

## Supplementary Online Content

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

## eMethods 1. Polytherapy Combinations

Of the 65 patients on polytherapy, 33 patients were on drug combinations involving lamotrigine, 21 involving carbamazepine, 19 involving valproate sodium, 19 involving levetiracetam, 15 involving oxcarbazepine, 10 involving topiramate, 9 involving clonazepam, 2 involving phenytoin sodium, 3 involving gabapentin, 2 involving vigabatrin, and 1 involving primidone, clobazam, pregabalin or eslicarbazepine. The combinations were distributed as follows:

Carbamazepine + lamotrigine = 8  
Lamotrigine + valproate = 7  
Lamotrigine + levetiracetam = 5  
Levetiracetam + carbamazepine = 4  
Valproate + carbamazepine = 4  
Topiramate + oxcarbazepine = 4  
Lamotrigine + oxcarbazepine = 3  
Clonazepam + valproate = 3  
Levetiracetam + oxcarbazepine = 3  
Lamotrigine + clonazepam = 2  
Lamotrigine + gabapentin = 2  
Topiramate + carbamazepine = 2  
Levetiracetam + topiramate = 1  
Lamotrigine + topiramate = 1  
Carbamazepine + gabapentin = 1  
Carbamazepine + phenytoin = 1  
Topiramate + clonazepam = 1  
Valproate + oxcarbazepine = 1  
Oxcarbazepine + clonazepam = 1  
Primidone + phenytoin = 1  
Vigabatrin + oxcarbazepine = 1  
Vigabatrin + valproate = 1  
Carbamazepine + clonazepam = 1  
Eslicarbazepine + lamotrigine + valproate = 1  
Clobazam + lamotrigine + levetiracetam = 1  
Lamotrigine + levetiracetam + clonazepam = 1  
Levetiracetam + oxcarbazepine + pregabalin = 1  
Valproate + levetiracetam + topiramate = 1  
Oxcarbazepine + levetiracetam + lamotrigine = 1  
Lamotrigine + levetiracetam + valproate = 1

### **eMethods 2. Antiepileptic Drug Types Used by Mothers on High-Dose Folic Acid Supplements**

Monotherapy was used by 80 % of those taking high dose supplements and antiepileptic drugs: lamotrigine was used by 36 %, carbamazepine by 19 %, levetiracetam by 6 %, topiramate by 6 %, valproate by 5 %, oxcarbazepine by 4 %, clonazepam by 2 %, gabapentin by 1 % and primidone by 1 %.

Polytherapy combinations involving lamotrigine were found in 13 %, levetiracetam in 8 %, carbamazepine in 6 %, oxcarbazepine in 6 %, valproate in 5 %, topiramate in 2 %, clonazepam in 2 % and vigabatrin in 1 %.

### **eMethods 3. Biobank Analysis of Blood Samples and Folate**

The samples were centrifuged within 30 minutes after collection and stored at 4°C until they were sent to the MoBa Biobank at the Norwegian Institute of Public Health{Paltiel, 2014 #147;Paltiel, 2014 #147}. Within 2 days after sampling plasma samples were aliquoted and kept at -80°C until delivery for antiepileptic drug and folate analysis, respectively.

For the folate analyses, the limits of quantification were 0.13, 0.40, 0.17, 0.27, and 0.53 nmol/L for mTHF, hmTHF, pABG, apABG and folic acid, respectively. The assay was linear for all analytes up to 140 nmol/L, and the coefficients of variation were < 10 %.{Hannisdal, 2009 #148}

**eTable 1. Modified Checklist for Autism in Toddlers (M-CHAT)—Child Age 18 Months**

<i>Alternatives: Yes/No</i>
1. Does your child enjoy being swung, bounced on your knee, etc.?
2. Does your child take interest in other children?
3. Does your child like climbing on things, such as up stairs?
4. Does your child enjoy playing peek-a-boo/hide-and-seek?
5. Does your child ever pretend, for example, to talk on the phone or take care of dolls, or pretend other things?
6. Does your child ever use his/her index finger to point, to ask for something?
7. Does your child ever use his/her index finger to point, to indicate interest in something?
8. Can your child play properly with small toys (e.g. cars or bricks) without just mouthing, fiddling, or dropping them?
9. Does your child ever bring objects over to you (parent) to show you something?
10. Does your child look you in the eye for more than one second or two?
11. Does your child ever seem oversensitive to noise? (E.g. plugging ears)
12. Does your child smile in response to your face or your smile?
13. Does your child imitate you? (E.g. you make a face – will your child imitate it?)
14. Does your child respond to his/her name when you call?
15. If you point at a toy across the room, does your child look at it?
16. Does your child walk?
17. Does your child look at things you are looking at?
18. Does your child make unusual finger movements near his/her face?
19. Does your child try to attract your attention to his/her own activity?
20. Have you ever wondered if your child is deaf?
21. Does your child understand what people say?
22. Does your child sometimes stare at nothing or wander with no purpose?
23. Does your child look at your face to check your reaction when faced with something unfamiliar?

