Using the Norwegian Mother and Child Cohort Study to determine risk factors for delayed development and neuro-psychiatric symptoms in the offspring of parents with epilepsy

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ABSTRACT

Introduction: Antiepileptic drug (AED) teratogenicity is suspected to be the main cause of impaired development in children of women with epilepsy. However, many factors may confound the reported risks. The purpose of this review is to characterize the epilepsy cohort in the Norwegian Mother and Child Cohort Study (MoBa) and show how it can be used to detangle various risk factors for adverse outcome in children of mothers with epilepsy.

Methods: MoBa is a large, long-term prospective, family-based cohort study. The database is linked to the Medical Birth Registry of Norway. The epilepsy cohort consists of mothers and their children representing more than 700 pregnancies. Blood samples were obtained from the mother during pregnancy and from the umbilical cord after delivery, and AED concentrations were measured. Validated screening tools determined the frequency of maternal confounding risk factors and adverse offspring outcomes. Risk estimates were reported as adjusted odds ratios with confidence intervals using the remaining MoBa cohort as a reference (n=107,597). Outcome in offspring of women with epilepsy without AED treatment in pregnancy and of fathers with epilepsy were used to separate the effect of epilepsy from the effect of *in utero* exposure to AEDs.

Results: Socioeconomic and psychiatric risk factors for adverse offspring outcomes were more frequent in mothers with epilepsy. The frequency of adverse offspring outcome was increased at 6, 18 and 36 months for verbal, motor and social development. Children of women with epilepsy without AED treatment and of fathers with epilepsy were generally similar to children of women without epilepsy.

Conclusion: Children of mothers with epilepsy are at risk of adverse outcomes. AED exposure emerges as the most important risk factor.

INTRODUCTION

Women with epilepsy account for 0.8% of all deliveries in Norway, and 0.3-0.5% of newborns have been exposed to antiepileptic drugs (AEDs) *in utero* (1). Although AED exposure increases the malformation risk (2,3), women with epilepsy are usually encouraged to continue their medication during pregnancy since severe epileptic seizures may cause fetal hypoxia and death, and also be harmful to the mother. Reportedly, 5% of maternal deaths during pregnancy are caused by epilepsy (4).

Recently, a possible association between fetal AED exposure and cognitive deficits and behavioral disorders has emerged (5). In utero AED exposure has been linked to lower IQ, delayed motor, verbal and social development, and neuropsychiatric disease such as autistic disorders and attention deficit hyperactive disorder (ADHD) (6-8). However, three major methodological challenges render the interpretation of these findings difficult. First, subjects with epilepsy may differ from the reference population socioeconomically and genetically. Second, the impact of epileptic seizures in pregnancy on the developing child is uncertain (9). Third, the relationship between the degree of AED exposure and the risk of adverse outcome is not well known. In most studies where maternal pre-pregnancy AED doses have been included, a dose-effect relationship has been demonstrated (6,10). However, due to pharmacokinetic variability, AED blood levels in the mother and fetus are not closely correlated to the reported dose (11-13). Hence, the degree of fetal AED exposure is unpredictable, even when dose information is available.

The Norwegian Mother and Child Cohort Study (MoBa) is well suited to target these three challenges. Prospective registration of a multitude of possible risk factors for adverse outcomes in the child has been included. Through validated screening tools (14-16), the woman is evaluated for psychiatric disorders. Hence, it

Table 1 Numbers of study subjects according to data file and publication

Datafile version	Publications	Pregnancies in mothers without epilepsy	Pregnancies in mothers with epilepsy	Mothers with epilepsy	Children by mothers with epilepsy	Children by fathers with epilepsy
V	Veiby et al (45, 46)	107 597[1]	711	634	726[3]	653[4]
VI	Reiter et al (21)	106 224	711	-	-	-
VII	Bjørk et al (23) Kolstad et al (22)	106 511	713 (706[2])	-	-	-

[1]Twins included
[2]Seven patients excluded due to denial of an diagnosis of epilepsy in the retrospective epilepsy questionaire
[3]333 exposed to antiepileptic drugs
[4] In 242 cases, the father used antiepileptic drugs

is possible to adjust for and evaluate the effect of numerous potential confounding factors. Most importantly, the population-based family-centered design of MoBa enables comparisons with a large, representative reference group, as well as with women with epilepsy not using AED treatment in pregnancy, and with fathers with epilepsy. It is thereby possible to partially adjust for the potential genetic and socioeconomic impact of epilepsy, isolating the *in utero* risk factors, e.g. AED exposure. Other large studies of *in utero* AED exposure often determine the effects on the developing child by comparing the offspring of women using different types of AEDs (3,6,7) or by using children of mothers without epilepsy as the only reference (17,18), possibly introducing systematic bias.

Furthermore, from material stored in the MoBa Biobank it is possible to measure AED concentrations and other factors possibly involved in AED-induced teratogenesis.

