug K, Nystad W, Skjaerven R, Stoltenberg C. Cohort profile: the Norwegian Mother and Child Cohort Study (MoBa). *Int J Epidemiol* 2006;35:1146-1150.
196. The Norwegian Institute of Public Health. The Norwegian Mother, Father and Child Cohort Study (MoBa): Revised protocol. End of enrollment - Protocol II. Available at https://www.fhi.no/globalassets/dokumenterfiler/studier/den-

norske-mor-far-og-barn--undersokelsenmoba/protokoll/2012-moba-protokoll.pdf.
197. Paltiel L, Haugan A, Skjerden T, Harbak K, Bækken S, Stensrud NK, et al. The biobank of the Norwegian Mother and Child Cohort Study - present status. *The Norwegian Journal of Epidemiology* 2014;24 (1-2):29-35.
198. Bjork MH, Veiby G, Spigset O, Gilhus NE. Using the Norwegian Mother and Child Cohort Study to determine risk factors for delayed development and neuropsychiatric symptoms in the offspring of parents with epilepsy. *The Norwegian Journal of Epidemiology* 2014;24:79-89.
199. Bjorke-Monsen AL, Roth C, Magnus P, Midttun O, Nilsen RM, Reichborn-Kjennerud T, et al. Maternal B vitamin status in pregnancy week 18 according to reported use of folic acid supplements. *Mol Nutr Food Res* 2013;57:645-652.
200. Roth C, Bjorke-Monsen AL, Reichborn-Kjennerud T, Nilsen RM, Smith GD, Stoltenberg C, et al. Use of folic acid supplements in early pregnancy in relation to maternal plasma levels in week 18 of pregnancy. *Mol Nutr Food Res* 2013;57:653-660.
201. Hannisdal R, Ueland PM, Svardal A. Liquid chromatography-tandem mass spectrometry analysis of folate and folate catabolites in human serum. *Clin Chem* 2009;55:1147-1154.
202. Hannisdal R, Ueland PM, Eussen SJ, Svardal A, Hustad S. Analytical recovery of folate degradation products formed in human serum and plasma at room temperature. *J Nutr* 2009;139:1415-1418.
203. Midttun O, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 2009;23:1371-1379.
204. Ulvik A, Midttun Ø, McCann A, Meyer K, Tell G, Nygård O, et al. Tryptophan catabolites as metabolic markers of vitamin B-6 status evaluated in cohorts of healthy adults and cardiovascular patients. *Am J Clin Nutr* 2020;111:178-186.
205. Midttun Ø, Kvalheim G, Ueland P. The B-vitamin network with ramification to the tryptophan-niacin pathway. Available at https://folk.uib.no/mfapu/Pages/BV/BVSite/pdf_files/literature/Midttun%20FASEB%202012.pdf.
206. Richter J, Janson H. A validation study of the Norwegian version of the Ages and Stages Questionnaires. *Acta Paediatr* 2007;96:748-752.
207. Squires J, Bricker D, Potter L. Revision of a parent-completed development screening tool: Ages and Stages Questionnaires. *J Pediatr Psychol* 1997;22:313-328.
208. Dale PS, Price TS, Bishop DV, Plomin R. Outcomes of early language delay: I. Predicting persistent and transient language difficulties at 3 and 4 years. *J Speech Lang Hear Res* 2003;46:544-560.
209. Hadley PA, Rice ML. Parental Judgments of Preschoolers' Speech and Language Development: A Resource for Assessment and IEP Planning. *Semin Speech Language* 1993;14:278-288.

210. Ottem E. 20 spørsmål om språkferdigheter– en analyse av sammenhengen mellom observasjonsdata og testdata. *Skolepsykologi* 2009;1.
211. Hawa VV, Spanoudis G. Toddlers with delayed expressive language: an overview of the characteristics, risk factors and language outcomes. *Res Dev Disabil* 2014;35:400-407.
212. Schjolberg S, Eadie P, Zachrisson HD, Oyen AS, Prior M. Predicting language development at age 18 months: data from the Norwegian Mother and Child Cohort Study. *J Dev Behav Pediatr* 2011;32:375-383.
213. Strand BH, Dalgard OS, Tambs K, Rognerud M. Measuring the mental health status of the Norwegian population: a comparison of the instruments SCL-25, SCL-10, SCL-5 and MHI-5 (SF-36). *Nord J Psychiatry* 2003;57:113-118.
214. Lash TL, VanderWeele TJ, Haneuse S, Rothman KJ. Modern Epidemiology. Forth Edition. 2021:Pages 3-6.
215. Olsen J, Christensen K, Murray J, Ekbom A. An Introduction to Epidemiology for Health Professionals. 2010:Pages 51-57.
216. Lash TL, VanderWeele TJ, Haneuse S, Rothman KJ. Modern Epidemiology. Forth Edition. 2021:Pages 105-121.
217. Lash TL, VanderWeele TJ, Haneuse S, Rothman KJ. Modern Epidemiology. Forth Edition. 2021:Pages 143-159.
218. Nilsen RM, Vollset SE, Gjessing HK, Skjaerven R, Melve KK, Schreuder P, et al. Self-selection and bias in a large prospective pregnancy cohort in Norway. *Paediatr Perinat Epidemiol* 2009;23:597-608.
219. Olsen J, Christensen K, Murray J, Ekbom A. An Introduction to Epidemiology for Health Professionals. 2010:Page 79.
220. Lash TL, VanderWeele TJ, Haneuse S, Rothman KJ. Modern Epidemiology. Forth Edition. 2021:Pages 333-364.
221. Olsen J, Christensen K, Murray J, Ekbom A. An Introduction to Epidemiology for Health Professionals. 2010:Pages 103-105.
222. Tu Y-K, Greenwood DC. Modern Methods for Epidemiology 2012:Pages 57-71.
223. Lash TL, VanderWeele TJ, Haneuse S, Rothman KJ. Modern Epidemiology. Forth Edition. 2021:Pages 287-314.
224. Lash TL, VanderWeele TJ, Haneuse S, Rothman KJ. Modern Epidemiology. Forth Edition. 2021:Pages 315-331.
225. Olsen J, Christensen K, Murray J, Ekbom A. An Introduction to Epidemiology for Health Professionals. 2010:Pages 119-122.
226. The Norwegian Mother Father and Child Cohort Study. Invitation to participate. Available at https://www.fhi.no/globalassets/dokumenterfiler/studier/den-norske-mor-far-og-barn--undersokelsenmoba/info-til-deltakere/mobabrosjyreinvitasjon2006.pdf.
227. Vejrup K, Magnus P, Magnus M. Lost to follow-up in the Norwegian mother, father and child cohort study. *Paediatr Perinat Epidemiol* 2021.
228. Lash TL, VanderWeele TJ, Haneuse S, Rothman KJ. Modern Epidemiology. Forth Edition. 2021:Pages 367-393.
229. Lupattelli A, Wood ME, Nordeng H. Analyzing Missing Data in Perinatal Pharmacoepidemiology Research: Methodological Considerations to Limit the Risk of Bias. *Clin Ther* 2019;41:2477-2487.

230. Lash TL, VanderWeele TJ, Haneuse S, Rothman KJ. Modern Epidemiology. Forth Edition. 2021:Pages 263-286.
231. Olsen J, Christensen K, Murray J, Ekbom A. An Introduction to Epidemiology for Health Professionals. 2010:Pages 145-150.
232. Olsen J, Christensen K, Murray J, Ekbom A. An Introduction to Epidemiology for Health Professionals. 2010:Pages 107-111.
233. Pallant J. SPSS Survival Manual. 6th edition. 2016:Pages 214-215.
234. Skovlund E, Fenstad GU. Should we always choose a nonparametric test when comparing two apparently nonnormal distributions? *J Clin Epidemiol* 2001;54:86-92.
235. Gaily E, Kantola-Sorsa E, Hiilesmaa V, Isoaho M, Matila R, Kotila M, et al. Normal intelligence in children with prenatal exposure to carbamazepine. *Neurology* 2004;62:28-32.
236. McNulty H, Rollins M, Cassidy T, Caffrey A, Marshall B, Dornan J, et al. Effect of continued folic acid supplementation beyond the first trimester of pregnancy on cognitive performance in the child: a follow-up study from a randomized controlled trial (FASSTT Offspring Trial). *BMC Med* 2019;17:196.
237. Gasperi V, Sibilano M, Savini I, Catani MV. Niacin in the Central Nervous System: An Update of Biological Aspects and Clinical Applications. *Int J Mol Sci* 2019;20.
238. Tam C, O'Connor D, Koren G. Circulating unmetabolized folic Acid: relationship to folate status and effect of supplementation. *Obstet Gynecol Int* 2012;2012:485179.
239. Obeid R, Kasoha M, Kirsch SH, Munz W, Herrmann W. Concentrations of unmetabolized folic acid and primary folate forms in pregnant women at delivery and in umbilical cord blood. *Am J Clin Nutr* 2010;92:1416-1422.
240. Yang X, Sun W, Wu Q, Lin H, Lu Z, Shen X, et al. Excess Folic Acid Supplementation before and during Pregnancy and Lactation Alters Behaviors and Brain Gene Expression in Female Mouse Offspring. *Nutrients* 2021;14.
241. Silveira JS, Ramires Júnior OV, Schmitz F, Ferreira FS, Rodrigues FC, Silva RC, et al. Folic acid supplementation during pregnancy alters behavior in male rat offspring: nitrative stress and neuroinflammatory implications. *Mol Neurobiol* 2022.
242. Di Y, Li Z, Li J, Cheng Q, Zheng Q, Zhai C, et al. Maternal folic acid supplementation prevents autistic behaviors in a rat model induced by prenatal exposure to valproic acid. *Food Funct* 2021;12:4544-4555.

Original publications

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Verbal abilities in children of mothers with epilepsy

Association to maternal folate status

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Neurology® 2018;91:e811-e821. doi:10.1212/WNL.0000000000006073

Abstract

Objective

To examine the effect of maternal folic acid supplementation and maternal plasma folate and antiepileptic drug (AED) concentrations on language delay in AED-exposed children of mothers with epilepsy.

Methods

Children of mothers with and without epilepsy enrolled from 1999 to 2008 in the Norwegian Mother and Child Cohort study were included. Information on medical history, AED use, and folic acid supplementation during pregnancy was collected from parent-completed questionnaires. Maternal plasma folate and maternal plasma and umbilical cord AED concentrations were measured in blood samples from gestational weeks 17 to 19 and immediately after birth, respectively. Language development at 18 and 36 months was evaluated by the Ages and Stages Questionnaires.

Results

A total of 335 AED-exposed children of mothers with epilepsy and 104,222 children of mothers without epilepsy were surveyed. For those with no maternal periconceptional folic acid supplementation, the fully adjusted odds ratio (OR) for language delay in AED-exposed children compared to the controls at 18 months was 3.9 (95% confidence interval [CI] 1.9–7.8, $p < 0.001$) and at 36 months was 4.7 (95% CI 2.0–10.6, $p < 0.001$). When folic supplementation was used, the corresponding ORs for language delay were 1.7 (95% CI 1.2–2.6, $p = 0.01$) and 1.7 (95% CI 0.9–3.2, $p = 0.13$), respectively. The positive effect of folic acid supplement use on language delay in AED-exposed children was significant only when supplement was used in the period from 4 weeks before the pregnancy and until the end of the first trimester.

Conclusion

Folic acid use early in pregnancy may have a preventive effect on language delay associated with in utero AED exposure.

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Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

The Article Processing Charge was funded by the authors.

Glossary

AED = antiepileptic drug; **AR** = attributable risk; **ASQ** = Ages and Stages Questionnaires; **CI** = confidence interval; **hmTHF** = 4-alfa-hydroxy-5-methyltetrahydrofolate; **MBRN** = Medical Birth Registry of Norway; **MoBa** = Norwegian Mother and Child Cohort Study; **mTHF** = 5-methyltetrahydrofolate; **OR** = odds ratio; **RR** = relative risk.

Most women with epilepsy are dependent on treatment with antiepileptic drugs (AEDs) throughout their pregnancy to prevent epileptic seizures.[1] AEDs increase the risk of congenital malformations in a dose-dependent manner.[2] Some AEDs have also been associated with impaired neurodevelopment and behavioral disorders in the offspring.[1,2] Hence, it is crucial to identify factors that modulate the risk of AED-related fetal harm.

Folate is a B vitamin important for normal brain development.[3] Many AEDs interact with folate metabolism and have been associated with reduced plasma folate.[4,5] There is growing evidence of a positive association between maternal folate status during pregnancy and neurodevelopmental outcome in the offspring.[3,6–8] Few studies have examined whether folic acid supplementation protects against impaired neurodevelopment after AED exposure in utero. Some studies have indicated that folic acid may have a positive effect on IQ and verbal abilities in children exposed to AEDs in utero,[9,10] but the results are conflicting.[11,12] We have previously found that AED-exposed children have fewer autistic traits if their mothers used folic acid supplements in the periconceptional period.[13] Women in Norway are recommended to use 0.4 mg folic acid daily in the periconceptional period only, while women with epilepsy who use AEDs usually are recommended to use 1 to 5 mg daily in the periconceptional period and 0.4 mg daily in the second and third trimesters. There is no mandatory folic acid food fortification in Norway.[14]

The aim of our study was to investigate the effect of maternal folic acid supplement use, maternal plasma folate, and AED concentrations during pregnancy on language development in AED-exposed children of mothers with epilepsy.

Methods

Study population

The study population consisted of women and children included in the Norwegian Mother and Child Cohort Study (MoBa). MoBa is a prospective, ongoing population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health and is linked to the compulsory Medical Birth Registry of Norway (MBRN).[15] Norwegian-speaking women were invited to participate from 1999 to 2008. The participation rate was 41%. Information on background, medical history, medication use, vitamin and folic acid intake, and child development, including language function, was obtained by parent-completed questionnaires. The questionnaires were answered in gestational weeks 17 to 19 (Q1) and 30 (Q2) and when the child was 18 and 36 months old (Q3 and Q4; response rates, 72% and 56%, respectively). Maternal blood samples were collected at week 17 to 19 of gestation and from the umbilical cord immediately after delivery.

The epilepsy diagnosis is based on self-reported information from the MoBa questionnaires and information from the MBRN registered by the family doctor or midwife.[16] We have previously validated the epilepsy cohort in MoBa (data available from Dryad, Methods, doi.org/10.5061/dryad.1237b6m), and the validity was very good.[17]

Our material is based on version VIII of the MoBa databank and consisted of 724 children of 616 mothers with epilepsy and 104,222 children of 86,443 mothers without epilepsy with available information on maternal folic acid supplement use during pregnancy (figure 1). The children of mothers with epilepsy were further classified into 2 groups: 1 group exposed to AEDs in utero (n = 335), our main study group, and another group not exposed to AEDs in utero (n = 389). We have previously reported on general development after in utero and breastmilk exposure to AED in this cohort.[18,19]

Variables

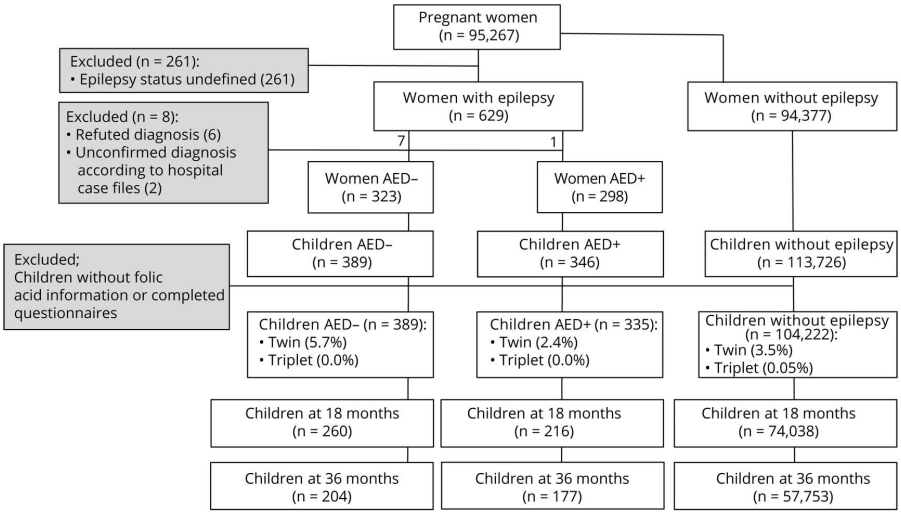
Maternal folic acid supplementation

Intake of folic acid before and during pregnancy was reported in gestational week 17 to 19 (Q1) for the following time intervals: >5 weeks before pregnancy, 4 weeks before pregnancy (preconception), and use during gestational weeks 0 to 4, 5 to 8, 9 to 12, and 13+. Folic acid use in gestational weeks 13 to 16, 17 to 20, 21 to 24, 25 to 29, and 29+ was reported in gestational week 30 (Q2) (data available from Dryad, Methods, doi.org/10.5061/dryad.1237b6m). Folic acid doses were obtained for 139 AED-exposed children and 160 AED-unexposed children by a separate retrospective questionnaire to women with epilepsy in our previous validation study (Q5; response rate 50%).[17] In 84 children (25%) in the AED-exposed group and 21 children (5%) in the AED-unexposed group, the mothers reported a daily intake of folic acid of ≥1 mg. We defined periconceptional folic acid use as maternal intake of folic acid supplements from 4 weeks before the start of the pregnancy and/or during the first trimester.

AED use

Information on AED use and type of medication was collected from self-reported information in Q1 and the MBRN data registered by the family doctor or midwife.[15] There was 100% agreement between self-reported AED use in MoBa and the

Figure 1 Flowchart of excluded and included cases



AED+ = antiepileptic drug use/exposure; AED− = no antiepileptic drug use/exposure.

reported AED use in hospital records in our previous validation study.[17]

Measurement of plasma folate and AED concentrations

From the MoBa biobank,[20] folate was available in maternal plasma samples obtained at gestational week 17 to 19 for 228 AED-exposed children (68%). Analysis included the biologically active 5-methyltetrahydrofolate (mTHF) and the degradation product 4-alfa-hydroxy-5-methyltetrahydrofolate (hmTHF). mTHF represents the prevailing folate form in plasma. This form is unstable in blood samples kept at room temperature, but is largely recovered as hmTHF. Hence, maternal plasma folate is given as the sum of the concentration of mTHF and hmTHF.[21,22]

The concentrations of valproate, lamotrigine, carbamazepine, carbamazepine10,11-epoxide, levetiracetam, topiramate, and the oxcarbazepine monohydroxy derivative metabolite were analyzed in 226 maternal plasma samples obtained at gestational week 17 to 19 and in 198 samples from the umbilical cord, as described previously,[17] for a total of 255 AED-exposed children (76%). In 238 of these samples (93%), the reported AED was detected. For the statistical analysis, the plasma concentrations were normalized relative to the ranges observed within each group according to the following formula: 100 × (observed concentration − minimum concentration)/concentration range.[10,23] The mean of the normalized plasma concentrations was calculated for each child on the basis of both the concentration from the maternal sample and the umbilical cord sample if both were present. If only one of the samples was available, this concentration was used. If a child was exposed to AED polytherapy, the mean normalized concentrations of each AED were added together.

Language delay

Global language delay

In Q3 and Q4, mothers completed a 3-item and a 6-item version, respectively (data available from Dryad, table 1, doi.org/10.5061/dryad.1237b6m), of the 18 months' and 36 months' communication scale from the Ages and Stages Questionnaires (ASQ).[24] ASQ is considered a reliable screening tool with high concurrent validity.[24,25] Each item had the following answer options: yes (10 points), sometimes (5 points), and not yet (0 points). The maximum score reflecting no language delay was 30 and 60 points at 18 and 36 months, respectively. Children with missing answers in Q3 were excluded. If only 1 answer was missing in Q4, this was imputed with the estimation-maximization procedure in SPSS (IBM, Armonk, NY). Children were defined as having global language delay when the mothers had reported an ASQ score >1.5 SD below the mean ASQ score in the total MoBa cohort.[24,26]

Expressive language delay

In Q4, a 1-item question regarding expressive language skills has shown acceptable validity as an indicator of the grammatical complexity level of 3-year-old children (data available from Dryad, table 2, doi.org/10.5061/dryad.1237b6m).[27] The maximum score reflecting no expressive language delay was 6 points. Children talking in 2- to 3-word phrases or less were classified as having expressive language delay.

Covariates

Relevant covariates were selected from the MoBa questionnaires and from the MBRN[6,28]: parental higher education (≥17 years of schooling), maternal low education (≤9 years of schooling), total household income <400,000 Norwegian kroner annually (equals approximately €42,000), unplanned pregnancy, smoking and alcohol use (consumption ≥1 per month) in pregnancy, parity (number of previous pregnancies with >21 gestation weeks), maternal age, maternal depression and anxiety symptoms during pregnancy (mean score >1.75 on the Hopkins symptom checklist[29] at gestational week 17–19), single mother, maternal prepregnancy body mass index, seizures during pregnancy, tonic-clonic seizures during pregnancy (data available from Dryad, Methods, doi.org/10.5061/dryad.1237b6m), AED polytherapy, twin or triplet children, Apgar score 5 minutes after birth, gestational age, and offspring sex.

Statistical analysis

The statistical analysis was performed with IBM SPSS software version 24. AED-exposed and -unexposed children of mothers with epilepsy were compared to a control group of children of mothers without epilepsy. Each of the 3 groups was stratified by periconceptional folic acid use. Groups with similar periconceptional folic acid supplementation status were compared. We also compared the supplemented group with the unsupplemented group within each of the 3 groups. Categorical variables were compared with the χ^2 test for independence or Fisher exact test when appropriate. Continuous variables were compared with the Mann-Whitney *U* test because of violation of the assumption of normal distribution. The risk for delayed language outcome was investigated with logistic regression. The relationship between maternal plasma folate status/AED concentrations and language outcome was examined by a multivariable linear regression model and by correlation analysis. Values of $p < 0.05$ were considered statistically significant. We hypothesized a causal relationship between no periconceptional folic acid supplementation and language delay to calculate the attributable risk (AR) of no periconceptional folic acid supplementation on language delay in each of the 3 groups (data available from Dryad, Methods, doi.org/10.5061/dryad.1237b6m). This was done by calculating relative risk (RR) in a 2 × 2 table and then the AR with the formula AR = RR − 1/RR.[30]

Standard protocol approval, registration, and patient consent

The establishment and data collection in MoBa obtained a license from the Norwegian Data Inspectorate and approval from the Regional Committee for Medical Research Ethics.

The current study was approved by the Regional Committee for Medical Research Ethics (reference No. 2011/1616). Written informed consent was obtained from all participating parents in MoBa.

Data availability

Data from MoBa and the MBRN used in this study are managed by the national health register holders in Norway and can be made available to researchers, provided that necessary approval is obtained from the Regional Ethics Committees in Norway and from the data owners. The Norwegian Institute of Public Health has a general contact point for data access at the following e-mail address: datatilgang@fhi.no.

Results

Characteristics of the children, their parents, and the pregnancies stratified by periconceptional folic acid use are presented in table 1 (full version: data available from Dryad, table 3, doi.org/10.5061/dryad.1237b6m). A total of 268 children were exposed to AED monotherapy in utero, and 65 children were exposed to AED polytherapy (data available from Dryad, table 4). In children exposed to monotherapy, the most frequently used AEDs were lamotrigine (39%), carbamazepine (26%), valproate (15%), levetiracetam (6%), topiramate (4%), and oxcarbazepine (3%). In the polytherapy group, the most frequently used AEDs were lamotrigine (51%), carbamazepine (32%), valproate (29%), levetiracetam (29%), oxcarbazepine (23%), and topiramate (15%). For 2 children, the AED drug regimen was unspecified.

Folic acid supplementation and language delay

Without periconceptional folic acid supplementation, 34% of the AED-exposed children had global language delay at 18 months compared to 11% in the control group without maternal epilepsy ($p < 0.001$) (table 2). The fully adjusted odds ratio (OR) was 3.9 (95% confidence interval [CI] 1.9–7.8, $p < 0.001$) (table 3). At 36 months, 24% of AED-exposed children had expressive language delay compared to only 6% in the control group ($p < 0.001$). The fully adjusted OR was 4.7 (95% CI 2.0–10.6, $p < 0.001$). In the children of mothers who had used folic acid periconceptionally, 17% of AED-exposed children had global language delay at 18 months compared to 11% in the control group ($p = 0.01$). The fully adjusted OR was 1.7 (95% CI 1.2–2.6, $p = 0.01$). For expressive language delay at 36 months with folic acid, 7% of AED-exposed children had a delay compared to 4% in the control group ($p = 0.08$). The fully adjusted OR was 1.7 (95% CI 0.9–3.2, $p = 0.13$). There were no significant differences between AED-unexposed children of mothers with epilepsy and the control group (tables 2 and 3).

Within the group of AED-exposed children, the proportion of children with language delay was higher in the no supplementation group than in the supplemented group (table 2). A difference was also found in children of women without epilepsy, but it was much smaller than for the AED-exposed children. Stratification by AED revealed that the number of lamotrigine-exposed children with language delay was significantly higher in the no supplementation group compared to the supplemented group (table 4). The same tendency was seen for children exposed to valproate and carbamazepine, but this was not significant (table 4).

Mothers of AED-exposed children with language delay started with folic acid later in pregnancy. The median start of folic acid supplementation was gestational week 6.5 for AED-

Table 1 Overview of the data material

Characteristics	AED-exposed children of mothers with epilepsy		AED-unexposed children of mothers with epilepsy		Children of mothers without epilepsy	
	Periconceptional folic acid[a]		Periconceptional folic acid[a]		Periconceptional folic acid[a]	
	Yes (n = 260, 79%)	No (n = 68, 21%)	Yes (n = 289, 74%)	No (n = 100, 26%)	Yes (n = 77,929, 76%)	No (n = 25,222, 25%)
Plasma folate,[b] median (minimum, maximum), nmol/L	67.5 (11, 141)	67.6 (9, 117)	—	—	—	—
Gestational age[c] at birth, median (minimum, maximum), wk	40.0 (16, 43)	39.0 (25, 42)[k,p]	39.0 (25, 43)[n]	39.0 (34, 42)	40.0 (16, 47)	40.0 (16, 47)[r]
Apgar score at 5 min, median (minimum, maximum)	10.0 (0, 10)	9.5 (0, 10)	10.0 (0, 10)	9.5 (7, 10)	10.0 (0, 10)	10.0 (0, 10)
Maternal age, median (minimum, maximum), y	29.0 (18, 42)[j]	30.0 (18, 38)	29.0 (19, 41)[m]	29.0 (16, 39)	30.0 (14, 47)	30.0 (14, 53)[r]
Single mother, n (%)	10 (4)[j]	5 (7)	8 (3)	9 (9)[m,p]	1,421 (2)	1,002 (4)[r]
Maternal higher education,[d] n (%)	42 (16)[l]	4 (6)[p]	43 (15)[o]	9 (9)	19,553 (25)	3,349 (13)[r]
Low total household income,[e] n (%)	27 (11)[l]	8 (13)	18 (6)	17 (19)[n,r]	4,126 (5)	2,342 (10)[r]
Unplanned pregnancy, n (%)	53 (21)	23 (34)[p]	57 (20)	33 (34)[q]	12,974 (17)	6,760 (27)[r]
Alcohol use,[f] n (%)	6 (2)	6 (9)[j,p]	5 (2)	4 (4)	1867 (2)	804 (3)[r]
Smoking, n (%)	24 (9)[k]	11 (16)	12 (4)	20 (20)[r]	4,116 (5)	3,380 (13)[r]
Anxiety/depression,[g] n (%)	52 (21)[l]	11 (17)	39 (14)	17 (18)	7,803 (10)	3,080 (13)[r]
TC seizure(s),[h] n (%)	17 (15)	3 (13)	3 (3)	2 (5)	—	—
AED polytherapy, n (%)	45 (17)	20 (29)[p]	—	—	—	—
Plasma AED,[i] median (minimum, maximum), μmol/L	40.2 (0, 258)	33.0 (0, 159)	—	—	—	—
Valproate use, n (%)	42 (16)	14 (21)	—	—	—	—
Carbamazepine use, n (%)	68 (26)	22 (32)	—	—	—	—
Lamotrigine use, n (%)	108 (42)	27 (40)	—	—	—	—
Levetiracetam use, n (%)	30 (12)	5 (7)	—	—	—	—
Topiramate use, n (%)	16 (6)	3 (4)	—	—	—	—
Oxcarbazepine use, n (%)	17 (7)	7 (10)	—	—	—	—

Abbreviations: AED = antiepileptic drug; TC = tonic-clonic.
The number (n) may vary within the groups because of missing data. The χ^2 test or Fisher exact test was used for comparing categorical variables; Mann-Whitney *U* test was used for comparing continuous variables because of violation of the assumption of normal distribution. Clinical characteristics of parents and children in the 2 epilepsy groups (children exposed and not exposed to AEDs) and the control group. All groups are stratified by periconceptional folic acid supplementation.
[a] Use of folic acid supplementation 4 weeks before the start of the pregnancy and/or during the first trimester.
[b] Plasma folate concentration: sum of maternal 5-methyltetrahydrofolate and 4-alfa-hydroxy-5-methyl-tetrahydrofolate in plasma at gestational week 17 to 19.
[c] Calculated from the ultrasonographic measurements performed at 18 to 19 weeks of gestation. When ultrasound data were unavailable, gestational age was estimated on basis of the first day of the last menstrual period.
[d] Seventeen or more years of schooling.
[e] Less than 400,000 Norwegian kroner (equals approximately €42,000) annually.
[f] Alcohol consumption ≥1 time per month during pregnancy.
[g] Maternal anxiety/depression during pregnancy (mean score >1.75 on the Hopkins symptom checklist in gestational week 17 to 19).
[h] N = 140 for AED-exposed children; n = 161 for AED-unexposed children.
[i] Median of standardized concentration (see text) in maternal plasma at gestational week 17 to 19 and umbilical cord blood.
Children of mothers with epilepsy using AED in pregnancy compared to children of mothers without epilepsy stratified by folic acid use: [j]$p < 0.05$, [k]$p < 0.01$, and [l]$p < 0.001$.
Children of mothers with epilepsy not using AED in pregnancy compared to children of mothers without epilepsy stratified by folic acid use: [m]$p < 0.05$, [n]$p < 0.01$, and [o]$p < 0.001$.
Children of mothers with no periconceptional folic acid supplement use compared to children of mothers with periconceptional folic acid supplement use within each of the 3 groups (stratified by maternal epilepsy and AED exposure): [p]$p < 0.05$, [q]$p < 0.01$, and [r]$p < 0.001$.

Table 2 Children with language delay stratified by maternal periconceptional folic acid use

	AED-exposed children of mothers with epilepsy, n (%)		AED-unexposed children of mothers with epilepsy, n (%)		Children of mothers without epilepsy, n (%)	
	Periconceptional folic acid[a]		Periconceptional folic acid[a]		Periconceptional folic acid[a]	
Language delay	Yes	No	Yes	No	Yes	No
Global language delay 18 mo	30 of 178 (17)[b]	13 of 38 (34)[c,d]	20 of 194 (10)	5 of 66 (8)	5,982 of 56,947 (11)	1856 of 16,659 (11)[d]
Global language delay 36 mo	8 of 143 (6)	4 of 34 (12)	9 of 162 (6)	3 of 42 (7)	2,275 of 45,437 (5)	858 of 11,930 (7)[f]
Expressive language delay 36 mo	10 of 144 (7)	8 of 33 (24)[c,e]	7 of 161 (4)	1 of 42 (2)	1844 of 45,536 (4)	753 of 11,931 (6)[f]

Abbreviation: AED = antiepileptic drug.
The χ^2 test or Fisher exact test was used for comparing categorical variables. Language delay in relation to periconceptional folic acid supplementation in the 2 epilepsy groups and in children of mothers without epilepsy. The epilepsy groups were compared to children of mothers without epilepsy and stratified by periconceptional folic acid supplementation. Children of mothers with and without periconceptional folic acid supplementation were compared.
[a] Use of folic acid supplementation 4 weeks before the start of the pregnancy and/or during the first trimester.
Delayed language function in children of mothers with epilepsy compared to children of mothers without epilepsy stratified by folic acid use: [b]$p < 0.01$ and [c]$p < 0.001$.
Delayed language function in children of mothers with no periconceptional folic acid supplement use compared to children of mothers with periconceptional folic acid supplement use within each of the 3 groups (stratified by maternal epilepsy and AED exposure): [d]$p < 0.05$, [e]$p < 0.01$, and [f]$p < 0.001$.

exposed children with language delay at 18 months and week 4.3 for AED-exposed children with language delay at 36 months. Mothers of AED-exposed children without language delay most often started supplementation 3 weeks before conception ($p = 0.01$ for 18 months and $p = 0.05$ for 36 months) (figure 2). When we analyzed supplementation intake in different gestational weeks, the proportion using folic acid before the start of the pregnancy and during the first trimester was higher for AED-exposed children without language delay than in children with delay (figure 2).