**eTable 2. Social Communication Questionnaire (SCQ)—Child Age 36 Months**

<i>Alternatives: Yes/No</i>
1. Does he/she talk using short phrases or sentences?
2. Can you now have a to and from "conversation" with him that involves taking turns or building on what you have said?
3. Has he/she ever used odd phrases or said the same thing over and over in almost exactly the same way? That is, either phrases he/she has heard other people use or the ones he/she has made up?
4. Has he/she ever used socially inappropriate questions or statements? For example, has he/she ever regularly asked personal questions or made personal comments at awkward times?
5. Does he/she ever get his pronouns the wrong way round, (i.e., saying "you" or "he" for "I")?
6. Has he/she ever used words that he/she seems to have invented or made up himself, or ever put things in odd, indirect ways, or metaphorical ways of saying things? For example, saying "hot rain" for "steam"
7. Has he/she ever said the same thing over and over in exactly the same way, or insist on you saying the same things over and over again?
8. Has he/she ever had things that he/she seemed to have to do in a very particular way or order, or rituals that he/she has to have you do?
9. Does his/her facial expression usually seem appropriate to the particular situation, as far as you can tell?
10. Has he/she ever used you hand like a tool, or as if it were part of his own body (e.g., pointing with your finger, putting your hand on a doorknob to get you to open the door)?
11. Has he/she ever had any interests that preoccupied him/her and might have seemed odd to other people (e.g. traffic lights, drainpipes or timetables)?
12. Has he/she ever seemed to be more interested in a certain part of a toy (e.g., spinning the wheels of a car) or an object than using the object as it was intended?
13. Has he/she ever had any special interests that were unusual in their intensity by otherwise appropriate for his/her age and peer group (e.g., trams, dinosaurs)?
14. Has he/she ever seemed to be unusually interested in the sight, feel, sound, taste or smell of things or people?
15. Has he/she ever had any mannerisms or odd ways of moving his/her hands or fingers, such as flapping or moving his/her fingers in front of his eyes?
16. Has he/she ever had any complicated movements of his whole body, such as spinning or repeatedly bouncing up an own?
17. Does he/she ever injure him-/herself deliberately, such as by biting his/her arm or banging his/her head?
18. Does he/she ever have any objects (other than a soft toy or comfort blanket) that he/her has to carry around with him/her?
19. Does he have any particular friend, or a best friend?
20. Does he/she ever talk with you just to be friendly (rather than to get something)?

21. Does he/she ever spontaneously copy you (or other people), or what you are doing (such as vacuuming, gardening, mending things)?
22. Does he/she ever spontaneously point at things around him just to show you things (not because he/she wants them)?
23. Does he/she ever use gestures, other than pointing or pulling your hand, to let you know what he/she wants?
24. Does he/she nod his head to mean "yes"?
25. Does he/she shake his head to mean "no"?
26. Does he/she usually look at you directly in the face when doing things with you or talking with you?
27. Does he/she smile back if someone smiles at him/her?
28. Does he/she ever show you things that interest him/her to engage your attention?
29. Does he/she ever offer to share things other than food with you?
30. Does he/she ever seem to want you to join in his/hers enjoyment of something?
31. Does he/she ever try to comfort you if you were sad or hurt?
32. When he/she wants something or wants help, does he/she look at you and use gestures with sounds or words to get your attention?
33. Does he/she show a normal range of facial expression?
34. Does he/she ever spontaneously join in and try to copy actions in social games -such as The Mulberry Bush or The Farmer's in His Den?
35. Does he/she play any pretend or make-believe games?
36. Does he/she seem interested in other children of approximately the same age whom he/she does not know?
37. Does he/she respond positively when another child approaches him/her?
38. If you came into a room and started talking to him/her without calling his/hers name, does he/she usually look up and pay attention to you?
39. Does he/she ever play imaginative games with another child in such a way that you can tell they understood what each other is pretending?
40. Does he/she play cooperatively in games that need some form of joining in with a group of other children, such as hide and seek or ball games?