Investigating the effect of these potential risk factors on pregnancy complications and offspring outcomes may help to answer *why* some fetuses are vulnerable to unfavorable effects of AED exposure whilst others are protected. Studies targeting individual susceptibility and teratogenic mechanisms are lacking and highly called for (3). Such knowledge will help the clinician to identify women with a high risk and guide therapy decisions and pre-pregnancy counselling.

The purpose of this review is to describe pregnancies in women with epilepsy in the MoBa study and summarize findings from research published from this cohort so far, as well as to provide an overview of ongoing research. We will show how the MoBa study can be used to detangle various risk factors for adverse outcomes in children born to mothers with epilepsy and shed light on groups of mothers at particular risk.

MATERIAL AND METHODS

Subjects

The MoBa study is a prospective population-based cohort study conducted by the Norwegian Institute of Public Health (19). From 1999 to 2008 pregnant women were invited to participate. There were no exclusion criteria. A detailed study protocol is available at www.fhi.no/moba-en. The women consented to participation in 40.6% of the pregnancies. Follow-up is still ongoing and conducted by extensive questionnaires and sampling of biobank material. Information on maternal epilepsy, other somatic disorders, and medication reported by doctors and midwives is also available through a linkage to the mandatory Medical Birth Registry of Norway (MBRN) (20).

Our published studies are based on version V-VII of the quality-assured data files released for research on mothers with epilepsy. Information on the epilepsy diagnosis, AEDs and anti-depressive medication was obtained from the antenatal MoBa questionnaires Q1 (gestational weeks 17-19) and Q3 (gestational week 30) and the MBRN. The epilepsy cohort consisted of more than 700 pregnancies. The number of study subjects according to data file version and publication is given in Table 1.

Validation of the epilepsy cohort and clinical epilepsy variables

A questionnaire was sent to mothers with epilepsy in version VII of the data files (n=604) for validation of the epilepsy diagnosis and to retrospectively obtain supplementary information on pregnancy AED use, AED doses, and seizures during pregnancy. To further validate the diagnosis and medication use, hospital records of the MoBa mothers with epilepsy residing in Western Norway were examined (n=40).

Outcome variables

Psychosocial characteristics and psychiatric disease

We have investigated differences in the occurrence of low level of education (12 years or less), low income (199,999 NOK or less/yr, equal to 24 000 Euro), unemployment due to disability, single parenting, depression and/or anxiety from early pregnancy to 3 years after delivery, as well as eating disorders before and after pregnancy in in the MoBa cohort (21-23). Currently, we are investigating the impact of body weight increase during pregnancy on pregnancy complications in women with epilepsy, including overweight and obesity. We are also investigating socioeconomic factors and psychiatric disease in fathers with epilepsy, as well as quality of life parameters and relationship satisfaction in women with epilepsy.

Validated screening tools to assess psychiatric symptoms included short versions of the Hopkins Symptom Checklist (SCL-5 and SCL-8) (14,24,25), which is designed to detect depression and anxiety in population surveys, widely used, and sensitive to psychological distress in epilepsy (26-28). A mean score greater than 1.75 was defined as presence of depression and anxiety as this cut-off has been established for the longer version SCL-25 (27).

A history of major depression was assessed by the Life Time Major Depression Scale (29). Bulimia nervosa and binge eating disorder were diagnosed by questions based on the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) (30).

Child development

Assessment of child development was based on short versions of validated screening tools completed by the mothers at child ages 6, 18 and 36 months (31-44). The instruments measure whether the child has reached critical developmental milestones, or show signs of abnormal behavior. To improve the construct validity of these scores, we applied cut-off values distant (2 to 3 standard deviations) from the mean to detect children where true impairment was likely. For scales without predefined cut-offs, definition of abnormal outcomes was based on the 90-95 percentile for the 6-months-old children (45) and the 95-98 percentile for 18- and 36-months-old children (46). Previous MoBa studies using the applied screening instruments have shown good reliability (32,35,47). At 6 months of age social skills, fine and gross motor development and temperament were evaluated. At 18 and 36 months of age social skills, communication skills, gross and fine motor development, as well as autistic traits were assessed. At 36 months ADHD and aggressive symptoms were recorded as well. The screening tools are described in detail by Veiby et al. (45,46). The impact of breastfeeding on development in children of mothers with epilepsy according to AED use was also studied (45).

Biobank material

Blood was drawn from the mother at weeks 17-19 and from the umbilical cord immediately after birth. The MoBa Biobank extracts plasma and stores the samples (48). Analyses of carbamazepine, carbamazepine-10,11-epoxide, the oxcarbazepine monohydroxyderivative metabolite (MHD), lamotrigine, levetiracetam and topiramate were performed in 100 µl plasma with liquid chromatography mass spectrometry (LC-MS) methods developed at the Department of Clinical Pharmacology, St. Olav University Hospital, Trondheim, using an Agilent Technologies LC-MSD 1100-system (Agilent, Palo Alto, California, USA).