The interaction between periconceptional folic acid use and AED exposure after adjustment for relevant covariates was significant for global language score at 18 months ($p = 0.04$) and both global and expressive language score at 36 months ($p < 0.001$ and $p = 0.01$, respectively) (data available from Dryad, figure 1, doi.org/10.5061/dryad.1237b6m). In AED-exposed children, the AR of no periconceptional folic acid intake was 0.51 for global language delay at 18 months and 0.52 at 36 months and 0.71 for expressive language delay at 36 months without adjustment for covariates. In children of mothers without epilepsy, the corresponding ARs were 0.06, 0.30, and 0.36. The ARs were similar after adjustment for relevant covariates (data available from Dryad, table 5).There was no significant relationship between language score and maternal plasma folate concentrations (data available from Dryad, table 6 and figure 2A) or folic acid dose (≥1 mg [n = 84] vs 0.4 mg [n = 55]) (data not shown) for AED-exposed children. Sensitivity analyses were done with the mothers using valproate or AED polytherapy excluded from the calculations. However, the effects of folic acid on AED-related language delay were similar or strengthened (data available from Dryad, table 7).

AED concentration and language delay

Higher maternal plasma valproate concentration was significantly correlated with a lower global language score at age 18 months ($r = -0.50$, $p = 0.04$) (data available from Dryad, figure 2, B and C, doi.org/10.5061/dryad.1237b6m). No other significant correlations between language score and maternal or umbilical cord AED concentrations were found (data available from Dryad, table 8 and figure 2, A and C).

Discussion

We found that in AED-exposed children maternal periconceptional folic acid supplementation was associated with better language outcome compared to children of mothers not using folic acid in the periconceptional period. The apparent protective effect of periconceptional folic acid supplementation was striking in the AED-exposed children compared to the AED-unexposed children of mothers with epilepsy and to children of mothers without epilepsy. For all language outcomes, the adjusted ORs for language delay were lower for AED-exposed children when folic acid supplementation was used compared to no supplementation. The interaction analysis between AED exposure and periconceptional folic acid use showed a synergistic effect on the degree of language delay: no folic acid supplementation had more consequences for language scores in AED-exposed children than in children with no AED exposure. The AR of no folic acid supplementation on language delay was >50% in AED-exposed children, whereas it was of modest importance in the control group.

Our results showing the importance of folic acid for language development are in line with 2 studies that found higher mean verbal index scores at 3 years and higher mean IQ at 6 years age in AED-exposed children of periconceptionally folic acid–supplemented mothers vs those without such supplementation.[9,10] We have recently found that periconceptional folic acid supplementation and plasma folate status in pregnancy also were associated with fewer autistic

Table 3 Crude and adjusted ORs (95% CIs) for language delay in children of mothers with epilepsy stratified by periconceptional folic acid use

	AED-exposed children of mothers with epilepsy		AED-unexposed children of mothers with epilepsy	
	Periconceptional folic acid[a]		Periconceptional folic acid[a]	
	Yes	No	Yes	No
Global language delay 18 mo				
Crude	1.7 (1.2–2.6)[b]	4.1 (2.1–8.1)[c]	1.0 (0.6–1.6)	0.7 (0.3–1.6)
Adjusted model 1	1.7 (1.1–2.5)[b]	4.2 (2.1–8.2)[c]	0.9 (0.6–1.5)	0.7 (0.3–1.7)
Adjusted model 2	1.7 (1.2–2.6)[b]	4.4 (2.2–8.7)[c]	1.0 (0.6–1.5)	0.7 (0.3–1.8)
Adjusted model 3	1.7 (1.2–2.6)[b]	3.9 (1.9–7.8)[c]	0.9 (0.6–1.5)	0.7 (0.3–1.7)
Global language delay 36 mo				
Crude	1.1 (0.6–2.3)	1.7 (0.6–4.9)	1.1 (0.6–2.2)	1.0 (0.3–3.2)
Adjusted model 1	1.1 (0.5–2.2)	1.8 (0.6–5.1)	1.1 (0.6–2.1)	1.0 (0.3–3.3)
Adjusted model 2	1.1 (0.5–2.2)	1.8 (0.6–5.2)	1.1 (0.6–2.2)	1.0 (0.3–3.3)
Adjusted model 3	1.1 (0.5–2.2)	1.6 (0.6–4.7)	1.1 (0.5–2.1)	1.1 (0.3–3.5)
Expressive language delay 36 mo				
Crude	1.8 (0.9–3.4)	4.8 (2.1–10.6)[c]	1.1 (0.5–2.3)	0.4 (0.1–2.6)
Adjusted model 1	1.7 (0.9–3.2)	5.0 (2.2–11.3)[c]	1.0 (0.5–2.2)	0.4 (0.0–2.6)
Adjusted model 2	1.7 (0.9–3.2)	5.1 (2.3–11.5)[c]	1.1 (0.5–2.3)	0.4 (0.0–2.6)
Adjusted model 3	1.7 (0.9–3.2)	4.7 (2.0–10.6)[c]	1.0 (0.5–2.2)	0.4 (0.1–2.8)

Abbreviation: AED = antiepileptic drug; CI = confidence interval; OR = odds ratio.
The full model containing all predictors (adjusted model 3) was statistically significant ($p < 0.0001$) for all language outcomes stratified by periconceptional folic acid supplementation. Covariates in the adjusted models: model 1: maternal age, parental socioeconomic status (single mother, low maternal education [≤9 years], low household income [<400,000 Norwegian kroner, equals approximately €42,000 per year]), parity (prior pregnancies >21 gestation weeks), smoking during pregnancy, alcohol use (consumption ≥1 time per month) during pregnancy, and maternal anxiety/depression symptoms (mean score >1.75 on the Hopkins symptom checklist in gestational week 17 to 19) during pregnancy; model 2: all covariates in model 1 plus maternal prepregnancy body mass index; and model 3: all covariates in models 1 and 2 plus Apgar score at 5 minutes after birth and gestational age (calculated from the ultrasonographic measurements performed at 18 to 19 weeks of gestation. When ultrasound data were unavailable, gestational age was estimated on basis of the first day of the last menstrual period). Crude and adjusted ORs [95% CIs]) for language delay in children of mothers with epilepsy compared to children of mothers without epilepsy stratified by periconceptional folic acid supplementation.
[a] Use of folic acid supplementation 4 weeks before the start of the pregnancy and/or during the first trimester.
Children of mothers with epilepsy compared to children of mothers without epilepsy stratified by folic acid use: [b]$p < 0.01$ and [c]$p < 0.001$.

traits in AED-exposed children from the same epilepsy cohort.[13] A modest effect of folic acid supplementation on risk of autism was also seen in children of mothers without epilepsy.[8] However, other studies did not find an association between folic acid supplementation and child IQ,[12] verbal comprehensive intelligence,[11] or general language function in AED-exposed children.[31,32] The discrepancy could be due to type of AED exposure and the timing or dose of folic acid supplementation. In addition, different folic acid food fortification practices between countries could blur the association between folic acid supplement and language outcome. Although there is some overlap between autism and language delay, language delay is multifactorial, complex, and much more common than autism.[33–35] We thus believe only a minor amount of the language delay found in our study might have been attributed to autistic traits.

We found that the critical period for maternal folic acid supplementation to prevent language delay in AED-exposed children was from 4 weeks before the start of the pregnancy and until the end of the first trimester. There was no significant association between language delay and folic acid supplementation later in pregnancy. Previous studies in the general population similarly highlight the periconceptional period for folic acid supplementation to prevent language delay.[3,6]

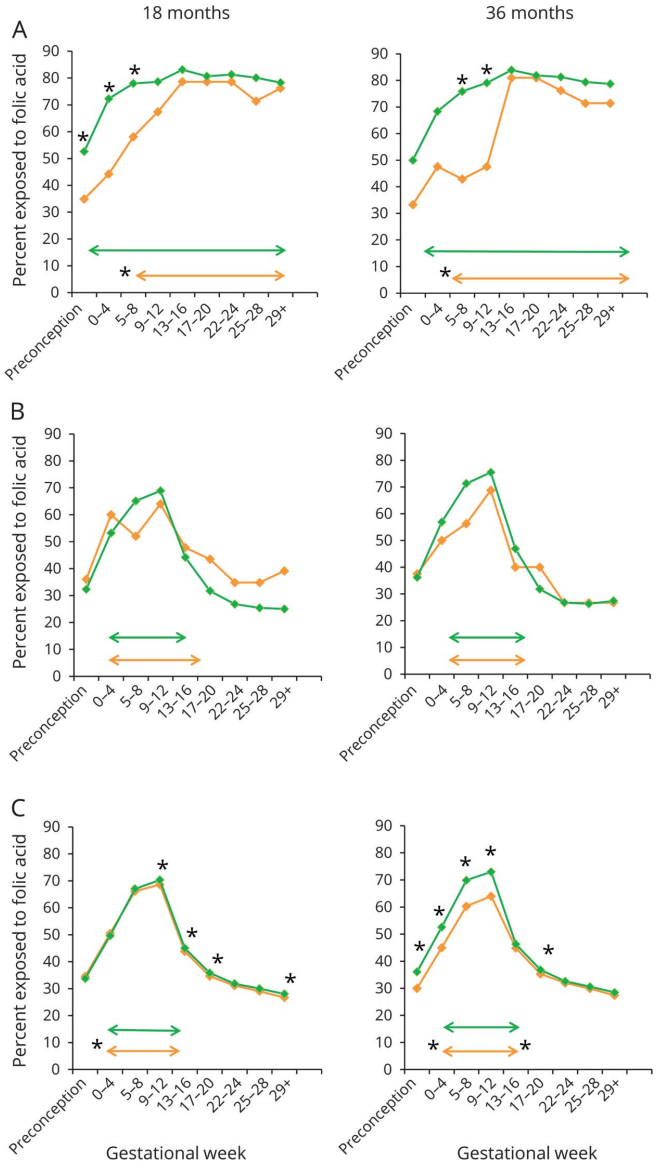
The larger proportion of language delay in lamotrigine-exposed children with no folic acid supplementation compared to those with supplementation has not been reported previously. However, mean IQ was higher in lamotrigine-exposed children who had been supplemented with folic acid compared to those who had not.[9] In rodents given lamotrigine, folic acid supplementation improved their epilepsy, mood, and memory.[36] Low serum folate concentrations have been reported after lamotrigine therapy.[37] Impaired neurodevelopment after lamotrigine exposure in utero has been discussed, but data have been conflicting.[38,39] A particularly

Table 4 Delayed language function with and without periconceptional folic acid supplementation for various AEDs

	AED-exposed children of mothers with epilepsy, n (%)	
	Periconceptional folic acid[a]	
	Yes	No
Valproate		
Global language delay 18 mo	9 of 30 (30)	2 of 6 (33)
Global language delay 36 mo	4 of 27 (15)	0 of 5 (0)
Expressive language delay 36 mo	4 of 27 (15)	1 of 4 (25)
Lamotrigine		
Global language delay 18 mo	13 of 77 (17)	7 of 16 (44)[b]
Global language delay 36 mo	4 of 58 (7)	2 of 16 (13)
Expressive language delay 36 mo	3 of 59 (5)	6 of 16 (38)[c]
Levetiracetam		
Global language delay 18 mo	3 of 21 (14)	0 of 2 (0)
Global language delay 36 mo	1 of 21 (5)	0 of 2 (0)
Expressive language delay 36 mo	2 of 21 (10)	0 of 1 (0)
Topiramate		
Global language delay 18 mo	2 of 9 (22)	0 of 2 (0)
Global language delay 36 mo	0 of 6 (0)	0 of 2 (0)
Expressive language delay 36 mo	1 of 5 (20)	0 of 1 (0)
Oxcarbazepine		
Global language delay 18 mo	5 of 12 (42)	1 of 5 (20)
Global language delay 36 mo	1 of 10 (10)	1 of 4 (25)
Expressive language delay 36 mo	1 of 10 (10)	1 of 4 (25)
Carbamazepine		
Global language delay 18 mo	4 of 43 (9)	3 of 12 (25)
Global language delay 36 mo	0 of 34 (0)	1 of 11 (9)
Expressive language delay 36 mo	1 of 34 (3)	1 of 11 (9)
AED monotherapy		
Global language delay 18 mo	22 of 149 (15)	9 of 28 (32)
Global language delay 36 mo	6 of 120 (5)	3 of 23 (13)
Expressive language delay 36 mo	8 of 121 (7)	6 of 23 (26)[b]
AED polytherapy		
Global language delay 18 mo	8 of 28 (29)	3 of 9 (33)
Global language delay 36 mo	2 of 22 (9)	1 of 10 (10)
Expressive language delay 36 mo	2 of 22 (9)	2 of 9 (22)

Abbreviation: AED = antiepileptic drug.
The χ^2 test or Fishes exact test was used for comparing categorical variables. The number of children exposed to each AED includes both monotherapy and polytherapy treatment.
[a] Use of folic acid supplementation 4 weeks before the start of the pregnancy and/or during the first trimester.
Delayed language function in children of mothers with no periconceptional folic acid supplement use compared to children of mothers with periconceptional folic acid supplement use stratified by maternal epilepsy (and specific AED exposure): [b]$p < 0.05$ and [c]$p < 0.01$.

Figure 2 Relationship between language delay and timing of maternal folic acid intake



Graphs illustrate the proportion of children (percent) exposed to maternal folic acid supplementation at different time intervals during pregnancy and the relationship to language delay (orange lines) and no language delay (green lines) at 18 and 36 months. Language delay at 36 months includes global language delay and expressive language delay. Arrows illustrate median start and median stop of maternal folic acid supplementation during pregnancy. Significant differences in folic acid supplementation (χ^2 test for independence) and median start/stop of folic acid supplementation (Mann-Whitney *U* test) are marked with asterisks. (A) Antiepileptic drug (AED)–exposed children of mothers with epilepsy at 18 months (n = 216) and 36 months (n = 179). (B) AED–unexposed children of mothers with epilepsy at 18 months (n = 260) and 36 months (n = 204). (C) Children of mothers without epilepsy at 18 months (n = 73,606) and 36 months (n = 57,715). Statistically significant differences were seen even with minor or no differences in percentages (*p* values between 0.02 and 0.05) or medians because of a high number of observations.

beneficial effect of periconceptional folic acid supplementation on language function in lamotrigine-exposed children is possible and could explain previous discordant results.

We did not find any correlation between folic acid doses or plasma folate concentrations and language delay. The maternal plasma samples were obtained during gestational week 17 to 19, which may not reflect accurately the folic acid supplement use reported before and very early in the pregnancy.[40] The exact dose of folic acid recommended to women with epilepsy who use AEDs has not been established.[2] The safety of high-dose folic acid supplement use in women with epilepsy and in the general population is still debated.[41,42] Folic acid dose recommendations cannot yet be specified for individual AEDs, although several AEDs interact with folic acid metabolism.[4,5,37]

We found a correlation between high maternal plasma valproate concentrations and low language score in children 18 months of age. This is in line with previous data showing a dose-dependent increased risk of language delay after valproate exposure in utero.[38,43,44] Maternal drug dose has been used as a proxy for child exposure, but valproate use in women of childbearing age has shown an extensive interindividual pharmacokinetic variability, with dose being a poor reflector of concentration.[45]

Strengths of our study are a large data collection including 2 different epilepsy groups. Both the maternal diagnosis of epilepsy and the type of AEDs have been validated. Maternal plasma folate and AED concentrations in umbilical cord and maternal blood were measured. Selection bias in the MoBa is moderate and does not affect exposure-outcome association analysis.[46] We adjusted for relevant confounders. Sensitivity analyses confirmed that the association of no use of folic acid with delayed language was not confounded by the frequency of polytherapy or valproate users. Our data have been obtained from parental reporting, and the interobserver reliability between parents and professional examiners for ASQ has been validated as high.[24] Parents are good evaluators of language abilities of their children.[47]

Weaknesses of our study include relatively low numbers of children exposed to specific AEDs and different doses of folic acid. This limits the interpretation of folate effects linked to individual AEDs and the effects of AED concentrations on language development; both are areas for future research. There were some loss to follow-up at 18 and 36 months of age. We do not have data on language development in the nonresponding group and do not know whether language delay in the child influenced the mother's motivation for continued participation. None of the children were assessed blindly because the language delay relied on maternal report only, not on a formal neuropsychologist review. Although the participants were included from 1999 to 2008 when less was known about the potential harmful effects of AEDs on language development, mothers who used AEDs during the pregnancy might have been more vigilant when reporting language skills than mothers with epilepsy not using AEDs. The mothers reported folic acid use before pregnancy during gestational week 17 to 19, and this may have an effect on the accuracy of these estimates. We do not have data on parental IQ or familial risk of language delay and therefore could not adjust for these factors in our analyses. Plasma folate concentrations were not measured at the most critical point for child development. The lack of mandatory folic acid fortification in Norway may have accentuated our results. Thus, our findings may not be generalizable to countries with a mandatory folic acid food fortification practice.

We found an apparent extensive protective effect of maternal folic acid supplementation from 4 weeks before the start of the pregnancy and during the first trimester on language delay at age 18 and 36 months in AED-exposed children of mothers with epilepsy. This effect was much stronger in AED-exposed children compared to children of mothers without epilepsy because no folic acid supplementation had more consequences for language scores in AED-exposed children compared to children not exposed to AEDs. From these findings, we advocate daily folic acid intake in all women on AEDs who are likely to become pregnant to decrease the risk of AED-mediated language delay.

Author contributions

Elisabeth Synnøve Nilsen Husebye: study design, analysis and interpretation of data, statistical analysis, writing manuscript. Nils Erik Gilhus: data acquisition, writing manuscript, critical revision of manuscript, study supervision, obtainment of funding. Bettina Riedel: interpretation of data, critical revision of manuscript. Olav Spigset: data acquisition, critical revision of manuscript. Anne Kjersti Daltveit: statistical advice and interpretation of data, critical revision of manuscript. Marte Helene Bjørk: study concept and design, data acquisition, analysis and interpretation of data, critical revision of manuscript, obtainment of funding.

Acknowledgment

The authors are grateful to all the participating families in Norway who take part in MoBa. They acknowledge Ragnhild Lervik, Hege Marit Nyberg, and Åshild Wæhre for excellent laboratory assistance and Gyri Veiby, MD, PhD, for contributing to data acquisition.

Study funding

The MoBa is supported by the Norwegian Ministry of Health and Care Services and the Ministry of Education and Research, NIH/National Institute of Environmental Health Sciences (contract N01-ES-75558), and NIH/National Institute for Neurological Disorders and Stroke (grant 1 UO1 NS 047537-01 and grant 2 UO1 NS 047537-06A1).

Disclosure

E. Husebye, N. Gilhus, B. Riedel, O. Spigset, and A. Daltveit report no disclosures relevant to the manuscript. M. Bjørk has received speaking and consultant honoraria from Novartis. Go to Neurology.org/N for full disclosures.

Received October 5, 2017. Accepted in final form May 24, 2018.

References

1. Gerard EE, Meador KJ. Managing epilepsy in women. Continuum 2016;22:204–226.
2. Harden CL. Pregnancy and epilepsy. Continuum 2014;20:60–79.
3. McGarel C, Pentieva K, Strain JJ, McNulty H. Emerging roles for folate and related B-vitamins in brain health across the lifecycle. Proc Nutr Soc 2015;74:46–55.
4. Linnebank M, Moskau S, Semmler A, et al. Antiepileptic drugs interact with folate and vitamin B12 serum levels. Ann Neurol 2011;69:352–359.
5. Belcastro V, Striano P, Gorgone G, et al. Hyperhomocysteinemia in epileptic patients on new antiepileptic drugs. Epilepsia 2010;51:274–279.
6. Roth C, Magnus P, Schjolberg S, et al. Folic acid supplements in pregnancy and severe language delay in children. JAMA 2011;306:1566–1573.
7. Gao Y, Sheng C, Xie RH, et al. New perspective on impact of folic acid supplementation during pregnancy on neurodevelopment/autism in the offspring children: a systematic review. PLoS One 2016;11:e0165626.
8. Suren P, Roth C, Bresnahan M, et al. Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. JAMA 2013;309:570–577.

9. Meador KJ, Baker GA, Browning N, et al. Fetal antiepileptic drug exposure and cognitive outcomes at age 6 years (NEAD study): a prospective observational study. Lancet Neurol 2013;12:244–252.
10. Meador KJ, Baker GA, Browning N, et al. Foetal antiepileptic drug exposure and verbal versus non-verbal abilities at three years of age. Brain 2011;134:396–404.
11. Kasradze S, Gogatishvili N, Lomidze G, et al. Cognitive functions in children exposed to antiepileptic drugs in utero: study in Georgia. Epilepsy Behav 2017;66:105–112.
12. Baker GA, Bromley RL, Briggs M, et al. IQ at 6 years after in utero exposure to antiepileptic drugs: a controlled cohort study. Neurology 2015;84:382–390.
13. Bjork M, Riedel B, Spigset O, et al. Association of folic acid supplementation during pregnancy with the risk of autistic traits in children exposed to antiepileptic drugs in utero. JAMA Neurol 2018;75:160–168.
14. Daltveit AK, Vollset SE, Lande B, Oien H. Changes in knowledge and attitudes of folate, and use of dietary supplements among women of reproductive age in Norway 1998-2000. Scand J Public Health 2004;32:264–271.
15. Magnus P, Birke C, Vejrup K, et al. Cohort profile update: the Norwegian Mother and Child Cohort Study (MoBa). Int J Epidemiol 2016;45:382–388.
16. Fisher RS, van Emde Boas W, Blume W, et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia 2005;46:470–472.
17. Bjork MH, Veiby G, Spigset O, Gilhus NE. Using the Norwegian Mother and Child Cohort Study to determine risk factors for delayed development and neuropsychiatric symptoms in the offspring of parents with epilepsy. Norwegian J Epidemiol 2014;24:79–89.
18. Veiby G, Daltveit AK, Schjolberg S, et al. Exposure to antiepileptic drugs in utero and child development: a prospective population-based study. Epilepsia 2013;54:1462–1472.
19. Veiby G, Engelsen BA, Gilhus NE. Early child development and exposure to antiepileptic drugs prenatally and through breastfeeding: a prospective cohort study on children of women with epilepsy. JAMA Neurol 2013;70:1367–1374.
20. Paltiel L, Haugan A, Skjerden T, et al. The biobank of the Norwegian Mother and Child Cohort Study: present status. Norwegian J Epidemiol 2014;24:29–35.
21. Hannisdal R, Ueland PM, Eussen SJ, Svardal A, Hustad S. Analytical recovery of folate degradation products formed in human serum and plasma at room temperature. J Nutr 2009;139:1415–1418.
22. Hannisdal R, Ueland PM, Svardal A. Liquid chromatography-tandem mass spectrometry analysis of folate and folate catabolites in human serum. Clin Chem 2009;55:1147–1154.
23. Bjork MH, Veiby G, Reiter SC, et al. Depression and anxiety in women with epilepsy during pregnancy and after delivery: a prospective population-based cohort study on frequency, risk factors, medication, and prognosis. Epilepsia 2015;56:28–39.
24. Squires J, Bricker D, Potter L. Revision of a parent-completed development screening tool: Ages and Stages Questionnaires. J Pediatr Psychol 1997;22:313–328.
25. Richter J, Janson H. A validation study of the Norwegian version of the Ages and Stages Questionnaires. Acta Paediatr 2007;96:748–752.
26. Zambrana IM, Pons F, Eadie P, Ystrom E. Trajectories of language delay from age 3 to 5: persistence, recovery and late onset. Int J Lang Commun Disord 2014;49:304–316.
27. Dale PS, Price TS, Bishop DV, Plomin R. Outcomes of early language delay: I. predicting persistent and transient language difficulties at 3 and 4 years. J Speech Lang Hear Res 2003;46:544–560.
28. Schjolberg S, Eadie P, Zachrisson HD, Oyen AS, Prior M. Predicting language development at age 18 months: data from the Norwegian Mother and Child Cohort Study. J Dev Behav Pediatr 2011;32:375–383.
29. Strand BH, Dalgard OS, Tambs K, Rognerud M. Measuring the mental health status of the Norwegian population: a comparison of the instruments SCL-25, SCL-10, SCL-5 and MHI-5 (SF-36). Nord J Psychiatry 2003;57:113–118.
30. LaMorte WWM. Measures of association, attributable proportion [online]. Boston University School of Public Health. Available at: sphweb.bumc.bu.edu/otlt/mph-modules/ep/ep713_association/EP713_Association6.html. Accessed December 14, 2017.
31. Thomas SV, Sukumaran S, Lukose N, George A, Sarma PS. Intellectual and language functions in children of mothers with epilepsy. Epilepsia 2007;48:2234–2240.
32. Thomas SV, Indrani L, Devi GC, et al. Pregnancy in women with epilepsy: preliminary results of Kerala Registry of Epilepsy and Pregnancy. Neurol India 2001;49:60–66.
33. Hawa VV, Spanoudis G. Toddlers with delayed expressive language: an overview of the characteristics, risk factors and language outcomes. Res Dev Disabil 2014;35:400–407.
34. O'Hare A, Bremner L. Management of developmental speech and language disorders: part 1. Arch Dis Child 2016;101:272–277.
35. Reilly S, McKean C, Morgan A, Wake M. Identifying and managing common childhood language and speech impairments. BMJ 2015;350:h2318.
36. Ali A, Pillai KK, Pal SN. Effects of folic acid and lamotrigine therapy in some rodent models of epilepsy and behaviour. J Pharm Pharmacol 2003;55:387–391.
37. Ni G, Qin J, Li H, et al. Effects of antiepileptic drug monotherapy on one-carbon metabolism and DNA methylation in patients with epilepsy. PLoS One 2015;10:e0125656.
38. Bromley RL, Baker GA. Fetal antiepileptic drug exposure and cognitive outcomes. Seizure 2017;44:225–231.
39. Velez-Ruiz NJ, Meador KJ. Neurodevelopmental effects of fetal antiepileptic drug exposure. Drug Saf 2015;38:271–278.
40. Roth C, Bjorke-Monsen AL, Reichborn-Kjennerud T, et al. Use of folic acid supplements in early pregnancy in relation to maternal plasma levels in week 18 of pregnancy. Mol Nutr Food Res 2013;57:653–660.
41. Asadi-Pooya AA. High dose folic acid supplementation in women with epilepsy: are we sure it is safe? Seizure 2015;27:51–53.
42. Choi JH, Yates Z, Veysey M, Heo YR, Lucock M. Contemporary issues surrounding folic acid fortification initiatives. Prev Nutr Food Sci 2014;19:247–260.
43. Shallcross R, Bromley RL, Cheyne CP, et al. In utero exposure to levetiracetam vs valproate: development and language at 3 years of age. Neurology 2014;82:213–221.
44. Nadebaum C, Anderson VA, Vajda F, Reutens DC, Barton S, Wood AG. Language skills of school-aged children prenatally exposed to antiepileptic drugs. Neurology 2011;76:719–726.
45. Johannessen Landmark C, Burns ML, Baftiu A, et al. Pharmacokinetic variability of valproate in women of childbearing age. Epilepsia 2017;58:e142–e146.
46. Nilsen RM, Vollset SE, Gjessing HK, et al. Self-selection and bias in a large prospective pregnancy cohort in Norway. Paediatr Perinat Epidemiol 2009;23:597–608.
47. Sachse S, Von Suchodoletz W. Early identification of language delay by direct language assessment or parent report? J Dev Behav Pediatr 2008;29:34–41.

Verbal abilities in children of mothers with epilepsy: Association to maternal folate status

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Neurology 2018;91;e811-e821 Published Online before print August 1, 2018
DOI 10.1212/WNL.0000000000006073

Supplemental data

e-Methods

Validation of the epilepsy cohort

The epilepsy cohort in MoBa has been validated by examining hospital records of women with epilepsy residing in Western Norway (n= 40) and by sending out an additional questionnaire to all women with epilepsy in MoBa, collecting retrospectively more detailed information on AED use, AED doses, seizures during pregnancy and folic acid doses (n= 300).[1] The prevalence of mothers with epilepsy was the same in MoBa and the population-based, compulsory Medical Birth Registry of Norway.[2] The women with epilepsy in MoBa are representative for women with epilepsy in general, as reported in our previous validation study.[1]

Folic acid supplement use

Over the counter folic acid tablets in Norway usually contain 0.4 mg folic acid, if part of a multivitamin they usually contain 0.2-0.4 mg. The use of folic acid and other vitamins in the MoBa cohort has been described and validated previously.[3-6] The frequency of intake of folic acid during the time intervals was reported in a second question in Q1 with the following answer options; daily, 4-6 times per week, 1-3 times per week. Mothers reporting folic acid use reported intake as frequently. For those children where the mothers reported folic acid use, a daily intake or an intake of 4-6 times per week was reported for 99% and 97% of children of mothers with epilepsy and children of mothers without epilepsy, respectively (data not shown).

Tonic-clonic (TC) seizures

Information on number of tonic-clonic (TC) seizures during pregnancy in women with epilepsy was retrospectively collected and available for 339 women in the validation study.[1] For AED exposed children, 12 children (4%) were exposed to one, 6 (2%) to two, one (0.3%)

to three and one (0.3%) to twenty TC seizures, respectively. For AED unexposed children, 4 children (1%) were exposed to one and 1 child (0.3%) to two TC seizures, respectively.

Adjusted attributable risk (AR)

We have chosen to present attributable risks based on observed relative risks. Adjusted estimates of attributable risks can be calculated based on adjusted odds ratios (OR), given by the formula AR=OR-1/OR[7]. The adjusted ORs were calculated in a logistic regression model (table e-5), adjusting for maternal age, parental socioeconomic status (single mother, low maternal education (≤ 9 years), low household income (≤ app. 42,000 EUR/year)), parity (prior pregnancies >21 gestation weeks), smoking and alcohol use during pregnancy, maternal depression and anxiety symptoms during pregnancy (mean score >1.75 on the Hopkins symptom check list in gestational week 17-19), maternal prepregnancy Body Mass Index, Apgar score 5 minutes after birth and gestational age (calculated from the ultrasonographic measurements performed at 18-19 weeks of gestation. When ultrasound data were unavailable, gestational age was estimated on basis of the first day of the last menstrual period).

Table e-1

3-item and 6-item version of Ages and Stages Questionnaires (ASQ) for children aged 18 months and 36 months respectively.

Alternatives: Yes (10 points), Sometimes (5 points), Not yet (0 points)
18 months
1. When you ask your child to, does he/she go into another room to find a familiar toy or object?
2. Does he/she say eight or more words in addition to “Mama” and “Dada”?
3. Without showing him/her first, does your child point to the correct picture when you say, “Where is the cat” or “Where is the dog”?
36 months
1. Without showing him/her first, does your child point to the correct picture when you say, “Where is the cat” or “Where is the dog”?
2. When you ask your child to point to his/her eyes, nose, hair, feet, ears, etc., does he/she point correctly to at least seven parts of the body?
3. Does your child use sentences that are made up of three of four words?
4. Without giving him/her help by pointing or using gestures, ask your child to “Put the shoe on the table” and “Put the book under the chair”. Does your child carry out both of these directions correctly?
5. When looking at a picture book, does your child tell you what is happening or what action is taking place in the picture (for example, “barking”, “running”, “eating”, or “crying”)? You may ask, “What is the dog (or boy) doing”?
6. Can your child tell you at least two things about an object he/she is familiar with?

Table e-2

1-item question on expressive language skills.

	Alternatives
About your child's language skills (Enter a cross for the option which best describes the way your child talks.)	1 = Not yet talking 2 = He/she is talking, but you can't understand him/her 3 = Talking in one-word utterances, such as "milk" or "down" 4 = Talking in 2- to 3-word phrases, such as "me got ball" or "give doll" 5= Talking in fairly complete sentences, such as "I got a doll" or "can I go outside?" 6 = Talking in long and complicated sentences, such as "when I went to the park, I went on the swings" or "I saw a man standing on the corner"

Table e-3

Overview of the data material. Clinical characteristics of parents and children in the two epilepsy groups (children exposed and not exposed to antiepileptic drugs (AEDs), respectively) and the control group. All groups are stratified for periconceptional folic acid supplementation.