**eTable 3. Multivariable Linear Regression Analysis Adjusted for Confounding Factors**

<b>Factor</b>	<b><math>\beta</math> Coefficient</b>	<b><i>P</i> Value</b>
Constant	NA	.03
Plasma folate level <sup>a</sup>	-0.25	.03
Plasma AED level <sup>b</sup>	0.10	.41
Maternal depression in pregnancy <sup>c</sup>	-0.02	.84
Smoking in pregnancy (yes or no)	-0.05	.67
Socioeconomic factors <sup>d</sup>	0.18	.14
Maternal age, y	0.11	.42
Alcohol consumed in pregnancy, U/mo <sup>e</sup>	0.15	.23
Maternal parity, No. of prior pregnancies in the past 21 gestational weeks	-0.23	.14
No. of epileptic seizures in pregnancy <sup>f</sup>	0.19	.12

Abbreviations: AED, antiepileptic drug; NA, not applicable.

<sup>a</sup>Indicates maternal folate concentration (sum of active 5-methyltetrahydrofolate [mTHF] and the degradation products 4- $\alpha$ -hydroxy-mTHF levels).

<sup>b</sup>Indicates plasma concentration of AEDs (mean of maternal and umbilical cord concentrations).

<sup>c</sup>Indicates mean score on the Hopkins Symptom Checklist (range, 1-4, with a score of >1.75 indicating depression).

<sup>d</sup>Indicates composite variable consisting of low income, low educational attainment, and single mother (sum range, 0-3, with 3 indicating presence of all 3 factors).

<sup>e</sup>Indicates mean number of units consumed from month of conception to week 19 of pregnancy.

<sup>f</sup>Indicates generalized tonic-clonic seizures during pregnancy.

**eTable 4. Autistic traits (Social Communication Questionnaire Score) in Relation to Quartiles of Plasma Folate**

Concentration	No. of individuals	Mean (SD)	p-value vs. 4 <sup>th</sup> quartile <sup>1</sup>	Autistic traits <sup>2</sup> n (%)
1 <sup>st</sup> quartile (low)	22	<b>7.91 (4.3)</b>	<b>0.04*</b>	3 (13.6 %)
2 <sup>nd</sup> quartile	26	6.70 (3.1)	0.27	1 (3.8 %)
3 <sup>rd</sup> quartile	38	6.66 (3.8)	0.29	4 (10.5 %)
4 <sup>th</sup> quartile (high)	34	<b>5.79 (3.2)</b>	-	1 (2.9 %)

Folate: plasma concentration of the biologically active 5-methyl-tetrahydrofolate (mTHF) and its degradation product 4-alpha-hydroxy-5-methyl-tetrahydrofolate (hmTHF).  
 1<sup>st</sup> quartile: Folate < 24.20 nmol/L. 2<sup>nd</sup> quartile: Folate ≥ 24.20 nmol/L and < 67.45 nmol/L. 3<sup>rd</sup> quartile: Folate ≥ 67.45 nmol/L and < 85.18 nmol/L. 4<sup>th</sup> quartile: Folate ≥ 85.18 nmol/L.

- 1) Student's t-test, p-values are two sided
- 2) Frequency of children with autistic traits (Social Communication Questionnaire Score > 13). Statistical comparison not possible due to low statistical power.

**eTable 5. Autistic Traits (Social Communication Questionnaire Score) in Relation to Plasma Concentration Quartiles of Antiepileptic Drugs**

	n	Mean (SD)	p-value vs. 4 <sup>th</sup> quartile <sup>1</sup>	Autistic traits <sup>2</sup> n (%)
1 <sup>st</sup> quartile (low)	22	6.49 (3.6)	0.80	2 (6.3 %)
2 <sup>nd</sup> quartile	30	6.50 (4.0)	0.82	3 (10.0 %)
3 <sup>rd</sup> quartile	34	6.66 (3.6)	0.95	2 (5.9 %)
4 <sup>th</sup> quartile (high)	31	6.72 (3.5)	-	2 (6.5 %)

Antiepileptic drug concentration: mean over standardized concentrations for all types of antiepileptic drugs used (100 x (observed value minus minimum value)/ maximum value minus minimum value). {Björk, 2013 #2084}  
 1<sup>st</sup> quartile: < 17.04, 2<sup>nd</sup> quartile: 17.04 ≥ and < 36.24. 3<sup>rd</sup> quartile: ≥ 36.24 and < 81.22 nmol/L. 4<sup>th</sup> quartile: ≥ 81.22

- 1) Student's t-test, p-values are two-sided.
- 2) Frequency of children with autistic traits (Social Communication Questionnaire Score > 13). Statistical comparison not possible due to low statistical power.