Carbamazepine, carbamazepine-10,11-epoxide and MHD were extracted by a liquid-liquid method and separated on a Zobrax SB-C18 (150 x 4.6 mm) column (Agilent), using carbamazepine-d_{10} as internal standard. The LC-MS was operated in the positive ionization mode, using mass transitions of *m/z* 237.1>194.1 for carbamazepine, 253.1>180.1 for carbamazepine-10,11-epoxide, 237.1>194.1 for MHD and 247.3 for carbamazepine-d_{10}. The limits of quantification were 2.5 µmol/l for carbamazepine, 0.25 µmol/l for carbamazepine-10,11-epoxide and 2.5 µmol/l for MHD, and the method was linear at least up to 200, 20 and 200 µmol/l, respectively, for these analytes. Within-day coefficients of variation were better than 3.5% and between-day coefficients of variation were better than 11.7% for the three analytes.

Lamotrigine was extracted by a liquid-liquid method and separated on a Supelguard Discovery 18 (20 x 4 mm) column (Supelco/Sigma-Aldrich, St. Louis, Missouri, USA), using minoxidil as internal standard. The LC-MS was operated in the positive ionization mode, using mass transitions of *m/z* 256.0>211.0 for lamotrigine and 210.1>164.1 for minoxidil. The limit of quantification was 0.5 µmol/l and the method was linear at least up to 100 µmol/l. Within-day coefficients of variation were better than 0.7% and between-day coefficients of variation were better than 6.3%.

Levetiracetam and topiramate were extracted by a liquid-liquid method and separated on a Zobrax Eclipse XDB-C8 (150 x 4.6 mm) column (Agilent), using levetiracetam-d_6 and topiramate-d_{12} as internal standards. The LC-MS was operated in the positive ionization mode, using mass transitions of *m/z* 171.1>126.1 for levetiracetam, 357.2>340.1 for topiramate, 132.0 for levetiracetam-d_6 and 369.2>352.2 for topiramate-d_{12}. The limits of quantification were 5 µmol/l for levetiracetam and 1 µmol/l for topiramate, and the method was linear at least up to 500 and 250 µmol/l, respectively. Within-day coefficients of variation were better than 11.3% and between-day coefficients of variation were better than 16.9% for both analytes.

Valproate was analysed by a commercial kit using a Cobas Integra 400 plus system (Roche Diagnostics, Rotkreuz, Switzerland). The limit of quantification was 25 µmol/l and the method was linear at least up to 1000 µmol/l.

Statistics

Statistical analyses were performed with SPSS for Windows. Categorical characteristics of the epilepsy group were compared to the reference group by

Pearson's chi-square test or Fisher's exact test. Continuous variables were compared by independent samples t-tests. Dichotomized outcome variables were analyzed by logistic regression. Risk estimates were presented as crude and adjusted odds ratios (OR) with 95% confidence intervals (CI) and p-values. The outcomes were analyzed according to maternal and paternal epilepsy, with and without prenatal exposure to AEDs. To assess potential effects of baseline differences between the AED exposed and reference groups, propensity-score matching was also performed as post hoc analyses. Each child in the drug-exposed group was matched to a child with very similar propensity score, and the risk of adverse outcomes was calculated comparing the matched groups.

Doses and plasma concentrations were correlated with the outcome variables using Spearman's rank correlation test. Two-sided p-values < 0.05 were regarded as statistically significant.

Ethics

Informed written consent was obtained from all the participants. The MoBa study has been approved by the Norwegian Data Inspectorate. All sub-studies presented were approved by The Regional Committee for Medical Research Ethics (REK).

RESULTS: PUBLISHED DATA AND ONGOING RESEARCH

The MoBa epilepsy cohort

In the validation study, questionnaires retrospectively obtaining supplementary data on women with epilepsy were completed for 300 pregnancies corresponding to 306 live born children (50% of the epilepsy cohort). According to the questionnaires 98% of the women who reported having epilepsy in the original MoBa questionnaire confirmed having epilepsy in the retrospective survey. The single most frequent self-reported type of epilepsy was juvenile myoclonic epilepsy. Generalized tonic clonic attacks were reported by 85%, whereas 52% had experienced absence seizures. Seizures during pregnancy and/or birth were reported by 17% and 3% respectively. Twenty-one patients described generalized tonic clonic (GTC) seizures, however only 1 patient had more than 3 such seizures in the period. Disease severity was unchanged during pregnancy for 86%, whereas 9% claimed that their epilepsy worsened relative to the pre-pregnancy period. Self-reported clinical epilepsy characteristics are further summarized in Table 2. Characteristics according to use of AEDs during pregnancy have also been reported (23).

In a subcohort of the women, self-reported epilepsy in the questionnaires was compared with hospital case records. The epilepsy diagnosis was verified for 38 of 40 mothers. The two patients whose diagnostic confirmation could not be obtained were both in the group not treated with AEDs. According to the hospital files, mothers not treated with AEDs (47.5%) generally had inactive epilepsy, as only one of them had experienced seizures within 2 years prior to the pregnancy. There was 100% agreement between the mothers' reporting of AED use during pregnancy and the drug use registered in the hospital records (46). Seizure occurrence during pregnancy reported by the women was confirmed by hospital records in 75% of the cases. Seizure freedom was confirmed in 89% of the cases. Age of epilepsy debut listed in the hospital records correlated strongly with the women's self-reported data in MoBa ($r=0.87$, $p<0.01$, $n = 24$).

