Valproate EU consortium

A post-authorisation safety study (PASS) to evaluate the paternal exposure to valproate and the risk of neurodevelopmental disorders including autism spectrum disorder as well as congenital abnormalities in offspring – a population-based retrospective study

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Abstract

Title

A post-authorisation safety study (PASS) to evaluate the paternal exposure to valproate and the risk of neurodevelopmental disorders including autism spectrum disorder as well as congenital abnormalities in offspring – a population-based retrospective study

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Keywords

Valproate; paternal exposure; neurodevelopmental disorders; congenital malformations; post-authorisation safety study

Rationale and Background

Valproate-containing medicines are approved and marketed in more than 120 countries worldwide, and in the European Union for epilepsy and for bipolar disorders in case of contraindication or intolerance to lithium. In recent years, due to an increased risk of neurodevelopmental disorders (NDD), including autism spectrum disorders (ASD), and congenital malformations (CM) in offspring after valproate exposure in utero, the use of valproate has been restricted to cases in which no other effective or tolerated treatment is available in women of childbearing potential suffering from epilepsy or bipolar disorder or in pregnant women suffering from epilepsy; it has been contraindicated in pregnant women suffering from bipolar disorder. There is currently scarce real-world evidence of an increased risk of NDD including ASD, or CM in offspring following paternal exposure to antiepileptic drugs (AEDs) at the time of conception. Following the Pharmacovigilance Risk Assessment Committee request dated 8 February 2018, a PASS was conducted aiming to evaluate the association between paternal exposure to valproate at the time of conception and risk of NDD, including ASD, and CM in offspring, in comparison to paternal exposure to lamotrigine or levetiracetam at the time of conception.

Research Questions and Objectives

The aim of this retrospective cohort study was to examine the association between paternal exposure to valproate at conception and the risk of NDD, including ASD, and CM in offspring, from data in Denmark, Sweden, and Norway. Paternal exposure to valproate was compared to paternal exposure to lamotrigine/levetiracetam at the time of conception.

The primary objective was:

1. To investigate the risk of NDD, including ASD, in offspring paternally exposed to valproate (monotherapy), compared to lamotrigine/levetiracetam (composite monotherapy) treatment at the time of conception.

The secondary objectives were:

2. To investigate the risk of CM in live and non-live offspring paternally exposed to valproate (monotherapy), compared to lamotrigine/levetiracetam (composite monotherapy) treatment at the time of conception, in Denmark and Norway.
3. To describe AED exposure (posology and duration) data and health characteristics of male patients prescribed AEDs (including valproate and lamotrigine/levetiracetam) in treatment of epilepsy and other indications at the time of conception of their offspring, for NDD and CM cohort.
4. To identify potentially important risk factors for outcomes of interest, in offspring paternally exposed to valproate (monotherapy) or lamotrigine/levetiracetam (composite monotherapy) at the time of conception, by examining AED exposure and health characteristics of the offspring and their mothers.

The exploratory objectives were:

5. To describe the putative risk factors and frequency of NDD, including ASD, as well as CM in offspring paternally exposed to valproate in combination with other AEDs, excluding lamotrigine/levetiracetam, and to lamotrigine/levetiracetam in combination with other AEDs, excluding valproate, at the time of conception.
6. To describe the risk factors and frequency of NDD, including ASD, as well as CM in paternally and maternally matched exposure-discordant (valproate vs. lamotrigine/levetiracetam monotherapy) siblings at conception.
7. To investigate the risk of CM in live offspring paternally exposed to valproate (monotherapy), compared to lamotrigine/levetiracetam (composite monotherapy) treatment at the time of conception in Sweden.
8. To describe the frequency of CM by target body system organ class in live and non-live offspring paternally exposed to valproate (monotherapy), and to lamotrigine/levetiracetam treatment (composite monotherapy) at the time of conception.

Besides the main and exploratory analyses listed above, several sensitivity analyses were performed, to assess the robustness of the methodology considered and allow a better understanding of the results. Among those, only 3 are presented in this abstract, given their importance and impact on the interpretation of the full study results, which consisted in repeating the statistical analysis for the primary objective and secondary objective 2: sensitivity analysis 2 restricted outcome of interest to ASD specifically, ignoring all other NDD diagnoses; sensitivity analysis 10 investigated the risk of outcome associated with exposure using a continuous measure of cumulative exposure to treatment; and sensitivity analysis 11 used a narrower definition of NDD as outcome of interest.