**eTable 6. Characteristics in Mothers of Children Without and With Autistic Traits at 18 and 36 Months or at Both Measurement Times**

Data are presented as number (percent) for categorical data or median (range) for continuous data.

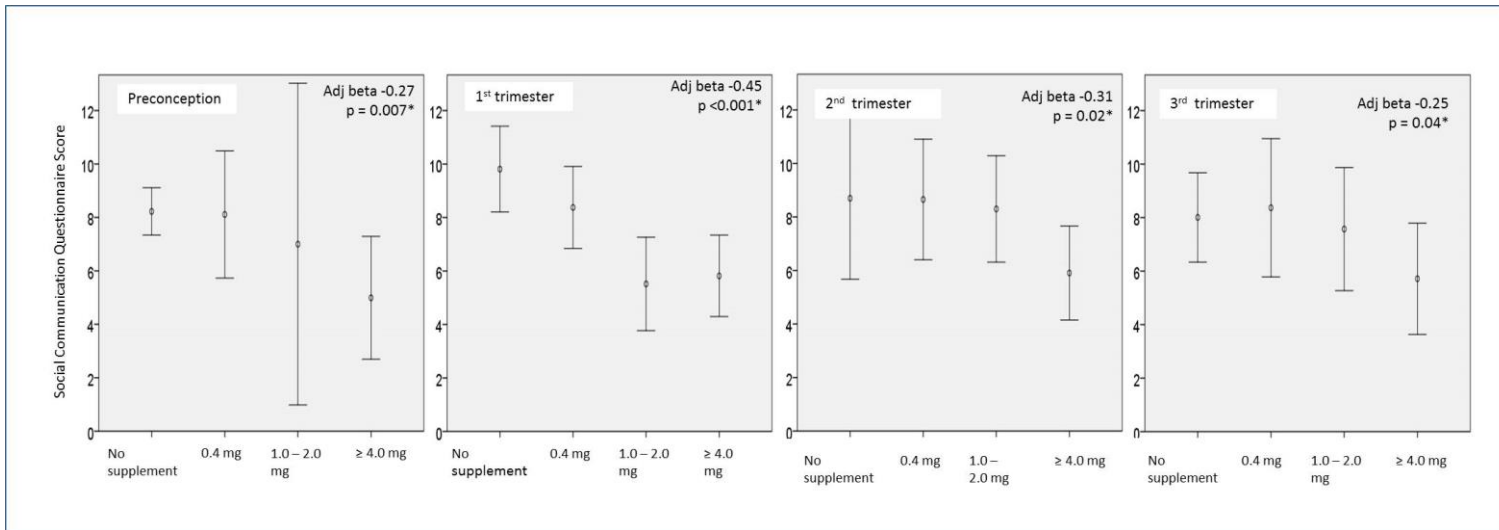
Autistic traits	Maternal epilepsy, AED n = 223			Maternal epilepsy, no AED n = 272			No maternal epilepsy n = 75,976		
	Never	at 18 or 36 months	At 18 and 36 months	Never	at 18 or 36 months	At 18 and 36 months	Never	at 18 or 36 months	At 18 and 36 months
N (%) within epilepsy subgroup→	184 (83%) <sup>1</sup>	35 (16 %) <sup>1</sup>	4 (2 %) <sup>1</sup>	245 (90%) <sup>1</sup>	25 (9 %) <sup>1</sup>	2 (1 %) <sup>1</sup>	69,021 (91 %) <sup>2</sup>	6452 (9 %) <sup>2</sup>	503 (1 %) <sup>2</sup>
M-CHAT sum	0 (2)	3 (11)	6 (9)***	0 (2)	3 (5)***	5 (0) <sup>5</sup>	0 (2.8)	3 (20)	4 (18)***
SCQ sum	6 (12)	13 (13)	18 (8)***	6 (12)	8 (12)	14 (1) <sup>5</sup>	6 (14)	10 (32)	15 (23)***
Folic acid supplementation <sup>3</sup>	97 (53 %)	13 (37 %)	0 (0 %)*	88 (36 %)	3 (12 %)	1 (50 %)	23,160(34%)	1900 (30 %)	113 (22 %)**
Plasma folate (nmol/L)	74 (127)	68 (115)	35 (0) <sup>4,5</sup>	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Folic acid dose (mg) 1 <sup>st</sup> trimester	2.0 (4.0)	0.0 (4.0)	0.0 (0.0)**	0.0 (4.0)	0 (2.0)	n.a.	n.a.	n.a.	n.a.
Start of folic acid (gestational week)	-1 (31)	7 (16)	15 (0)**	2 (16)	2 (16)	1 (3) <sup>5</sup>	2 (31)	2 (31)	2 (31)
Alcohol	7 (4 %)	1 (3 %)	0 (0 %)	3 (1 %)	1 (4 %)	0 (0 %)	1673(2 %)	164 (3 %)	19 (4 %)
Unplanned pregnancy (n)	40 (22 %)	8 (24 %)	3 (75 %)	50 (21 %)	6 (24 %)	1 (50 %)	11,764 (17 %)	1318 (21 %)	125 (25 %)**
Maternal depression (n)	31 (17 %)	23 (34 %)	1 (25 %)*	34 (14 %)	1 (4 %)	1 (50 %)	6122 (9 %)	907 (15 %)	94 (20 %)**
Smoking (n)	14 (8 %)	3 (9 %)	0 (0 %)	15 (6 %)	1 (4 %)	0 (0 %)	4038 (6 %)	577 (9 %)	44 (9 %)**
Low SES	21 (11 %)	7 (20 %)	1 (25 %)	32 (13 %)	2 (8 %)	1 (50 %)	5506 (8 %)	783 (12 %)	87 (17 %)**
Polytherapy	31 (17 %)	10 (29 %)	1 (25 %)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Standardized AED plasma concentration	38 (258)	25 (167)	21 (0) <sup>4,5</sup>	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Maternal age (years)	30 (23)	30 (18)	28 (15)	29 (24)	30 (17)	24 (3) <sup>5</sup>	30 (32)	30 (32)	29 (27)**
Parity	2 (4)	1 (3)	1 (2)	1 (4)	1 (3)	2 (0) <sup>5</sup>	2 (4)	1 (4)	1(4)**
GTC sum	0 (2)	0 (20)	0 (0) <sup>4,5</sup>	0 (1)	0 (1)	n.a.	n.a.	n.a.	n.a.