AEDs were detected in 95% of the plasma samples where the mother reported use of such medication during pregnancy. Maternal and umbilical cord mean plasma concentrations were in the middle of the reference interval for carbamazepine (Table 3). In contrast, mean levetiracetam, valproate, lamotrigine, oxcarbazepine and topiramate concentrations were in the lower part of the reference interval (Table 3). The mean daily dose was higher than recommended according to Norwegian guidelines (49) in the non-pregnant population for lamotrigine (259 mg vs. a dose interval in monotherapy of 100-200 mg) and topiramate (217 mg vs. 100-200 mg), but lower for carbamazepine (574 mg vs. 800-1200 mg). The dose was in the normal range for valproate (754 mg vs. 10 mg/kg), oxcarbazepine (1125 mg vs. 600-2400 mg) and levetiracetam (1405 mg vs. 1000-3000 mg). The correlations between dose and plasma concentrations in maternal and umbilical cord samples were 0.37 and 0.65 for carbamazepine ($p=0.14$ and < 0.01, respectively), 0.40 and 0.43 for lamotrigine ($p=0.05$ and 0.03, respectively), 0.55 and 0.14 for levetiracetam ($p=0.10$ and 0.80, respectively), 0.43 and 0.35 for valproate ($p=0.29$ and 0.49, respectively) and 0.57 for the maternal topiramate samples ($p=0.23$). For oxcarbazepine and the umbilical cord topiramate samples the number of cases with both dose and concentration data available was too few to make meaningful correlations.

Maternal risk factors for adverse offspring outcome

Women with epilepsy treated with AEDs more often had low income and low education, were more often unemployed due to disability, single mothers, and smokers during pregnancy than women without epilepsy. Use of alcohol and illicit drugs was similar between the groups (21).

Compared to women without epilepsy, women with epilepsy more often reported current or previous psychiatric disease (13.4% vs. 10.1%, $p<0.01$). Using screening tools, 19.4% of the AED treated and 14.0% of the untreated women with epilepsy screened positive for early pregnancy depression and/or anxiety, compared to 10.8% in the reference population ($p<0.01$ and $p=0.05$ respectively). For the AED treated women the risk was still significant after adjustment for age, education, income, unemployment, and single parenting (21). The point prevalence of depression and

Table 2 Self-reported epilepsy characteristics

Epilepsy etiology	
Temporal lobe epilepsy (n = 88), no (%)	15 (17.0)
Juvenile myoclonic epilepsy (n = 100), no (%)	26 (26.0)
Epilepsy due to brain tumor (n = 91), no (%)	6 (6.6)
Epilepsy due to head trauma (n = 100), no (%)	22 (22.0)
Age when epilepsy was diagnosed (n = 285) mean yrs (SD)	13.1 (8.3)
Seizure type	
Generalized tonic clonic seizures (n = 210), no (%)	179 (85.2)
Absence seizures (n = 115), no (%)	60 (52.2)
Non-convulsive seizures (n = 100), no (%)	81 (58.7)
Seizures during pregnancy and birth	
Reported seizure(s) during pregnancy (n = 291), no (%)	49 (16.8)
Proportion with convulsive seizures[1] (n = 41), no (%)	19 (46.3)
Frequent seizures (> 10)[3], (n=290) no (%)	11 (3.8)
Total number of seizures (n = 46) median (range)	2 (296)
Seizures during birth (n = 292), no (%)	9 (3.1)
Proportion with convulsive seizures during birth[1] (n = 9), no (%)	6 (66.7)
Disease modification during pregnancy	
Improved (n=277), no (%)	15 (5.4)
Worsened (n=277), no (%)	24 (8.7)
Unchanged (n=277), no (%)	238 (85.9)

SD= standard deviation
More than one seizure type and cause of epilepsy may be reported for the same person
[1]Proportion of the attacks during pregnancy/birth reported to have been generalized tonic clonic attacks
[3]Myoclonic seizures excluded. Only 1 woman had more than 3 tonic clonic seizures

Table 3 Antiepileptic drug doses and plasma concentrations (n =255)

