U.PORTO

FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2022/2023

Roana Josephine Pratsivnik Soulé

Prenatal exposure to valproate and the risk of autism spectrum disorder: A systematic review.

Exposição pré-natal ao valproato e risco de perturbações do espetro do autismo: Uma revisão sistemática.

MARÇO, 2023

Roana Josephine Pratsivnik Soulé

Prenatal exposure to valproate and the risk of autism spectrum disorder: A systematic review.

Exposição pré-natal ao valproato e risco de perturbações do espetro do autismo: Uma revisão sistemática.

Mestrado Integrado em Medicina

Área: Neurociências Clínicas e Saúde Mental

Tipologia: Dissertação

Trabalho efetuado sob a Orientação de:

Prof. Doutora Irene Maria Palmares Dias Carvalho

Trabalho organizado de acordo com as normas da revista: Epilepsia

MARÇO, 2023

UC Dissertação/Projeto (6º Ano) - DECLARAÇÃO DE INTEGRIDADE

Eu, Roana Josephine Pratsivnik Soulé, abaixo assinado, nº mecanográfico 201708388, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 09/03/2023

Assinatura conforme cartão de identificação:
Roana Josephine Pratsivnik Soulé

UC Dissertação/Projeto (6º Ano) – DECLARAÇÃO DE REPRODUÇÃO

NOME

Roana Josephine Pratsivnik Soulé

NÚMERO DE ESTUDANTE	E-MAIL
201708388	roanasoule@gmail.com

DESIGNAÇÃO DA ÁREA DO PROJECTO

Neurociências Clínicas e Saúde Mental

TÍTULO DISSERTAÇÃO/~~MONOGRAFIA~~ (riscar o que não interessa)

Prenatal exposure to valproate and the risk of autism spectrum disorder: A systematic review.
Professora Doutora Irene Maria Palmares Dias Carvalho

ORIENTADOR

COORIENTADOR (se aplicável)

ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
É AUTORIZADA A REPRODUÇÃO PARCIAL DESTE TRABALHO (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
DE ACORDO COM A LEGISLAÇÃO EM VIGOR, (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTE TRABALHO.	☒

Faculdade de Medicina da Universidade do Porto, 09/03/2023

Assinatura conforme cartão de identificação: Roana Josephine Pratsivnik Soulé

AGRADECIMENTOS

A realização desta dissertação de Mestrado Integrado em Medicina contou com a colaboração imprescindível, de forma direta ou indireta, de várias pessoas às quais estarei eternamente grata.

À Professora Doutora Irene Maria Palmares Dias Carvalho, pela orientação, total apoio e disponibilidade até ao último minuto, sabedoria transmitida, sugestões e críticas construtivas, incentivo e exigência de qualidade que em muito enriqueceram este trabalho.

Aos meus dois grandes amigos Cristie e Ricardo, que durante estes anos de faculdade estiveram sempre ao meu lado, agradeço para sempre pelo companheirismo, tempo e paciência demonstrados nos meus momentos de aflição.

Por último, dirijo um especial agradecimento aos meus pais, irmãos, avós e namorado por todo o apoio incondicional, carinho e compreensão que demonstraram durante esta fase da minha vida. A eles dedico esta dissertação, bem como todas as minhas demais conquistas.

Prenatal exposure to valproate and the risk of autism spectrum disorder: A systematic review.

Roana Soulé[1], Irene Palmares Carvalho[1 2 3]

Affiliations:

[1] Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal.

[2] Medical Psychology Unit, Department of Clinical Neurosciences and Mental Health, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal.

[3] CINTESIS@RISE, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal.

Corresponding author: Roana Soulé

E-mail: roanasoule@gmail.com

ABSTRACT

Objective: To determine whether children prenatally exposed to valproate (VPA) have increased risk of autism spectrum disorders (ASD).

Methods: The literature search was conducted in PubMed, Web of Science, Scopus, and PsycINFO with the following keywords combined with the Boolean operators 'AND' and 'OR': "valproate in pregnancy", "prenatal exposure to valproate" and "offspring autism", "autism spectrum disorder" or "autism". Inclusion criteria were research studies reporting results on the use of VPA in pregnant women and its relationship with ASD, resulting in a total of 260 articles from which 9 were included in this review.