Children of mothers that answered the questionnaires at 18 and/or 36 months of age are included.

- 1) Proportion of total cases without autistic traits, with autistic traits at 18 or 36 months or at 18 and 36 months within children of women with epilepsy with and without antiepileptic drug (AED) treatment. The distribution was significantly different between the exposed and unexposed groups (p=0.045). The 4 children with autistic traits at both time points used lamotrigine, one in combination with valproate.
- 2) Proportion of cases without autistic traits, with autistic traits at 18 or 36 months or at 18 and 36 months within children of mothers without epilepsy, significant different from the AED exposed group (p < 0.001).
- 3) Periconceptual folic acid supplement use
- 4) Only one observation available
- 5) Statistics could not be computed due to low numbers.
- 6) Categorical variables: numbers (%) presented. The Chi-square test for trend (linear-by-linear association). \*p<0.05 \*\*p>0.01 \*\*\*p>0.001
- 7) Continuous variables: median (range). Jonckheere-Terpstra test for ordered alternatives (non-parametric test for trend) \*p<0.05 \*\*p>0.01 \*\*\*p>0.001

n.a. not available/not applicable. Plasma folate: sum of 5-methyl-tetrahydrofolate and 4-alpha-hydroxy-5-methyl-tetrahydrofolate. Alcohol use (any). Maternal depression: measured in gestational week 17-19 : mean score >1.75 on the Hopkins symptom check list (range 1-4 with higher score indicating depression).{Strand, 2003 #154} Smoking (any). Low SES= low socioeconomic status (0-3 with 3 representing presence of all three factors: Low education ( $\leq 9$  years), low household income ( $\leq 42404$  Euros/399 999 kr/y/\$49336) or single mother). AED concentration: mean over standardized data (see text). Parity (prior pregnancies past 21 gestational weeks), GTC sum = numbers of generalized tonic clonic seizures.