	Number of subjects[1]	Number of subjects treated with monotherapy (%)[4]	Dose (mg/d) Mean (min-max)	Reference interval (µmol/l)	Maternal plasma concentration week 18 (µmol/l) Mean (min-max)[5]	Umbilical cord plasma concentration at delivery (µmol/l) Mean (min-max)[5]
Lamotrigine	114	85 (75 %)	259 (100-750)	10-50	10.2 (0.8-52.1)	7.9 (0.7-26.8)
Carbamazepine	72	56 (77 %)	574 (25-1200)	15-45	29.6 (2.0-47.0)	19.2 (5.0-29.0)
Carbamazepine 10,11-epoxide[2]	72	56 (77 %)	-	-	3.8 (0.3-10.8)	3.1 (0.3-8.4)
Oxcarbazepine[3]	9	7 (77 %)	1125 (300-2400)	15-45	40.8 (11.8-40.1)	17.0 (3.9-28-8)
Valproate	44	34 (77 %)	754 (200-1200)	350-700	306.0 (31.0-538.0)	275.8 (90.0-497.0)
Topiramate	15	7 (46 %)	217 (100-400)	15-60	15.2 (2.0-38.0)	13.0 (6.0-38.0)
Levetiracetam	29	13 (45 %)	1405 (200-3000)	30-240	94.9 (21.0-366.0)	65.4 (11.0-193)

[1] Subjects with dose and/or plasma concentration available. Dose was reported in the retrospective questionnaire, information was available for 96 subjects Plasma concentrations were available for 237 subjects. Information on both dose and plasma concentration was available for 78 subjects.
[2] Carbamazepine 10,11-epoxide is the active metabolite of carbamazepine
[3] Plasma concentrations of oxcarbazepine were analyzed as the oxcarbazepine monohydroxy derivative metabolite (MHD)
[4] 202 (79.2 %) used one antiepileptic drug, 53 (20.8 %) used several antiepileptic drugs concomitantly
[5]Conversion factors from µmol/l to µg/ml: 0.256 for lamotrigine, 0.236 for carbamazepine, 0.252 for carbamazepine-10,11-epoxide, 0.254 for the oxcarbazepine monohydroxy derivative metabolite (MHD), 0.144 for valproate, 0.339 for topiramate, 0.170 for levetiracetam