	AED exposed children of mothers with epilepsy		**AED unexposed children of mothers with epilepsy**		**Children of mothers without epilepsy**	
Characteristics	**Periconceptional folic acid[a]**		**Periconceptional folic acid[a]**		**Periconceptional folic acid[a]**	
	YES n = 260 (79%)	NO n = 68 (21%)	YES n = 289 (74%)	NO n = 100 (26%)	YES n = 77,929 (76%)	NO n = 25,222 (25%)
Maternal plasma folate (nmol/L)[1]; median (min,max)	67.5 (11,141)	67.6 (9, 117)	-	-	-	-
Male offspring; n(%)	122 (48)	35 (52)	143 (50)	53 (54)	39,660 (51)	12,890 (52)
Gestational age at birth[2] (weeks); median (min,max)	40.0 (16,43)	39.0 (25,42)[**, ¤]	39.0 (25,43)[##]	39.0 (34,42)	40.0 (16,47)	40.0 (16,47)[¤¤¤]
Apgar score 5 minutes after birth; median (min,max)	10.0 (0,10)	9.5 (0,10)	10.0 (0,10)	9.5 (7,10)	10.0 (0,10)	10.0 (0,10)
Twin or triplet child; n(%)	6 (2)	3 (4)	20 (7)[##]	1 (1)[¤]	2835 (4)	808 (3)[¤¤]
Parity[3]; median (min,max)	1.0 (1,5)	2.0 (1, 4)	1.0 (1, 5)	2.0 (1, 5)[¤]	2.0 (1,5)	2.0 (1,5)[¤¤¤]

Maternal age (years); median (min,max)	29.0 (18,42)*	30.0 (18,38)	29.0 (19, 41)#	29.0 (16,39)	30.0 (14,47)	30.0 (14,53)¤¤¤
Maternal prepregnancy BMI; median (min,max)	23.9 (16,43)***	22.9 (17,38)	23.6 (17,43)##	23.6 (18,42)	23.0 (13,59)	23.4 (13,54)¤¤¤
Single mother; n(%)	10 (4)*	5 (7)	8 (3)	9 (9)#,¤	1421 (2)	1002 (4)¤¤¤
Paternal higher education[4]; n(%)	41 (16)**	6 (9)	47 (16)##	11 (11)	18,722 (24)	3398 (14)¤¤¤
Maternal higher education[4]; n(%)	42 (16)***	4 (6)¤	43 (15)###	9 (9)	19,553 (25)	3349 (13)¤¤¤
Maternal low education[5]; n(%)	8 (3)	4 (6)	12 (4)##	9 (9)	1352 (2)	1476 (6)¤¤¤
Low total household income[6]; n(%)	27 (11)***	8 (13)	18 (6)	17 (19)##, ¤¤¤	4126 (5)	2342 (10)¤¤¤
Unplanned pregnancy; n(%)	53 (21)	23 (34)¤	57 (20)	33 (34)¤¤	12,974 (17)	6760 (27)¤¤¤
Alcohol consumption[7]; n(%)	6 (2)	6 (9)*, ¤	5 (2)	4 (4)	1867 (2)	804 (3)¤¤¤
Smoking during pregnancy; n(%)	24 (9)**	11 (16)	12 (4)	20 (20)¤¤¤	4116 (5)	3380 (13)¤¤¤
Maternal anxiety/depression during pregnancy[8]; n(%)	52 (21)***	11 (17)	39 (14)	17 (18)	7803 (10)	3080 (13)¤¤¤
≥1 epileptic seizure during pregnancy; n(%)	35 (27)	6 (20)	8 (6)	6 (14)	-	-

TC seizure(s) during pregnancy[9]; n(%)	17 (15)	3 (13)	3 (3)	2 (5)	-	-
AED polytherapy during pregnancy; n(%)	45 (17)	20 (29)[¤]	-	-	-	-
Plasma AED (µmol/L)[10]; median (min,max)	40.2 (0, 258)	33.0 (0, 159)	-	-	-	-
Valproate use; n(%)	42 (16)	14 (21)	-	-	-	-
Carbamazepine use; n(%)	68 (26)	22 (32)	-	-	-	-
Lamotrigine use; n(%)	108 (42)	27 (40)	-	-	-	-
Levetiracetam use; n(%)	30 (12)	5 (7)	-	-	-	-
Topiramate use; n(%)	16 (6)	3 (4)	-	-	-	-
Oxcarbazepine use; n(%)	17 (7)	7 (10)	-	-	-	-

AED = antiepileptic drug. BMI = Body Mass Index. TC seizure(s) = Tonic-clonic seizure(s). N may vary within the groups due to missing data. Chi-square test or Fisher's exact test was used for comparing categorical variables, Mann-Whitney U test was used for comparing continuous variables due to violation of the assumption of normal distribution. [a] Use of folic acid supplementation four weeks before the start of the pregnancy and/or during the first trimester [1] Plasma folate concentration: sum of 5-methyltetrahydrofolate (MTHF) and 4-alfa-hydroxy-5-methyl-tetrahydrofolate (hmTHF) in plasma at gestational week 17-19 [2] Calculated from the ultrasonographic measurements performed at 18-19 weeks of gestation. When ultrasound data were unavailable, gestational age was estimated on basis of the first day of the last menstrual period. [3] Number of all pregnancies >21 gestation weeks [4] 17 or more years of schooling [5] 9 or less years of schooling [6]< 400 000 NOK (equals approximately 42,000 EUR) annually [7] Alcohol consumption ≥1 time per month during pregnancy [8] Mean score >1.75 on the Hopkins symptom check list in gestational week 17-19 [9] n=140 for AED exposed children, n=161 for AED unexposed children [10] Median of standardized concentration (see text) in maternal plasma at gestational week 17-19 and umbilical cord blood

[*]Children of mothers with epilepsy using AED in pregnancy compared to children of mothers without epilepsy, stratified for folic acid use, [*]p<0.05

[**] p<0.01 [***] p<0.001.

[#] Children of mothers with epilepsy not using AED in pregnancy compared to children of mothers without epilepsy, stratified for folic acid use,

[#] p<0.05 [##] p<0.01 [###] p<0.001.

[¤] Children of mothers with no periconceptional folic acid supplement use compared to children of mothers with periconceptional folic acid supplement use within each of the three groups (stratified for maternal epilepsy and AED exposure), [¤] p<0.05 [¤¤] p<0.01 [¤¤¤] p<0.001.

Table e-4

Overview of drug combinations of the 65 AED polytherapy exposed children.

Drug combination	n
Lamotrigine + valproate	7
Lamotrigine + levetiracetam	5
Lamotrigine + carbamazepine	8
Lamotrigine + oxcarbazepine	3
Lamotrigine + clonazepam	2
Lamotrigine + gabapentin	2
Lamotrigine + topiramate	1
Levetiracetam + carbamazepine	4
Levetiracetam + topiramate	1
Levetiracetam + oxcarbazepine	3
Valproate + carbamazepine	4
Valproate + oxcarbazepine	1
Valproate + clonazepam	3
Topiramate + oxcarbazepine	4
Topiramate + carbamazepine	2
Topiramate + clonazepam	1
Carbamazepine + gabapentin	1
Carbamazepine + phenytoin	1
Carbamazepine + clonazepam	1
Oxcarbazepine + clonazepam	1
Primidone + phenytoin	1
Vigabatrin + oxcarbazepine	1
Vigabatrin + valproate	1
Lamotrigine + levetiracetam + clonazepam	1
Lamotrigine + levetiracetam + valproate	1

Lamotrigine + clobazam + levetiracetam	1
Lamotrigine + ethosuximide + valproate	1
Lamotrigine + oxcarbazepine + levetiracetam	1
Levetiracetam + oxcarbazepine + pregabalin	1
Levetiracetam + valproate + topiramate	1

Table e-5

Adjusted attributable risk (AR) of no folic acid supplementation on language delay. The adjusted AR is calculated based on the adjusted odds ratio (OR) from the formula AR=OR-1/OR, as described in eMethods. The relative risk (RR) of language delay and the corresponding unadjusted and adjusted odds ratio (OR) of language delay when no folic acid supplementation are also presented.

AED exposed children of mothers with epilepsy				
	RR	OR	Adjusted OR	Adjusted AR
Global language delay 18 months	2.03	2.57	2.32	0.57
Global language delay 36 months	2.10	2.25	2.09	0.52
Expressive language delay 36 months	3.49	4.28	3.76	0.73

Children of mothers without epilepsy				
	RR	OR	Adjusted OR	Adjusted AR
Global language delay 18 months	1.06	1.07	1.01	0.01
Global language delay 36 months	1.44	1.47	1.31	0.23
Expressive language delay 36 months	1.56	1.60	1.37	0.27

Table e-6

Language delay in relation to plasma concentration quartiles of folate in AED exposed children of mothers with epilepsy. There were no significant differences in language delay across the different plasma folate quartiles.

Plasma folate	n	Median score global language delay 18 months[a]	p-value vs. 4th quartile[b]	Global language delay 18 months; n(%)[c]
1st quartile (low)	29	25.0	0.70	6 (21)
2nd quartile	40	25.0	0.93	5 (13)
3rd quartile	42	20.0	0.21	8 (19)
4th quartile (high)	40	25.0	-	8 (20)

Plasma folate: Concentration of 5-methyltetrahydrofolate (mTHF) and 4-alpha-hydroxy-5-methyl-tetrahydrofolate (hmTHF). 1st quartile: < 42.20 nmol/L. 2nd quartile: ≥ 42.20 nmol/L and < 67.45 nmol/L. 3rd quartile: ≥ 67.45 nmol/L and < 85.18 nmol/L. 4th quartile: ≥ 85.18 nmol/L.

[a] Maximum score 30 points equivalent to normal language/no global language delay
[b] Mann-Whitney U test
[c] The total amount (n and %) of children with global language delay in each plasma folate quartile

Plasma folate	n	Median score global language delay 36 months[a]	p-value vs. 4th quartile[b]	Global language delay 36 months; n(%)[c]
1st quartile (low)	22	55.0	0.32	1 (5)
2nd quartile	27	60.0	0.41	1 (4)
3rd quartile	40	55.0	0.26	3 (8)
4th quartile (high)	33	59.9	-	1 (3)

Plasma folate: Concentration of 5-methyltetrahydrofolate (mTHF) and 4-alpha-hydroxy-5-methyl-tetrahydrofolate (hmTHF). 1st quartile: < 42.20 nmol/L. 2nd quartile: ≥ 42.20 nmol/L and < 67.45 nmol/L. 3rd quartile: ≥ 67.45 nmol/L and < 85.18 nmol/L. 4th quartile: ≥ 85.18 nmol/L.

[a] Maximum score 60 points equivalent to normal language/no global language delay
[b] Mann-Whitney U test
[c] The total amount (n and %) of children with global language delay in each plasma folate quartile

Plasma folate	n	Median score expressive language delay 36 months[a]	p-value vs. 4th quartile[b]	Expressive language delay 36 months; n(%)[c]
1st quartile (low)	21	6.0	0.95	3 (14)
2nd quartile	27	6.0	0.65	2 (7)
3rd quartile	40	6.0	0.85	5 (13)
4th quartile (high)	34	6.0	-	1 (3)

Plasma folate: Concentration of 5-methyltetrahydrofolate (mTHF) and 4-alpha-hydroxy-5-methyl-tetrahydrofolate (hmTHF). 1st quartile: < 42.20 nmol/L. 2nd quartile: ≥ 42.20 nmol/L and < 67.45 nmol/L. 3rd quartile: ≥ 67.45 nmol/L and < 85.18 nmol/L. 4th quartile: ≥ 85.18 nmol/L.

[a] Maximum score 6 points equivalent to normal expressive language/no expressive language delay
[b] Mann-Whitney U test
[c] The total amount (n and %) of children with global language delay in each plasma folate quartile

Table e-7

Sensitivity analysis. Delayed language function with and without periconceptional folic acid supplementation in AED exposed children after exclusion of children exposed to valproate and AED polytherapy, and in children exposed to lamotrigine monotherapy only.

	AED exposed children of mothers with epilepsy n (%)	
	Periconceptional folic acid[a]	
	YES	NO
Monotherapy, valproate excluded[b]		
Global language delay 18 months	16 of 125 (13)	8 of 25 (32)¤
Global language delay 36 months	3 of 100 (3)	3 of 21 (14)
Expressive language delay 36 months	5 of 101 (5)	6 of 21 (29)¤¤
Monotherapy, lamotrigine only		
Global language delay 18 months	8 of 62 (13)	5 of 11 (46)¤
Global language delay 36 months	3 of 46 (7)	2 of 11 (18)
Expressive language delay 36 months	2 of 47 (4)	5 of 11 (46)¤¤

AED = antiepileptic drug. Chi-square test or Fisher's exact test was used for comparing categorical variables. [a] Use of folic acid supplementation four weeks before the start of the pregnancy and/or during the first trimester [b] The two children with unspecified AED drug regime are not included.

¤ Delayed language function in children of mothers with no periconceptional folic acid supplement use compared to children of mothers with periconceptional folic acid supplement use, stratified for maternal epilepsy, ¤ $p<0.05$ ¤¤ $p<0.01$ ¤¤¤ $p<0.001$.

Table e-8

Language delay in relation to AED plasma concentration quartiles in AED exposed children of mothers with epilepsy. There were no significant differences in language delay across the different quartiles of plasma AED concentration.

Plasma AED concentration	n	Median score global language delay 18 months[a]	p-value vs. 4th quartile[b]	Global language delay 18 months; n(%)[c]
1st quartile (low)	42	25.0	1.00	6 (14)
2nd quartile	39	25.0	0.90	5 (13)
3rd quartile	41	20.0	0.44	9 (22)
4th quartile (high)	41	25.0	-	5 (12)

AED concentration: Mean of normalized plasma concentrations for all types of antiepileptic drugs according to the formula 100 x (observed concentration – minimum contration) / concentration range. 1st quartile: < 17.04 µmol/l. 2nd quartile: 17.04 µmol/l ≥ and < 36.24 µmol/l. 3rd quartile: ≥ 36.24 µmol/l and < 81.22 µmol/l. 4th quartile: ≥ 81.22 µmol/l.

[a] Maximum score 30 points equivalent to normal language/no global language delay
[b] Mann-Whitney U test
[c] The total amount (n and %) of children with global language delay in each plasma AED concentration quartile

Plasma AED concentration	n	Median score global language delay 36 months[a]	p-value vs. 4th quartile[b]	Global language delay 36 months; n(%)[c]
1st quartile (low)	31	60.0	0.86	1 (3)
2nd quartile	31	60.0	0.34	2 (7)
3rd quartile	36	55.0	0.37	2 (6)
4th quartile (high)	32	59.0	-	1 (3)

AED concentration: Mean of normalized plasma concentrations for all types of antiepileptic drugs according to the formula 100 x (observed concentration – minimum contration) / concentration range. 1st quartile: < 17.04 µmol/l. 2nd quartile: 17.04 µmol/l ≥ and < 36.24 µmol/l. 3rd quartile: ≥ 36.24 µmol/l and < 81.22 µmol/l. 4th quartile: ≥ 81.22 µmol/l.

[a] Maximum score 60 points equivalent to normal language/no global language delay
[b] Mann-Whitney U test
[c] The total amount (n and %) of children with global language delay in each plasma AED concentration quartile

Plasma AED concentration	n	Median score expressive language delay 36 months[a]	p-value vs. 4th quartile[b]	Expressive language delay 36 months; n(%)[c]
1st quartile (low)	32	6.0	0.51	3 (9)
2nd quartile	31	6.0	0.79	3 (10)
3rd quartile	36	6.0	0.66	2 (6)
4th quartile (high)	32	6.0	-	1 (3)

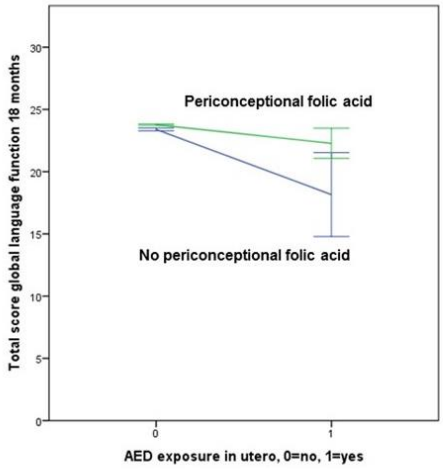
AED concentration: Mean of normalized plasma concentrations for all types of antiepileptic drugs according to the formula 100 x (observed concentration – minimum contration) / concentration range. 1st quartile: < 17.04 µmol/l. 2nd quartile: 17.04 µmol/l ≥ and < 36.24 µmol/l. 3rd quartile: ≥ 36.24 µmol/l and < 81.22 µmol/l. 4th quartile: ≥ 81.22 µmol/l.

[a] Maximum score 6 points equivalent to normal expressive language/no expressive language delay
[b] Mann-Whitney U test
[c] The total amount (n and %) of children with global language delay in each plasma AED concentration quartile

Figure e-1

The interaction between maternal periconceptional folic acid supplement use and in utero exposure to antiepileptic drugs (AEDs). The results are presented as interaction plots (left) and multivariable linear regression analysis results (right). AED exposed children are compared to children not exposed to AED.

Interaction plot with 95% CI

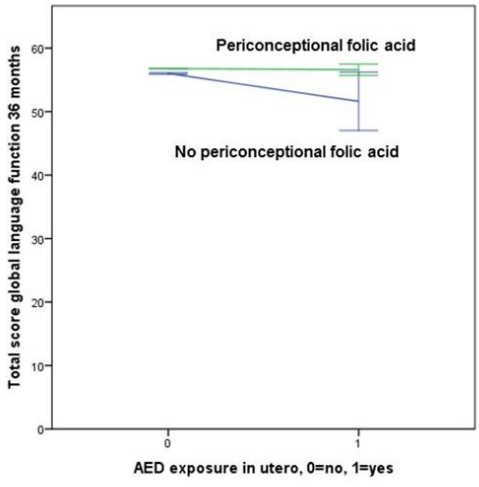


	B	Std. Error (B)	Beta	P value
Constant	23.41	0.06	-	<0.001
AED exposure in utero	-4.30	1.10	-0.03	<0.001
Periconceptional folic acid	0.37	0.07	0.02	<0.001
AED exposure in utero × periconceptional folic acid	2.87	1.25	0.02	0.022

AED = antiepileptic drug
Periconceptional folic acid = maternal use of folic acid supplementation four weeks before start of the pregnancy and/or during the first trimester
B= the regression coefficient
Std. Error = standard error
Beta = standardized regression coefficient

Adjusting for covariates (data not shown): We adjusted for maternal age, parental socioeconomic status (single mother, low maternal education (≤9 years), low household income (<400 000 NOK (equals approximately 42,000 EUR)/year), parity (prior pregnancies >21 weeks), smoking (any), alcohol use (any), maternal anxiety/depression symptoms (mean score >1.75 on the Hopkins symptom check list in gestional week 17-19), prepregnancy body mass index, gestational age, Apgar score 5 minutes after birth and exposure to AED polytherapy. With exception of gestational age and Apgar score 5 minutes after birth, all covariates had a negative effect on langauge score. With exception of exposure to AED polytherapy (p=0.08) and smoking (p=0.15), all effects were statistically significant (p<0.05). Adjusting for covariates did not change the significant interaction in our initial regression analysis above.

Interaction plot with 95% CI

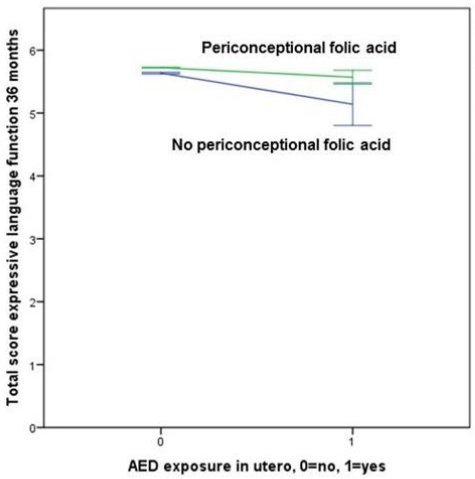


	B	Std. Error (B)	Beta	P value
Constant	56.07	0.05	-	<0.001
AED exposure in utero	-3.99	0.96	-0.04	<0.001
Periconceptional folic acid	0.73	0.06	0.05	<0.001
AED exposure in utero × periconceptional folic acid	3.82	1.09	0.03	<0.001

AED = antiepileptic drug
Periconceptional folic acid = maternal use of folic acid supplementation four weeks before start of the pregnancy and/or during the first trimester
B= the regression coefficient
Std. Error = standard error
Beta = standardized regression coefficient

Adjusting for covariates (data not shown): We adjusted for maternal age, parental socioeconomic status (single mother, low maternal education (≤9 years), low household income (<400 000 NOK (equals approximately 42,000 EUR)/year), parity (prior pregnancies >21 weeks), smoking (any), alcohol use (any), maternal anxiety/depression symptoms (mean score >1.75 on the Hopkins symptom check list in gestional week 17-19), prepregnancy body mass index, gestational age, Apgar score 5 minutes after birth and exposure to AED polytherapy. With exception of maternal age, alcohol use, gestational age and Apgar score 5 minutes after birth, all covariates had a negative effect on language score. With exception of exposure to AED polytherapy (p=0.56) and alcohol use (p=0.50), the effects were significant (p<0.05). Adjusting for covariates did not change the significant interaction in our initial regression analysis above.

Interaction plot with 95% CI



	B	Std. Error (B)	Beta	P value
Constant	5.64	0.01	-	<0.001
AED exposure in utero	-0.43	0.10	-0.04	<0.001
Periconceptional folic acid	0.08	0.01	0.06	<0.001
AED exposure in utero × periconceptional folic acid	0.28	0.11	0.02	0.013

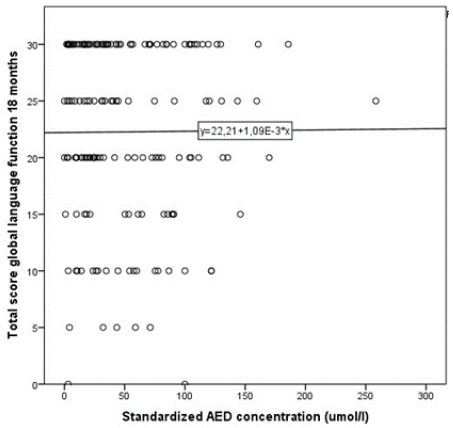
AED = antiepileptic drug
Periconceptional folic acid = maternal use of folic acid supplementation four weeks before the start of the pregnancy and/or during the first trimester
B= the regression coefficient
Std. Error = standard error
Beta = standardized regression coefficient

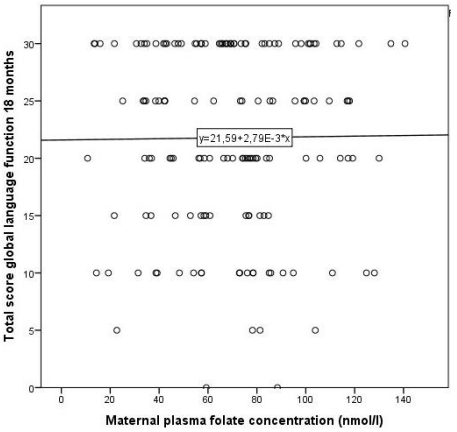
Adjusting for covariates (data not shown): We adjusted for maternal age, parental socioeconomic status (single mother, low maternal education (≤9 years), low household income (<400 000 NOK (equals approximately 42,000 EUR)/year), parity (prior pregnancies >21 weeks), smoking (any), alcohol use (any), maternal anxiety/depression symptoms (mean score >1.75 on the Hopkins symptom check list in gestional week 17-19), prepregnancy body mass index, gestational age, Apgar score 5 minutes after birth and exposure to AED polytherapy. With exception of gestional age and Apgar score 5 minutes after birth, all covariates had a negative effect on language score. With exception of exposure to AED polytherapy (p=0.67) and alcohol use (p=0.85), the effects were significant (p<0.05). Adjusting for covariates did not change the significant interaction in our initial regression analysis above.

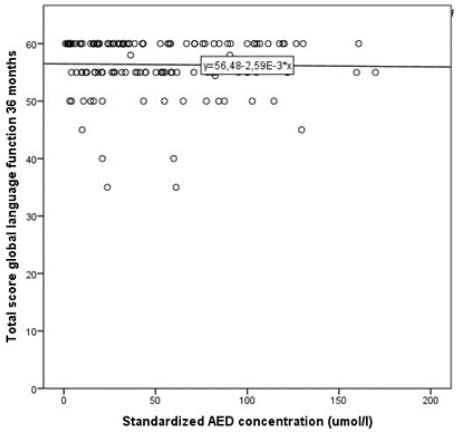
Figure e-2

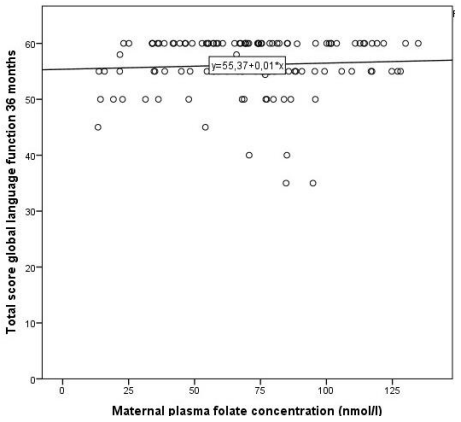
Scatter plots and correlation analysis.

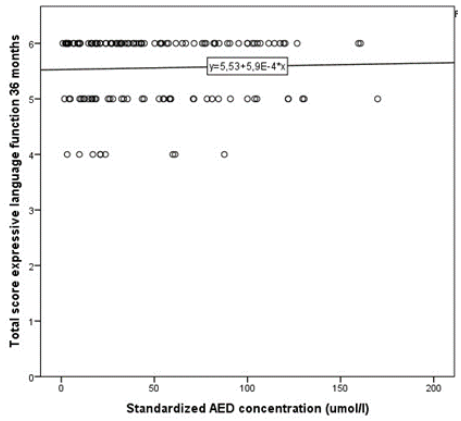
A. *Correlation between language delay and standardized antiepileptic drug (AED) concentration (maternal plasma and umbilical cord blood, µmol/l), and between language delay and maternal plasma folate concentration (nmol/l) in AED exposed children. None of the correlations were statistically significant. For calculation of the standardized AED concentration, see text.*

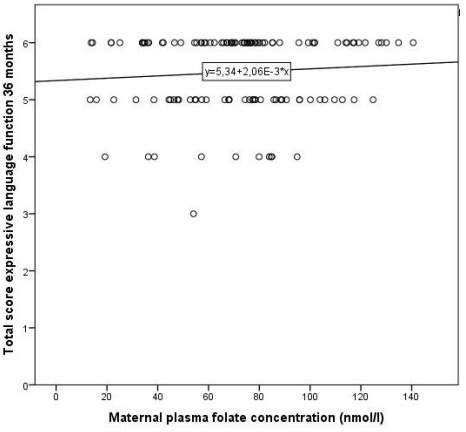




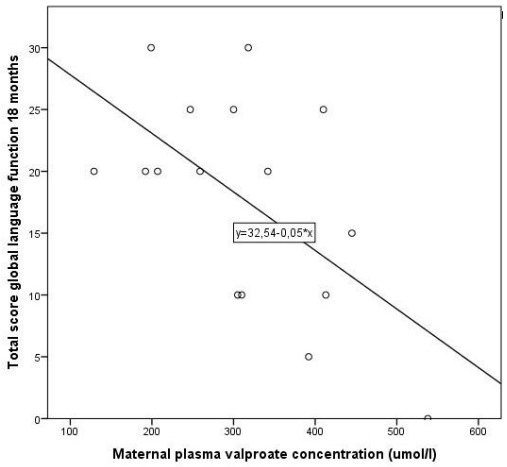


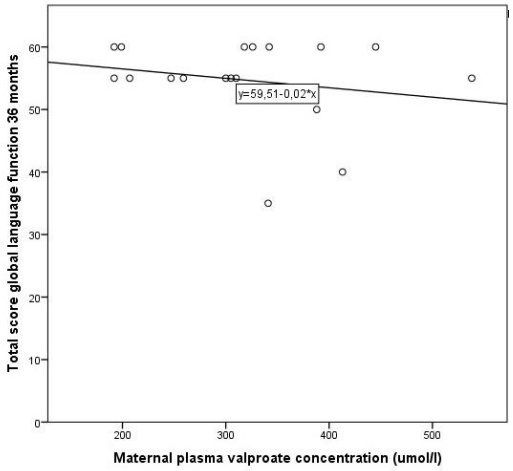


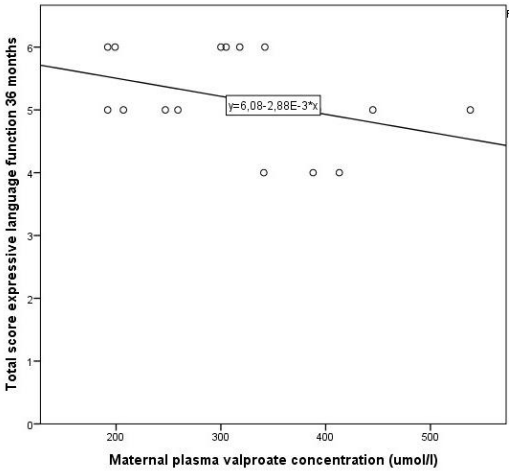




B. *Correlation between language delay and maternal plasma valproate concentration (µmol/l) in children exposed to valproate in utero.*







C. *Correlation between language delay and specific AED concentrations (µmol/l) in maternal plasma and umbilical cord. Significant results are highlighted.*

AED concentration (µmol/l)		**Total score global language function 18 months**	**Total score global language function 36 months**	**Total score expressive language function 36 months**
Carbamazepine (maternal)	n	46	35	35
	r value	0.28	0.26	0.21
	p value	0.06	0.14	0.22
Carbamazepine (umbilical cord)	n	42	33	33
	r value	0.28	0.27	0.14
	p value	0.07	0.13	0.43
Oxcarbazepine (maternal)[a]	n	4	3	3
	r value	0.74	-	-
	p value	0.26	-	-
Oxcarbazepine (umbilical cord)[a]	n	4	3	3
	r value	–0.32	-	-
	p value	0.68	-	-
Lamotrigine (maternal)	n	57	44	45
	r value	–0.13	–0.17	–0.11
	p value	0.33	0.27	0.50
Lamotrigine (umbilical cord)	n	54	42	43
	r value	–0.06	0.001	–0.02
	p value	0.65	1.00	0.91
	n	16	17	17

Levetiracetam (maternal)	r value	–0.33	–0.14	0.16
	p value	0.21	0.58	0.53
Levetiracetam (umbilical cord)	n	12	11	11
	r value	0.39	0.08	0.32
	p value	0.21	0.83	0.33
Valproate (maternal)	n	17	18	18
	r value	**–0.50**	–0.09	–0.43
	p value	**0.04**	0.71	0.08
Valproate (umbilical cord)	n	18	18	18
	r value	–0.05	–0.11	–0.31
	p value	0.84	0.66	0.21
Topiramate (maternal)	n	7	4	4
	r value	0.15	0.78	0.26
	p value	0.75	0.23	0.74
Topiramate (umbilical cord)	n	6	3	3
	r value	0.75	0.87	0.87
	p value	0.08	0.33	0.33

[a] Correlation analysis not possible for 36 months age

References

1. Bjork MH, Veiby G, Spigset O, Gilhus NE. Using the Norwegian Mother and Child Cohort Study to determine risk factors for delayed development and neuropsychiatric symptoms in the offspring of parents with epilepsy. The Norwegian Journal of Epidemiology 2014;24:79-89.
2. Nilsen RM, Vollset SE, Gjessing HK, et al. Self-selection and bias in a large prospective pregnancy cohort in Norway. Paediatr Perinat Epidemiol 2009;23:597-608.
3. Haugen M, Brantsaeter AL, Alexander J, Meltzer HM. Dietary supplements contribute substantially to the total nutrient intake in pregnant Norwegian women. Annals of nutrition & metabolism 2008;52:272-280.
4. Nilsen RM, Vollset SE, Gjessing HK, et al. Patterns and predictors of folic acid supplement use among pregnant women: the Norwegian Mother and Child Cohort Study. Am J Clin Nutr 2006;84:1134-1141.
5. Roth C, Bjorke-Monsen AL, Reichborn-Kjennerud T, et al. Use of folic acid supplements in early pregnancy in relation to maternal plasma levels in week 18 of pregnancy. Molecular nutrition & food research 2013;57:653-660.
6. Brantsaeter AL, Haugen M, Hagve TA, et al. Self-reported dietary supplement use is confirmed by biological markers in the Norwegian Mother and Child Cohort Study (MoBa). Annals of nutrition & metabolism 2007;51:146-154.
7. LaMorte WWM, PhD, MPH, Boston University School of Public Health. Measures of Association, Measuring Association in Case-Control Studies [online]. Available at: http://sphweb.bumc.bu.edu/otlt/mph-modules/ep/ep713_association/EP713_Association8.html. Accessed December 15, 2017.

II

ORIGINAL ARTICLE

Language impairment in children aged 5 and 8 years after antiepileptic drug exposure *in utero* – the Norwegian Mother and Child Cohort Study

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Keywords:
anticonvulsants, antiepileptic drugs, epilepsy, language impairment, MoBa, seizures

Received 12 July 2019
Accepted 21 November 2019

European Journal of Neurology 2020, **27:** 667–675

doi:10.1111/ene.14140

Background and purpose: The purpose was to examine the consequences of antiepileptic drug (AED) exposure during pregnancy on language abilities in children aged 5 and 8 years of mothers with epilepsy.

Methods: The study population included children of mothers with and without epilepsy enrolled in the Norwegian Mother and Child Cohort Study 1999–2008. Mothers prospectively provided information on epilepsy diagnosis, AED use during pregnancy and the child's language abilities at age 5 and 8 years, in questionnaires with validated language screening tools. AED concentrations in gestation week 17–19 and in the umbilical cord were measured.

Results: The study population included 346 AED-exposed and 388 AED-unexposed children of mothers with epilepsy, and 113 674 children of mothers without epilepsy. Mothers of 117 and 121 AED-exposed children responded to the questionnaires at age 5 and 8 years, respectively. For AED-exposed children, the adjusted odds ratio for language impairment was 1.6 [confidence interval (CI) 1.1–2.5, $P = 0.03$] at age 5 years and 2.0 (CI 1.4–3.0, $P < 0.001$) at age 8 years, compared to children of mothers without epilepsy. Children exposed to carbamazepine monotherapy had a significantly increased risk of language impairment compared to control children at age 8 years (adjusted odds ratio 3.8, CI 1.6–9.0, $P = 0.002$). Higher maternal valproate concentrations correlated with language impairment at age 5 years. Periconceptional folic acid supplement use protected against AED-associated language impairment.