Results: Of the nine articles included in this review, eight were population-based cohort studies and one was a population-based case-control study. Prenatal exposure to VPA was associated with ASD development in six studies, whereas three reported no association between VPA exposure and ASD, possibly due to sample size and ASD assessment method. Among five studies examining VPA dose effects, two reported a dose-response relationship with ASD, whereas one reported that the risk of ASD was similarly increased with both high and low VPA doses, and another two showed no association, possibly due to a limited range of doses in the samples. No difference was found between exposure to VPA monotherapy and polytherapy on ASD in one study, whereas another three reported differences, but in opposite directions. Two studies were concordant in that there was no difference in the risk of ASD between offspring of women who took VPA early and later in pregnancy. Results by sex (three studies) were inconsistent, with one study concluding that ASD was more frequent in VPA-exposed boys than girls, one showing no evidence of sex differences, and the other showing that ASD is more frequent in females than in males VPA-exposed children.

Significance: The results suggests that the use of VPA during any pregnancy trimester, alone or in combination with other antiepileptic drug, can be associated with a significantly greater risk of ASD in the offspring, comparing with unexposed children.

Keywords: Valproate; Autism Spectrum Disorder; Pregnancy.

RESUMO

Objetivo: Determinar se crianças com exposição pré-natal ao valproato (VPA) têm risco aumentado de perturbações do espetro do autismo (PEA).

Métodos: A pesquisa bibliográfica foi realizada na PubMed, Web of Science, Scopus e PsycINFO com os seguintes termos combinados com os operadores booleanos 'AND' e 'OR': “valproate in pregnancy”, “prenatal exposure to valproate” and “offspring autism”, “autism spectrum disorder” or “autism”. Os critérios de inclusão foram estudos que relatam resultados sobre o uso de VPA em mulheres grávidas e sua relação com PEA, resultando num total de 260 artigos, dos quais 9 foram incluídos nesta revisão.

Resultados: Dos nove artigos incluídos nesta revisão, oito eram estudos de coorte de base populacional e um era estudo de caso-controle de base populacional. A exposição pré-natal ao VPA foi associada com o desenvolvimento de PEA em seis estudos, enquanto três não relataram qualquer associação entre a exposição ao VPA e PEA, possivelmente devido ao tamanho da amostra e ao método de avaliação de PEA. Dos cinco estudos que examinaram os efeitos da dose de VPA, dois relataram uma relação dose-resposta com PEA, enquanto um relatou que o risco de PEA aumentava de forma semelhante com doses altas e baixas de VPA, e outros dois não mostraram nenhuma associação, possivelmente devido à limitação de doses nas amostras. Nenhuma diferença foi encontrada entre a exposição à monoterapia com VPA e à politerapia no desenvolvimento de PEA em um estudo, enquanto outros três relataram diferenças, mas em direções opostas. Dois estudos foram concordantes em que não houve diferença no risco de PEA entre filhos de mulheres que tomaram VPA no início e no final da gravidez. Os resultados por sexo (três estudos) foram inconsistentes, com um estudo concluindo que PEA eram mais frequentes crianças do sexo masculino expostos ao VPA do que nas do sexo feminino, outro não mostrava evidências de diferenças entre os sexos, e o outro mostrava que PEA eram mais frequentes em crianças do sexo feminino do que nas do sexo masculino expostos ao VPA.

Significado: Os resultados sugerem que o uso de VPA durante qualquer trimestre da gravidez, isoladamente ou em combinação com outro antiepilético, pode estar associado a um risco significativamente maior de desenvolvimento de PEA na prole, em comparação com crianças não expostas.

INTRODUCTION

Valproate (VPA) is a well-established anticonvulsant drug that is used to treat conditions such as epilepsy. Available as valproic acid, sodium valproate and semisodium valproate, VPA acts on dopamine, GABA and glutamate neurotransmission and intracellular signaling. It has been used increasingly to manage conditions other than epilepsy, particularly bipolar and other affective disorders, and, as a result, more women of childbearing age are likely to be exposed to this drug [1].