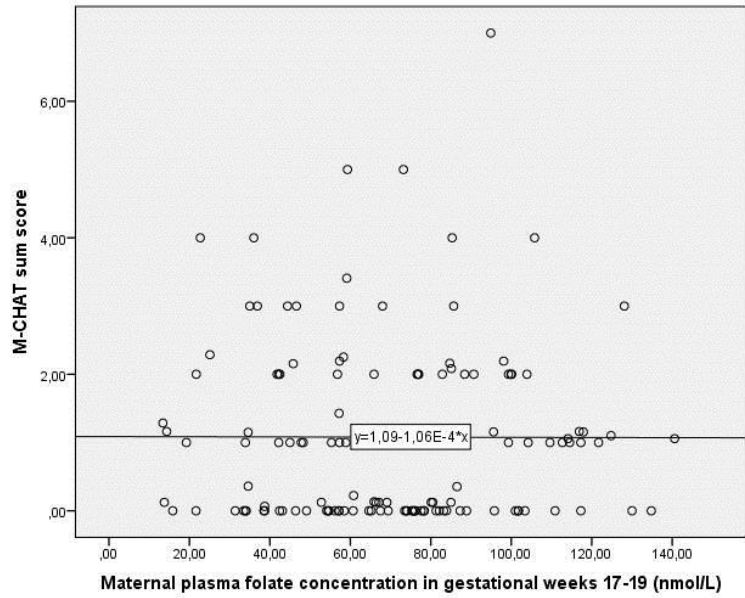
**eFigure 1. Degree of Autistic Traits at 36 Months of Age According to Folic Acid Dose Used Before and During Pregnancy**



Plot: mean autistic traits score and 95 % error bars.

Adj beta: the standardized regression coefficient from a linear regression model adjusted for parity, socioeconomic factors, antiepileptic drug serum level, maternal smoking and number of generalized tonic clonic seizures during pregnancy. The model is also adjusted for valproate sodium use, as valproate users in particular are recommended high folic acid supplement doses according to Norwegian treatment guidelines.

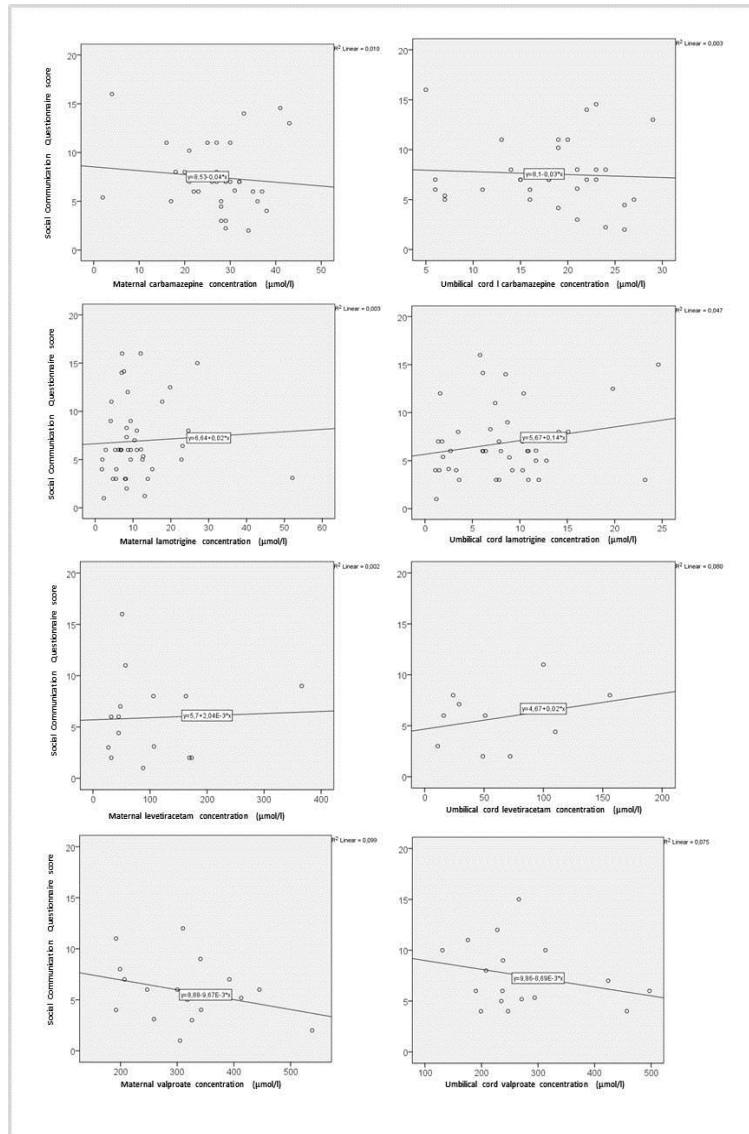
**eFigure 2. Scatterplot Between Total M-CHAT Score in the Child and Maternal Plasma Folate Concentration in Gestational Week 17-19**



Spearman's correlation: rho = 0.01, p = 0.9.