Conclusion: Foetal AED exposure *in utero* is associated with an increased risk of language impairment in children aged 5 and 8 years of mothers with epilepsy. Periconceptional folic acid use had a protective effect on AED-associated language impairment.

Introduction

Foetal antiepileptic drug (AED) exposure *in utero* is associated with adverse neurodevelopmental effects in infancy and early childhood in children of mothers with epilepsy, particularly after valproate exposure [1–3]. Such effects include low intelligence quotient (IQ), poor language abilities and an increased risk of behavioural disorders such as autism spectrum disorder and attention deficit and hyperactivity disorder [1,2]. Evidence on the long-term effects of foetal AED exposure on language abilities is emerging, but data are limited, particularly for newer AEDs such as lamotrigine, levetiracetam and topiramate [1,3]. After AED exposure *in utero*, verbal abilities and verbal IQ can be reduced in children aged 5–9 years, particularly

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for valproate [4–8] and this reduction might be permanent [9]. Data regarding carbamazepine exposure and language performance are conflicting [1,2]. Few studies have examined the influence of AED concentrations during pregnancy on language abilities in older children. It has been shown previously that language impairment and risk of autistic traits at age 1.5 and 3 years depend on maternal plasma AED concentrations and folate status [10–12].

This study presents data from the epilepsy population in the Norwegian Mother and Child Cohort Study (MoBa) after 8 years of follow-up. The aim of the study was to examine the effect of foetal AED exposure and plasma AED concentrations during pregnancy on language abilities in children at age 5 and 8 years of mothers with epilepsy.

Material and methods

Study population

The study population consisted of children of mothers with and without epilepsy included in the MoBa. This is an ongoing, prospective, population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health and linked to the compulsory Medical Birth Registry of Norway (MBRN) [13]. The overall participation rate was 41%. The mothers answered questionnaires during week 17–19 (Q1) and week 30 (Q2) in the pregnancy, and after the child was born at age 6 months (Q3), 1.5 years (Q4), 3 years (Q5), 5 years (Q5Y) and 8 years (Q8Y) (Fig. 1). The questionnaires obtained information regarding maternal medical and social background, medication and vitamin use and maternal health during the pregnancy, and detailed information regarding child development, including language abilities. Blood samples were collected from the mothers in gestation week 17–19 and from the umbilical cord immediately after birth [13,14].

Our data were based on version X of the MoBa databank and included 734 children of 620 mothers with epilepsy and 113 674 children of 94 338 mothers without epilepsy. The epilepsy diagnosis [15] was based on self-reported information from the MoBa questionnaires as well as information from the MBRN registered by the family doctor or midwife. The epilepsy cohort in MoBa has been validated previously (Appendix S1: Methods S1), and the validity is very good [16].

Variables

Antiepileptic drug use and plasma AED concentrations
Information on type of AED use during the pregnancy was obtained from Q1 and Q2, and from MBRN data [13]. Plasma concentrations of valproate, lamotrigine, carbamazepine, oxcarbazepine monohydroxyderivative metabolite, levetiracetam and topiramate were analysed in 226 maternal blood samples (gestational week 17–19) and 198 umbilical cord samples for altogether 254 AED-exposed children (73%) [14,16]. The reported AED was detected in samples from 237 of the 254 children (93%) (Appendix S1: Methods S1).

Maternal folate status
Mothers in the MoBa reported on the use and frequency of intake of folic acid supplement during pregnancy in Q1 and Q2 (Appendix S1: Methods S1). Periconceptional folic acid supplement use was defined as use 4 weeks before pregnancy and/or during the first trimester. There are no compulsory folic acid food fortifications in Norway. Plasma folate concentrations were analysed in maternal blood samples from gestation week 17–19 (Appendix S1: Methods S1) [14].

Language impairment
The definition of language impairment was based on the following parent-reported screening instruments in the MoBa: the communication scale from the Ages and Stages Questionnaires (ASQ) [17,18] the Speech and Language Assessment Scale (SLAS) [19] and the Norwegian instrument Twenty Statements about Language-related Difficulties (Language 20) (Appendix S1: Methods S1 and Table S1) [20]. All three instruments were available in Q5Y, only the latter being available in Q8Y. Children at age 5 years with results outside the cut-off for at least one of the three parent-reported instruments in Q5Y were defined to have language impairment. Similarly, children at age 8 years with a result above cut-off for the Language 20 semantic subscale in Q8Y were classified with language impairment. Criteria for language impairment at age 1.5 and 3 years have been described elsewhere [12]. Children who fill criteria for language impairment by any of the screening instruments are recommended for referral for clinical assessment and diagnosis [18–20]

Covariates

Covariates from the MoBa questionnaires and MBRN based on clinical relevance were included [12,21] (Table 1, Appendix S1: Methods S1 and Fig. S1).

Statistical analysis

Missing data analyses were performed by comparing the clinical characteristics of children who responded and did not respond to Q5Y and Q8Y (Appendix S1: Methods S1, Tables S2 and S3). AED-exposed and

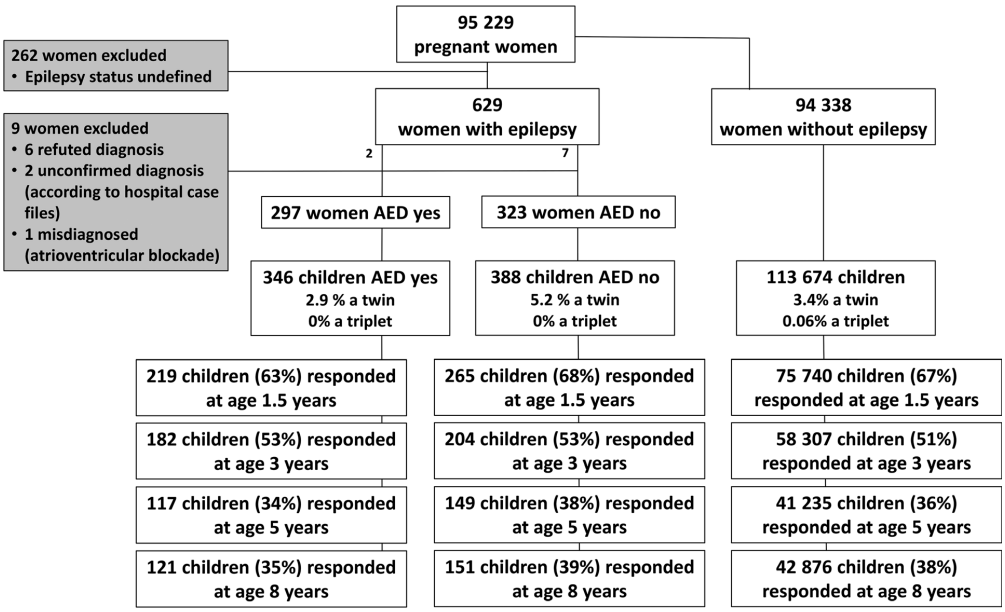

Figure 1 Flow chart of included and excluded cases. AED yes, AED-exposed children, AED no, AED-unexposed children.

AED-unexposed children of mothers with epilepsy were compared with the control group of all children of mothers without epilepsy (Appendix S1: Methods S1).

Ethics

This study was approved by the Regional Committee for Medical Research Ethics (reference number 2011/1616) (Appendix S1: Methods S1).

Results

Sample characteristics

The study population consisted of 346 AED-exposed and 388 AED-unexposed children of mothers with epilepsy, and a control group of 113 674 children of mothers without epilepsy (Table 1 and Fig. 1). Of the AED-exposed children, 280 children were exposed to AED monotherapy and 64 to AED polytherapy (Appendix S1: Table S4). For two children, the AED regime was unspecified. The most frequent AED exposures in monotherapy were lamotrigine (n = 112), carbamazepine (n = 72) and valproate (n = 40). Of the early pregnancy folic acid users, 97% of the total study population used it four times per week or more (Q1), and the same was true for 91% of the late users (Q2). Mothers of 117 and 121 of the AED-exposed children responded to Q5Y and Q8Y (Fig. 1). For AED-exposed children, no periconceptional folic acid supplementation, smoking during pregnancy, male offspring and previous language impairment at an earlier age were predictors of language impairment at age 5 or 8 years (Table 2). Of the AED-exposed children with language impairment at age 1.5 or 3 years, 41% (n = 22) continued to have such at age 5 or 8 years (Appendix S1: Fig. S2).

Antiepileptic drug use and language impairment

At age 5 years, 30% (n = 35) of AED-exposed children had language impairment compared to 22% (n = 9011) amongst children of mothers without epilepsy (P = 0.04). The adjusted odds ratio (aOR) for language impairment was 1.6 [confidence interval (CI) 1.1–2.5, P = 0.03) (Table 3). At age 8 years, 32% (n = 38) of AED-exposed children had language impairment compared to 19% (n = 8250) in the control group (P < 0.001); the aOR was 2.0 (CI 1.4–3.0, P < 0.001) (Table 3). There were no significant differences in language impairment between AED-unexposed children of mothers with epilepsy and the control group.

Children exposed to carbamazepine monotherapy had significantly higher risk of language impairment compared to control children at age 8 years (aOR 3.8,

Table 1 Characteristics of children of mothers with and without epilepsy

Characteristics	AED-exposed children of mothers with epilepsy n = 346	AED-unexposed children of mothers with epilepsy n = 388	Children of mothers without epilepsy n = 113 674
Male offspring; n (%)	168 (49)	195 (51)	57 958 (51)
Gestational age at birth[a] (weeks); median (range)	40.0 (27.0)	39.0 (18.0)	40.0 (32.0)
Apgar score 5 min after birth; median (range)	10 (10.0)	10 (10.0)	10 (10.0)
Twin or triplet child; n (%)	10 (3)	20 (5)	3918 (4)
Parity[b]; median (range)	1.0 (4.0)	1.0 (4.0)	1.0 (4.0)
Maternal age (years); median (range)	29.0 (24.0)	29.0 (25.0)	30.0 (39.0)
Maternal prepregnancy BMI (kg/m^2); median (range)	23.6 (26.4)	23.6 (25.9)	23.1 (47.0)
Periconceptional folic acid use[c]	259 (79)	289 (75)	77 895 (76)
Single mother; n (%)	15 (5)	17 (4)	2418 (2)
Paternal low education[d]; n (%)	20 (6)	23 (6)	4692 (4)
Maternal low education[d]; n (%)	12 (4)	21 (5)	2825 (3)
Low total household income[e]; n (%)	35 (11)	35 (10)	6464 (7)
Unplanned pregnancy; n (%)	76 (24)	90 (24)	19 725 (19)
Alcohol during pregnancy[f]; n (%)	12 (4)	9 (2)	2670 (3)
Smoking during pregnancy; n (%)	40 (12)	32 (8)	9525 (9)
Maternal anxiety/depression during pregnancy[g]; n (%)	62 (20)	56 (15)	10 821 (11)
≥1 epileptic seizure during pregnancy; n (%)	42 (26)	13 (7)	NA
TC seizure(s) during pregnancy; n (%)	20 (12)	5 (3)	NA
Maternal report of familial language impairment[h] at age 5 years	47 (41)	63 (42)	14 163 (35)
Maternal report of seldom/never helping their child read letters and sounds during a typical week at age 5 years	17 (15)	22 (15)	6675 (16)
Maternal report of never reading to their child at age 8 years	6 (5)	9 (6)	2959 (7)
Language impairment age 1.5 years[i]	43 (20)	24 (9)	7896 (11)
Language impairment age 3 years[j]	22 (12)	14 (7)	4182 (7)

AED, antiepileptic drug; BMI, body mass index; TC seizure(s), tonic–clonic seizure(s). N may vary within the groups due to missing data. The chi-squared test for independence or Fisher's exact test was used for categorical variables, the Mann–Whitney U test for continuous variables due to violation of the assumption of normal distribution. AED-exposed and AED-unexposed children are compared to children of mothers without epilepsy. [a]Calculated from the ultrasonographic measurements performed at 18–19 weeks of gestation. When ultrasound data were unavailable, gestational age was estimated on the basis of the first day of the last menstrual period. [b]Number of all pregnancies >21 gestation weeks including the current pregnancy. Maximum value is 5, representing a parity of 5 or more. [c]Maternal folic acid supplement use 4 weeks before pregnancy and/or during the first trimester. [d]9 or fewer years of schooling. [e] < 400 000 NOK (equals approximately 41 000 EUR) annually. [f]Alcohol consumption ≥1 time per month. [g]Mean score >1.75 on the Hopkins symptom check list in gestational week 17–19. [h]Maternal report of a biological relative (sibling, parent, grandparent, aunt, uncle or cousin) who was a late talker or had difficulties with either reading or writing or pronunciation. [i]Defined as children who score 1.5 standard deviations or more below the mean score of the three-item version of the communication scale from the Ages and Stages Questionnaires at age 1.5 years. [j]Defined as children who score 1.5 standard deviations or more below the mean score of the six-item version of the communication scale from the Ages and Stages Questionnaires at age 3 years and/or are talking in two- to three-word phrases or fewer (expressive language impairment) at age 3 years.

CI 1.6–9.0, P = 0.002, n = 23) but not at age 5 years (aOR 1.9, CI 0.6–5.6, P = 0.26, n = 17) (Table 3). Children exposed to valproate monotherapy *in utero* had the poorest mean language scores at both ages (Table 4).

Antiepileptic drug concentrations and language impairment

Higher maternal plasma valproate concentration during pregnancy correlated with a lower ASQ score (Spearman's rho −0.77, P = 0.02, n = 9) and a higher Language 20 score (Spearman's rho 0.82, P = 0.01, n = 9), both indicating language impairment at age 5 years (Fig. 2 and Appendix S1: Table S5). A significant correlation between higher maternal carbamazepine concentration and lower Language 20 score (Spearman's rho −0.47, P = 0.04, n = 19), indicating less language impairment, appeared at age 5 years, but the scatter plot contained outliers influencing the association (Appendix S1: Table S5).

Maternal folate status and language impairment

In the mothers not taking folic acid supplementation, 63% (n = 5) of AED-exposed children had language

Table 2 Clinical characteristics of AED-exposed and AED-unexposed children of mothers with epilepsy and children of mothers without epilepsy with language impairment at age 5 or 8 years

	AED-exposed children of mothers with epilepsy*n* total = 164		AED-unexposed children of mothers with epilepsy*n* total = 202		Children of mothers without epilepsy*n* total = 55 192	
Language impairment[a]	Yes*n* = 57 (35%)	OR (95% CI)	Yes*n* = 66 (33%)	OR (95% CI)	Yes*n* = 14 392 (26%)	OR (95% CI)
Male offspring; *n* (%[b])	39 (47)	**3.1 (1.6–6.1)***	34 (33)	1.0 (0.6–1.9)	8503 (30)	**1.6 (1.5–1.6)***
Gestational age at birth[c] (weeks); median (range)	40.0 (17.0)	1.0 (0.9–1.2)	39.0 (14.0)	0.9 (0.8–1.0)	40.0 (22.0)	**1.0 (1.0–1.0)***
Apgar score 5 min after birth; median (range)	9.0 (5.0)	1.0 (0.7–1.4)	9.0 (10.0)	0.9 (0.7–1.1)	10.0 (10.0)	**0.9 (0.9–1.0)***
Twin or triplet child; *n* (%)	2 (33)	0.9 (0.2–5.3)	3 (38)	1.2 (0.3–5.3)	485 (31)	**1.3 (1.1–1.4)***
Parity[d]; median (range)	2.0 (4.0)	1.3 (0.9–1.8)	1.0 (3.0)	1.0 (0.7–1.5)	2.0 (4.0)	**1.0 (0.9–1.0)***
Maternal age (years); median (range)	31.0 (21.0)	1.0 (0.9–1.1)	29.0 (20.0)	1.0 (0.9–1.0)	30.0 (32.0)	**1.0 (1.0–1.0)***
Maternal prepregnancy BMI (kg/m^2); median (range)	23.2 (23.7)	1.0 (0.9–1.1)	22.8 (24.0)	1.0 (0.9–1.1)	23.2 (38.2)	**1.0 (1.0–1.0)***
Periconceptional folic acid use[e]	42 (31)	**0.4 (0.2–0.9)***	54 (34)	1.2 (0.6–2.6)	11,538 (26)	1.0 (0.9–1.0)
Single mother; *n* (%)	2 (50)	1.9 (0.3–14.2)	1 (33)	1.0 (0.1–11.3)	298 (31)	**1.3 (1.1–1.5)***
Maternal low education[f]; *n* (%)	0 (0)	NA	4 (80)	8.7 (1.0–79.5)	323 (41)	**2.0 (1.7–2.3)***
Low total household income[g]; *n* (%)	5 (50)	2.2 (0.6–8.0)	5 (46)	1.8 (0.5–6.0)	851 (32)	**1.4 (1.3–1.5)***
Unplanned pregnancy; *n* (%)	12 (36)	1.1 (0.5–2.5)	11 (37)	1.2 (0.5–2.8)	2599 (28)	**1.1 (1.1–1.2)***
Alcohol during pregnancy[h]; *n* (%)	1 (50)	1.9 (0.1–31.4)	2 (67)	4.2 (0.4–47.4)	358 (27)	1.0 (0.9–1.2)
Smoking during pregnancy; *n* (%)	8 (62)	**3.3 (1.0–10.7)***	4 (40)	1.4 (0.4–5.1)	856 (31)	**1.3 (1.2–1.4)***
Maternal anxiety/depression during pregnancy[i]; *n* (%)	8 (36)	1.1 (0.4–2.7)	10 (40)	1.5 (0.6–3.5)	1737 (35)	**1.6 (1.5–1.7)***
≥1 epileptic seizure during pregnancy; *n* (%)	13 (41)	1.4 (0.6–3.2)	7 (70)	**5.8 (1.4–23.9)***	NA	NA
TC seizure(s) during pregnancy; *n* (%)	6 (35)	1.0 (0.3–3.0)	4 (100)	NA	NA	NA
AED polytherapy during pregnancy; *n* (%)	11 (33)	0.9 (0.4–2.1)	NA	NA	NA	NA
AED monotherapy during pregnancy; *n* (%)	45 (35)	1.0 (0.5–2.3)	NA	NA	NA	NA
Plasma AED (μmol/l)[j]; median (range)	53.2 (170)	1.0 (1.0–1.0)	NA	NA	NA	NA
Maternal report of familial language impairment[k] at age 5 years	17 (36)	1.4 (0.6–3.0)	24 (38)	1.4 (0.7–2.8)	4844 (34)	1.6 (1.6–1.7)
Maternal report of seldom/never reading to their child[l]	9 (39)	1.2 (0.5–3.1)	12 (43)	1.7 (0.7–3.7)	3354 (37)	**1.8 (1.8–1.9)***
Language impairment age 1.5 years[m]	17 (59)	**3.9 (1.7–9.2)***	12 (67)	**4.8 (1.7–13.6)***	2608 (51)	**3.4 (3.2–3.6)***
Language impairment age 3 years[n]	14 (82)	**12.0 (3.2–44.4)***	3 (33)	1.0 (0.2–4.0)	2080 (71)	**7.9 (7.3–8.6)***

AED, antiepileptic drug; BMI, body mass index; CI, confidence interval; OR, odds ratio; TC seizure(s), tonic–clonic seizure(s). *N* may vary slightly within the groups due to missing data. Significant results are marked with bold values. [a]Language impairment at age 5 years according to the Ages and Stages Questionnaires, the Speech and Language Assessment Scale or the Twenty Statements about Language-related Difficulties or at age 8 years according to the Twenty Statements about Language-related Difficulties semantic subscale. [b]Percentage was calculated from the number of children with that characteristic and language impairment out of all children with that characteristic in each of the three groups. [c]Calculated from the ultrasonographic measurements performed at 18–19 weeks of gestation. When ultrasound data were unavailable, gestational age was estimated on the basis of the first day of the last menstrual period. [d]Number of all pregnancies >21 gestation weeks including the current pregnancy. Maximum value is 5, representing a parity of 5 or more. [e]Folic acid supplement use 4 weeks before pregnancy and/or during the first trimester. [f]9 or fewer years of schooling. [g]<400 000 NOK (equals approximately 41 000 EUR) annually. [h]Alcohol consumption ≥1 time per month. [i]Mean score >1.75 on the Hopkins symptom check list in gestational week 17–19. [j]Median of standardized concentration (see text) in maternal plasma at gestational week 17–19 and umbilical cord blood. [k]Maternal report of a biological relative (sibling, parent, grandparent, aunt, uncle or cousin) who was a late talker or had difficulties with either reading or writing or pronunciation. [l]Maternal report of seldom/never helping their child read letters and sounds during a typical week at age 5 years or maternal report of never reading to their child age 8 years. [m]Defined as children who score 1.5 standard deviations or more below the mean score of the three-item version of the communication scale from the Ages and Stages Questionnaires at age 1.5 years. [n]Defined as children who score 1.5 standard deviations or more below the mean score of the six-item version of the communication scale from the Ages and Stages Questionnaires at age 3 years and/or are talking in two- to three-word phrases or fewer (expressive language impairment) at age 3 years. **P* value <0.05.

impairment at age 5 years compared to 23% ($n = 1422$) of children of mothers without epilepsy ($P = 0.02$) (Appendix S1: Table S6). Similarly, at age 8 years, 52% ($n = 11$) of AED-exposed children had language impairment compared to 22% ($n = 1712$) in the non-supplemented control group ($P = 0.002$) (Appendix S1: Table S6). The aORs for language impairment in AED-exposed children compared to control children with no folic acid use were 10.5 (CI 1.9–56.3, $P = 0.006$) at age 5 years and 3.8 (CI 1.6–9.1, $P = 0.003$) at age 8 years, respectively. When the mothers were using periconceptional folic acid

Table 3 Language impairment for specific AED therapies in monotherapy, AED polytherapy and any AED exposure

	n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
Language impairment age 5 years[a]			
Children of mothers without epilepsy	9011 of 41 194 (22)	NA	NA
Valproate	5 of 14 (36)	2.0 (0.7–5.9)	2.2 (0.7–7.0)
Carbamazepine	6 of 17 (35)	1.9 (0.7–5.3)	1.9 (0.6–5.6)
Lamotrigine	9 of 39 (23)	1.1 (0.5–2.3)	1.0 (0.5–2.3)
Levetiracetam	2 of 9 (22)	1.0 (0.2–4.9)	1.0 (0.2–5.3)
Topiramate	2 of 4 (50)	3.6 (0.5–25.4)	5.8 (0.5–64.0)
AED monotherapy	28 of 91 (31)	**1.6 (1.0–2.5)***	**1.7 (1.0–2.7)***
AED polytherapy	7 of 26 (27)	1.3 (0.6–3.1)	1.4 (0.6–3.4)
Any AED	35 of 117 (30)	**1.5 (1.0–2.3)***	**1.6 (1.1–2.5)***
Language impairment age 8 years[b]			
Children of mothers without epilepsy	8250 of 42 550 (19)	NA	NA
Valproate	5 of 16 (31)	1.9 (0.7–5.4)	2.2 (0.7–6.4)
Carbamazepine	10 of 23 (43)	**3.2 (1.4–7.3)****	**3.8 (1.6–9.0)****
Lamotrigine	9 of 41 (22)	1.2 (0.6–2.4)	1.2 (0.6–2.6)
Levetiracetam	1 of 6 (17)	0.8 (0.1–7.1)	0.7 (0.1–6.0)
Topiramate	1 of 4 (25)	1.4 (0.1–13.3)	1.1 (0.1–10.9)
AED monotherapy	30 of 97 (31)	**1.9 (1.2–2.9)****	**2.0 (1.3–3.0)****
AED polytherapy	7 of 21 (33)	2.1 (0.8–5.2)	2.4 (0.9–6.1)
Any AED	38 of 120 (32)	**1.9 (1.3–2.8)*****	**2.0 (1.4–3.0)*****

AED, antiepileptic drug; CI, confidence interval; OR, odds ratio. AED-exposed children were compared to children of mothers without epilepsy. Significant results are marked with bold values. [a]Language impairment at age 5 years according to the Ages and Stages Questionnaires, the Speech and Language Assessment Scale or the Twenty Statements about Language-related Difficulties. [b]Language impairment at age 8 years according to the semantic subscale of the Twenty Statements about Language-related Difficulties. Covariates in the model: maternal age, parental socioeconomic status (single mother, low maternal education (≤9 years), low household income [<400 000 NOK (equals approximately 41 000 EUR)/year]), parity (pregnancies >21 gestation weeks), maternal prepregnancy body mass index, maternal report of familial language delay [sibling, parent, grandparent, aunt, uncle or cousin who was a late talker or had difficulties with either reading or writing or pronunciation (only in 5 years model)], smoking during pregnancy, alcohol use during pregnancy (consumption ≥1 time per month) (only 8 years model), maternal anxiety/depression symptoms (mean score >1.75 on the Hopkins symptom check list in gestational week 17–19) during pregnancy, Apgar score 5 min after birth, gestational age (calculated from the ultrasonographic measurements performed at 18–19 weeks of gestation; when ultrasound data were unavailable, gestational age was estimated on the basis of the first day of the last menstrual period), maternal report of seldom/never helping their child read letters and sounds during a typical week at age 5 years (5 years model) or maternal report of never reading to their child at age 8 years (8 years model). *P value < 0.05. **P value < 0.01 ***P value < 0.001

supplement, the corresponding aORs for language impairment were 1.4 (CI 0.9–2.2, $P = 0.14$) at age 5 years and 1.7 (CI 1.1–2.6, $P = 0.02$) at age 8 years, respectively.

After adjustment for covariates, a significant interaction was found between periconceptional folic acid use and AED exposure for ASQ score at age 5 years ($P = 0.009$, standardized beta 0.03), but not for SLAS or Language 20 scores at age 5 or 8 years. In the linear regression model, maternal folate concentration correlated with Language 20 score at age 5 years in the unadjusted, but not in the adjusted, model.

Discussion

An increased risk of language impairment was found in AED-exposed children at age 5 and 8 years of mothers with epilepsy compared to children of mothers without epilepsy. Valproate and carbamazepine exposure affected language outcome the most. Periconceptional folic acid use had a protective effect on the risk of language impairment. There was no increased risk of language impairment in AED-unexposed children of mothers with epilepsy compared to children of mothers without epilepsy. A large number of other possible confounders was considered, and these did not have any consistent effect on language outcome. AED exposure, and especially if the mother did not take folic acid, was the single most important factor for language outcome.

The AED-associated risk of language impairment was particularly evident in the carbamazepine monotherapy group at age 8 years. The aOR for language impairment in this group was almost fourfold compared to children of mothers without epilepsy. Poor neurodevelopmental outcomes after AED exposure *in utero* have mostly been associated with valproate exposure [1,2]. For children exposed to carbamazepine and other AEDs such as lamotrigine, levetiracetam and topiramate, evidence regarding any effect on specific cognitive skills in older children is lacking [1,2]. Valproate and carbamazepine exposure in monotherapy have been associated with language impairment in several studies [4–9,11,22] but other

Table 4 Mean language scores in children of mothers with and without epilepsy

	ASQ score[a] 5 years Mean (SD, 95% CI)	Language 20 score[b] 5 years Mean (SD, 95% CI)	SLAS score[c] 5 years Mean (SD, 95% CI)	Language 20 score[b]8 years Mean (SD, 95% CI)
Children of mothers without epilepsy	66.0 (6.7, 65.9–66.1)	25.3 (8.3, 25.3–25.4)	3.5 (0.6, 3.5–3.6)	10.6 (3.8, 10.6–10.6)
AED-unexposed children	66.2 (5.2, 65.4–67.1)	26.1 (8.3, 24.8–27.5)	3.5 (0.5, 3.4–3.6)	**11.3 (4.1, 10.6–11.9)***
AED-exposed children	65.7 (7.9, 64.3–67.2)	**27.1 (9.0, 25.4–28.7)****	**3.4 (0.6, 3.3–3.5)***	**12.1 (5.2, 11.2–13.0)****
Valproate monotherapy	64.3 (6.2, 60.7–67.8)	29.4 (12.8, 22.1–36.8)	**3.1 (0.6, 2.8–3.5)***	**13.0 (6.4, 9.6–16.4)***
Carbamazepine monotherapy	65.3 (7.9, 61.2–69.3)	**28.6 (8.1, 24.4–32.8)***	3.3 (0.5, 3.1–3.6)	**12.9 (5.3, 10.7–15.2)****
Lamotrigine monotherapy	65.3 (11.4, 61.6–69.1)	24.7 (6.1, 22.7–26.7)	3.4 (0.7, 3.2–3.7)	10.9 (3.7, 9.7–12.1)
Levetiracetam monotherapy	68.0 (2.3, 66.2–69.7)	24.1 (7.0, 18.7–29.5)	3.7 (0.8, 3.1–4.4)	10.0 (2.5, 7.4–12.7)
Topiramate monotherapy	64.6 (6.7, 53.9–75.2)	27.8 (11.2, 10.1–45.6)	3.5 (0.4, 2.9–4.1)	11.0 (3.5, 5.5–16.5)
AED monotherapy	65.6 (8.8, 63.8–67.5)	27.0 (9.4, 25.0–29.0)	3.4 (0.6, 3.3–3.6)	**11.9 (4.8, 11.0–12.9)****
AED polytherapy	66.1 (3.5, 64.7–67.5)	**27.4 (7.8, 24.2–30.6)***	3.4 (0.5, 3.2–3.6)	13.0 (6.8, 9.9–16.1)

AED, antiepileptic drug; ASQ, Ages and Stages Questionnaires; CI, confidence interval; Language 20, Twenty Statements about Language-related Difficulties; SLAS, Speech and Language Assessment Scale. AED-exposed and AED-unexposed children were compared to children of mothers without epilepsy by using the Mann–Whitney *U* test due to violation of the assumption of normal distribution. Significant results are marked with bold values. [a]Ages and Stages Questionnaires score (0–70 points). A low score indicates language impairment. [b]Twenty Statements about Language-related Difficulties score (0–100 points total score, 0–40 points semantic subscale). A high score indicates language impairment. [c]Composite scale mean score (1–5 points) from the Speech and Language Assessment Scale. A score below 3 points indicates language impairment. **P* value < 0.05. ***P* value < 0.01.

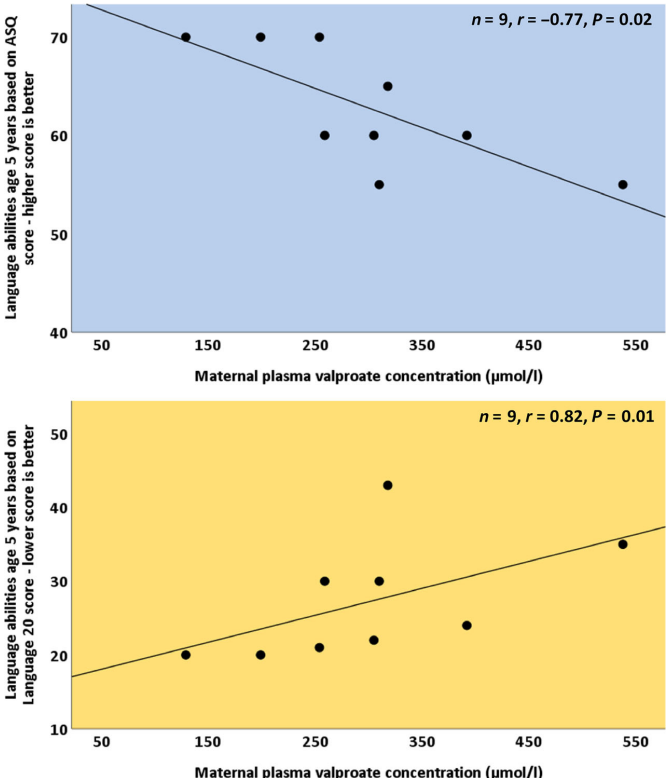

Figure 2 The correlation between language score and maternal plasma valproate concentration (µmol/l) during pregnancy at age 5 years. [Colour figure can be viewed at wileyonlinelibrary.com]

studies reported no such association for carbamazepine [8,9]. Two studies based on neuropsychological assessment reported reduced verbal abilities in children aged 3 and 6 years after foetal carbamazepine exposure [6,22]. Our results are based on validated screening instruments. Different study designs and different maternal folate status [23] could explain divergent results between studies. The lack of significantly increased aORs for language impairment after valproate exposure in monotherapy at age 5 and 8 years is probably due to small numbers of children and low concentrations of valproate [16]. Nevertheless, a correlation was found between maternal valproate concentrations during pregnancy and language impairment. This is consistent with our previous study of children aged 18 months in the MoBa [12] and with previous reports on dose-dependent valproate-mediated language impairment [4,5,8].