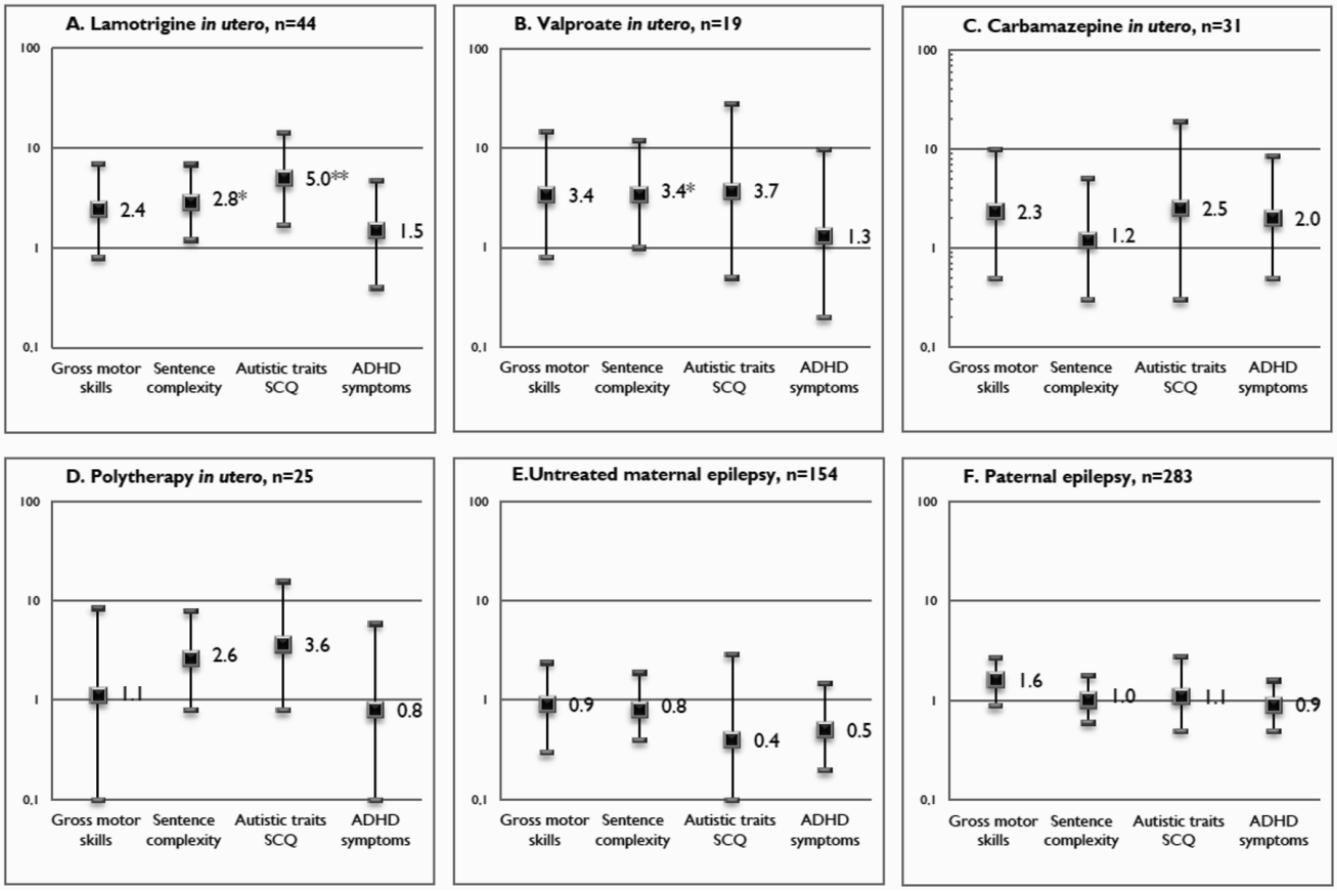

Figure 1. Adjusted odds ratio with 95% confidence interval (log scale) for adverse development in children of parents with epilepsy compared to the reference group. SCQ: Social Communication Questionnaire (previously Autism Screening Questionnaire). * P value < 0.05. ** P value < 0.01. Reprinted with kind permission from Epilepsia. Copyright John Wiley and Sons 2013.

anxiety was also increased late in pregnancy, in the postpartum period as well as 18 and 36 months after delivery for mothers with epilepsy using AEDs compared to women without epilepsy. No specific AED type was found to protect against peripartum depression. High seizure frequency and previous anxiety and/or depression were the strongest risk factors (23). Women with epilepsy had a higher life-time prevalence of eating disorders, and more often had binge eating disorder during pregnancy than women without epilepsy (21,22).

Adverse offspring outcomes

At 6 months of age, children of mothers reporting AED use during pregnancy (n=223) had a higher risk of impaired fine motor skills compared to the reference group (n=77,770) (OR 2.1, 95% CI 1.3–3.2 when adjusted for maternal age, education, folate supplement, smoking, breastfeeding and child malformation) (45). The risk was similar for monotherapy with lamotrigine, valproate, or carbamazepine. Children of mothers reporting use of multiple AEDs had the highest risk. In this group, 25% had impaired fine motor skills and 23% had impaired social skills, in contrast to 4.8% and 10%, respectively, in the reference group ($p<0.05$ after adjustment). Continuous breastfeeding during the first 6 months after delivery was associated with a tendency towards improved outcome for all the developmental domains, regardless of maternal AED treatment (45).

At 18 months of age, children of mothers reporting AED use (n=184) had increased risk of autistic features (adjusted OR 2.7, 95% CI 1.1–6.7), impaired fine motor skills (adjusted OR 1.8, 95% CI 1.0–3.4), gross motor skills (adjusted OR 2.0, 95% CI 1.1–3.7) and social skills (adjusted OR 2.2, 95% CI 1.3-3.6, Table 4) compared to the reference group (n=60, 583) (46). At 36 months of age, these children (n=139) had a higher frequency of abnormal gross motor skills (adjusted OR 2.2, 95% CI 1.1–4.2), poor sentence skills (adjusted OR 2.1, 95% CI 1.2–3.6), and autistic traits (adjusted OR 3.4, 95% CI 1.6–7.0, Figure 1) than the reference group (n=43,571). The frequency of ADHD symptoms was similar between groups, but more aggressive behavior was seen in children of mothers reporting AED use (adjusted OR 1.8, 95% CI 1.0–3.4) (46). Compared to the reference group, risk estimates (confidence intervals) for all individual AED types were overlapping at 18 and 36 months, hence the individual AEDs were not different from each other. Children of fathers with epilepsy had a higher risk of

Table 4 Risk for adverse development score at 18 months in children of parents with epilepsy [1] compared to a reference group of children of parents without epilepsy