Study Design

This was a multi-country, population-based, retrospective cohort study using data from national registries in Denmark, Sweden, and Norway. A cohort of offspring paternally exposed to valproate was compared to a cohort of offspring paternally exposed to lamotrigine/levetiracetam, at the time of conception, to investigate the risk of NDD, including ASD, as the primary outcome of interest and the risk of CM (as a composite of major and/or minor CM) as a secondary outcome.

Setting

The study period began on 1 January 1997 (1 April 2004 for the secondary outcome) in Denmark, 1 January 2007 in Sweden and 1 January 2010 in Norway, and, based on the availability of information from national registries. The study time period ended on 31 December 2018 for Denmark and 31 December 2019 for both Sweden and Norway.

Subjects and Study Size

Pregnancies were included if they met all the following inclusion criteria:

- Singleton pregnancies, with known pregnancy-length of at least 12 weeks within the study time period
- Pregnancies linked to both mother and father within the study time period
- Father with a continuous enrolment in the database for ≥12 months prior to linked mother at the date of the last menstrual period plus 2 weeks (LMP2)
- Father with at least one AED in the data available

Pregnancies were excluded if they met any of the following exclusion criteria:

- Adopted children
- Pregnancy associated with in vitro fertilization
- Pregnancies with missing gestational age and/or missing maternal LMP2 (for these pregnancies it will not be possible to identify the exposure window for the study)
- Different cohorts were constructed for analysis with further inclusion/exclusion criteria to address the research questions related to the primary (NDD including ASD), and secondary (CM) outcomes

For the primary outcome, NDD including ASD, to observe a hazard ratio (HR) of 2.00 with 5% significance and 80% power, a sample size of 1178 offspring within the family linked unit was needed across all 3 countries. This required a minimum of 589 offspring within a family linked unit with paternal exposure to valproate (monotherapy) and a minimum of 589 offspring within a family linked unit with paternal exposure to lamotrigine/levetiracetam (composite monotherapy).

For the secondary outcome, CM, assuming to observe an odds ratio (OR) of 2.5 with 5% significance and 80% power, sample size of 826 offspring within the family linked unit was needed across all 3 countries. This required a minimum of 413 offspring within a family linked unit with paternal exposure to valproate (monotherapy) and a minimum of 413 offspring within a family linked unit with paternal exposure to lamotrigine/levetiracetam (composite monotherapy).

Variables and Data Sources

The primary outcome of interest was NDD, including ASD, and the secondary outcome of interest was a composite of CM (major and/or minor), in offspring up to 12 years of age for both outcomes, based on International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) diagnostic codes.

The primary exposure of interest was paternal use of valproate during the spermatogenic risk window prior to conception of the offspring (defined by the first day of the LMP2 date of the mother within the linked family unit). Exposure information was derived from prescription data, as recorded in the National Prescription Registries for each country (from 1995 in Denmark, 2005 in Sweden and 2008 in Norway). Country-specific cohorts of eligible linked family units were then identified.

The data sources used to retrieve this information were national registries in Denmark, Sweden and Norway:

- Denmark: Danish civil registration system, Register of medicinal product statistics, National patient registry, Cause of death register, Medical birth registry, The in vitro fertilization register
- Sweden: Multigenerational register, Cause of death register, National prescription registry, National patient registry, Medical birth registry
- Norway: Central person register, Norwegian prescription database, Norwegian patient registry, Medical birth registry, Cause of death register

Results

Primary outcome, NDD including ASD

Primary outcome, descriptive results

The Primary outcome cohort for descriptive analyses included 2,031 offspring (respectively, 832 and 1,199 paternally exposed to valproate and to lamotrigine/levetiracetam) in Denmark, 2,451 (respectively, 968 and 1,483) in Sweden, and 1,471 (respectively, 413 and 1,058) in Norway.