In this study, the previously reported AED-associated poorer language abilities in preschool years [11] have been shown to persist into school age in AED-exposed children of mothers with epilepsy. Our data shows that the language impairment at age 5 and 8 years may develop early or emerge later in the preschool years in children of mothers with epilepsy, as in children from the general population [24]. Our findings suggest a possible long-term AED-associated effect on language abilities in offspring after foetal AED exposure. Reduced total brain volume and grey matter volume compared to healthy children have been reported in valproate-exposed children aged 10 years with low IQ and language dysfunction [25]. Similarly, adults aged 23 years with antenatal exposure to mainly valproate, carbamazepine, phenytoin or primidone in monotherapy or polytherapy had reduced grey matter volume compared to healthy controls, and particularly in the left hemisphere controlling language [26]. These findings support the notion of permanently affected language abilities associated with foetal AED exposure. The protective effect of periconceptional folic acid supplementation on AED-associated language impairment was highly evident in our study. This protection, previously reported for age 1.5 and 3 years [12] remained equally evident at age 5 and 8 years. This strongly suggests a role of folate in the mechanism of AED-associated language impairment. Furthermore, these findings emphasize the importance of folic acid supplement use in the periconceptional period in all women with epilepsy who use AEDs [5,10,12,22]. Language impairment in children requires intervention, as it may have severe consequences for language skills in adulthood, academic achievements, mental health, behaviour and social life [21,27].

The strengths of our study include a validated, large dataset comprising epilepsy groups with and without AED exposure and a control group without epilepsy. Plasma AED and folate concentrations were analysed in the mothers during the pregnancy, as well as umbilical cord AED concentrations. Relevant covariates were adjusted for. Selection bias in the MoBa has been reported as moderate and with no or minimal effects on exposure–outcome association measurements [28]. The response rates for the two epilepsy groups and the control group were similar, and thus the exposure–outcome association measurements are unlikely to be biased.

Our missing data analyses indicated that children with normal language may be overrepresented. Children where mothers reported language impairment at age 1.5 and 3 years, no exposure to folic acid supplementation and with a lower socioeconomic status were more often missing from the study at age 5 and 8 years. Our findings may have been even more pronounced without any such selection bias. Mothers who used AEDs during pregnancy may be aware of the potential neurodevelopmental effects of AED exposure and might therefore have reported their children's language skills more vigilantly. If true, this would make the true results less pronounced. However, the association between foetal AED exposure, poor language abilities and maternal folate status was not well known during the inclusion period of the MoBa and is therefore unlikely to have influenced our results. There was no formal neuropsychological assessment of the children, but parents are considered good evaluators of the language abilities of their children [29]. Mothers who use AEDs during pregnancy are likely to have more severe epilepsy with a higher risk of epileptic seizures than untreated mothers with epilepsy. It cannot be excluded that such non-AED factors contribute to our observed language impairment. Information on maternal IQ was not available and this variable could not be controlled for, but education was controlled for in the analyses.

In this study, an association between language impairment and foetal AED exposure in children aged 5 and 8 years of mothers with epilepsy was found. Children of mothers with untreated epilepsy had no increased risk of language impairment. Periconceptional folic acid supplement use had a protective effect on AED-associated language impairment. Clinicians should be aware of the risk of poor language abilities in children exposed to AEDs *in utero*, particularly valproate and carbamazepine, with early intervention for signs of language impairment. The importance of folic acid supplement use in all AED-using women with epilepsy with a chance of becoming pregnant is emphasized.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Supplementaty data.

References

1. Bromley RL, Baker GA. Fetal antiepileptic drug exposure and cognitive outcomes. *Seizure* 2017; **44:** 225–231.
2. Kellogg M, Meador KJ. Neurodevelopmental effects of antiepileptic drugs. *Neurochem Res* 2017; **42:** 2065–2070.
3. Fujimura K, Mitsuhashi T, Takahashi T. Adverse effects of prenatal and early postnatal exposure to antiepileptic drugs: validation from clinical and basic researches. *Brain Dev* 2017; **39:** 635–643.
4. Nadebaum C, Anderson VA, Vajda F, Reutens DC, Barton S, Wood AG. Language skills of school-aged children prenatally exposed to antiepileptic drugs. *Neurology* 2011; **76:** 719–726.
5. Meador KJ, Baker GA, Browning N, *et al*. Fetal antiepileptic drug exposure and cognitive outcomes at age 6 years (NEAD study): a prospective observational study. *Lancet Neurol* 2013; **12:** 244–252.
6. Baker GA, Bromley RL, Briggs M, *et al*. IQ at 6 years after *in utero* exposure to antiepileptic drugs: a controlled cohort study. *Neurology* 2015; **84:** 382–390.
7. Bromley RL, Calderbank R, Cheyne CP, *et al*. Cognition in school-age children exposed to levetiracetam, topiramate, or sodium valproate. *Neurology* 2016; **87:** 1943–1953.
8. Gaily E, Kantola-Sorsa E, Hiilesmaa V, *et al*. Normal intelligence in children with prenatal exposure to carbamazepine. *Neurology* 2004; **62:** 28–32.
9. Vinten J, Adab N, Kini U, Gorry J, Gregg J, Baker GA. Neuropsychological effects of exposure to anticonvulsant medication *in utero*. *Neurology* 2005; **64:** 949–954.
10. Bjork M, Riedel B, Spigset O, *et al*. Association of folic acid supplementation during pregnancy with the risk of autistic traits in children exposed to antiepileptic drugs *in utero*. *JAMA Neurol* 2018; **75:** 160–168.
11. Veiby G, Daltveit AK, Schjolberg S, *et al*. Exposure to antiepileptic drugs *in utero* and child development: a prospective population-based study. *Epilepsia* 2013; **54:** 1462–1472.
12. Husebye ESN, Gilhus NE, Riedel B, Spigset O, Daltveit AK, Bjork MH. Verbal abilities in children of mothers with epilepsy: association to maternal folate status. *Neurology* 2018; **91:** e811–e821.
13. Magnus P, Birke C, Vejrup K, *et al*. Cohort profile update: the Norwegian Mother and Child Cohort Study (MoBa). *Int J Epidemiol* 2016; **45:** 382–388.
14. Paltiel L, Haugan A, Skjerden T, *et al*. The biobank of the Norwegian Mother and Child Cohort Study – present status. *Nor J Epidemiol* 2014; **24:** 29–35.
15. Fisher RS, van Emde Boas W, Blume W, *et al*. Epileptic seizures and epilepsy: definitions proposed by the International League against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* 2005; **46:** 470–472.
16. Bjork MH, Veiby G, Spigset O, Gilhus NE. Using the Norwegian Mother and Child Cohort Study to determine risk factors for delayed development and neuropsychiatric symptoms in the offspring of parents with epilepsy. *Nor J Epidemiol* 2014; **24:** 79–89.
17. Richter J, Janson H. A validation study of the Norwegian version of the Ages and Stages Questionnaires. *Acta Paediatr* 2007; **96:** 748–752.
18. Squires J, Bricker D, Potter L. Revision of a parent-completed development screening tool: Ages and Stages Questionnaires. *J Pediatr Psychol* 1997; **22:** 313–328.
19. Hadley PA, Rice ML. Parental judgments of preschoolers' speech and language development: a resource for assessment and IEP planning. *Semin Speech Lang* 1993; **14:** 278–288.
20. Ottem E.20 spørsmål om språkferdigheter – en analyse av sammenhengen mellom observasjonsdata og testdata. Skolepsykologi. 2009. 1.
21. Hawa VV, Spanoudis G. Toddlers with delayed expressive language: an overview of the characteristics, risk factors and language outcomes. *Res Dev Disabil* 2014; **35:** 400–407.
22. Meador KJ, Baker GA, Browning N, *et al*. Foetal antiepileptic drug exposure and verbal versus non-verbal abilities at three years of age. *Brain* 2011; **134:** 396–404.
23. Linnebank M, Moskau S, Semmler A, *et al*. Antiepileptic drugs interact with folate and vitamin B12 serum levels. *Ann Neurol* 2011; **69:** 352–359.
24. Reilly S, McKean C, Morgan A, Wake M. Identifying and managing common childhood language and speech impairments. *BMJ* 2015; **350:** h2318.
25. Sreedharan RM, Sheelakumari R, Anila KM, Kesavadas C, Thomas SV. Reduced brain volumes in children of women with epilepsy: a neuropsychological and voxel based morphometric analysis in pre-adolescent children. *J Neuroradiol* 2018; **45:** 380–385.
26. Ikonomidou C, Scheer I, Wilhelm T, *et al*. Brain morphology alterations in the basal ganglia and the hypothalamus following prenatal exposure to antiepileptic drugs. *Eur J Paediatr Neurol* 2007; **11:** 297–301.
27. O'Hare A, Bremner L. Management of developmental speech and language disorders: Part 1. *Arch Dis Child* 2016; **101:** 272–277.
28. Nilsen RM, Vollset SE, Gjessing HK, *et al*. Self-selection and bias in a large prospective pregnancy cohort in Norway. *Paediatr Perinat Epidemiol* 2009; **23:** 597–608.
29. Sachse S, Von Suchodoletz W. Early identification of language delay by direct language assessment or parent report? *J Dev Behav Pediatr* 2008; **29:** 34–41.

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Supplemental data

Contents

e-Methods - Supplement to material and methods

Acknowledgements

The Norwegian Mother and Child Cohort Study is supported by the Norwegian Ministry of Health and Care Services and the Ministry of Education and Research. We are grateful to all the participating families in Norway who take part in this on-going cohort study. Ragnhild Lervik, Hege Marit Nyberg and Åshild Wæhre are acknowledged for excellent laboratory assistance and Gyri Veiby for contributing in data acquisition.

Funding

We acknowledge the following sources of funding: Norwegian Epilepsy Foundation, Norwegian Chapter International League Against Epilepsy, The General Medical Research Fund, The Fund of Dr. Nils Henrichsen and Anna Henrichsen, and The Fund of Lawyer Rolf Sandberg Reberg and Ellen Marie Reberg. The sources of funding had no role in the design of the study nor the interpretation of the results.

Disclosure of Conflicts of Interest

Anne Kjersti Daltveit has received project funding from Pfeizer Inc. Marte Helene Bjørk has received project funding, speaking and consultant honoraria from Novartis. The remaining authors have no conflicts of interest.

Data accessibility statement

Researchers who want access to data sets for replication should submit an application to datatilgang@fhi.no. Access to data sets requires approval from the Regional committees for medical and health research ethics in Norway and a formal contract with MoBa. The consent

given by the participants does not open for storage of data on an individual level in repositories or journals.

Author contributions

Elisabeth Synnøve Nilsen Husebye, study design, analysis and interpretation of data, writing manuscript, funding.

Nils Erik Gilhus, data acquisition, critical revision of manuscript, study supervision, funding.

Olav Spigset, data acquisition, critical revision of manuscript.

Anne Kjersti Daltveit, statistical advice and interpretation of data, critical revision of manuscript.

Marte Helene Bjørk, study design, data acquisition, analysis and interpretation of data, critical revision of manuscript, funding.

Validation

We have previously validated the epilepsy cohort in MoBa by sending out questionnaires regarding type of epilepsy, seizures during pregnancy and AED use (n=300, response rate 50%) and by examination of hospital records of women with epilepsy in the Western part of Norway (n=40).[1] We have also analyzed plasma concentrations of AEDs in maternal blood and umbilical cord blood samples obtained from the MoBa biobank.[2] We found that the validity of the epilepsy diagnosis in MoBa was very good, as 98% of the women that reported an epilepsy diagnosis in MoBa confirmed this in the retrospective survey.[1] There was 100% agreement between self-reported AED use and AED use registered in the hospital records.[1] Women with epilepsy in MoBa seem to be representative of women with epilepsy in general in Norway, as the characteristics reported by mothers with epilepsy in MoBa were similar to

the Norwegian cohort included in the European and International Registry of Antiepileptic Drugs in Pregnancy (EURAP) study.[1]

Variables

AED use and plasma AED concentrations

For the statistical analysis, we calculated standardized AED concentrations (µmol/l) by normalizing the plasma concentration relative to the ranges observed within each group according to the formula *100 x (observed concentration – minimum concentration) / concentration range.*[3, 4] We calculated the mean standardized AED concentration for each child based on both the maternal and umbilical cord AED concentration. If only one of these was available, that one was used. For children exposed to AED polytherapy, we added the mean of each normalized AED concentration together.

Maternal folate status

Mothers in MoBa reported whether they used folic acid supplement or not more than 5 weeks before pregnancy, 4 weeks before pregnancy (preconception), and during gestational week 0-4, 5-8, 9-12, 13+ (Q1), and gestational week 13-16, 17-20, 21-24, 25-29, 29+ (Q2). They also reported their frequency of folic acid intake (daily, 4-6 times per week, 1-3 times per week) in both questionnaires. Plasma folate concentrations in maternal blood samples from gestation week 17-19 were measured by analyzing the biologically active 5-methyltetrahydrofolate (mTHF) and the degradation product 4-alfa-hydroxy-5-methyltetrahydrofolate (hmTHF) for altogether 227 AED-exposed children (66%).[3, 5] mTHF represents the prevailing plasma folate form. It is unstable in samples kept at room temperature, but largely recovered as hmTHF. Hence, we calculated plasma folate concentrations as the sum of hmTHF and mTHF.[6, 7]

Language impairment

ASQ has high concurrent validity and is effective as a diagnostic tool for identifying language developmental delay.[8, 9] For age 5 years, a 7-item version of the ASQ communication scale was included in Q5Y, 6 items from the 5-year ASQ version and 1 item from the 4-year ASQ version. Each item was scored 10 (“yes”), 5 (“sometimes”) or 0 (“not yet”), with a higher score reflecting no language delay. Maximum score at age 5 years was 70. We defined language impairment as a total ASQ score of 1.5 standard deviations (SD) or more below the mean for the total study population.[9] The SLAS is a 14-item valid screening tool assessing age-appropriate language skills used to identify children with language disorders.[10] A 13-item version of the 14-item instrument was included in the Q5Y. Each item was scored from 1 (“very much lower”) to 5 (“very much higher”), with a score of 3 (“typical for age”) or higher reflecting no language disorder. We added the score for each item and divided the sum by the total number of items to make a SLAS composite scale mean score, with a maximum of 5.[10] We defined language impairment as a SLAS composite scale mean score below 3.[10] The Language 20 is a validated Norwegian 20-item parental-reported screening instrument to identify children with language impairment.[11] The instrument consists of three subscales: semantic (8 items), receptive (6 items) and expressive (6 items). Each item was scored from 1 (“doesn’t fit the child, absolutely wrong”) to 5 (“fits well with the child, absolutely right”), with a lower score reflecting no language impairment. The full 20-item version of the instrument was included in Q5Y. In Q8Y, the semantic subscale only was included. Minimum and maximum scores on Language 20 at age 5 years were 20 (definite normal language) and 100 (definite language impairment). Corresponding scores at age 8 years were 8 (normal) and 40 (impaired). The validated cut-off for language impairment for both the 20-item and 8-item instrument was a total score of 31%

or more of the maximum possible score.[11] We defined children with such scores as having language impairment.

Covariates

We included the following covariates from Q1 and MBRN (figure e-1): maternal age, single mother, low maternal education (9 years or less of schooling), low total household income (<400,000 NOK annually - equals approximately 41,000 EUR), parity (number of previous pregnancies > 21 gestation weeks), maternal prepregnancy Body Mass Index (BMI), smoking (any) during pregnancy, alcohol consumption during pregnancy (≥1 time per month), maternal report of anxiety or depression symptoms in gestational week 17-19 (mean score >1.75 on the Hopkins symptom check list[12]), offspring sex, twin or triplet child, unplanned pregnancy, Apgar score 5 minutes after birth, and gestational age. Gestational age was calculated from the ultrasonographic measurements performed at 18-19 weeks of gestation. When ultrasound data were unavailable, gestational age was estimated on basis of the first day of the last menstrual period. Covariates included from Q5Y and Q8Y were: maternal report of familial language impairment (a sibling, parent, grandparent, aunt, uncle or cousin who was a late talker or had difficulties with reading, writing or pronunciation, Q5Y), maternal report of seldom/never helping their child read during a typical week at age 5 years (Q5Y), or never reading for their child at age 8 years (Q8Y). Other covariates were: epileptic seizures during pregnancy and tonic-clonic (TC) seizures during pregnancy.

Missing data

181 AED-exposed children and 185 AED-unexposed children of mothers with epilepsy, and 58,338 children of mothers without epilepsy did not complete either Q5Y or Q8Y. Many children completed only one of the questionnaires. For AED exposed children, 44 children

completed Q5Y, but not Q8Y. 48 children completed Q8Y, but not Q5Y. 73 children completed both Q5Y and Q8Y. For AED unexposed children, 52 children completed Q5Y, but not Q8Y and 54 children completed Q8Y, but not Q5Y. 97 children completed both questionnaires. In the control group, the corresponding numbers were 12,460 children who completed Q5Y, but not Q8Y and 14,101 children who completed Q8Y, but not Q5Y. 28,775 children of mothers without epilepsy completed both questionnaires.

AED-exposed children with missing data on Q5Y had significantly more often a mother who was younger, single, reported symptoms of anxiety and depression during the pregnancy, belonged to a low income household, reported fewer TC seizures, and an unplanned pregnancy compared to mothers of the children who responded to the questionnaire (table e-2). Children with missing data were also significantly more often exposed to alcohol or smoking during pregnancy, and less to periconceptional folic acid supplement use. The same differences were seen for AED-unexposed children and the control group, but not as pronounced (table e-2). In Q8Y, the differences between children with and without missing data in the three groups were less prominent. AED-exposed children with missing data had significantly younger mothers who reported more anxiety and depression symptoms during pregnancy, compared to children who responded to Q8Y (table e-3).

Statistical analysis

We used IBM SPSS Software version 24 to perform the statistical analyses. We compared categorical variables by using the Chi-square test for independence or Fisher's exact test when appropriate. Continuous variables were compared by using Mann-Whitney U test due to violation of the assumption of normal distribution. We applied the estimation-maximization procedure in SPSS to impute missing answers regarding language impairment.

Imputation was performed if ≤2 of 7 answers (ASQ Q5Y), ≤3 of 13 answers (SLAS Q5Y), ≤4 of 20 answers (Language 20 Q5Y) and ≤2 of 8 answers (Language 20 Q8Y), respectively, were missing. To assess the risk of language impairment after fetal AED exposure, we performed a logistic regression analysis with adjustment for the relevant covariates. We analyzed each covariate in the regression model separately initially, and only included variables with p-values <0.1 in the final model. We used the same procedure to calculate the risk of language impairment stratified for AED treatment group. We examined the effect of each covariate on language impairment at age 5 or 8 years by logistic regression for each of the three groups. Adjusted odds ratios (ORs) for language impairment for AED-exposed children compared to control children after stratification for periconceptional folic acid supplement use were calculated. We examined the effect of plasma AED concentrations and maternal plasma folate concentrations on language score by correlation analyses, in a linear regression model, and by including an interaction term (periconceptional folic acid supplement use x AED use) in a multivariable linear regression model. A p-value <0.05 was considered statistically significant.

Ethics

The establishment of MoBa and initial data collection was based on a license from the Norwegian Data Protection Agency and approval from The Regional Committee for Medical Research Ethics. The MoBa cohort is currently regulated by the Norwegian Health Registry Act. All parents in MoBa have given written consent to participate.

Table e-1: Screening instruments

Parental-reported screening instruments. The table includes the 7-item version of the Ages and Stages Questionnaires (ASQ) communication scale (6 items from the 5 year ASQ version and 1 item from the 4 year ASQ version), the 13-item version of the Speech and Language Assessment Scale (SLAS), and the Twenty Statements about Language-related Difficulties (Language 20) from the 5 years questionnaire. The semantic subscale (8 items) from the Language 20 instrument was in the 8 years questionnaire.

ASQ 5 years
The child's ability to understand and tell. Response options: "Yes", "Sometimes", "Not yet"
1. Can your child tell you at least two things about common object? For example, if you say to your child, "Tell me about the ball", does he say something like, "It is round. I throw it. It is big"?
2. Without giving your child help by pointing or repeating directions, does your child follow three directions that are *unrelated* to one another? Give all three directions before your child starts. For example, you may ask your child to "Clap your hands, walk to the door, and sit down" or "Give me the pen, open the book, and stand up."
3. Does your child use four- and five- word sentences? For example, does your child say, "I want the car"?
4. When talking about something that already happened, does your child use words that end in "ed" such as *walked, jumped* or *played*? Ask your child questions, such as "How did you get to the store?" ("We walked.") "What did you do at your friend's house?" ("We played.")
5. Does your child use comparison words, such as *heavier, stronger* or *shorter*? Ask your child questions, such as "A car is *big*, but a bus is " (bigger); "A cat is *heavy*, but a man is " (heavier); A TV is *small*, but a book is " (smaller).
6. Does your child answer the following questions: 1) "What do you do when you are hungry?" (Acceptable answers include: "Get food", "Eat", "Ask for something to eat", and "Have a snack".) 2) "What do you do when you are tired?" (Acceptable answers include: "Take a nap", "Rest", "Go to sleep", "Go to bed", "Lie down", and "Sit down.")
7. Does your child repeat the sentences shown below back to you, without any mistakes? You may repeat each sentence one time. Mark "yes" if your child repeats both sentences without mistakes or "sometimes" if your child repeats one sentence without mistakes. "Jane hides her shoes for Maria to find." "Al read the blue book under his bed."
SLAS 5 years
About the child's abilities and skills compared with peers. Enter a cross from 1-5 for each line according to how well the statement fits your child. Response options: 1-"very much lower", 2-2, 3-"typical for age", 4-4, 5-"very much higher"
1. My child's ability to ask questions properly is…
2. My child's ability to answer questions properly is…
3. My child's ability to say sentences clearly enough to be understood by strangers is…
4. The number of words my child knows is…
5. My child's ability to use his/her words correctly is…
6. My child's ability to get his/her message across to others when talking is…
7. My child's ability to use proper words when talking to others is…
8. My child's ability to get what he/she wants by talking is…
9. My child's ability to start a conversation going with other children is…
10. My child's ability to keep a conversation going with other children is…
11. The length of this child's sentences is…
12. My child's ability to make 'grown up' sentences is…
13. My child's ability to correctly say the sounds in individual words is…
Language 20 5 years (20 items) and 8 years (8 items from the semantic subscale)

How do these statements fit the child? Response options: 1-" doesn't fit the child, absolutely wrong", 2-2, 3-"both yes and no", 4-4, 5-" fits well with the child, absolutely right"
Semantic subscale
1. Forgets words s/he knows the meaning of
2. Confuses words with similar meaning (e.g. shirt, sweater, jacket)
3. Has difficulty understanding the meaning of common words
4. Has difficulty answering questions as quickly as other children
5. Is often searching for the right words
6. Uses incomplete sentences
7. Uses short sentences when s/he answers questions
8. Has difficulty retelling a story s/he has heard
Receptive subscale
9. It doesn't seem like what s/he is learning is remembered
10. Has difficulty remembering things
11. Has difficulty understanding what others are saying
12. Misconceive instructions and messages
13. Has problems remembering messages
14. Misunderstands context and what is going on
Expressive subscale
15. Is difficult to understand
16. Has difficulty expressing wishes and needs
17. Is not understood by others
18. Seldom initiates conversation with others
19.Has difficulties in pronunciation
20. Is not able to have a dialogue with peers
ASQ = the Ages and Stages Questionnaires. SLAS = the Speech and Language Assessment Scale. Language 20 = Twenty Statements about Language-related Difficulties.

Table e-2: Clinical characteristics of children with missing data at age 5 years

The table includes clinical characteristics of children with and without completed questionnaires at age 5 years (Q5Y). Antiepileptic drug (AED)-exposed and –unexposed children of mothers with epilepsy are compared with children of mothers without epilepsy stratified for completed Q5Y. Children who responded to the questionnaire are also compared with children with missing data within each of the three groups.

	AED-exposed children of mothers with epilepsy		**AED-unexposed children of mothers with epilepsy**		**Children of mothers without epilepsy**	
Completed 5 years questionnaire	***YES n=117***	***NO n=229***	***YES n=149***	***NO n=239***	***YES n=41,235***	***NO n=72,439***
Male offspring; n (%)	58 (50)	110 (49)	78 (53)	117 (50)	21,001 (51)	36,957 (52)
Gestational age at birth[a] (weeks); median (range)	40.0 (11.0)	39.0 (27.0)*	40.0 (15.0)	39.0 (18.0) ##,¤	40.0 (32.0)	40.0 (32.0)¤¤¤
Apgar score 5 minutes after birth; median (range)	9.0 (10.0)	10.0 (10.0)	10.0 (10.0)	9.0 (10.0)	10.0 (10.0)	10.0 (10.0)¤¤¤
Twin or triplet child; n (%)	4 (3)	6 (3)	8 (5)	12 (5)	1124 (3)	2794 (4)¤¤¤
Parity[b]; median (range)	2.0 (4.0)	1.0 (3.0)	1.0 (3.0) #	2.0 (4.0)¤	2.0 (4.0)	1.0 (4.0)¤¤¤
Maternal age (years); median (range)	30.0 (23.0)	28.0 (22.0)**,¤¤	29.0 (22.0) #	29.0 (24.0)	30.0 (32.0)	30.0 (39.0)¤¤¤
Maternal prepregnancy BMI; median (range)	23.3 (23.8)	23.8 (26.4)**	23.6 (24.9)	23.6 (25.9) #	23.0 (42.2)	23.1 (46.3)¤¤¤
Periconceptional folic acid use[c]	108 (93)*	151 (71)¤¤¤	122 (82)	167 (70)¤¤	34,723 (85)	43,172 (69)¤¤¤
Single mother; n (%)	1 (1)	14 (7)***,¤	3 (2)	14 (6)##	687 (2)	1731 (3)¤¤¤
Maternal low education[d]; n (%)	1 (1)	11 (5)	5 (3)#	16 (7)##	518 (1)	2307 (3)¤¤¤
Low total household income[e] n (%)	6 (5)	29 (15)***,¤	9 (6)	26 (12)#	1833 (5)	4631 (8)¤¤¤
Unplanned pregnancy; n (%)	19 (17)	57 (27)*,¤	25 (17)	65 (28)#,¤	6611 (16)	13,114 (21)¤¤¤
Alcohol during pregnancy[f]; n (%)	0 (0)	12 (6)*,¤	2 (1)	7 (3)	888 (2)	1782 (3)¤¤¤
Smoking during pregnancy; n (%)	7 (6)	33 (15)¤	7 (5)	25 (11)¤	1764 (4)	7761 (11)¤¤¤
Maternal anxiety/depression during pregnancy[g]; n (%)	15 (13)	47 (23)***,¤	22 (15) #	34 (15)	3652 (9)	7169 (12)¤¤¤
≥1 epileptic seizure during pregnancy; n (%)	26 (28)	16 (23)	7 (7)	6 (8)	NA	NA
TC seizure(s) during pregnancy; n (%)	16 (17)	4 (6)¤	3 (3)	2 (3)	NA	NA
AED polytherapy during pregnancy; n (%)	26 (22)	38 (17)	NA	NA	NA	NA
AED monotherapy during pregnancy; n (%)	91 (78)	189 (83)	NA	NA	NA	NA
Plasma AED (µmol/L)[h]; median (range)	30.3 (258)	44.6 (186)	NA	NA	NA	NA
Valproate use; n (%)	19 (16)	40 (18)	NA	NA	NA	NA
Carbamazepine use; n (%)	25 (21)	68 (30)	NA	NA	NA	NA
Lamotrigine use; n (%)	55 (47)	90 (39)	NA	NA	NA	NA
Levetiracetam use; n (%)	20 (17)	16 (7)¤¤	NA	NA	NA	NA
Topiramate use; n (%)	7 (6)	13 (6)	NA	NA	NA	NA

Oxcarbazepine use; n (%)	9 (8)	14 (6)	NA	NA	NA	NA
Language impairment age 1.5 years[i]	19 (18)*	24 (22)***	14 (11)	10 (8)	3916 (11)	3980 (11)
Language impairment age 3 years[j]	8 (9)	14 (16)**	6 (5)	8 (10)	2166 (6)	2016 (8)[¤¤¤]

AED = antiepileptic drug. BMI = Body Mass Index. TC seizure(s) = Tonic-clonic seizure(s). ASQ = the Ages and Stages Questionnaires. N may vary slightly within the groups due to missing data. Chi-square test for independence or Fisher's exact test was used for categorical variables, Mann-Whitney U test for continuous variables due to violation of the assumption of normal distribution

* AED-exposed children of mothers with epilepsy compared to children of mothers without epilepsy stratified for completed questionnaire, * p<0.05 ** p<0.01 *** p<0.001.
[#] AED-unexposed children of mothers with epilepsy compared to children of mothers without epilepsy stratified for completed questionnaire, [#] p<0.05 [##] p<0.01 [###] p<0.001
[¤] Children who responded to the questionnaires compared to children who did not respond in each of the three groups, [¤] p<0.05 [¤¤] p<0.01 [¤¤¤] p<0.001

[a] Calculated from the ultrasonographic measurements performed at 18-19 weeks of gestation. When ultrasound data were unavailable, gestational age was estimated on basis of the first day of the last menstrual period [b] Number of all pregnancies >21 gestation weeks including the current pregnancy. Maximum value is 5, representing parity of 5 or more [c] Folic acid supplement use four weeks before pregnancy and/or during the first trimester [d] 9 or less years of schooling [e] <400 000 NOK (equals approximately 41,000 EUR) annually [f] Alcohol consumption ≥1 time per month [g] Mean score >1.75 on the Hopkins symptom check list in gestational week 17-19 [h] Median of standardized concentration (see text) in maternal plasma at gestational week 17-19 and umbilical cord blood [i] Defined as children who score 1.5 standard deviations or more below the mean score of the 3-item version of the communication scale from the Ages and Stages Questionnaires (ASQ) at age 1.5 years [j] Defined as children who score 1.5 standard deviations or more below the mean score of the 6-item version of the communication scale from the Ages and Stages Questionnaires (ASQ) at age 3 years and/or are talking in two- to three-word phrases or less (expressive language impairment) at age 3 years

Table e-3: Clinical characteristics of children with missing data at age 8 years

The table includes clinical characteristics of children with and without completed questionnaires at age 8 years (Q8Y). Antiepileptic drug (AED)-exposed and –unexposed children of mothers with epilepsy are compared with children of mothers without epilepsy stratified for completed Q8Y. Children who responded to the questionnaire are also compared with children with missing data within each of the three groups.