		MATERNAL EPILEPSY : Antiepileptic drug exposure in utero [2]							PATERNAL EPILEPSY [2]	
ADVERSE SCORE	**Reference**	**All exposures**	**Monotherapy**	**Lamotrigine**	**Valproate**	**Carbamazepine**	**Polytherapy**	**Unexposed**	**No treatment [4]**	**Treatment [4]**
Age 18 months	n = 60,583	n = 184	n = 158	n = 65	n = 25	n = 41	n = 26	n = 221	n = 216	n = 147
Gross motor skills [5]	**2.9 %**	* **7.1 %** (13)	**5.7 %** (9)	**7.8 %** (5)	* **16.0 %** (4)	**0.0 %** (0)	* **15.4 %** (4)	**3.2 %** (7)	**3.7 %** (8)	**4.1 %** (6)
OR (95 % CI) [3]		2.0 (1.1-3.7)	1.6 (0.8-3.4)	1.7 (0.6-5.1)	7.0 (2.4-21.0)	NA	4.1 (1.3-13.3)	1.2 (0.6-2.6)	1.3 (0.7-2.7)	1.6 (0.7-3.6)
Fine motor skills [5]	**3.1 %**	* **6.1 %** (11)	**4.5 %** (7)	**3.1 %** (2)	**4.0 %** (1)	* **10.0 %** (4)	* **15.4 %** (4)	**5.1 %** (11)	* **5.6 %** (12)	**3.5 %** (5)
OR (95 % CI) [3]		1.8 (1.0-3.4)	1.4 (0.7-3.0)	0.9 (0.2-3.7)	1.3 (0.2-9.7)	3.3 (1.1-9.2)	4.3 (1.4-13.0)	1.7 (0.9-3.1)	1.9 (1.0-3.4)	1.0 (0.4-2.6)
Personal-Social skills [5]	**4.2 %**	* **9.4 %** (17)	**6.5 %** (10)	**3.1 %** (2)	**0.0 %** (0)	***12.2 %** (5)	* **26.9 %** (7)	**3.7 %** (8)	**5.6 %** (12)	* **10.3 %** (15)
OR (95 % CI) [3]		2.2 (1.3-3.6)	1.5 (0.8-2.9)	0.6 (0,2-2.7)	NA	3.2 (1.3-8.3)	7.1 (2.9-17.8)	0.9 (0.4-1.8)	1.4 (0,8-2.5)	2.3 (1.3-4.1)
Autism checklist [6]	**7.8 %**	* **14.0 %** (24)	**10.9 %** (16)	**15.6 %** (10)	**8.3 %** (2)	**8.8 %** (3)	* **33.3 %** (8)	**10.0 %** (20)	**11.1 %** (24)	**11.0 %** (16)
OR (95 % CI) [3]		1.7 (1.1-2.6)	1.3 (0.7-2.2)	1.8 (0.9-3.8)	1.0 (0.2-4.5)	1.1 (0.3-3.6)	4.5 (1.8-11.1)	1.3 (0.8-2.0)	1.4 (0.9-2.2)	1.6 (1.0-2.7)
Autistic traits [6]	**0.9 %**	* **3.5 %** (6)	**2.0 %** (3)	**3.1 %** (2)	**0.0 %** (0)	**2.9 %** (1)	* **12.5 %** (3)	**0.5 %** (1)	**1.4 %** (3)	* **2.8 %** (4)
OR (95 % CI) [3]		2.7 (1.1-6.7)	1.4 (0.3-5.6)	1.5 (0.2-11.0)	NA	3.3 (0.5-24.8)	8.3 (2.3-30.0)	0.5 (0.1-3.7)	1.6 (0.5-5.0)	3.7 (1.4-10.1)

[1] Each cell contains the percentage (No.) of adverse outcomes within groups and corresponding odds ratio (OR) with 95 % CI
[2] Numbers may not equal 100 % within groups due to variation of missing values. NA = Not applicable. * *P* value < 0.05
[3] ORs are adjusted for maternal age, parity, education, smoking, anxiety/depression, periconceptional folate use, and child low birth weight and malformation
[4] Antiepileptic drug use by father within 6 months prior to conception
[5] The Ages and Stages questionnaire
[6] Assessable for 92 % of the 18 months' cohort. Autism checklist: Modified Checklist for Autism in Toddlers (MCHAT). Autistic traits: Early Screening of Autistic Traits (ESAT)

abnormal social skills (adjusted OR 2.3, 95% CI 1.3–4.1) and autistic traits (adjusted OR 3.7, 95% CI 1.4–10.1) at 18 months of age compared to children of fathers without epilepsy, but this finding was not reproduced at 36 months of age (Figure 1).

Children of women with epilepsy who did not report use AEDs had normal development at all assessment points (Figure 1).

DISCUSSION

The MoBa epilepsy cohort

The validity of self-reported epilepsy diagnosis and AED use was very good. Consequently, information on maternal disease and medication use during pregnancy registered in the MoBa database and the MBRN appears to be highly reliable. These results are of importance for researchers investigating other disorders in the MoBa cohort, as the reliability might also apply for similar, chronic conditions, such as multiple sclerosis. As expected, AED plasma concentrations showed a low correlation to AED dose, which can be attributed to intraindividual variability in drug metabolism and considerable changes in pharmacokinetics during pregnancy (11,12). Differences in drug-adherence and recall bias may also play a role. The results indicate that plasma concentrations are a more reliable measure for AED exposure than dose. In previous studies, dose at the start of pregnancy has usually been used as a measure for degree of AED exposure (6,7,10).

The MoBa epilepsy cohort was representative for women with epilepsy in general. The self-reported epilepsy characteristics were similar to the cohort of Norwegian women included in the European and International Registry of Antiepileptic Drugs in Pregnancy (EURAP) database. The proportion of women with self-reported localization-related epilepsy in our cohort was 53% in the hospital records. The corresponding proportion in EURAP was 46% (50). However, the proportion of women with juvenile myoclonic epilepsy (22% in the self-reported group, 15% in hospital records) was larger than the 5-10% normally seen in non-pregnant epilepsy populations. (51). This overrepresentation probably reflects that the MoBa cohort mainly consists of young (between 19 and 41 years) and female participants, as there is a female preponderance in juvenile myoclonic epilepsy (51). Fewer women had seizures during pregnancy according to our studies (17% in the self-reported group, 15% in hospital records) than among the Norwegian women in EURAP (37%) (50). However, the women in EURAP were recruited from neurological departments and a tertiary epilepsy center; hence the patients probably had more severe epilepsy compared to those in the population based MoBa cohort. However, the proportion of women having seizures during birth was similar between our data and the EURAP data (3.1% vs. 2.7%).