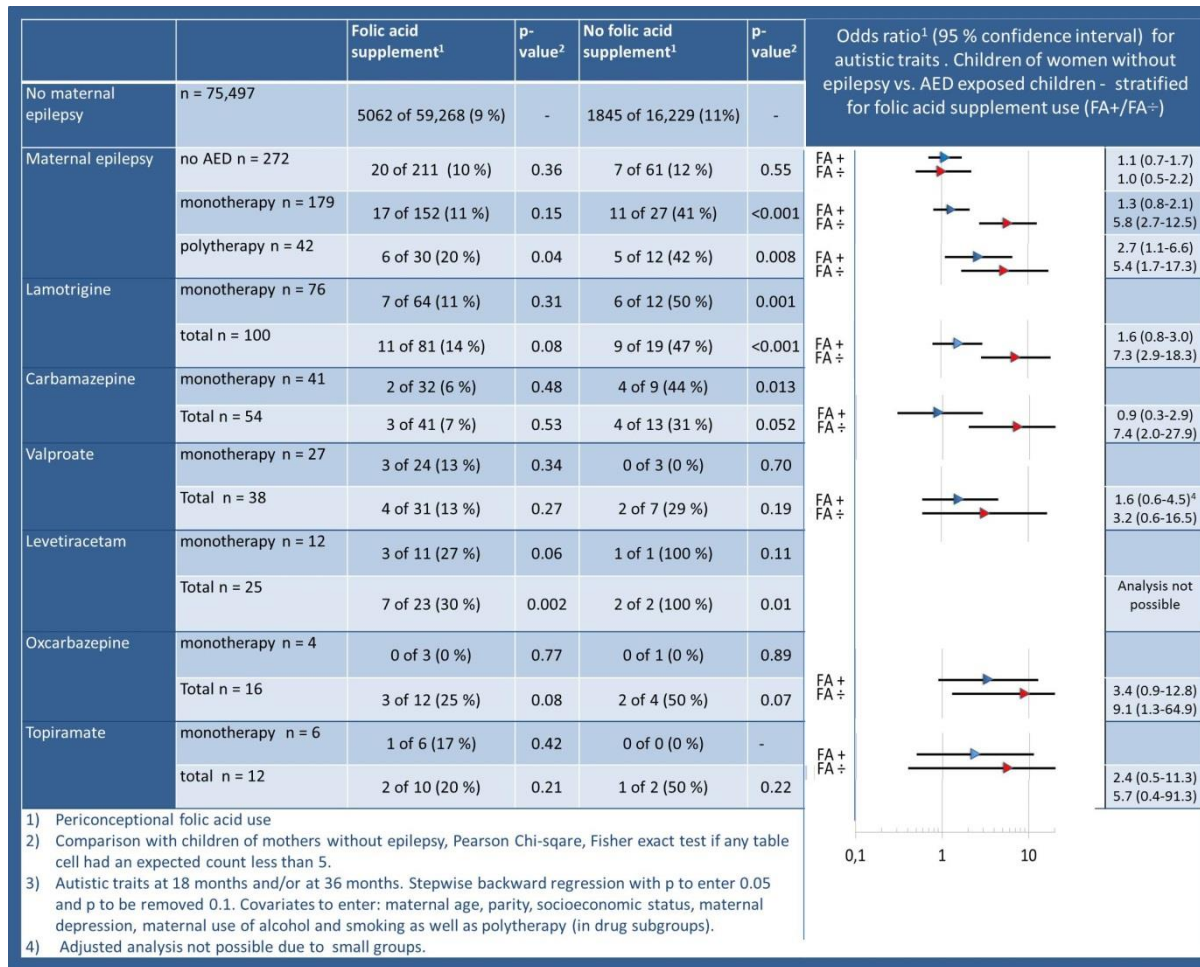
**eFigure 3. Degree of Autistic Traits According to Antiepileptic Drug Concentrations during Gestational Weeks 17-19 and in the Umbilical Cord After Delivery**