	AED-exposed children of mothers with epilepsy		**AED-unexposed children of mothers with epilepsy**		**Children of mothers without epilepsy**	
Completed 8 years questionnaire	***YES n=121***	***NO n=225***	***YES n=151***	***NO n=237***	***YES n=42,876***	***NO n=70,798***
Male offspring; n (%)	64 (53)	104 (47)	77 (51)	118 (51)	21,838 (51)	36,120 (52)
Gestational age at birth[a] (weeks); median (range)	40.0 (17.0)	39.0 (27.0)**	40.0 (14.0)	39.0 (18.0)$^{\#,¤}$	40.0 (32.0)	40.0 (32.0)$^{¤¤¤}$
Apgar score 5 minutes after birth; median (range)	10.0 (10.0)	10.0 (10.0)	10.0 (8.0)	9.0 (10.0)	10.0 (10.0)	10.0 (10.0)$^{¤¤¤}$
Twin or triplet child; n (%)	2 (2)	8 (4)	4 (3)	16 (7)$^{\#}$	1201 (3)	2717 (4)$^{¤¤¤}$
Parity[b]; median (range)	2.0 (4.0)	1.0 (4.0)	1.0 (4.0)	1.0 (4.0)	2.0 (4.0)	1.0 (4.0)$^{¤¤¤}$
Maternal age (years); median (range)	30.0 (22.0)	28.0 (22.0)**,$^{¤¤}$	30.0 (22.0)	29.0 (25.0)$^{\#}$	30.0 (33.0)	30.0 (38.0)$^{¤¤¤}$
Maternal prepregnancy BMI; median (range)	23.3 (21.9)	24.0 (26.4)**	23.7 (24.9)$^{\#}$	23.5 (25.9)$^{\#}$	23.0 (41.5)	23.2 (47.0)$^{¤¤¤}$
Periconceptional folic acid use[c]	100 (83)	159 (77)	117 (78)	172 (73)	34,448 (81)	43,447 (72)$^{¤¤¤}$
Single mother; n (%)	3 (3)	12 (6)*	2 (1)	15 (6)$^{\#\#,¤}$	719 (2)	1699 (3)$^{¤¤¤}$
Maternal low education[d]; n (%)	1 (1)	11 (5)	5 (3)	16 (7)$^{\#\#}$	583 (1)	2242 (3)$^{¤¤¤}$
Low total household income[e] n (%)	8 (7)	27 (14)**	8 (6)	27 (12)$^{\#,¤}$	2022 (5)	4442 (8)$^{¤¤¤}$
Unplanned pregnancy; n (%)	24 (20)	52 (26)	19 (13)	71 (30)$^{\#\#\#,¤¤¤}$	7048 (17)	12,677 (21)$^{¤¤¤}$
Alcohol during pregnancy[f]; n (%)	2 (2)	10 (5)*	2 (1)	7 (3)	1104 (3)	1566 (3)
Smoking during pregnancy; n (%)	12 (10)*	28 (13)	6 (4)	26 (11)$^{¤}$	2085 (5)	7440 (11)$^{¤¤¤}$
Maternal anxiety/depression during pregnancy[g]; n (%)	14 (12)	48 (24)***,$^{¤¤}$	20 (14)$^{\#}$	36 (16)	3726 (9)	7095 (12)$^{¤¤¤}$
≥1 epileptic seizure during pregnancy; n (%)	24 (25)	18 (28)	10 (10)	3 (4)	NA	NA
TC seizure(s) during pregnancy; n (%)	12 (13)	8 (12)	4 (4)	1 (1)	NA	NA
AED polytherapy during pregnancy; n (%)	21 (17)	43 (19)	NA	NA	NA	NA
AED monotherapy during pregnancy; n (%)	98 (81)	182 (81)	NA	NA	NA	NA
Plasma AED (µmol/L)[h]; median (range)	33.3 (258)	36.4 (186)	NA	NA	NA	NA
Valproate use; n (%)	23 (19)	36 (16)	NA	NA	NA	NA
Carbamazepine use; n (%)	33 (27)	60 (27)	NA	NA	NA	NA
Lamotrigine use; n (%)	55 (46)	90 (40)	NA	NA	NA	NA
Levetiracetam use; n (%)	11 (9)	25 (11)	NA	NA	NA	NA

Topiramate use; n (%)	7 (6)	13 (6)	NA	NA	NA	NA
Oxcarbazepine use; n (%)	4 (3)	19 (8)	NA	NA	NA	NA
Language impairment age 1.5 years[i]	20 (18)*	23 (22)***	13 (11)	11 (8)	4201 (11)	3695 (10)¤¤
Language impairment age 3 years[j]	16 (16)***	6 (8)	8 (7)	6 (7)	2368 (7)	1814 (8)¤¤¤

AED = antiepileptic drug. BMI = Body Mass Index. TC seizure(s) = Tonic-clonic seizure(s). ASQ= the Ages and Stages Questionnaires. N may vary slightly within the groups due to missing data. Chi-square test for independence or Fisher's exact test was used for categorical variables, Mann-Whitney U test for continuous variables due to violation of the assumption of normal distribution

* AED-exposed children of mothers with epilepsy compared to children of mothers without epilepsy stratified for completed questionnaire, * $p<0.05$ ** $p<0.01$ *** $p<0.001$

[#] AED-unexposed children of mothers with epilepsy compared to children of mothers without epilepsy stratified for completed questionnaire, [#] $p<0.05$ [##] $p<0.01$ [###] $p<0.001$

[¤] Children who responded to the questionnaires compared to children who did not respond in each of the three groups, [¤] $p<0.05$ [¤¤] $p<0.01$ [¤¤¤] $p<0.001$

[a] Calculated from the ultrasonographic measurements performed at 18-19 weeks of gestation. When ultrasound data were unavailable, gestational age was estimated on basis of the first day of the last menstrual period [b] Number of all pregnancies >21 gestation weeks including the current pregnancy. Maximum value is 5, representing parity of 5 or more [c] Folic acid supplement use four weeks before pregnancy and/or during the first trimester [d] 9 or less years of schooling [e] <400 000 NOK (equals approximately 41,000 EUR) annually [f] Alcohol consumption ≥1 time per month [g] Mean score >1.75 on the Hopkins symptom check list in gestational week 17-19 [h] Median of standardized concentration (see text) in maternal plasma at gestational week 17-19 and umbilical cord blood [i] Defined as children who score 1.5 standard deviations or more below the mean score of the 3-item version of the communication scale from the Ages and Stages Questionnaires (ASQ) at age 1.5 years [j] Defined as children who score 1.5 standard deviations or more below the mean score of the 6-item version of the communication scale from the Ages and Stages Questionnaires (ASQ) at age 3 years and/or are talking in two- to three-word phrases or less (expressive language impairment) at age 3 years

Table e-4: Overview of antiepileptic drug (AED) exposure in utero

Overview of antiepileptic drug (AED) exposure in utero in the total study population and at age 5 and 8 years, respectively.

Type of antiepileptic drug	Total study population n = 346	Age 5 years n = 117	Age 8 years n = 120
AED monotherapy	280	91	97
AED polytherapy	64	26	21
AED drug regime unspecified	2	0	2
Lamotrigine monotherapy	112	39	41
Carbamazepine monotherapy	72	17	23
Valproate monotherapy	40	14	16
Levetiracetam monotherapy	17	9	6
Topiramate monotherapy	11	4	4
Oxcarbazepine monotherapy	8	1	1
Clonazepam monotherapy	7	3	2
Phenytoin monotherapy	4	1	1
Phenobarbital monotherapy	4	1	0
Gapapentin monotherapy	3	0	1
Primidone monotherapy	1	1	1
Clobazam monotherapy	1	1	1
Valproate + lamotrigine	7	3	4
Valproate + carbamazepine	4	0	0
Valproate + oxcarbazepine	1	0	1
Valproate + clonazepam	3	0	0
Valproate + vigabatrine	1	1	1
Valproate + levetiracetam + topiramate	1	0	1
Valproate + lamotrigine + ethosuximide	1	0	0
Valproate + lamotrigine + levetiracetam	1	1	0
Lamotrigine + levetiracetam	5	2	0
Lamotrigine + carbamazepine	8	3	6
Lamotrigine + oxcarbazepine	2	2	1
Lamotrigine + clonazepam	2	1	0
Lamotrigine + gabapentin	2	1	1
Lamotrigine + topiramate	1	0	0
Lamotrigine + levetiracetam + clonazepam	1	0	0
Lamotrigine + levetiracetam + clobazam	1	1	1
Lamotrigine + levetiracetam + oxcarbazepine	2	2	0
Levetiracetam + carbamazepine	4	3	2
Levetiracetam + oxcarbazepine	3	1	1
Levetiracetam + oxcarbazepine + pregabalin	1	1	0
Topiramate + oxcarbazepine	4	1	0
Topiramate + carbamazepine	2	2	2
Topiramate + clonazepam	1	0	0
Carbamazepine + gabapentin	1	0	0
Carbamazepine + phenytoin	1	0	0
Carbamazepine + clonazepam	1	0	0
Oxcarbazepine + clonazepam	1	1	0
Oxcarbazepine + vigabatrin	1	0	0
Primidone + phenytoin	1	0	0

Table e-5: Supplement to figure 2. The correlation between plasma antiepileptic drug (AED) concentrations and language score at age 5 and 8 years

The correlation between plasma antiepileptic drug (AED) concentrations (µmol/l) from maternal blood samples (gestational week 17-19) and umbilical cord samples, and language score at age 5 and 8 years. Significant results are highlighted and presented as scatter plots.

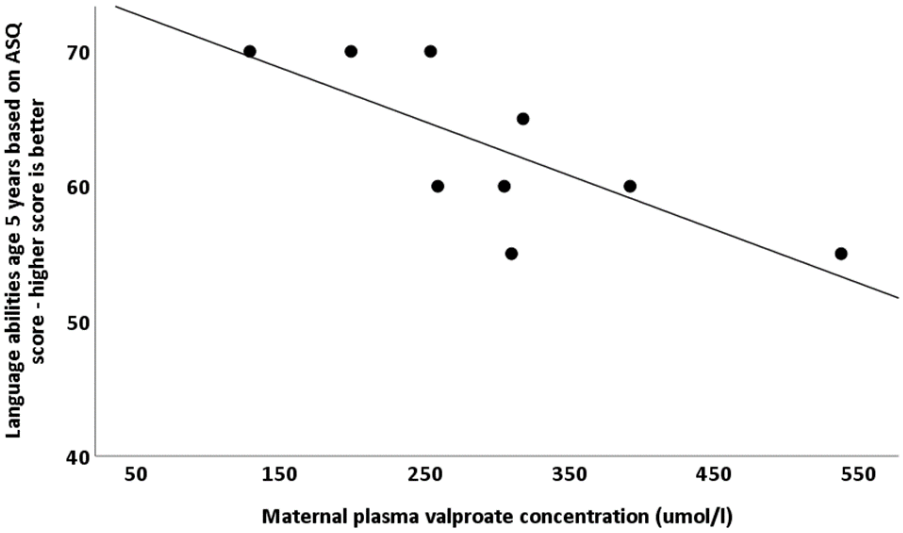
AED concentration (µmol/l)		**ASQ score[a] 5 years**	**Language 20 score[b] 5 years**	**SLAS score[c] 5 years**	**Language 20 score[b] 8 years**
Carbamazepine (maternal)	n	19	**19**	19	27
	r value	0.39	**-0.47**	0.06	-0.09
	p value	0.10	**0.04***	0.81	0.64
Carbamazepine (umbilical cord)	n	17	17	17	26
	r value	0.33	-0.31	0.45	0.13
	p value	0.19	0.23	0.07	0.53
Oxcarbazepine (maternal)[d]	n	0	0	0	1
	r value	NA	NA	NA	NA
	p value	NA	NA	NA	NA
Oxcarbazepine (umbilical cord)[1]	n	0	0	0	1
	r value	NA	NA	NA	NA
	p value	NA	NA	NA	NA
Lamotrigine (maternal)	n	33	33	33	32
	r value	-0.06	-0.05	-0.16	-0.23
	p value	0.76	0.80	0.38	0.21
Lamotrigine (umbilical cord)	n	30	31	31	33
	r value	-0.11	0.23	-0.16	0.05
	p value	0.56	0.22	0.39	0.79
Levetiracetam (maternal)	n	15	15	15	9
	r value	0.16	0.01	0.29	0.00
	p value	0.57	0.99	0.29	1.00
Levetiracetam (umbilical cord)	n	10	10	10	8
	r value	-0.04	0.23	0.06	0.24
	p value	0.92	0.52	0.87	0.56
Valproate (maternal)	n	**9**	**9**	8	13
	r value	**-0.77***	**0.82***	-0.52	0.11
	p value	**0.02***	**0.01***	0.18	0.71
Valproate (umbilical cord)	n	10	10	10	12
	r value	-0.06	0.50	-0.60	-0.06
	p value	0.88	0.14	0.07	0.85
Topiramate (maternal)	n	6	6	6	4

	r value	0.00	-0.77	-0.46	0.21
	p value	1.00	0.08	0.35	0.79
Topiramate (umbilical cord)	n	5	5	5	3
	r value	0.27	-0.81	0.58	-0.87
	p value	0.66	0.10	0.31	0.33
Standardized AED concentration	n	87	88	87	91
	r value	-0.04	0.13	-0.14	0.13
	p value	0.73	0.22	0.21	0.22

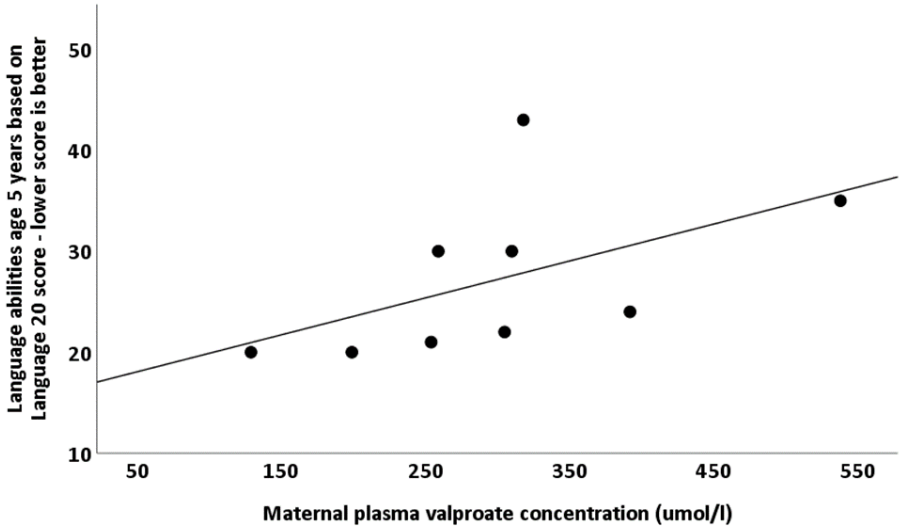
AED = Antiepileptic drug. r value is Spearman's rho due to violation of the assumption of normal distribution. *p-values <0.05

[a] The Ages and Stages Questionnaires score (0-70 points). A low score indicates language impairment [b] The Twenty Statements about Language-related Difficulties score (0-100 points total score, 0-40 points semantic subscale). A high score indicates language impairment [c] Composite scale mean score (1-5 points) from the Speech and Language Assessment Scale (SLAS). Score below 3 points indicates language impairment [d] Measured as the active monohydroxyderivative metabolite

A.



B.


Language abilities age 5 years based on Language 20 score - lower score is better
50
40
30
20
10
50
150
250
350
450
550
Maternal plasma valproate concentration (umol/l)

C.

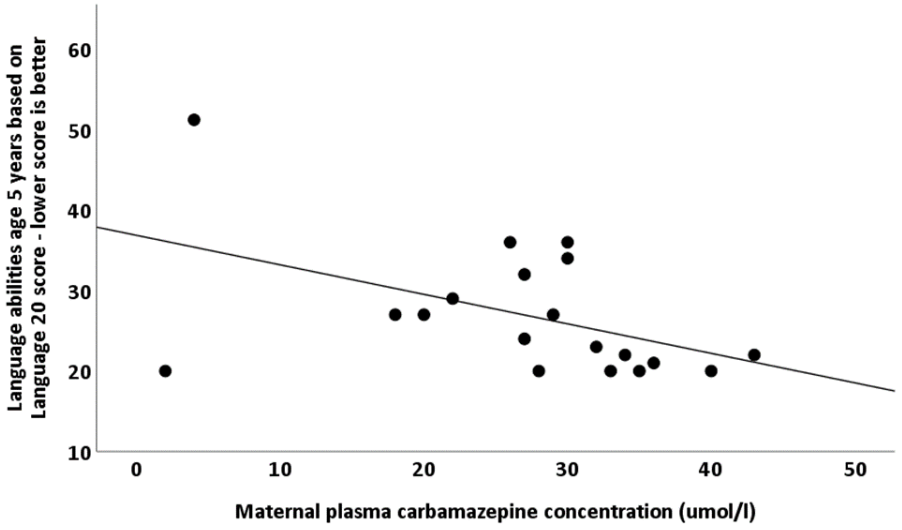

Language abilities age 5 years based on Language 20 score - lower score is better
60
50
40
30
20
10
0
10
20
30
40
50
Maternal plasma carbamazepine concentration (umol/l)

Table e-6: Language impairment stratified for periconceptional folic acid supplement use

Language impairment at age 5 and 8 years in AED-exposed and -unexposed children of mothers with epilepsy compared to children of mothers without epilepsy stratified for periconceptional folic acid supplement use (yes, no).

	AED-exposed children of mothers with epilepsy n (%)		**AED-unexposed children of mothers with epilepsy n (%)**		**Children of mothers without epilepsy n (%)**	
	Periconceptional folic acid[a]		**Periconceptional folic acid[a]**		**Periconceptional folic acid[a]**	
	YES	NO	YES	NO	YES	NO
Language impairment 5 years[b]	29 of 108 (27)	5 of 8 (63)*,¤	32 of 122 (26)	7 of 27 (26)	7510 of 34,697 (22)	1422 of 6096 (23)¤¤
Language impairment 8 years[c]	27 of 99 (27)*	11 of 21 (52)**,¤	29 of 116 (25)	6 of 34 (18)	6445 of 34,203 (19)	1712 of 7926 (22)¤¤¤

AED = antiepileptic drug. Chi-square test for independence or Fisher's exact test was used for categorical variables. * Delayed language function in children of mothers with epilepsy compared to children of mothers without epilepsy, * p<0.05 ** p<0.01 *** p<0.001 ¤ Delayed language function in children of mothers with no periconceptional folic acid supplement use compared to children of mothers with periconceptional folic acid supplement use within each of the three groups (stratified for maternal epilepsy and AED exposure), ¤ p<0.05 ¤¤ p<0.01 ¤¤¤ p<0.001

[a] Maternal folic acid supplement use four weeks before pregnancy and/or during the first trimester [b] Language impairment at age 5 years according to the Ages and Stages Questionnaires (ASQ) or the Speech and Language Assessment Scale (SLAS) or the Twenty Statements about Language-related Difficulties (Language 20) [c] Language impairment at age 8 years according to the Twenty Statements about Language-related Difficulties (Language 20) semantic subscale

Figure e-1: Direct acyclic graph (DAG)

Direct acyclic graph (DAG) of the relationship between antiepileptic drug (AED) exposure in utero and language impairment in the child. The graph illustrates hypothesized causal pathways (green arrows) with variables acting as possible intermediate variables or mediators, and biased pathways (pink and black arrows) with confounding variables. Variables in the biased pathways except male offspring have been adjusted for in the logistic regression analysis.

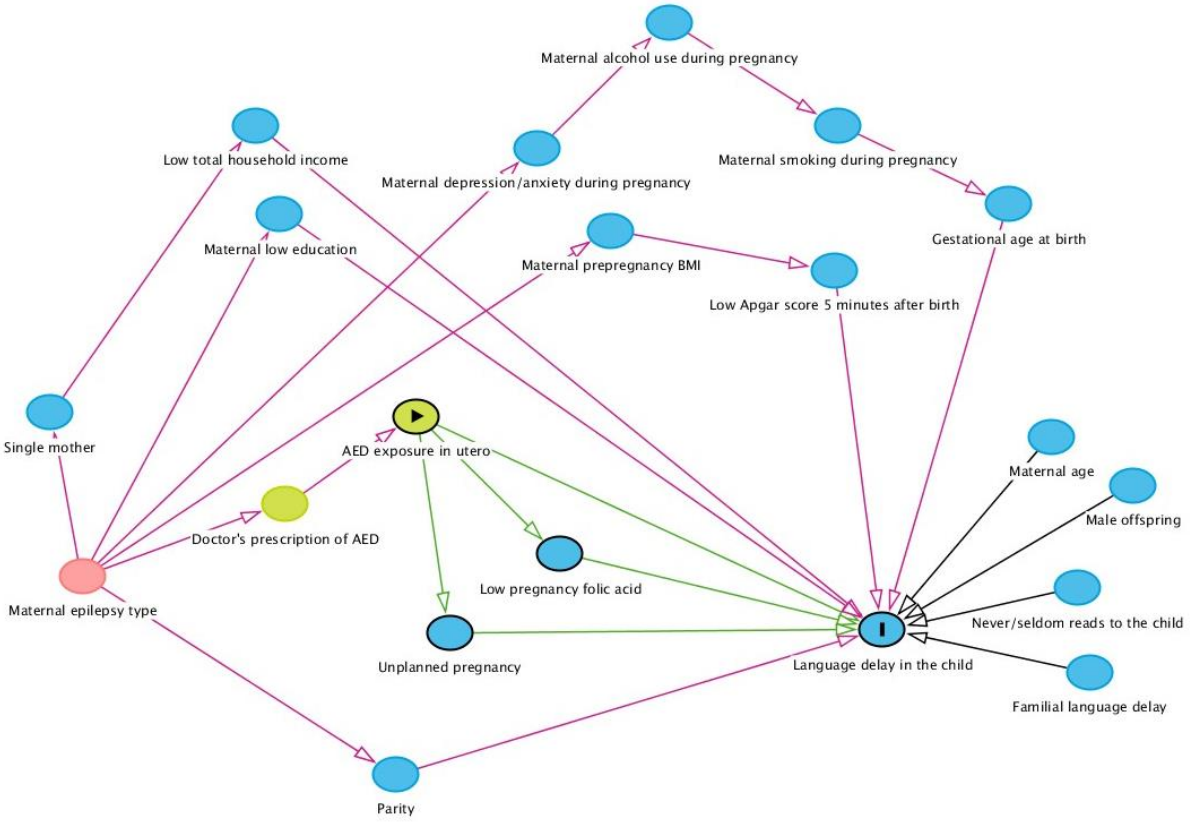
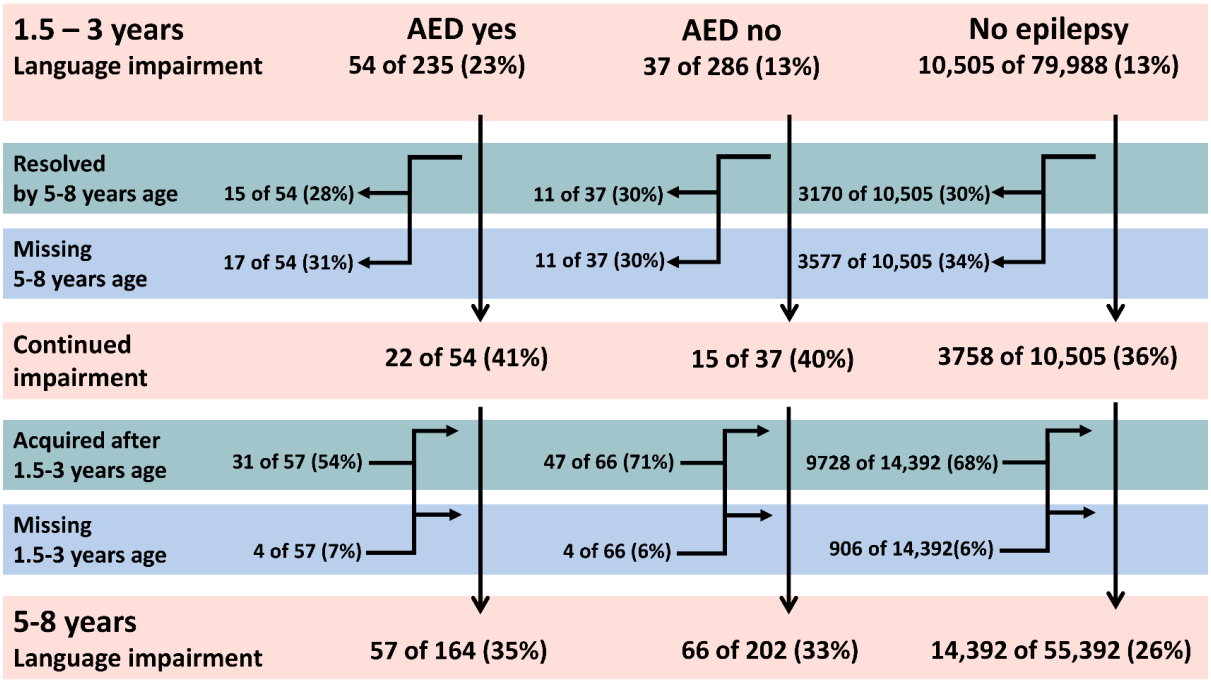


Figure e-2: Trajectories of language impairment in children aged 1.5-8 years

Trajectories of language impairment in children aged 1.5-8 years. AED yes = AED-exposed children of mothers with epilepsy, AED no = AED-unexposed children of mothers with epilepsy, no epilepsy = children of mothers without epilepsy.



References

[1]. Bjork MH, Veiby G, Spigset O, Gilhus NE. Using the Norwegian Mother and Child Cohort Study to determine risk factors for delayed development and neuropsychiatric symptoms in the offspring of parents with epilepsy. *The Norwegian Journal of Epidemiology*. 2014 **24:** 79-89.
[2]. Paltiel L, Haugan A, Skjerden T*, et al.* The biobank of the Norwegian Mother and Child Cohort Study - present status. *The Norwegian Journal of Epidemiology*. 2014 **24:** 29-35.
[3]. Husebye ESN, Gilhus NE, Riedel B, Spigset O, Daltveit AK, Bjork MH. Verbal abilities in children of mothers with epilepsy: Association to maternal folate status. *Neurology*. 2018 **91:** e811-e821.
[4]. Meador KJ, Baker GA, Browning N*, et al.* Foetal antiepileptic drug exposure and verbal versus non-verbal abilities at three years of age. *Brain*. 2011 **134:** 396-404.
[5]. Bjork M, Riedel B, Spigset O*, et al.* Association of Folic Acid Supplementation During Pregnancy With the Risk of Autistic Traits in Children Exposed to Antiepileptic Drugs In Utero. *JAMA Neurol*. 2018 **75:** 160-168.
[6]. Hannisdal R, Ueland PM, Eussen SJ, Svardal A, Hustad S. Analytical recovery of folate degradation products formed in human serum and plasma at room temperature. *J Nutr*. 2009 **139:** 1415-1418.
[7]. Hannisdal R, Ueland PM, Svardal A. Liquid chromatography-tandem mass spectrometry analysis of folate and folate catabolites in human serum. *Clin Chem*. 2009 **55:** 1147-1154.
[8]. Richter J, Janson H. A validation study of the Norwegian version of the Ages and Stages Questionnaires. *Acta Paediatr*. 2007 **96:** 748-752.
[9]. Squires J, Bricker D, Potter L. Revision of a parent-completed development screening tool: Ages and Stages Questionnaires. *J Pediatr Psychol*. 1997 **22:** 313-328.
[10]. Hadley PA, Rice ML. Parental Judgments of Preschoolers' Speech and Language Development: A Resource for Assessment and IEP Planning. *Semin Speech Language*. 1993 **14:** 278-288.
[11]. Ottem E. 20 spørsmål om språkferdigheter– en analyse av sammenhengen mellom observasjonsdata og testdata. *Skolepsykologi*. 2009 **1**.
[12]. Strand BH, Dalgard OS, Tambs K, Rognerud M. Measuring the mental health status of the Norwegian population: a comparison of the instruments SCL-25, SCL-10, SCL-5 and MHI-5 (SF-36). *Nord J Psychiatry*. 2003 **57:** 113-118.

III

Received: 9 June 2021 | Revised: 23 August 2021 | Accepted: 7 September 2021

DOI: 10.1111/epi.17076

Epilepsia®

FULL-LENGTH ORIGINAL RESEARCH

Vitamin B status and association with antiseizure medication in pregnant women with epilepsy

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Funding information
Norwegian Chapter International League Against Epilepsy; Dr. Nils Henrichsen og hustru Anna Henrichsens legat; Det alminnelige medisinske forskningsfond ved Universitetet i Bergen; Advokat Rolf Sandberg Reberg og Ellen Marie Rebergs Legat til Epilepsiforskning; Norwegian Epilepsy Foundation

Summary

Objective: Antiseizure medication (ASM) use interacts with vitamin B status in nonpregnant epilepsy populations. We aimed to examine the association between ASM and vitamin B status in pregnant women with epilepsy.

Methods: We performed a cross-sectional study of pregnancies in women with epilepsy enrolled in the Norwegian Mother, Father and Child Cohort Study from 1999 to 2008. Data on ASM and vitamin supplement use were collected from questionnaires. We analyzed maternal plasma concentrations of ASM and metabolites of folate, including unmetabolized folic acid (UMFA), riboflavin (vitamin B2), pyridoxine (vitamin B6), and niacin (vitamin B3) during gestational weeks 17–19.

Results: We included 227 singleton pregnancies exposed to ASM with available plasma samples (median maternal age 29 years, range 18 to 41 years). From the preconception period to gestational week 20, any supplement of folic acid was reported in 208 of pregnancies (94%), riboflavin in 72 (33%), pyridoxine in 77 (35%), and niacin in 45 (20%). High ASM concentrations correlated with high concentrations of UMFA and inactive folate metabolites, and with low concentrations of riboflavin and metabolically active pyridoxine. There was no association between ASM and niacin status.

Significance: ASM concentrations during pregnancy were associated with vitamin B status in pregnant women with epilepsy. Additional studies are needed to determine the clinical impact of these findings, and to define the optimal vitamin doses that should be recommended to improve pregnancy outcomes.

KEYWORDS
anticonvulsants, folic acid, MBRN, MoBa, pyridoxine, riboflavin

1 | INTRODUCTION

Exposure to antiseizure medication (ASM) during pregnancy is associated with an increased risk of congenital malformations and adverse neurodevelopment in the children.[1–4] Several ASMs interact with folate metabolism and reduce folate concentrations,[5–8] adding to the folate-lowering effect of pregnancy itself.[9] Chronic ASM use has been associated with increased folate catabolism.[10] Studies examining the interplay between folate metabolism and ASM use are needed in pregnant women. Women with epilepsy using ASMs are often recommended a high dose of folic acid supplement during pregnancy.[1,3,11] Studies of nonepilepsy populations show that excessive folic acid supplementation results in plasma accumulation of unmetabolized folic acid (UMFA).[12,13] The safety of high supplement doses has been questioned,[12–15] as studies in women without epilepsy have reported negative effects of high UMFA concentrations on neurodevelopment.[14–16]

In nonpregnant epilepsy populations, there is an association between chronic ASM use and low concentrations of non-folate B vitamins such as riboflavin (vitamin B2) and pyridoxine (vitamin B6).[5,7,17,18] Riboflavin and pyridoxine act in close interaction with folate in one-carbon metabolism, representing metabolic pathways fundamental for normal fetal development.[9,19] Niacin (vitamin B3) plays a key role in neuronal development and survival.[20] The association between ASM use and vitamin B status in pregnant women with epilepsy has not been examined in detail. One study reported an association between low folate concentrations and ASM polytherapy, and also with high phenytoin and phenobarbital concentrations.[21] Another study reported low concentrations of active folate metabolite during lamotrigine treatment.[22]

In this study, we aimed to examine the association between various ASM concentrations and vitamin B status during pregnancy in women with epilepsy. Such studies contribute important knowledge to aid decisions on recommendations for vitamin supplements in pregnancy for women with epilepsy using ASM.

Key point

- Antiseizure medication (ASM) use is associated with low concentrations of folate and other B vitamins in nonpregnant epilepsy populations
- High ASM concentrations correlated with high concentrations of unmetabolized folic acid in pregnant women with epilepsy
- High ASM concentrations correlated with high concentrations of inactive folate metabolites in pregnant women with epilepsy
- High ASM concentrations correlated with both low riboflavin and pyridoxine status in pregnant women with epilepsy

2 | MATERIAL AND METHODS

2.1 | Study population

The study population included singleton pregnancies of women with epilepsy using ASM with available plasma samples enrolled in the Norwegian Mother, Father and Child Cohort Study (MoBa). MoBa is a population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health and linked to the compulsory Medical Birth Registry of Norway (MBRN).[23] During the years 1999–2008, pregnant women were invited to participate in gestational weeks 17–19. The participation rate was 41%. Women answered questionnaires in gestational weeks 17–19 and 30 on medication use, vitamin use, social and medical background, and parameters related to current and previous pregnancies.[23] A maternal blood sample was collected during gestational weeks 17–19.[24] The current study is based on version 10 of the quality-assured MoBa data files.

We identified women with epilepsy based on self-reported information in the MoBa questionnaires and from diagnostic data registered by the primary care physician or midwife in the MBRN. The MoBa epilepsy cohort has been described elsewhere.[25–27] We collected information on ASM use from the two pregnancy questionnaires, and from the MBRN.[23] Response rates were 97% for the first questionnaire in gestational weeks 17–19, and 89% for the second in gestational week 30. The epilepsy cohort in MoBa has been validated by a retrospective survey (50% response rate), and in a hospital record examination of a subcohort (n = 40).[25] The validity was high, as 98% of the women who reported a diagnosis of epilepsy in MoBa confirmed this in the retrospective survey.[25] There was 100% agreement between the reported ASM use in MoBa and ASM use registered in the hospital records.[25]

2.2 | Vitamin supplement use

We obtained information on type, timing, and frequency of vitamin supplement use in the questionnaires from gestational weeks 17–19 and 30. The mothers reported on use of folic acid, riboflavin, pyridoxine, and niacin during the following gestational week intervals, with week 0 starting with the first day of the last menstrual period: −4 to 0, 0–4, 5–8, 9–12, 13+ (first questionnaire) and for gestational

weeks 13–16, 17–20, 21–24, 25–28, and 29+ (second questionnaire). Intake was reported as either daily, 4–6 times per week, or 1–3 times per week. Maternal intake of supplements in the first and second trimester has been associated previously with plasma concentrations in samples collected during gestational weeks 17–19.[28,29] We defined supplement use as any use of a supplement during gestational weeks −4 to 20.

We collected information on folic acid dose from 97 of 227 pregnancies (43%) with data from the retrospective validation questionnaire,[25] as this information was not included in the ordinary MoBa questionnaires. The women reported a folic acid dose of 0.4 mg, 1–2 mg, or ≥4 mg during gestational weeks −4 to 24. We grouped the pregnancies according to the highest reported dose during this period: low-dose folic acid (0.4–2 mg) and high-dose folic acid (≥4 mg).

2.3 | Plasma ASM concentrations

We analyzed plasma concentrations of valproate, carbamazepine, lamotrigine, levetiracetam, topiramate, and the oxcarbazepine monohydroxyderivative metabolite.[25] Standardized ASM concentrations were calculated by normalizing the plasma concentrations to the concentration range observed for that drug in the present study according to the formula *100 x (observed concentration − minimum concentration measured for that drug) / concentration range measured for that drug*.[26,30] For ASM polytherapy, the sum of all standardized ASM concentrations was given.