Offspring characteristics were similar in Denmark, Sweden, and Norway: mostly male (52.2%, 51.2%, and 52.1%, respectively), born at term between 37-41 weeks of gestational age (89.6%, 88.5% and 89.8%, respectively), and weighed ≥2500 grams at birth (96.5%, 96.3%, and 96.3%, respectively); similar in both exposure groups. Year of birth was unbalanced between the 2 paternal exposure groups. Across all 3 countries, more offspring in the valproate exposed group were conceived in the earliest years of the study period compared to those in the lamotrigine/levetiracetam group, although the variation observed for Norway was minor. As a result, the mean follow-up period in years per offspring was longer in offspring paternally exposed to valproate than those to lamotrigine/levetiracetam in Denmark (respectively, 9.2 and 6.6 years) and Sweden (6.7 and 5.1 years). However, the length of follow-up was comparable between offspring from fathers exposed to valproate and those exposed to lamotrigine/levetiracetam in Norway (5.0 and 4.8 years).

Mothers' age at childbirth was similar in Denmark, Sweden, and Norway (median of 30 years, 31 years, and 31 years, respectively); similar in both exposure groups. Across all 3 countries, mothers of offspring paternally exposed to valproate were less frequently affected by comorbidities prior to childbirth compared to those to lamotrigine/levetiracetam: neurotic disorder (respectively, 5.8% and 7.5% in Denmark, 9.8% and 13.1% in Sweden, 12.8% and 13.6% in Norway), gestational diabetes (respectively, 3.6% and 3.8% in Denmark, 2.7% and 3.2% in Sweden, 5.3% and 6.6% in Norway) and affective disorder (respectively, 2.6% and 5.0% in Denmark, 9.0% and 10.5% in Sweden, 5.8% and 10.9% in Norway). Maternal smoking during pregnancy was slightly more frequently recorded in the valproate exposure group compared to the lamotrigine/levetiracetam exposure group (respectively, 16.6% and 15.8% in Denmark, 7.9% and 5.5% in Sweden, 6.8% and 4.5% in Norway).

The maternal use of medications during pregnancy was similar across the 3 countries. A lower proportion of mothers was observed with a polypharmacy index between 1 and 4 during pregnancy in the valproate group compared to the lamotrigine/levetiracetam group (respectively, 44.0% and 51.6% in Denmark, 45.4% and 48.2% in Sweden, 44.8% and 47.3% in Norway); likewise for the concomitant use of medications associated with valproate-indicated psychiatric conditions during pregnancy (respectively 3.4% and 6.3% in Denmark, 6.4% and 8.4% in Sweden, 3.9% and 6.5% in Norway). The use of AEDs was very low (<1% for most individual AEDs), both before LMP2 and during pregnancy, irrespective of the exposure groups and the country.

Fathers' age at childbirth was also similar in Denmark, Sweden, and Norway (median of 33 years in all countries); similar in both exposure groups. Across all 3 countries, fathers of offspring paternally exposed to valproate were less frequently affected by comorbidities prior to childbirth compared to those exposed to lamotrigine/levetiracetam: neurotic disorders (respectively, 6.3% and 11.3% in Denmark, 13.5% and 27.4% in Sweden, 7.5% and 15.6% in Norway), affective disorder excluding bipolar disorder and mania (respectively, 3.7% and 13.0% in Denmark, 11.1% and 30.3% in Sweden, 7.8% and 22.6% in Norway) and bipolar affective disorder (respectively, 2.6% and 7.5 in Denmark, 12.9% and 29.5% in Sweden, 14.0% and 27.7% in Norway).

The paternal use of medication in the 3-month lookback from LMP2 was similar across the 3 countries. A lower proportion of fathers was observed with a polypharmacy index between 1 and 4 in the valproate group compared to the lamotrigine/levetiracetam group (respectively, 29.1% and 41.5% in Denmark, 30.5% and 47.7% in Sweden, 34.6% and 45.7% in Norway); likewise for the use of medications associated with neuropsychiatric adverse events (respectively, 49.3% and 56.0% in Denmark, 48.5% and 64.1% in Sweden, 56.4% and 64.6% in Norway). In all countries, the most common indication for AED use was epilepsy, both among fathers exposed to valproate and lamotrigine/levetiracetam (respectively, 70.0% and 59.0% in Denmark, 70.7% and 46.1% in Sweden, 57.9% and 41.5% in Norway).