There are no significant correlations (Pearsons correlation,  $p > 0.16$ )



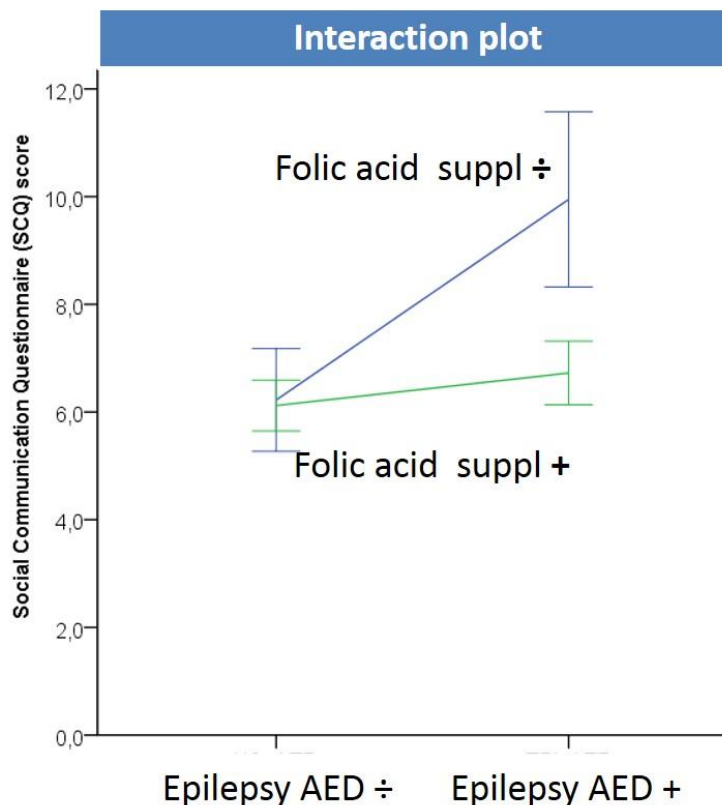
**eFigure 4. Effect of Folic Acid Supplement in Antiepileptic Drug Subgroups**

Risk of autistic traits according to type of antiepileptic treatment and folic acid supplement use (FA+) or no use (FA-). Children of mothers with epilepsy with different type of treatment are compared to children of mothers without epilepsy stratified for use of periconceptional folic acid supplement.



**eFigure 5. The Antiepileptic Drug and Folic Acid Supplement Interaction**

Interaction plot between folic acid (FA) supplement use and antiepileptic drug (AED) use on degree of autistic traits (sum SCQ score). Data from the multivariable linear analysis in table



Social Communication Score (95 % Confidence interval Error bars) for 36 months old children of mothers using and not using periconceptual folic acid supplements (green and blue lines). Points are shown for children not exposed (AED ÷) and exposed (AED +) for AED during pregnancy. All children of women with epilepsy are included in the analysis (n=231).

Interaction plot	B	Std. Error (B)	Beta	Significance
Constant	5.67	1.72	-	0.001
<b>AED</b>	<b>3.68</b>	<b>0.95</b>	<b>0.51</b>	<b>&lt;0.001</b>
FA supplement	- 0.21	0.71	- 0.02	0.77
<b>AED x FA suppl</b>	<b>- 3.13</b>	<b>1.07</b>	<b>-0.42</b>	<b>0.004</b>
<b>Depression</b>	<b>1.32</b>	<b>0.48</b>	<b>0.17</b>	<b>0.007</b>
Alcohol	0.67	1.33	0.03	0.62
Socioeconomic	0.28	0.54	0.04	0.60
Parity	- 0.23	0.28	- 0.06	0.41
GTC (No.)	0.20	0.20	0.06	0.33
Age	- 0.03	0.05	- 0.04	0.58
Smoking	- 0.03	0.78	- 0.00	0.10

Multivariate linear analysis of the interaction between antiepileptic drug (AED) and folic acid (FA) supplement use adjusted for confounding factors. B = the regression coefficient, Std Error = standard error and beta is the standardized regression coefficient. Supl= supplement.

Depression: mean Hopkins symptom checklist during pregnancy (range 1-4, higher score indicates depression). Alcohol during pregnancy. Socioeconomic low education, low economy, single parent (sum 1-3). GTC: number of generalized tonic clonic seizures during pregnancy. Maternal age and smoking during pregnancy (any).

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