2.4 | Vitamin and metabolite concentrations

We analyzed plasma vitamin and metabolite concentrations at Bevital Laboratory, Bergen (www.bevital.no). We examined folate status by analyzing the biologically active 5-methyltetrahydrofolate (mTHF) metabolite, the mTHF-derived 4-alfa-hydroxy-5-methyltetrahydrofolate (hmTHF) metabolite, and the inactive metabolites para-aminobenzoylglutamate (pABG) and acetamidobenzoylglutamate (apABG),[31] as well as unmetabolized folic acid (UMFA).[31] UMFA values below the limit of quantification (LOQ, 0.53 nmol/L)[31] were reported as 0.0 nmol/L. Metabolically active folate concentration was given as the sum of mTHF and hmTHF ("folate").[26,31–33] We calculated the ratio between the active (mTHF plus hmTHF) and inactive (pABG plus apABG) folate metabolites and used a low ratio as a marker of increased folate catabolism. We also calculated the ratio between UMFA and metabolically active folate to better separate the effect of UMFA from the effect of folate.[31,34]

We analyzed plasma riboflavin to examine riboflavin status.[35] We examined pyridoxine status by analyzing metabolically active pyridoxine (pyridoxal-5-phosphate [PLP]) and a functional marker of pyridoxine status, HKr, described in detail elsewhere.[36,37] High HKr indicates low pyridoxine status.[37] We analyzed plasma nicotinamide to examine niacin status.[36]

2.5 | Statistical analysis

We used IBM SPSS Software version 25 for the statistical analyses. We categorized the pregnancies according to ASM monotherapy and ASM polytherapy. Pregnancies where none of the reported ASMs could be detected were categorized into a separate group of suspected low ASM adherence pregnancies. We recorded relevant covariates from the questionnaire in gestational weeks 17–19, and from the MBRN stratified for ASM group[28,29]: maternal age, parity, maternal education, maternal prepregnancy body mass index (BMI), smoking during pregnancy, unplanned pregnancy, epileptic seizures during pregnancy, and tonic-clonic (TC) epileptic seizures during pregnancy. For continuous variables, we reported median values with range. We analyzed vitamin and metabolite concentrations stratified for ASM group and vitamin supplement use, and folate status stratified for ASM group and folic acid dose. Two-sided p-values <0.05 were considered statistically significant. The different ASM groups were compared with the nonparametric Kruskal-Wallis test, due to violation of the assumption of normal distribution and low number of pregnancies in each group. Adjustment for multiple testing was done by multiplying the observed p-value by the number of comparisons made (Dunn-Bonferroni post hoc method). This Bonferroni corrected p-value was considered statistically significant when <0.05. We used Mann-Whitney U test to compare vitamin and metabolite concentrations between supplemented and nonsupplemented pregnancies and between high-dose and low-dose folic acid supplement, stratified for ASM group. We examined the associations between ASM concentrations and vitamin and metabolite concentrations in a nonparametric correlation analysis (Spearman rank correlation). We performed sensitivity analyses by excluding supplement users from the correlation analyses for riboflavin, niacin, and pyridoxine. For folate status, high-dose folic acid supplement users were excluded, because exclusion of nonsupplemented folic acid pregnancies (n = 13) was not meaningful.

2.6 | Standard protocol approvals, registrations, and patient consents

The establishment of MoBa and initial data collection were based on a license from The Norwegian Data Protection Agency and approval from The Regional Committee for Medical Research Ethics. The MoBa cohort is regulated by the Norwegian Health Registry Act. All parents in MoBa have given written consent to participate. The current study was approved by The Regional Committee for Medical Research Ethics (reference 2011/1616).

3 | RESULTS

We identified 227 singleton pregnancies in 203 mothers with epilepsy who had available plasma samples from gestational weeks 17–19 (Figure 1 and Table 1). The mothers used ASM monotherapy in 183 pregnancies and ASM polytherapy in 44 pregnancies (Table S1). The reported ASM used during pregnancy was detected in 199 pregnancies (88%) (Table S1). We studied eight ASM groups: six monotherapy groups with the reported ASM detected in plasma for valproate (n = 24), lamotrigine (n = 65), carbamazepine (n = 48), levetiracetam (n = 11), topiramate (n = 8), and oxcarbazepine (n = 5); one polytherapy group with at least one of the reported ASMs detected in plasma (n = 40); and one low-adherence group with none of the reported ASMs detected in plasma (n = 26) (Table 1). Most women in the latter group admitted low adherence, because only 25% reported regular ASM intake in gestational week 13+ in the first questionnaire, compared to 78%-90% in the other ASM groups.

In 221 pregnancies with available supplement data, the women reported any folic acid supplement use in 208 (94%), riboflavin supplement in 72 (33%), niacin in 45 (20%), and pyridoxine in 77 pregnancies (35%) (Table 1). Intake was reported as ≥4–6 times per week or daily in ≥90% of the pregnancies for all supplements.

Among the included pregnancies from the retrospective validation survey, 76 (33%) had precise information on folic acid dose from gestational weeks −4 to 24. High-dose folic acid (≥4 mg) was reported in 39 pregnancies, and low-dose (0.4–2 mg) was reported in 37 pregnancies.

3.1 | Folate status and association with ASM concentrations

High ASM concentrations correlated with high concentrations of UMFA and inactive folate metabolites, and with a low ratio between active and inactive folate metabolites (Figure 2 and Table S2).

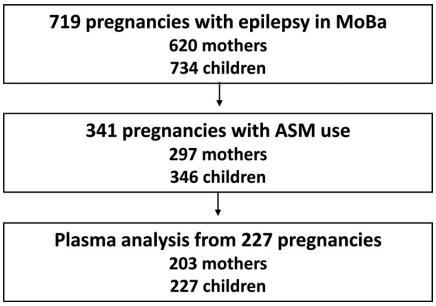

FIGURE 1 Flow chart of excluded and included cases. The flow chart shows excluded and included cases. MoBa, The Norwegian Mother, Father and Child Cohort Study; ASM, antiseizure medication.

The low-adherence group had the lowest folate concentrations; otherwise there were few differences in folate between the different ASM (Table 2). The UMFA concentrations were higher in mothers using valproate, lamotrigine, carbamazepine, or ASM polytherapy, respectively, compared to the low-adherence group (Table 2). The concentrations of the inactive folate metabolites and ratio between active and inactive folate metabolites differed between individual ASMs (Table 2). The concentrations of inactive folate metabolites were higher in ASM polytherapy users compared to lamotrigine users, levetiracetam users, and the low-adherence group, and in valproate users compared to the low-adherence group and levetiracetam users (Table 2). Mothers using levetiracetam had the highest ratio between active and inactive folate metabolites (Table 2). Women using carbamazepine monotherapy or ASM polytherapy had a lower ratio between active and inactive folate metabolites compared to mothers using lamotrigine (Table 2).

In particular, high valproate concentrations correlated with high concentrations of inactive folate (Figure 3 and Table S2). The correlation strength remained unchanged after removing high-dose folic acid users (Table S2). High topiramate concentrations correlated with high UMFA concentrations, but the correlation strength was reduced after removal of high-dose supplement users (Table S2).

High-dose folic acid supplement users had higher folate and UMFA concentrations compared to low-dose users (Figure S1). High folate concentrations correlated with high UMFA concentrations (Figure S1). High-dose users did not differ in ASM concentrations compared to low-dose users (data not shown). After stratification for ASM group, the concentrations of the different folate metabolites in high-dose users compared to low-dose users were essentially the same across ASM groups (Figure S2).

TABLE 1 Clinical characteristics stratified for antiseizure medication (ASM) treatment.

	Valproate n = 24	Lamotrigine n = 65	Carbamazepine n = 48	Levetiracetam n = 11	Topiramate n = 8	Oxcarbazepine n = 5	Polytherapy n = 40	Low-adherence group[1] n = 26
Maternal age, y; median (range)	27.0 (19.0)	29.0 (21.0)	30.0 (23.0)	30.0 (14.0)	28.5 (12.0)	29.0 (5.0)	28.0 (20.0)	29.0 (19.0)
Parity[2]; median (range)	1.0 (2.0)	0.0 (4.0)	1.0 (4.0)	0.0 (3.0)	0.5 (2.0)	0.0 (1.0)	1.0 (3.0)	0.5 (4.0)
Maternal prepregnancy BMI; median (range)	23.3 (13.4)	22.9 (20.2)	24.7 (18.0)	24.4 (18.4)	20.4 (6.1)	23.9 (13.5)	24.1 (18.8)	24.5 (22.8)
Maternal low education[3]; n (%)	0 (0)	0 (0)	2 (4)	0 (0)	0 (0)	0 (0)	2 (6)	2 (8)
Smoking during pregnancy; n (%)	8 (33)	9 (14)	7 (15)	1 (9)	1 (13)	2 (40)	7 (18)	3 (12)
Unplanned pregnancy; n (%)	3 (14)	16 (25)	11 (23)	0 (0)	0 (0)	2 (40)	10 (26)	4 (17)
≥1 epileptic seizure during pregnancy; n (%)	3 (30)	6 (18)	3 (11)	4 (44)	1 (17)	0 (0)	10 (50)	0 (0)
TC seizure(s) during pregnancy; n (%)	2 (20)	4 (12)	2 (7)	1 (11)	0 (0)	0 (0)	5 (25)	0 (0)
Plasma ASM concentration (μmol/L)[4]; median (range)	314.0 (409.0)	7.6 (26.2)	29.5 (39.0)	57.0 (146.0)	12.0 (17.0)	35.4 (56.3)	NA	0.0 (0.0)[1]
Folic acid supplement use[5]; n (%)	21 (96)	62 (97)	45 (94)	11 (100)	7 (100)	4 (80)	38 (95)	20 (83)
Riboflavin supplement use[5]; n (%)	3 (14)	30 (47)	10 (21)	3 (27)	4 (57)	2 (40)	14 (35)	6 (25)
Pyridoxine supplement use[5]; n (%)	3 (14)	32 (50)	11 (23)	3 (27)	4 (57)	2 (40)	15 (38)	7 (29)
Niacin supplement use[5]; n (%)	2 (9)	17 (27)	6 (13)	2 (18)	3 (43)	2 (40)	9 (23)	4 (17)

Abbreviations: ASM, antiseizure medication; BMI, body mass index; maternal low education, total missing n = 19; N may vary due to missing data: maternal prepregnancy BMI, total missing n = 14; SD, standard deviation; seizures during pregnancy, total missing n = 115 (due to validation study data, see Methods); smoking during pregnancy, total missing n = 1; TC seizure(s), tonic-clonic seizure(s); unplanned pregnancy, total missing n = 11; vitamin supplement use, total missing n = 6.

[1]Consist of pregnancies where the mother reported ASM use, but no ASM was detected in plasma samples.

[2]Number of all previous pregnancies >12 weeks of gestation, values from 0 (nulliparous) to 4, where 4 means 4 or more. When the median parity value is between 0 and 1, the median is given as 0.5.

[3]9 or fewer years of schooling.

[4]Maternal plasma concentration in gestational weeks 17–19 for each monotherapy group.

[5]Any supplement use during gestational weeks −4 to 20.

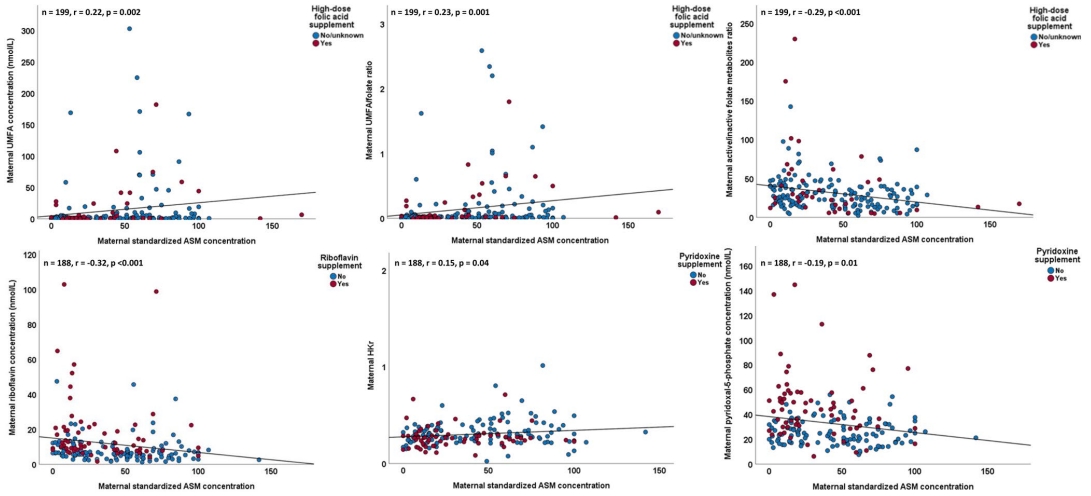

FIGURE 2 Correlation between maternal standardized antiseizure medication (ASM) concentrations and concentrations of vitamin B metabolites. The figure shows the correlation between standardized ASM and vitamin B metabolite concentrations. Blue dots represent no supplement or no or unknown folic acid supplement dose; red dots represent supplemented pregnancies or high-dose folic acid supplement. ASM, antiseizure medication. UMFA, unmetabolized folic acid; HKr, marker of pyridoxine deficiency; r, Spearman's rho; p, *p*-value.

3.2 | Riboflavin status and association with ASM concentrations

High ASM and high lamotrigine concentrations correlated with low concentrations of riboflavin (Figure 2 and Table S2). We observed minor changes in the correlation coefficients after removal of supplement users (Table S2). The riboflavin concentration did not differ between individual ASMs (Table 2 and Table 3).

3.3 | Pyridoxine status and association with ASM concentrations

High concentrations of ASM correlated with low concentrations of metabolically active pyridoxine, and with a high value for the marker of low pyridoxine status (HKr) (Figure 2 and Table S2). Removal of pyridoxine supplement users only slightly reduced the strength of the correlations (Table S2). The metabolically active pyridoxine concentration did not differ between individual ASMs (Table 2), but between supplement users and nonusers (Table 3). However, for individual ASMs, high valproate concentrations correlated with high HKr (Figure 3 and Table S2). In this group, the HKr was higher than in the other ASM groups (Table 2), particularly in those without pyridoxine supplement use (Table 3).

3.4 | Niacin status and association with ASM concentrations

We found no correlation between concentrations of ASM and nicotinamide (Table S2). The nicotinamide concentrations did not differ between different ASM groups (Table 2 and Table 3).

4 | DISCUSSION

In this cohort of pregnant women with epilepsy using ASMs, we found an association between plasma ASM concentrations and folate, riboflavin, and pyridoxine status, all of them part of one-carbon metabolism. High ASM concentrations were associated with high concentrations of inactive folate metabolites and UMFA, and with low concentrations of riboflavin and metabolically active pyridoxine. The associations were present even though many women used various types of vitamin B supplements during pregnancy.

The median folate concentration was lowest in the group with low adherence to ASM therapy and probably also to folic acid supplement use. Low folate concentrations during valproate, carbamazepine, oxcarbazepine, topiramate,[6,7] and lamotrigine treatment[8] have been reported in nonpregnant epilepsy populations compared to controls without ASM use. Folic acid supplement use in

TABLE 2 Maternal plasma vitamin B metabolite concentrations in gestational weeks 17–19 stratified for antiseizure medication (ASM) treatment

	Valproate n = 24	Lamotrigine n = 65	Carbamazepine n = 48	Levetiracetam n = 11	Topiramate n = 8	Oxcarbazepine n = 5	Polytherapy n = 40	Low-adherence group[1] n = 26
Plasma folate status								
Folate[2] (nmol/L); median (range)	**80.0 (84.7)**	**65.9 (119.0)**	**65.5 (103.8)**[b]	**73.9 (107.1)**	**66.9 (86.4)**	67.1 (68.4)	**74.2 (112.1)**[b]	**38.2 (96.7)**[a]
pABG (nmol/L); median (range)	**1.6 (19.5)**	1.1 (20.7)[b]	**1.3 (29.5)**	0.9 (17.4)	1.2 (9.2)	1.0 (5.3)	**1.5 (24.8)**[b]	**0.8 (9.7)**[a]
apABG (nmol/L); median (range)	**0.9 (2.4)**	0.8 (6.7)[b]	**0.9 (2.6)**	**0.6 (1.1)**[a]	**1.2 (1.3)**	0.6 (2.0)	**1.1 (2.4)**[b,c]	0.8 (2.2)[c]
Ratio active/inactive metabolites[3]; median (range)	**25.5 (76.6)**	27.5 (225.6)[b,c]	**22.7 (76.2)**[b]	**41.3 (168.7)**[a]	31.4 (58.1)	39.2 (32.7)	**19.4 (94.2)**[c]	**23.8 (180.4)**
UMFA (nmol/L); median (range)	**2.7 (303.0)**	**1.1 (169.0)**	**1.5 (182.0)**	0.8 (108.0)	1.9 (41.6)	0.8 (8.4)	**1.6 (167.0)**	**0.6 (63.8)**[a]
Ratio UMFA/folate[4]; median (range)	**0.03 (2.6)**	0.02 (1.6)	**0.03 (2.2)**	0.01 (0.8)	0.02 (0.3)	0.01 (0.1)	**0.02 (1.4)**	**0.009 (0.8)**[a]
Plasma riboflavin status								
Riboflavin (nmol/L); median (range)	5.9 (22.8)	10.0 (100.3)	6.6 (97.2)	9.6 (62.4)	7.4 (19.6)	6.4 (17.6)	6.9 (46.2)	7.6 (53.0)
Plasma pyridoxine status								
PLP (nmol/L); median (range)	24.7 (45.1)	32.7 (135.3)	23.1 (78.4)	24.9 (121.5)	31.1 (49.9)	27.8 (60.9)	28.9 (105.4)	27.2 (138.9)
HKr[5]; median (range)	**0.4 (0.9)**[a]	**0.3 (0.6)**	**0.3 (0.5)**	**0.3 (0.2)**	**0.3 (0.3)**	**0.2 (0.2)**	**0.3 (0.7)**	**0.3 (0.4)**
Plasma niacin status								
Nicotinamide (nmol/L); median (range)	485.3 (1058.5)	337.1 (1108.5)	370.2 (1652.3)	499.6 (501.2)	266.6 (526.7)	277.7 (1063.6)	412.9 (1099.0)	434.5 (1514.7)

All ASM groups are compared with each other by using Kruskal-Wallis test. Groups that differ significantly (p-value <0.05) from the group marked with an [a] are marked with bold text, additional groups that significantly differ from each other are both marked with [b] and than with [c]. Significant differences after Bonferroni correction for multiple tests are underlined.

Abbreviations: ASM, 3-hydroxyanthranilic acid; XA, 3-hydroxykynurenine; pABG, 4-alfa-hydroxy-5-methyltetrahydrofolate; hmTHF, 5-methyltetrahydrofolate; UMFA, acetamidobenzoylglutamate; HAA, anthranilic acid; antiseizure medication, N may vary slightly due to missing data for plasma riboflavin status, plasma niacin status and plasma pyridoxine status in the following ASM treatment groups: lamotrigine (n = 1), carbamazepine (n = 1), levetiracetam (n = 2), polytherapy (n = 7); kynurenic acid, AA; mTHF; para-aminobenzoylglutamate, apABG; pyridoxal-5-phosphate, HK; unmetabolized folic acid, PLP; xanthurenic acid, KA.

[1]Consists of pregnancies where the mother reported ASM use, but no ASM was detected in plasma samples.

[2]Based on sum of mTHF and hmTHF.

[3]Ratio between active and inactive folate metabolites; mTHF + hmTHF: pABG + apABG.

[4]Ratio between UMFA and mTHF + hmTHF.

[5]Ratio between HK: (KA + XA + AA + HAA). This is a functional marker of vitamin B6 status; a high ratio shows an inverse association to the concentration of PLP.

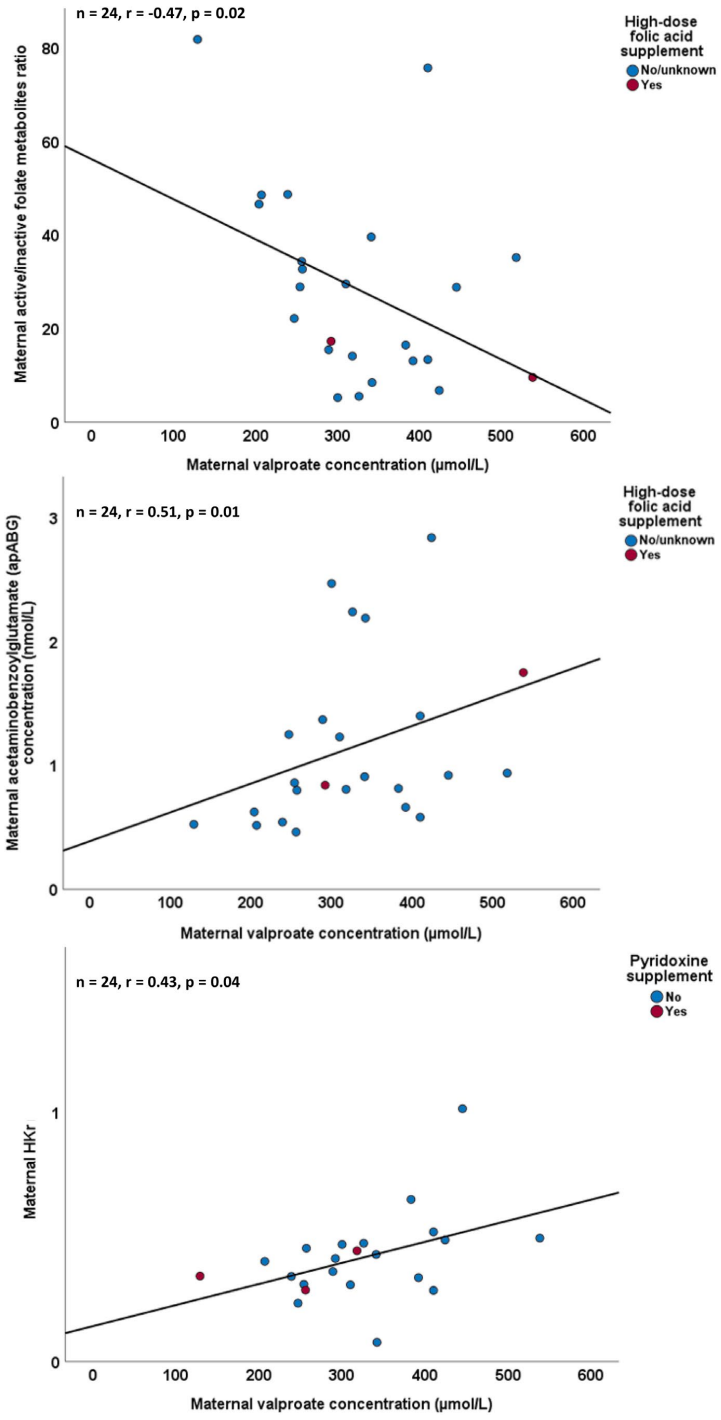

FIGURE 3 Correlation between maternal valproate concentrations and concentrations of vitamin B metabolites. The figure shows the correlation between maternal valproate concentrations in monotherapy users and vitamin B metabolites. Blue dots represent no supplement or no or unknown folic acid supplement dose; red dots represent supplemented pregnancies or high-dose folic acid supplement; HKr, marker of pyridoxine deficiency; r, Spearman's rho; p, *p*-value.

TABLE 3 Nonfolate vitamin B status stratified for antiseizure medication (ASM) treatment and supplement use in gestational weeks −4 to 20

	Supplement	Valproate	Lamotrigine	Carbamazepine	Levetiracetam	Topiramate	Oxcarbazepine	Polytherapy	Low-adherence group[5]
n riboflavin status[1]		n = 3/19	n = 30/33	n = 9/38	n = 3/6	n = 4/3	n = 2/3	n = 12/21	n = 6/18
Riboflavin, nmol/L *median (range)*	***Riboflavin***	22.6 (5.5)[f]	12.7 (97.6)[f]	8.8 (94.7)[f]	12.1 (56.3)	7.6 (19.6)	13.6 (17.6)	9.3 (14.6)[f]	9.7 (8.8)
	No riboflavin	5.5 (18.1)[f]	6.9 (20.6)[f]	6.0 (44.0)[f]	8.2 (14.6)	7.9 (7.3)	6.4 (14.4)	5.7 (46.2)[f]	6.9 (53.0)
n pyridoxine status[1]		n = 3/19	n = 32/31	n = 10/37	n = 3/6	n = 4/3	n = 2/3	n = 13/20	n = 7/17
PLP, nmol/L HKr[4] *median (range)*	***Pyridoxine***	51.1 (12.3)[f] 0.3 (0.2)	46.8 (129.3)[f] 0.3 (0.6)[f]	33.3 (76.9)[f] 0.3 (0.1)	57.7 (87.6)[f] 0.2 (0.1)	42.7 (45.0) 0.3 (0.3)	46.8 (60.9) 0.2 (0.0)	33.0 (103.6)[f] 0.3 (0.6)	43.8 (51.1) 0.3 (0.3)
	No pyridoxine	24.5 (36.8)[f] **0.4 (0.9)[a]**	24.1 (33.6)[f] **0.3 (0.5)[f]**	20.9 (45.0)[f] **0.3 (0.5)**	22.4 (18.6)[f] **0.3 (0.2)**	26.0 (39.4) **0.3 (0.2)**	27.8 (5.3) **0.2 (0.2)**	23.5 (40.7)[f] **0.3 (0.7)**	25.1 (138.9) **0.3 (0.4)**
n niacin status[1]		n = 2/20	n = 17/46	n = 5/42	n = 2/7	n = 3/4	n = 2/3	n = 7/26	n = 4/20
Nicotinamide, nmol/L *median (range)*	***Nicotinamide***	706.0 (11.2)	342.7 (1092.7)	185.7 (460.1)	529.4 (127.6)	416.6 (474.5)	215.2 (125.1)	432.9 (1099.0)	309.1 (442.4)
	No nicotinamide	420.7 (1058.5)	337.7 (882.3)	377.7 (1652.3)	499.6 (501.2)	219.1 (114.8)	694.3 (1063.6)	376.5 (795.1)	434.5 (1498.1)

Note: .: All ASM groups are compared with each other by using Kruskal-Wallis test stratified for supplement use. Groups that differ significantly (p-value <0.05) from the group marked with an [a] are marked with bold text, additional groups that significantly differ from each other are both marked with [b],[c],[d], and [e]. Significant differences after Bonferroni correction for multiple tests are underlined. Statistically significant (two-sided p-values <0.05) differences in concentrations between the two supplement groups stratified for ASM group by using Mann-Whitney U test are marked with [f].

Abbreviations: 3-hydroxyanthranilic acid, N may vary slightly due to missing data: supplemented pregnancies, n = 3 (carbamazepine n = 1, polytherapy n = 2); anthranilic acid, HAA; antiseizure medication, PLP; ASM; kynurenic acid, AA; non-supplemented pregnancies, n = 8 (lamotrigine n = 1, levetiracetam n = 2, polytherapy n = 5), Six pregnancies are not included in the table due to missing supplement data; pyridoxal-5-phosphate, HK;3-hydroxykynurenine, XA; xanthurenic acid, KA.

[1]Number of pregnancies for each supplement category (supplement/no supplement).

[2]Based on sum of mTHF and hmTHF.

[3]Ratio between active and inactive folate metabolites; mTHF + hmTHF: pABG + apABG.

[4]Ratio between HK: (KA + XA + AA + HAA). This is a functional marker of vitamin B6 status; a high ratio shows low functional PLP status.

[5]Consists of pregnancies where the mother reported ASM use, but no ASM was detected in plasma samples.

pregnancy has been associated with a higher IQ in children of mothers taking lamotrigine or carbamazepine treatment particularly.[38,39] In a study of pregnant women with epilepsy and levetiracetam or lamotrigine treatment, low concentrations of biologically active folate (mTHF) were found with lamotrigine, but folic acid supplement status was not reported.[22] Lamotrigine use was furthermore associated with changes in one-carbon metabolism, with altered pathways involving folate, purine, and sulfur amino acid metabolism.[22] Associations between ASM polytherapy and low folate concentrations, and high phenytoin and phenobarbital concentrations and low folate concentrations have been reported in another study in pregnant women with epilepsy.[21] Possibly, lamotrigine and carbamazepine particularly influence one-carbon metabolism in pregnancy, but concentrations of folate were not clearly different between the ASM groups in our study. Longitudinal studies with multiple sampling before and during pregnancy, and with different folic acid substitution regimens, are needed to fully understand the interplay between ASM, plasma folate status, and supplement use during pregnancy.

We found higher concentrations of inactive folate metabolites and a lower ratio between active and inactive folate metabolites in women using valproate, carbamazepine, and ASM polytherapy, compared to several of the other ASM groups. Chronic high-dose folic acid supplement use could partly explain higher concentrations of inactive folate metabolites, probably inducing an increase in folate catabolism. However, both high-dose use and any use of folic acid supplement were widespread across the ASM groups. Furthermore, there were no differences in ASM concentrations among high-dose users compared to low-dose users. Supplement use could therefore not fully explain the correlation between high ASM concentrations and high inactive folate metabolites. It is possible that valproate, carbamazepine, and ASM polytherapy use increase folate catabolism to a larger degree than other ASMs. In addition to ASMs, also pregnancy itself and chronic folic acid supplementation may influence folate catabolism.[10]

High-dose folic acid supplement users had high concentrations of UMFA. However, studies in both nonpregnant[40] and pregnant[34,41] women from the general population found that the UMFA concentration does not depend solely on the intake of folic acid, and suggested that there are mechanisms by which the body adapts to high supplement intake, thus limiting high plasma concentrations of UMFA. High ASM concentrations correlated with high UMFA concentrations in our study, and this indicates that these mechanisms may be influenced by ASM use. The optimal folic acid dose for women with epilepsy is not known,[4,42] and the safety of high supplement doses has been questioned.[12–15] Animal studies have reported adverse effects of high UMFA concentrations on genetic programming and neuronal development.[14,15] One study found an association between higher concentrations of cord blood UMFA and increased risk of autism spectrum disorder in some population groups in the United States.[16] The large range and high maximum UMFA concentrations in this study may point to a closer monitoring of folate status during pregnancy in women with epilepsy on ASMs in order to avoid unnecessary high folic acid doses.

We found an association between high ASM concentrations and low riboflavin and pyridoxine status. The findings persisted after excluding women with supplement use of these vitamins in a sensitivity analysis. Low riboflavin concentrations have been reported in patients using carbamazepine, phenobarbital, phenytoin, and primidone in nonpregnant epilepsy populations.[18] Low pyridoxine concentrations during ASM use have also been reported, but less consistently.[5,18,43] Few studies have examined the association between ASM and nonfolate B vitamins in pregnant women with epilepsy. Adequate riboflavin and pyridoxine status is important for optimal folate metabolic function in one-carbon metabolism, and for normal fetal brain development during pregnancy.[19] We found no association between ASM and niacin status, the only vitamin in our study not participating in one-carbon metabolism.[19] Our findings show an association between ASM and riboflavin and pyridoxine status. The benefit of multivitamin B supplements in women with epilepsy planning and undergoing pregnancy should be further investigated.

Strengths of our study include a validated epilepsy diagnosis, prospectively collected data on supplement use, and analyses of plasma ASMs and vitamin and metabolite concentrations in a large sample of pregnancies. Women with epilepsy in MoBa are representative of women with epilepsy in Norway.[25] Multivitamin and folic acid supplement users are overrepresented in MoBa, whereas smokers are underrepresented.[44] Hence, we assume that women included in this study had a healthier lifestyle than women refusing inclusion, and a better vitamin B status. This would bias our results towards the null. Limitations of the study include plasma concentrations being measured only once during the pregnancy in the second trimester, a limited numbers of pregnancies in the ASM groups with the exception of valproate, lamotrigine, and carbamazepine, and self-reported information on vitamin supplement use. Genetic factors influence vitamin concentrations, but we did not have access to genetic information. We did not adjust for multiple pregnancies in the same mother. Only 22 mothers contributed with more than one pregnancy. The variance in vitamin and metabolite concentrations explained by ASM concentrations was low in some of our analyses, illustrating that factors other

than ASM treatment also influence the vitamin status during pregnancy. We presented standardized maternal ASM concentrations in addition to the individual ASM concentrations. By using relative plasma concentrations, we adjusted for differences in pharmacokinetics between the ASMs. Even though different ASMs have different pharmacological mechanisms, the majority of the ASMs have been associated with low folate status and their direct mechanism of action related to vitamin B status could share common pathways.[5–8] The folic acid dose data were collected retrospectively and were not available for the entire cohort.

In conclusion, we found important associations between plasma ASM concentrations and maternal folate status in pregnant women with epilepsy. Interactions between ASM and folate metabolic pathways, the pregnancy itself, and use of high folic acid doses may explain these findings. Furthermore, we found an association between ASM concentrations and low riboflavin and pyridoxine status. Optimal concentrations of both these vitamins are required for normal folate metabolic functioning and are thus essential for normal fetal development. Our findings provide new information regarding the association between ASM and vitamin B status during pregnancy. Additional studies are needed to determine the clinical impact of these findings, and to define the optimal vitamin doses in pregnancy.

ACKNOWLEDGMENTS

The Norwegian Mother, Father and Child Cohort Study is supported by the Norwegian Ministry of Health and Care Services and the Ministry of Education and Research. We are grateful to all the participating families in Norway who take part in this ongoing cohort study. Ragnhild Lervik, Hege Marit Nyberg, and Åshild Wæhre (all bioengineers at St. Olav University Hospital, Trondheim, Norway) are acknowledged for their excellent laboratory assistance. Gyri Veiby (MD, PhD, Department of Neurology, Haukeland University Hospital, Bergen, Norway) is acknowledged for contributing to data acquisition. We acknowledge the following sources of support: Norwegian Epilepsy Foundation, Norwegian Chapter International League Against Epilepsy, *Det alminnelige medisinske forskningsfond ved Universitetet i Bergen*, *Dr. Nils Henrichsen og hustru Anna Henrichsens legat*, and *Advokat Rolf Sandberg Reberg og Ellen Marie Rebergs Legat til Epilepsiforskning*. The sources of funding had no role in the design of the study or the interpretation of the results.