The overall cumulative incidence proportions of NDD including ASD over the study follow-up period (0-12 years in Denmark and Sweden, and 0-10 years in Norway) were higher in offspring paternally exposed to valproate than in those to lamotrigine/levetiracetam in the 3 countries: respectively, 6.6% (95% confidence interval [CI]: 4.9, 8.3) and 3.7% (95% CI: 2.6, 4.7) in Denmark, 5.4% (95% CI: 4.0, 6.8) and 3.5% (95% CI: 2.6, 4.4) in Sweden, and 4.1% (95% CI: 2.2, 6.0) and 2.4% (95% CI: 1.5, 3.3) in Norway. The pooled ratio of the cumulative incidence proportions (valproate over lamotrigine/levetiracetam paternal exposure groups) across the 3 countries for the 0-10 years follow-up period was 1.58 (95% CI: 1.21, 2.05); no heterogeneity was observed between country-specific estimates (I^2=0.0, 95% CI: 0.0, 0.9).

The overall cumulative incidence rates of NDD including ASD over the study follow-up period (0-12 years in Denmark and Sweden, and 0-10 years in Norway) were also higher in offspring paternally exposed to valproate than in those to lamotrigine/levetiracetam in the 3 countries: respectively, 7.2 (95% CI: 5.4, 9.3) per 1000 person-years and 5.6 (95% CI: 4.0, 7.5) per 1000 person-years in Denmark, 8.0 (95% CI: 6.0, 10.5) per 1000 person-years and 6.9 (95% CI: 5.2, 9.1) per 1000 person-years in Sweden, and 8.3 (95% CI: 4.8, 13.2) per 1000 person-years and 4.9 (95% CI: 3.2, 7.2) per 1000 person--years in Norway. The pooled ratio of the cumulative incidence rates (valproate over lamotrigine/levetiracetam paternal exposure groups) across the 3 countries for the 0-10 years follow-up period was 1.26 (95% CI: 0.97, 1.64); no heterogeneity was observed between country-specific estimates (I^2=0.0, 95% CI: 0.0, 0.9).

Primary outcome, comparative results

From the Primary outcome cohort for descriptive analyses described above, 232 offspring in total across the 3 countries, who had a diagnosis of epilepsy and/or having received AEDs and/or from epileptic mother and/or being maternally exposed to AEDs (including valproate, lamotrigine or levetiracetam) in utero, or in the 3-month lookback from LMP2, were excluded to form the Primary outcome cohort for the comparative analyses. This comparative cohort consisted of 1,950 offspring (respectively, 793 and 1,157 paternally exposed to valproate and to lamotrigine/levetiracetam) in Denmark, 2,355 offspring (respectively, 930 and 1,425) in Sweden, and 1,416 offspring (respectively, 398 and 1,018) in Norway.

The risk of NDD including ASD associated with the paternal exposure to valproate compared to the paternal exposure to lamotrigine/levetiracetam was assessed using crude Cox regression models: occurrence of NDD was observed in 43 out of 793 (5.4%) and 41 out of 1,157 (3.5%) offspring in the valproate and in the lamotrigine/levetiracetam groups respectively in Denmark, in 49 out of 930 (5.3%) and 41 out of 1,425 (2.9%) offspring, respectively in Sweden, and in 0 out of 383 (0.0%) and 23 out of 1,018 (2.3%) offspring, respectively in Norway, after excluding influential subjects (specifically, those subjects that individually affect the model estimates above a pre-defined threshold, ie, the model coefficient changes substantially after removing/including them). The resulting HRs indicated no significant higher risk of NDD including ASD with the paternal exposure to valproate compared to lamotrigine/levetiracetam in Denmark and Sweden: 0.94 (95% CI: 0.60, 1.46) in Denmark, and 1.16 (95% CI: 0.76, 1.76) in Sweden. In Norway, no events were observed in the valproate paternal exposure group after the exclusion of influential subjects (N=15), which led to non-calculable HR. Therefore, for Norway, the crude Cox regression model was rerun without excluding the influential subjects (this model is a deviation from the protocol, adapted due to very small numbers); in Norway, results from the rerun crude adapted model indicated no significant higher risk of NDD including ASD with the paternal exposure to valproate compared to lamotrigine/levetiracetam in Norway (HR: 1.60, 95% CI: 0.81, 3.15) as for the other countries. In order to meta-analysis results from all the 3 countries, the pooled crude HR was estimated without excluding influential subjects (as no influential subjects were identified in the crude models for Denmark and Sweden). The pooled crude HR across the 3 countries was consistent with the country-specific estimates in terms of strength and non-significance of the risk: 1.13 (95% CI: 0.85, 1.49); no heterogeneity was observed between country-specific estimates (I^2=0.0, 95% CI: 0.0, 89.6).