The impact of AED exposure during pregnancy

Our studies showed that exposure to AEDs *in utero* was associated with adverse effects on several key developmental domains at all measured time points. The exposed group did not reach motor milestones at the expected age, had poorer language skills, and more autistic behavior. The risk of impaired motor skills was detectable already at 6 months of age. The risk was generally highest for children exposed to multiple drugs. There was no difference between the various drugs when used in monotherapy. This is in contrast to previous studies which have found adverse developmental outcomes mainly in relation to valproate exposure *in utero* (3). Adverse socioeconomic factors and psychiatric disease were more frequent in women with epilepsy. However, the effect of AED exposure on offspring development persisted even after adjusting for these factors.

Children of mothers with epilepsy who did not use AEDs scored within the normal range for all developmental domains, and children of fathers with epilepsy were mainly similar to the reference population. Thus, our results point to the *in utero* AED exposure as the main risk factor for adverse development in children of women with epilepsy. Maternal epilepsy severity was higher in the group using AEDs than in those not using AEDs (23). However, disease severity should be similar in fathers and mothers with epilepsy using AEDs, accounting for potential genetic or psychosocial effects on development in offspring of parents with active epilepsy. Frequent seizures during the pregnancy may affect later cognitive function (9). However, only 21 women had generalized tonic clonic seizures during pregnancy or birth, and of these only 1 woman had more than 3 seizures. Prior research has indicated that less than 5 tonic clonic seizures during pregnancy do not seem to affect developmental outcome (9). Women using AEDs should be encouraged to breastfeed, as no harmful effects from maternal AED use during the breastfeeding period were detected. Breastfeeding has also been shown to improve cognitive outcome (52,53).

The main strengths of the MoBa epilepsy cohort is the prospective study design, the ability to adjust for numerous possible confounders, and the recruitment of a large and representative reference group that is normally not available in such studies (5,54). Including fathers with epilepsy as well as women with epilepsy not treated with AEDs as internal control groups is a unique feature of our study design. Due to the population-based enrollment, selection bias in the MoBa epilepsy cohort is probably much lower than in clinic-based studies, thereby providing results that may be more representative for general epilepsy populations. Even so, some degree of selection is probably present. It has e.g. been shown that the subjects in the MoBa study are more resourceful than the general population; however this selection does not seem to affect exposure-outcome associations (55). Other weaknesses

in MoBa include a self-reported epilepsy diagnosis, developmental assessment based on maternal ratings, and a moderate response rate at 36 months. Even though the screening tools employed are validated, the short versions used in the questionnaires are not always so. The construct validity of the developmental MoBa screening tools has been criticized, e.g. with regard to what extent differences in scores actually represent meaningful clinical differences (56). This criticism does not necessarily apply to cut-offs that are set distant from the mean score, as performed in our studies. The construct validity is probably improved when the instruments are used to detect severe deviations, such as in relation to considerable developmental delay.

This paper reports previously unpublished data on AED plasma levels measured in maternal and umbilical cord plasma, and information on AED doses used during pregnancy. These data will be correlated to offspring developmental scores to search for dose/effect and concentration/effect relationships. The possibility to correlate the levels of AED exposure to future child outcomes from early age and throughout childhood in a population-based setting is unique. Moreover, the collection of clinical epilepsy data from a subcohort in MoBa has assured the quality of the dataset. Information on seizures during pregnancy and epilepsy subtype is now at our disposal and we will assess the effect of these variables on various offspring outcomes. Finally, we have found higher BMI in women with epilepsy before and during pregnancy (22,46) and are currently studying the frequency of and risk factors associated with overweight, obesity and weight gain during pregnancy in the cohort. These factors are of considerable interest, as they are known to increase pregnancy complications in women without epilepsy (57,58). We will assess their impact on pregnancy outcome in women with epilepsy in relation to AED use.

CONCLUSIONS

The validity of epilepsy-related information in the MoBa database was very good. The MoBa epilepsy cohort may be more representative for general epilepsy populations than studies based upon clinical materials. Use of the epilepsy cohort in MoBa has shown that AED exposure *in utero* is associated with impaired development and increased risk of autistic traits in the child. We will proceed with this cohort and analyze the impact of AED plasma concentrations and the contribution of seizures during pregnancy, socioeconomic variables, psychiatric disease, vitamin deficiency and maternal overweight on the developing children born to mothers with epilepsy. The combination of such data is not available in other population-based studies of women with epilepsy in pregnancy. Hence, the unique design of the MoBa study will enable us to expand the knowledge within this field, entangling the various risk factors for adverse outcomes in children of women with epilepsy.

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CONFLICTS OF INTEREST

Marte Helene Bjørk and Gyri Veiby have received lecture honoraria from Glaxo Smith Kline and congress travel support from UCB pharma. NE Gilhus has received lecture fee from Octapharma, Baxter, and Merck Serono. O Spigset has no conflict of interests.

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