CONFLICTS OF INTEREST

E. S. N. Husebye reports no disclosures relevant to the manuscript. B. Riedel reports no disclosures relevant to the manuscript. A.-L. Bjørke-Monsen reports no disclosures relevant to the manuscript. O. Spigset reports no disclosures relevant to the manuscript. A. K. Daltveit has received project funding from Pfizer Inc. N. E. Gilhus reports no disclosures relevant to the manuscript. Marte Helene Bjørk has received speaking and/or consultant honoraria from Novartis, Teva, Eisai, and Lilly, and project funding from Novartis unrelated to the present work.

AUTHOR CONTRIBUTIONS

Elisabeth Synnøve and Nilsen Husebye: designed and conceptualized study, analyzed the data, interpreted the data, drafted and revised the manuscript, and assisted with funding. Bettina Riedel: designed and conceptualized study, provided statistical advice, interpreted the data, and provided critical revision of manuscript. Anne-Lise Bjørke-Monsen: designed and conceptualized study, provided statistical advice, interpreted the data, and provided critical revision of manuscript. Olav Spigset: had a major role in the acquisition of data, interpreted the data, and provided critical revision of manuscript. Anne Kjersti Daltveit: provided methodological advice and critical revision of manuscript. Nils Erik Gilhus: had a major role in the acquisition of data, interpreted the data, provided critical revision of manuscript, and assisted with funding. Marte Helene Bjørk: had a major role in the acquisition of data, designed and conceptualized study, provided statistical advice, interpretation of data, and provided critical revision of manuscript and study supervision, and assisted with funding.

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REFERENCES

1. Harden Cynthia L. Pregnancy and Epilepsy. CONTINUUM: Lifelong Learning in Neurology. 2014;20(1):60–79. https://doi.org/10.1212/01.CON.0000443837.95260.af
2. Kellogg M, Meador KJ. Neurodevelopmental Effects of Antiepileptic Drugs. Neurochem Res. 2017;42:2065–70.
3. Whelehan A, Delanty N. Therapeutic strategies for treating epilepsy during pregnancy. Expert Opin Pharmacother. 2019;20:323–32.
4. Tomson T, Battino D, Bromley R, Kochen S, Meador K, Pennell P, et al. Management of epilepsy in pregnancy: a report from the International League Against Epilepsy Task Force on Women and Pregnancy. Epileptic Disord. 2019;21:497–517.
5. Belcastro V, Striano P. Antiepileptic drugs, hyperhomocysteinemia and B-vitamins supplementation in patients with epilepsy. Epilepsy Res. 2012;102:1–7.
6. Belcastro V, Striano P, Gorgone G, Costa C, Ciampa C, Caccamo D, et al. Hyperhomocysteinemia in epileptic patients on new antiepileptic drugs. Epilepsia. 2010;51:274–9.
7. Linnebank M, Moskau S, Semmler A, Widman G, Stoffel-Wagner B, Weller M, et al. Antiepileptic drugs interact with folate and vitamin B12 serum levels. Ann Neurol. 2011;69:352–9.

8. Ni G, Qin J, Li H, Chen Z, Zhou Y, Fang Z, et al. Effects of antiepileptic drug monotherapy on one-carbon metabolism and DNA methylation in patients with epilepsy. PLoS One. 2015;10:e0125656.
9. McGarel C, Pentieva K, Strain JJ, McNulty H. Emerging roles for folate and related B-vitamins in brain health across the lifecycle. Proc Nutr Soc. 2015;74:46–55.
10. Suh JR, Herbig AK, Stover PJ. New perspectives on folate catabolism. Annu Rev Nutr. 2001;21:255–82.
11. Tomson T, Battino D, Bromley R, Kochen S, Meador KJ, Pennell PB, et al. Global Survey of Guidelines for the Management of Epilepsy in Pregnancy: A report from the International League Against Epilepsy Task Force on Women and Pregnancy. Epilepsia Open. 2020;5:366–70.
12. Dolin CD, Deierlein AL, Evans MI. Folic Acid Supplementation to Prevent Recurrent Neural Tube Defects: 4 Milligrams Is Too Much. Fetal Diagn Ther. 2018;44:161–5.
13. Li K, Wahlqvist ML, Li D. Nutrition, One-Carbon Metabolism and Neural Tube Defects: A Review. Nutrients. 2016;8(11):741–https://doi.org/10.3390/nu8110741
14. Murray LK, Smith MJ, Jadavji NM. Maternal oversupplementation with folic acid and its impact on neurodevelopment of offspring. Nutr Rev. 2018;76:708–21.
15. Wiens D, DeSoto MC. Is High Folic Acid Intake a Risk Factor for Autism?-A Review. Brain Sci. 2017;7(12):149.
16. Raghavan R, Selhub J, Paul L, Ji Y, Wang G, Hong X, et al. A prospective birth cohort study on cord blood folate subtypes and risk of autism spectrum disorder. Am J Clin Nutr. 2020;112(5):1304–1317.
17. Apeland T, Mansoor MA, Pentieva K, McNulty H, Seljeflot I, Strandjord RE. The effect of B-vitamins on hyperhomocysteinemia in patients on antiepileptic drugs. Epilepsy Res. 2002;51:237–47.
18. Apeland T, Mansoor MA, Pentieva K, McNulty H, Strandjord RE. Fasting and post-methionine loading concentrations of homocysteine, vitamin B2, and vitamin B6 in patients on antiepileptic drugs. Clin Chem. 2003;49:1005–8.
19. McNulty H, Ward M, Hoey L, Hughes CF, Pentieva K. Addressing optimal folate and related B-vitamin status through the lifecycle: health impacts and challenges. Proc Nutr Soc. 2019;78(3):449–462.
20. Gasperi V, Sibilano M, Savini I, Catani MV. Niacin in the Central Nervous System: An Update of Biological Aspects and Clinical Applications. Int J Mol Sci. 2019;20(4):974.
21. Dansky LV, Andermann E, Rosenblatt D, Sherwin AL, Andermann F. Anticonvulsants, folate levels, and pregnancy outcome: a prospective study. Ann Neurol. 1987;21:176–82.
22. Walker DI, Perry-Walker K, Finnell RH, Pennell KD, Tran V, May RC, et al. Metabolome-wide association study of antiepileptic drug treatment during pregnancy. Toxicol Appl Pharmacol. 2019;363:122–30.
23. Magnus P, Birke C, Vejrup K, Haugan A, Alsaker E, Daltveit AK, et al. Cohort Profile Update: The Norwegian Mother and Child Cohort Study (MoBa). Int J Epidemiol. 2016;45:382–8.
24. Paltiel L, Haugan A, Skjerden T, Harbak K, Bækken S, Stensrud NK, et al. The biobank of the Norwegian Mother and Child Cohort Study - present status. The Norwegian Journal of Epidemiology. 2014;24(1–2):29–35.
25. Bjork MH, Veiby G, Spigset O, Gilhus NE. Using the Norwegian Mother and Child Cohort Study to determine risk factors for delayed development and neuropsychiatric symptoms in the offspring of parents with epilepsy. The Norwegian Journal of Epidemiology. 2014;24:79–89.
26. Husebye ESN, Gilhus NE, Riedel B, Spigset O, Daltveit AK, Bjork MH. Verbal abilities in children of mothers with epilepsy: Association to maternal folate status. Neurology. 2018;91:e811–21.
27. Husebye ESN, Gilhus NE, Spigset O, Daltveit AK, Bjørk MH. Language impairment in children aged 5 and 8 years after antiepileptic drug exposure in utero - the Norwegian Mother and Child Cohort Study. Eur J Neurol. 2020;27:667–75.
28. Bjorke-Monsen AL, Roth C, Magnus P, Midttun O, Nilsen RM, Reichborn-Kjennerud T, et al. Maternal B vitamin status in pregnancy week 18 according to reported use of folic acid supplements. Mol Nutr Food Res. 2013;57:645–52.
29. Roth C, Bjorke-Monsen AL, Reichborn-Kjennerud T, Nilsen RM, Smith GD, Stoltenberg C, et al. Use of folic acid supplements in early pregnancy in relation to maternal plasma levels in week 18 of pregnancy. Mol Nutr Food Res. 2013;57:653–60.
30. Meador KJ, Baker GA, Browning N, Cohen MJ, Clayton-Smith J, Kalayjian LA, et al. Foetal antiepileptic drug exposure and verbal versus non-verbal abilities at three years of age. Brain. 2011;134:396–404.
31. Hannisdal R, Ueland PM, Svardal A. Liquid chromatography-tandem mass spectrometry analysis of folate and folate catabolites in human serum. Clin Chem. 2009;55:1147–54.
32. Hannisdal R, Ueland PM, Eussen SJ, Svardal A, Hustad S. Analytical recovery of folate degradation products formed in human serum and plasma at room temperature. J Nutr. 2009;139:1415–8.
33. Bjork M, Riedel B, Spigset O, Veiby G, Kolstad E, Daltveit AK, et al. Association of Folic Acid Supplementation During Pregnancy With the Risk of Autistic Traits in Children Exposed to Antiepileptic Drugs In Utero. JAMA Neurol. 2018;75:160–8.
34. Plumptre L, Masih SP, Ly A, Aufreiter S, Sohn KJ, Croxford R, et al. High concentrations of folate and unmetabolized folic acid in a cohort of pregnant Canadian women and umbilical cord blood. Am J Clin Nutr. 2015;102:848–57.
35. Hustad S, McKinley MC, McNulty H, Schneede J, Strain JJ, Scott JM, et al. Riboflavin, flavin mononucleotide, and flavin adenine dinucleotide in human plasma and erythrocytes at baseline and after low-dose riboflavin supplementation. Clin Chem. 2002;48:1571–7.
36. Midttun O, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom. 2009;23:1371–9.
37. Ulvik A, Midttun Ø, McCann A, Meyer K, Tell G, Nygård O, et al. Tryptophan catabolites as metabolic markers of vitamin B-6 status evaluated in cohorts of healthy adults and cardiovascular patients. Am J Clin Nutr. 2020;111:178–86.
38. Meador KJ, Baker GA, Browning N, Cohen MJ, Bromley RL, Clayton-Smith J, et al. Fetal antiepileptic drug exposure and cognitive outcomes at age 6 years (NEAD study): a prospective observational study. Lancet Neurol. 2013;12:244–52.
39. Meador KJ, Pennell PB, May RC, Brown CA, Baker G, Bromley R, et al. Effects of periconceptional folate on cognition in

children of women with epilepsy: NEAD study. Neurology. 2020;94:e729–40.
40. Tam C, O'Connor D, Koren G. Circulating unmetabolized folic Acid: relationship to folate status and effect of supplementation. Obstet Gynecol Int. 2012;2012:485179.
41. Obeid R, Kasoha M, Kirsch SH, Munz W, Herrmann W. Concentrations of unmetabolized folic acid and primary folate forms in pregnant women at delivery and in umbilical cord blood. Am J Clin Nutr. 2010;92:1416–22.
42. Asadi-Pooya AA. High dose folic acid supplementation in women with epilepsy: are we sure it is safe? Seizure. 2015;27:51–3.
43. Sener U, Zorlu Y, Karaguzel O, Ozdamar O, Coker I, Topbas M. Effects of common anti-epileptic drug monotherapy on serum levels of homocysteine, vitamin B12, folic acid and vitamin B6. Seizure. 2006;15:79–85.
44. Nilsen RM, Vollset SE, Gjessing HK, Skjaerven R, Melve KK, Schreuder P, et al. Self-selection and bias in a large prospective pregnancy cohort in Norway. Paediatr Perinat Epidemiol. 2009;23:597–608.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Husebye ESN, Riedel B, Bjørke-Monsen A-L, Spigset O, Daltveit AK, Gilhus NE, et al. Vitamin B status and association with antiseizure medication in pregnant women with epilepsy. Epilepsia. 2021;62:2968–2980. https://doi.org/10.1111/epi.17076

Supplemental data

Contents

Table S1: Antiseizure medication (ASM) overview

Overview of antiseizure medication (ASM) use (n = 227).

Antiseizure medication (ASM)	Comments	Number of pregnancies
Valproate monotherapy	24 pregnancies: ASM analyzed and detected, 8 pregnancies[1]: ASM analyzed but not detected	32
Lamotrigine monotherapy	65 pregnancies: ASM analyzed and detected, 6 pregnancies[1]: ASM analyzed but not detected	71
Carbamazepine monotherapy	48 pregnancies: ASM analyzed and detected, 4 pregnancies[1]: ASM analyzed but not detected	52
Levetiracetam monotherapy	11 pregnancies: ASM analyzed and detected, 2 pregnancies[1]: ASM analyzed but not detected	13
Topiramate monotherapy	8 pregnancies: ASM analyzed and detected, 2 pregnancies[1]: ASM analyzed but not detected	10
Oxcarbazepine monotherapy	ASM analyzed and detected	5
Polytherapy including valproate	6 pregnancies: all ASMs analyzed and detected, 2 pregnancies: some of the ASMs analyzed and detected, 3 pregnancies[1]: none of the ASMs detected	11
Polytherapy including lamotrigine	8 pregnancies: all ASMs analyzed and detected, 10 pregnancies: some of the ASMs analyzed and detected	18
Polytherapy including levetiracetam	2 pregnancies: all ASMs analyzed and detected, 2 pregnancies: some of the ASMs analyzed and detected, 1 pregnancy[1]: none of the ASMs detected	5
Polytherapy including topiramate	1 pregnancy: all ASMs analyzed and detected, 4 pregnancies: some of the ASMs analyzed and detected	5
Polytherapy with other ASMs	3 pregnancies: some of the ASMs analyzed and detected, 2 pregnancies: none of the ASMs were analyzed	5

[1] Pregnancies included in the low-adherence group (n = 26)

Table S2: Spearman correlation coefficients between vitamin and metabolite concentrations and antiseizure medication (ASM) concentrations, supplement to figure 3 and 4

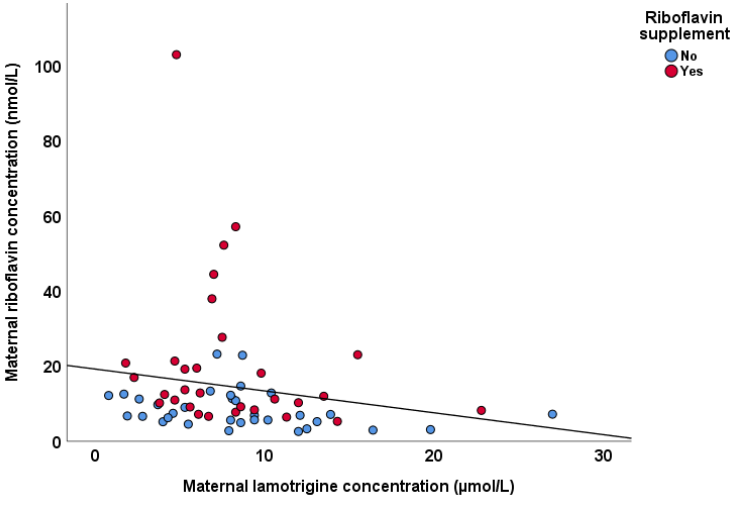
Spearman correlation coefficients between vitamin B concentrations (nmol/L) and ASM concentrations (µmol/L). Statistically significant results are in bold numbers, and presented with scatter plots below and in figure 3 and 4. Sensitivity analyses were performed by excluding high-dose folic acid supplement users (maternal folate status, n = 31 for monotherapy, n = 8 for polytherapy, and n = 39 for any ASM), riboflavin supplement users (maternal vitamin B2 status, n = 56 for monotherapy, n = 16 for polytherapy, and n = 72 for any ASM), niacin supplement users (maternal vitamin B3 status, n = 34 for monotherapy, n = 11 for polytherapy, and n = 45 for any ASM) and pyridoxine supplement users (maternal vitamin B6 status, n = 60 for monotherapy, n = 17 for polytherapy, and n =77 for any ASM) from the analyses. Significant results after sensitivity analyses are marked with an asterisk.

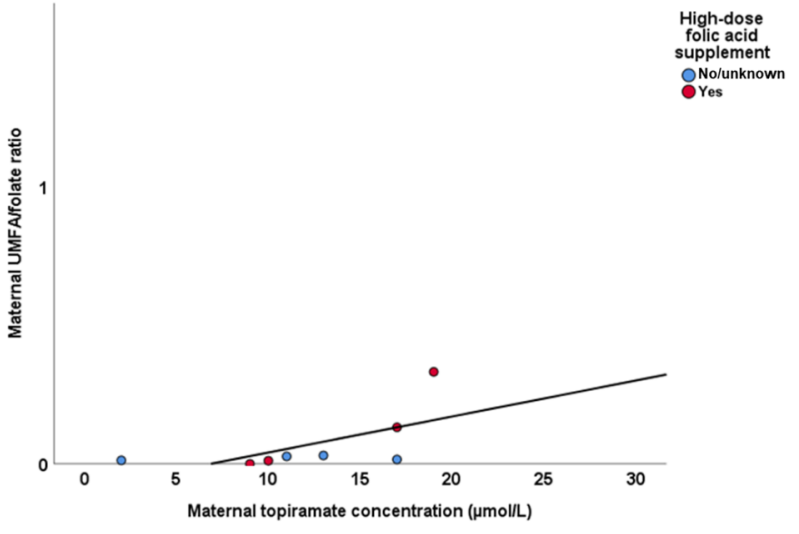
		All ASM[1] **n = 199**	**Valproate** **n = 24**	**Lamotrigine** **n = 65**	**Carbamazepine** **n = 48**	**Levetiracetam** **n = 11**	**Topiramate** **n = 8**	**Oxcarbazepine** **n = 5**	**Polytherapy[1]** **n = 38**
Plasma folate status									
Folate[2]	rho[6]	0.06	0.13	-0.03	0.20	-0.04	0.56	-0.30	0.12
	p-value	0.41	0.55	0.84	0.17	0.91	0.15	0.62	0.49
pABG	rho[6]	**0.23**	0.33	0.06	0.003	-0.19	0.55	-0.30	0.27
	p-value	**0.001***	0.12	0.61	0.99	0.57	0.16	0.62	0.10
apABG	rho[6]	**0.25**	**0.51**	-0.05	-0.04	0.06	-0.01	-0.30	0.16
	p-value	**<0.001***	**0.01***	0.70	0.77	0.87	0.98	0.62	0.33

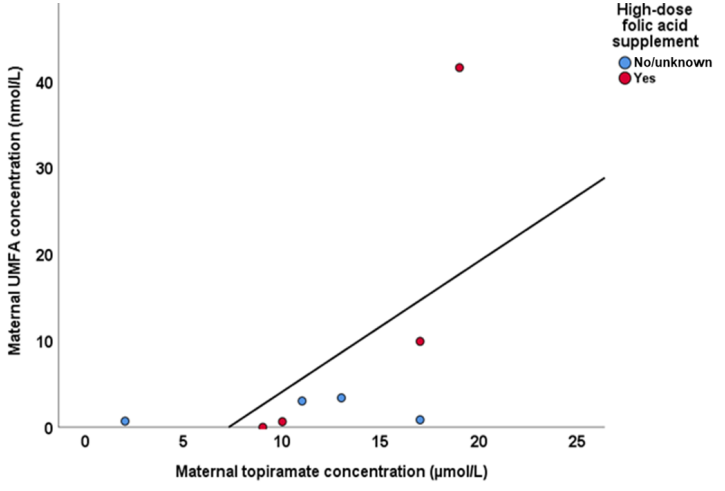
Ratio active/inactive metabolites[3]		**-0.29** **<0.001***	**-0.47** **0.02**	-0.13 0.31	0.13 0.36	0.17 0.62	-0.13 0.76	-0.30 0.62	-0.22 0.19
UMFA		**0.22** **0.002***	0.06 0.79	0.15 0.24	0.04 0.81	-0.01 0.97	**0.83** **0.01**	-0.15 0.81	0.22 0.19
Ratio UMFA/folate[4]		**0.23** **0.001***	0.07 0.76	0.17 0.17	-0.02 0.90	-0.01 0.97	**0.83** **0.01**	0.05 0.94	0.20 0.23
Plasma riboflavin status									
Riboflavin	rho[6] p-value	**-0.32** **<0.001***	-0.40 0.06	**-0.27** **0.03**	-0.01 0.93	-0.23 0.55	-0.23 0.59	-0.30 0.62	-0.14 0.47
Plasma pyridoxine status									
PLP (nmol/L)	rho[6] p-value	**-0.19** **0.01**	-0.31 0.14	-0.003 0.98	-0.11 0.47	0.33 0.38	-0.01 0.98	-0.40 0.51	-0.04 0.83
HKr[5]		**0.15** **0.04**	**0.43** **0.04***	0.05 0.72	0.25 0.09	0.23 0.55	0.18 0.67	0.10 0.87	-0.05 0.79
Plasma niacin status									
Nicotinamide	rho[6] p-value	0.05 0.48	-0.04 0.87	0.22 0.08	-0.17 0.26	0.07 0.87	0.35 0.40	-0.70 0.19	0.12 0.51

ASM = antiseizure medication. mTHF = 5-methyltetrahydrofolate. hmTHF = 4-alfa-hydroxy-5-methyltetrahydrofolate. pABG = para-aminobenzoylglutamate. apABG = acetamidobenzoylglutamate. UMFA = unmetabolized folic acid. PLP = pyridoxal-5-phosphate. HK = 3-hydroxykynurenine. XA = xanthurenic acid. KA = kynurenic acid. AA = anthranilic acid. HAA= 3-hydroxyanthranilic acid. Data were missing for plasma riboflavin status, plasma niacin status and plasma pyridoxine status in the following ASM treatment groups: any ASM (n = 11), lamotrigine (n = 1), carbamazepine (n = 1), levetiracetam (n = 2), polytherapy (n = 7).									

[1] Based on standardized ASM concentrations, see text [2] Based on sum of mTHF and hmTHF [3] Ratio between active and inactive folate metabolites: mTHF + hmTHF : pABG + apABG [4] Ratio between UMFA : mTHF + hmTHF [5] This is a functional marker of vitamin B6 status; a high concentration shows an inverse association to the concentration of PLP [6] Ratio between HK : (KA + XA + AA+ HAA). This is a functional marker of vitamin B6 status; a high ratio shows an inverse association to the concentration of PLP [6] Spearman's rho






High-dose folic acid supplement
No/unknown
Yes
Maternal UMFA concentration (nmol/L)
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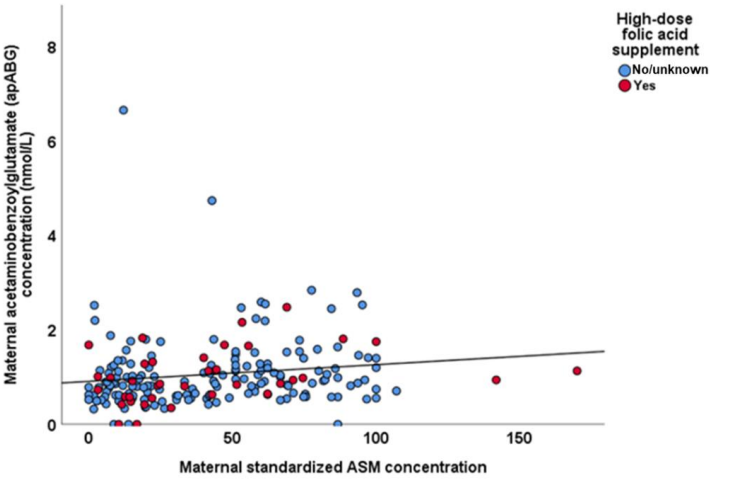
10
20
30
40
0
5
10
15
20
25
Maternal topiramate concentration (µmol/L)


High-dose folic acid supplement
No/unknown
Yes
Maternal acetaminobenzoylglutamate (apABG) concentration (nmol/L)
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2
4
6
8
0
50
100
150
Maternal standardized ASM concentration

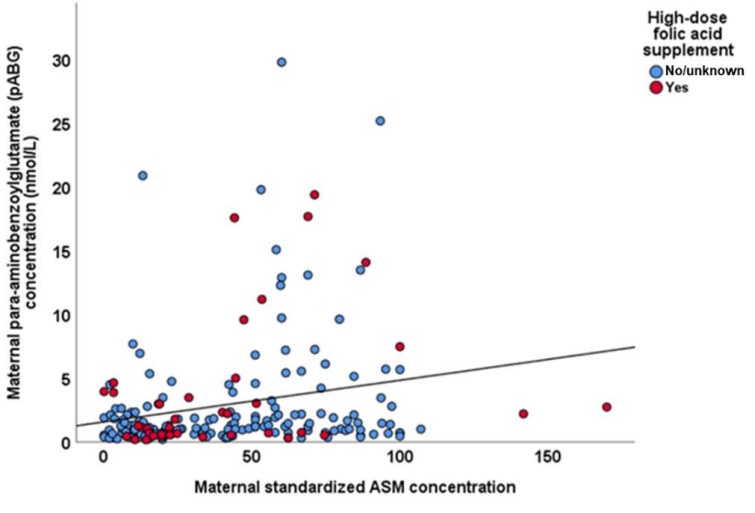

High-dose folic acid supplement
No/unknown
Yes
Maternal para-aminobenzoylglutamate (pABG) concentration (nmol/L)
0
5
10
15
20
25
30
0
50
100
150
Maternal standardized ASM concentration

Figure S1: Correlation between maternal folate concentration and unmetabolized folic acid (UMFA) concentrations, and association with folic acid dose during pregnancy

The figure shows the correlation between maternal folate concentrations and unmetabolized folic acid (UMFA) concentrations in gestational weeks 17-19, and the associations (Mann-Whitney U Test) between concentrations of UMFA and folate and high dose and low dose folic acid supplement use, presented as box plots. UMFA = unmetabolized folic acid. r = Spearman's rho. p = p-value.

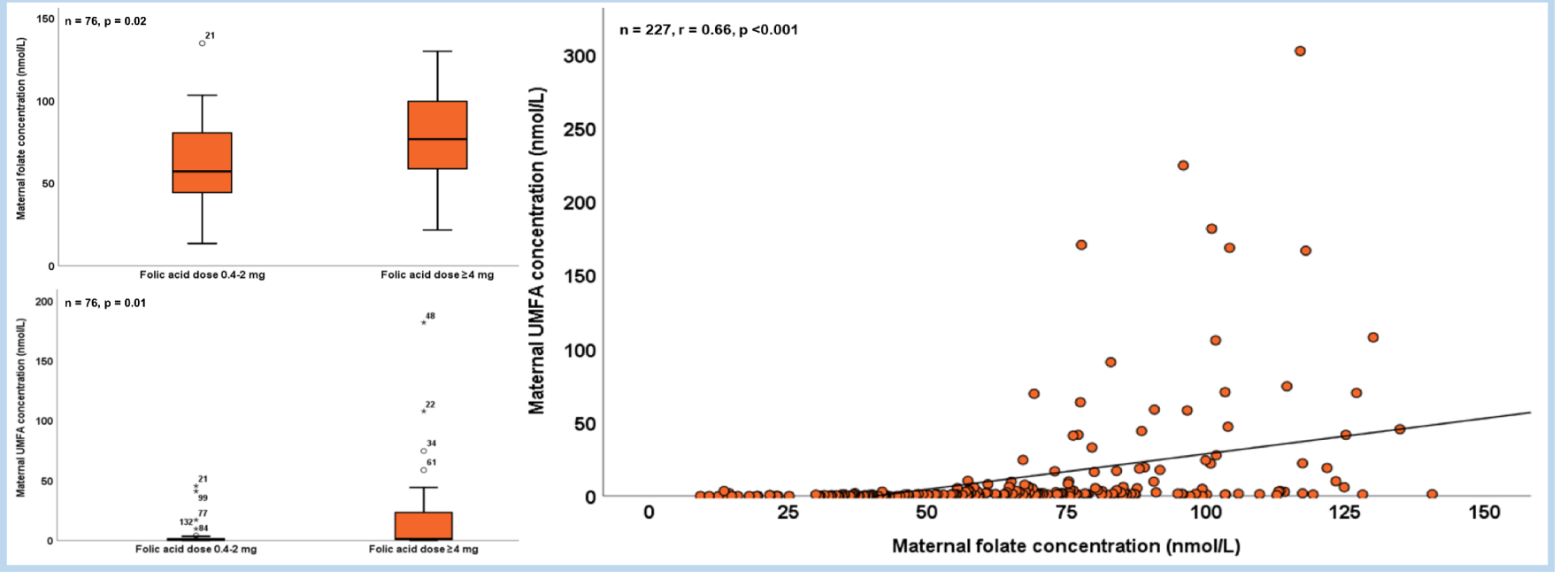
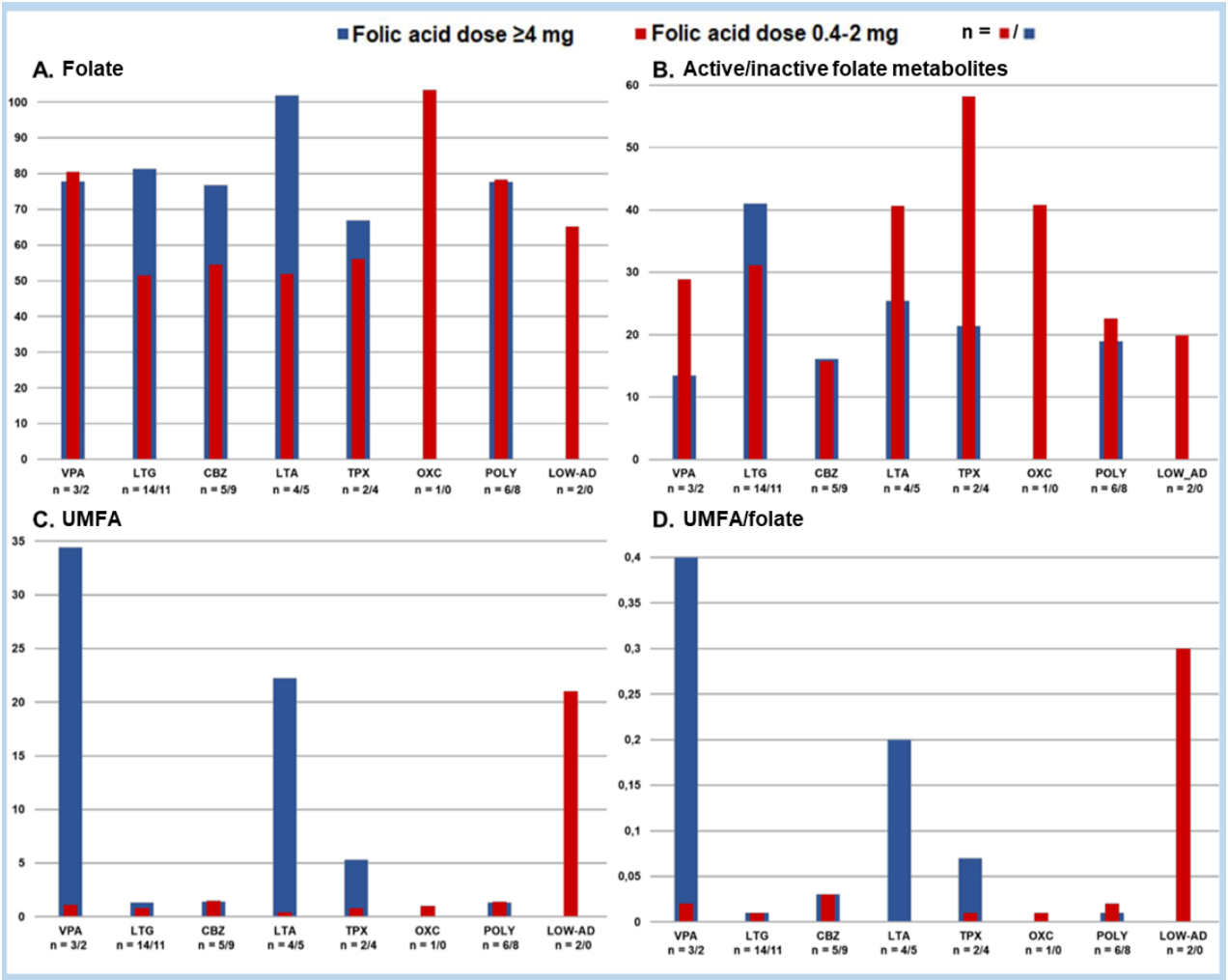


Figure S2: Maternal folate status stratified for antiseizure medication (ASM) and use of high dose (≥4mg) and low dose (0.4-2 mg) folic acid supplement during gestational week -4 to 24

The figure shows median maternal folate concentration (A), ratio between active and inactive folate metabolites (B), median unmetabolized folic acid (UMFA) concentration (C), and ratio between UMFA and folate (D) stratified for folic acid dose and ASM treatment. There were no significant differences in folate and folate metabolite concentrations between ASM groups stratified for folic acid dose (Kruskal-Wallis Test), or between high dose and low dose stratified for ASM group (Mann-Whitney U Test). VPA = valproate. LTG = lamotrigine. LTA = levetiracetam. TPX = topiramate. OXC = oxcarbazepine. POLY = polytherapy. LOW-AD = low-adherence group.



Graphic design: Communication Division, UiB / Print: Skipnes Kommunikasjon AS

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ISBN: 9788230850756 (print)
9788230852316 (PDF)