The risk of NDD including ASD associated with paternal exposure to valproate compared to that to lamotrigine/levetiracetam was further assessed using propensity score (PS)-weighted Cox regression models after the further exclusion of offspring with outlier weights: occurrence of NDD was observed in 35 out of 678 (5.6%) and 36 out of 1,118 (3.2%) offspring in the valproate and in the lamotrigine/levetiracetam groups respectively in Denmark, in 47 out of 841 (5.6%) and 34 out of 1,334 (2.5%) offspring respectively in Sweden, and in 13 out of 325 (4.0%) and 21 out of 910 (2.3%) offspring respectively in Norway. The HRs adjusted for PS-weighted were higher than crude HRs in all countries but remained non-significant: 1.34 (95% CI: 0.79, 2.25) (also adjusted for unbalanced risk factors: maternal affective disorders and maternal concomitant medications associated with valproate-indicated psychiatric conditions during pregnancy) in Denmark, 1.54 (95% CI: 0.95, 2.51) in Sweden, and 1.76 (95% CI: 0.83, 3.71) in Norway. However, the pooled PS-weighted adjusted HR across the 3 countries showed a significantly higher risk with the paternal exposure to valproate due to a more precise estimation as the population size increased: 1.50 (95% CI: 1.09, 2.07); no heterogeneity was observed between country-specific estimates (I^2=0.0, 95% CI: 0.0, 89.6).

Sensitivity analysis 11 (ie, considering a narrow case definition of NDD) indicated a significant association compared to that of the main analysis in Sweden (PS-weighted adjusted HR of NDD narrow definition: 1.70, 95% CI: 1.02, 2.81). Non-significant relative risk was observed in Denmark (PS-weighted adjusted HR of NDD: 1.59, 95% CI: 0.89, 2.86) (model also adjusted for Maternal affective disorder and Maternal concomitant medications associated with valproate-indicated psychiatric conditions during pregnancy - mothers with at least one prescription) and in Norway (PS-weighted adjusted HR of NDD narrow definition: 1.87, 95% CI: 0.86-4.08).

Sensitivity analysis 2 (ie, considering ASD alone as primary outcome) indicated a non-significant association in the opposite direction compared to that of the main analysis in Denmark (PS-weighted adjusted HR of ASD: 0.76, 95% CI: 0.30, 1.89) and a significant association in Sweden (PS-weighted adjusted HR of ASD: 2.70, 95% CI: 1.19, 6.17). In Norway, due to the low event count (<10), it was not possible to conduct the crude and adjusted Cox regression models for sensitivity analysis 2.

Sensitivity analysis 10 (ie, considering continuous measure of cumulative exposure) indicated a non-significant association in the opposite direction compared to that of the main analysis in Denmark (PS-weighted adjusted HR: 0.58, 95% CI: 0.31, 1.08). In Sweden, results were similar to the main analysis (PS-weighted adjusted HR: 1.17, 95% CI: 0.62-2.18). In Norway, due to the low event count (<10), it was not possible to conduct the adjusted Cox regression models for sensitivity analysis 10.

All other sensitivity analyses provided similar adjusted HRs as those of the main analysis, for the 3 countries.

Primary outcome, exploratory results

Exploratory analysis to address objective 5 consistently indicated, across the paternal exposure groups, similar distributions of risks factors and confounders (in all countries) and higher proportions of offspring presenting a NDD including ASD (in Sweden and Norway) (numbers were masked in Denmark), in offspring paternally exposed to polytherapy with AEDs compared to offspring paternally exposed to valproate or to lamotrigine/levetiracetam in monotherapy (ie, from the main analysis).

Exploratory analysis to address objective 6 was conducted in very few numbers of offspring in all countries, preventing the run of the analysis for associations of risk factors and confounders with the occurrence of NDD including ASD (0 out 21 offspring presented the outcome in Denmark; 3 out 29 offspring presented the outcome in Sweden, and 0 out 2 offspring presented the outcome in Norway).

Secondary outcome, CM

Secondary outcome, descriptive results

The Secondary outcome cohort for descriptive analyses included 1,655 offspring (respectively, 549 and 1,106 paternally exposed to valproate and to lamotrigine/levetiracetam) in Denmark, and 1,476 offspring (respectively, 416 and 1,060) in Norway.

Offspring, maternal and paternal demographic characteristics in the 2 exposure groups of the Secondary outcome cohorts were similar to those from the Primary outcome cohorts for descriptive analyses in all countries.

Consistently, across Denmark and Norway, mothers of offspring paternally exposed to valproate were similarly affected by comorbidities prior to childbirth as those to lamotrigine/levetiracetam: gestational diabetes (respectively, 3.6% and 3.4% in Denmark, 1.9% and 2.3% in Norway), diabetes (respectively, 0.9% and 1.2% in Denmark, 1.7% and 2.0% in Norway), obesity (respectively, 1.5% and 1.8% in

Denmark, 1.4% and 0.9% in Norway) and epilepsy (respectively, 2.7% and 1.6% in Denmark, 1.7% and 1.4% in Norway). They were less likely to use medications associated with teratogenic activity/fetal toxicity than mothers of offspring paternally exposed to lamotrigine/levetiracetam prior to LMP2 (respectively, 25.9% and 30.8% in Denmark, 28.1% and 29.5% in Norway) and/or during the pregnancy (respectively, 27.0% and 33.5% in Denmark, 27.9% and 32.5% in Norway).

Fathers of offspring paternally exposed to valproate were more likely to receive their AED to treat epilepsy than those of offspring paternally exposed to lamotrigine/levetiracetam in the 2 countries (respectively, 75.4% and 58.3% in Denmark, 57.7% and 41.6% in Norway).

The overall cumulative incidence proportion of reported CM diagnoses (major and minor as composite) over the study follow-up period (0-12 years for Denmark and 0-10 years for Norway) was lower in offspring paternally exposed to valproate compared to those to lamotrigine/levetiracetam in Denmark, while they were similar between the 2 paternal exposure groups in Norway: respectively, 9.3% (95% CI: 6.9, 11.7) and 14.1% (95% CI: 12.1, 16.2) in Denmark, and 15.1% (95% CI: 11.7, 18.6) and 14.5% (95% CI: 12.4, 16.7) in Norway.

Secondary outcome, comparative results

From the cohort described above, 1,970 offspring in total across the 2 countries were excluded as follows to form the Secondary outcome cohort for the comparative analysis: if they had a diagnosis of epilepsy, and/or received AEDs, and/or were born from a mother with epilepsy, and/or were maternally exposed to AEDs (including valproate, lamotrigine or levetiracetam) in utero, or in the 3-months lookback from LMP2, and/or their mother had been exposed to teratogenic drugs 3 months prior to conception or during pregnancy, and/or their father had been exposed to teratogenic drugs 3 months prior to conception. This comparative cohort consisted of 648 offspring (respectively, 259 and 389 paternally exposed to valproate and to lamotrigine/levetiracetam) in Denmark, and 513 offspring (respectively, 169 and 344) in Norway.

The risk of CM associated with paternal exposure to valproate compared with paternal exposure to lamotrigine/levetiracetam was assessed using crude logistic regression models. CM was found in 23 out of 259 (8.9%) and 53 out of 389 (13.6%) offspring in the valproate and the lamotrigine/levetiracetam groups respectively in Denmark, and in 24 out of 169 (14.2%) and 46 out of 344 (13.4%) offspring respectively in Norway. The resulting ORs indicated no significant association with paternal exposure to valproate in the 2 countries: 0.62 (95% CI: 0.37, 1.04) in Denmark, and 1.06 (95% CI: 0.62, 1.82) in Norway. The crude pooled OR across the 2 countries was estimated: 0.81, 95% CI: 0.48, 1.36); but moderate-to-substantial heterogeneity was observed between country-specific estimates (I^2=0.5, 95% CI: Not available).

The risk of CM associated with the paternal exposure to valproate compared with lamotrigine/levetiracetam was further assessed using PS-weighted logistic regression models in smaller populations due to exclusion of offspring with outlier weights: respectively, 21 out of 238 (8.8%) and 52 out of 381 (13.6%) offspring with the outcome in Denmark, and 14 out of 136 (10.3%) and 40 out of 301 (13.3%) offspring with the outcome in Norway. The OR adjusted for PS-weights was similar to the crude OR in Denmark: 0.61 (95% CI: 0.36, 1.06). In Norway, the PS-weighted logistic regression model did not converge due to a quasi-complete separation, exacerbated by the low event numbers. Therefore, the PS-weighted adjusted OR and the pooled PS-weighted adjusted OR could not be estimated.

All the sensitivity analyses generated similar PS-weighted adjusted ORs as that of the main analysis in Denmark. However, the comparison is limited in Norway, considering the adjusted logistic regression models did not converge.

Secondary outcome, exploratory results

Exploratory analyses to address exploratory objectives 5 and 6 were conducted on a very low number of offspring, either preventing the run of the analysis or limiting the interpretation of the results. Exploratory analysis to address exploratory objective 7 (risk of CM live offspring in Sweden) was conducted on 2,451 offspring (968 paternally exposed to valproate and 1,483 paternally exposed to lamotrigine/levetiracetam) for descriptive analyses and on 888 offspring (418 paternally exposed to valproate and 470 paternally exposed to lamotrigine/levetiracetam) for comparative analyses. Offspring, maternal and paternal characteristics were similar to those observed in Denmark and Norway with regards to the paternal exposure group. The overall cumulative incidence proportions of CM over the study follow-up in offspring paternally exposed to valproate was similar to that in offspring paternally exposed to lamotrigine/levetiracetam: respectively, 10.4% (95% CI: 8.5, 12.4) and 10.5% (95% CI: 9.0, 12.1). The crude and the PS-weights adjusted ORs of CM in offspring paternally exposed to valproate compared to offspring paternally exposed to lamotrigine/levetiracetam were in favor of an absence of an increased risk, being respectively 1.01 (95% CI: 0.66, 1.55) and 0.92 (95% CI: 0.59, 1.44).

Exploratory analysis to address objective 8 was conducted on 76 offspring that reported 111 CM diagnoses in Denmark and 70 offspring that reported 105 CM diagnoses in Norway. Proportion of major CM was higher in the reported CM, in both countries among offspring from fathers exposed to valproate and in Denmark among offspring from fathers exposed to lamotrigine/levetiracetam: 24 out 34 (70.6%) in the valproate group and 61 out of 77 (79.2%) in the lamotrigine/levetiracetam group were major CM diagnoses in Denmark, 25 out of 40 (62.5%) and 31 out of 65 (47.7%) respectively in Norway. Distribution of the most frequently reported target body organ classes according to the exposure group was not consistent between the 2 countries. In Norway, the most frequently reported target body organ classes was the digestive system in both groups (32.5% in valproate group, 30.8% in lamotrigine/levetiracetam group); the second was the limbs (25.0% and 24.6% respectively), and the third one was congenital heart defects (12.5% and 13.9% respectively). In Denmark, all numbers were masked in the valproate group except for limbs (17.7%) whereas in lamotrigine/levetiracetam group, the limbs were the second most reported (20.8%), the first one being congenital heart defects (26.0%).

Discussion

This comprehensive real-world retrospective study provides the first results on NDD including ASD and CM outcomes in offspring paternally exposed to valproate at the time of conception, compared to those exposed to lamotrigine/levetiracetam, in Denmark, Sweden and Norway.

A significantly increased risk of NDD including ASD associated with paternal exposure to valproate compared with paternal exposure to lamotrigine/levetiracetam at the time of conception was observed when pooling the country-specific adjusted risk estimates into a meta-analysis (PS-weighted adjusted HR: 1.5, 95% CI: 1.1, 2.1; I^2=0.0%). However, due to the observational nature of this study, no causal relationship can be established, nor the biological or the pharmacological mechanisms to explain the relationship.

The nature of the NDD and specific subtypes (ASD, intellectual disabilities, attention deficit hyperactivity disorder) was not assessed because the study was powered to investigate NDD as a composite

outcome. However, sensitivity analyses focusing on a narrow definition of NDD showed that the risk estimates varied in strength, and significance compared to those from the main analysis, and these variations were not consistent across the 3 countries. For example, with sensitivity analysis 2 focusing on ASD as primary outcome, the association reversed toward a non-significant reduced risk with the paternal exposure to valproate in Denmark, while the risk almost doubled and became significant with this exposure in Sweden. It is noteworthy that differences in the length of follow-up were observed between countries. In Denmark and Sweden, where offspring were followed from birth to 12 years of age, follow-up was shorter in Sweden, with 23.3% of the offspring in the lamotrigine/levetiracetam group followed-up more than 8 years *versus* 41.8% in the valproate group; it was longer in Denmark, with 40.2% lamotrigine/levetiracetam group followed-up more than 8 years *versus* 74.3% in the valproate group. This may explain the lower rate of ASD captured in the lamotrigine/levetiracetam group in Sweden compared to Denmark and may highlight the impact of the follow-up duration on the results. While these sensitivity analyses relied on lower number of events and estimates may be more prone to instability and lower reliability, they call for caution in the interpretation of the study results. It is worthwhile to note that offspring paternally exposed to valproate were systematically more frequently conceived in the earlier years of inclusion than those exposed to lamotrigine/levetiracetam, although this variation was minor in Norway. As a result, offspring paternally exposed to valproate had on average a longer follow-up time and a higher probability of presenting NDD, including ASD diagnoses. Considering that the risk of being diagnosed with NDD including ASD is not constant across ages but rather detected at later ages when children start school (ie, from 5 or 6 years old), this may have biased the risk estimates generated from Cox regression models.

In line with previously published studies, results from crude pooled OR suggested no increased risk of CM associated with the paternal exposure to valproate compared to the paternal exposure to lamotrigine/levetiracetam in the 3 months preconception period, consistent across Denmark and Norway (pooled OR: 0.81, 95% CI: 0.48, 1.36; I^2=49.6%). However, findings should be interpreted with caution since they are crude data that have not been adjusted for other variables and may reflect the observed heterogeneity. The presence of such heterogeneity may be due to the fact that only 2 estimates were pooled in the meta-analysis. Non-convergence of adjusted logistic regression models precluded the pooled PS-weighted adjusted OR to be estimated.

Additionally, some methodological limitations may be acknowledged. This study used secondary data that was not collected primarily for research purposes and therefore information on certain parameters, such as some known risk factors and/or causal factors (eg, genetic abnormalities, congenital infectious diseases, paternal condition severity that required AED use, lifestyle factors) which were not identified nor controlled for. These factors were assumed to be balanced between the 2 paternal exposure groups, but this assumption could not be verified, and unmeasured confounding may bias the risk estimates. Especially the type of epilepsy, which may not be balanced between the 2 paternal exposure groups; indeed, valproate is the treatment of choice (or first-line drug) for male patients with idiopathic generalized epilepsy, a type of epilepsy which could be associated with NDD and is known to have a genetic basis and as such can be found in several members of the same family.

This study found an increased risk of NDD, including ASD, with paternal exposure to valproate, compared to lamotrigine/levetiracetam at the time of conception. Due to methodological limitations, especially the difference in follow-up time between the 2 paternal exposure groups which may impact the interpretation of the results, these findings regarding risk of NDD should be interpreted with caution. While the study did not find any difference in risks of CM between the 2 paternal exposure groups,

findings were based on crude estimates which were potentially biased and also affected by moderate-to-substantial heterogeneity, thus these findings should also be interpreted with caution.

Marketing authorisation holders (MAH)

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