VPA is generally not indicated for fertile women but, because it is the most effective therapy in some epilepsy syndromes, the patient has the right to choose VPA therapy, thus undertaking the elevated risk of developmental abnormalities appearing in the offspring, for greater safety regarding seizures [2]. Becoming pregnant poses a challenging balance between optimizing seizure control and minimizing the potentially harmful effects of antiepileptic drugs (AEDs) on the fetus. Among the AEDs, VPA appears to induce developmental neurotoxicity in children's central nervous system [3], cognitive impairment and congenital malformations in the offspring [4,5]. Children exposed to VPA in utero might also develop Valproate fetopathy syndrome (FVS), a syndrome that correlates intellectual disability with malformations [6]. Case series of children with FVS presenting autism have been reported [7,8].

Autism spectrum disorder (ASD) refers to neurological and developmental disorders that can occur to different degrees and in a variety of forms. It can include childhood autism (autistic disorder), Asperger syndrome, atypical autism,

and other or unspecified pervasive developmental disorders. People with ASD frequently have difficulties with social communication and present stereotyped or repetitive behaviors and interests [9, 10]. Although ASD can be diagnosed at any age, symptoms generally appear in the first two years of life. According to data from the National Autism Association, about 1% of the world's population suffers from ASD, and the prevalence rate among boys is higher than it is among girls [11]. A variety of environmental factors may increase the occurrence of ASD, and prenatal VPA exposure would be a modifiable environmental factor [12]. In animals, prenatal exposure to VPA is known to cause ASD-like symptoms [13]. The prevalence of ASD in children prenatally exposed to VPA has been suggested to range from 8% to 15% [14,15], as opposed to a general population prevalence of 0.6%–1% [9,16,17].

In recent years, research on the pathophysiological mechanisms of ASD after VPA exposure has attracted extensive attention, and a significant number of research efforts have been attempted to define the contributory role of VPA to the impairment of normal prenatal growth and development [18]. Related studies have found that VPA can change genes transcription levels, causing abnormal signaling pathways, synaptic dysfunction, and neurogenesis deficits, which can lead to ASD in the offspring [19]. Previous systematic reviews have examined the association between in-utero VPA exposure and neurodevelopment, reporting a significant association of children's VPA exposure with lower IQ scores and poorer overall neurodevelopmental outcomes [20,21]. However, the role of VPA exposure in the development of ASD is lacking in previous systematic reviews. The aim of this systematic review was to synthetize

the most relevant research on this theme to provide an understanding on whether prenatal exposure to VPA is an important risk factor for the development of ASD in the offspring. VPA doses, uses (alone or in polytherapy), time (early *versus* later) in pregnancy, and sex differences in VPA-exposed children are also examined.

METHODS

This systematic review was conducted following the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The relevant databases used for the search were PubMed, Scopus, Web of Science and PsycINFO, without any publication year restrictions. The databases were search until October 2022, with keywords combined with the Boolean operators 'AND' and 'OR': "valproate in pregnancy", "prenatal exposure to valproate", and "offspring autism", "autism spectrum disorder" or "autism".

Inclusion and exclusion criteria

Eligible articles were research studies reporting results on the use of VPA in pregnant women and its relationship with ASD in their offspring. Animal studies, non-empirical research articles, and studies written in languages other than English were excluded.

Study selection and data extraction

The search retrieved a total of 260 articles from across the four databases. After removal of 54 duplicate articles, 206 were screened by title and abstract reading, leading to the exclusion of 171 documents. The remaining 35 articles were read in full, and 26 were excluded. The process of search, article screening, and data extraction was performed by the two authors independently, and questions arising in this process were

discussed and resolved through consensus. For the qualitative synthesis, the following details were extracted from all eligible studies: author's name, year of publication, study's country, research design, duration and sample size, population characteristics, measures and results. A flowchart of the selection process is shown in Figure 1.

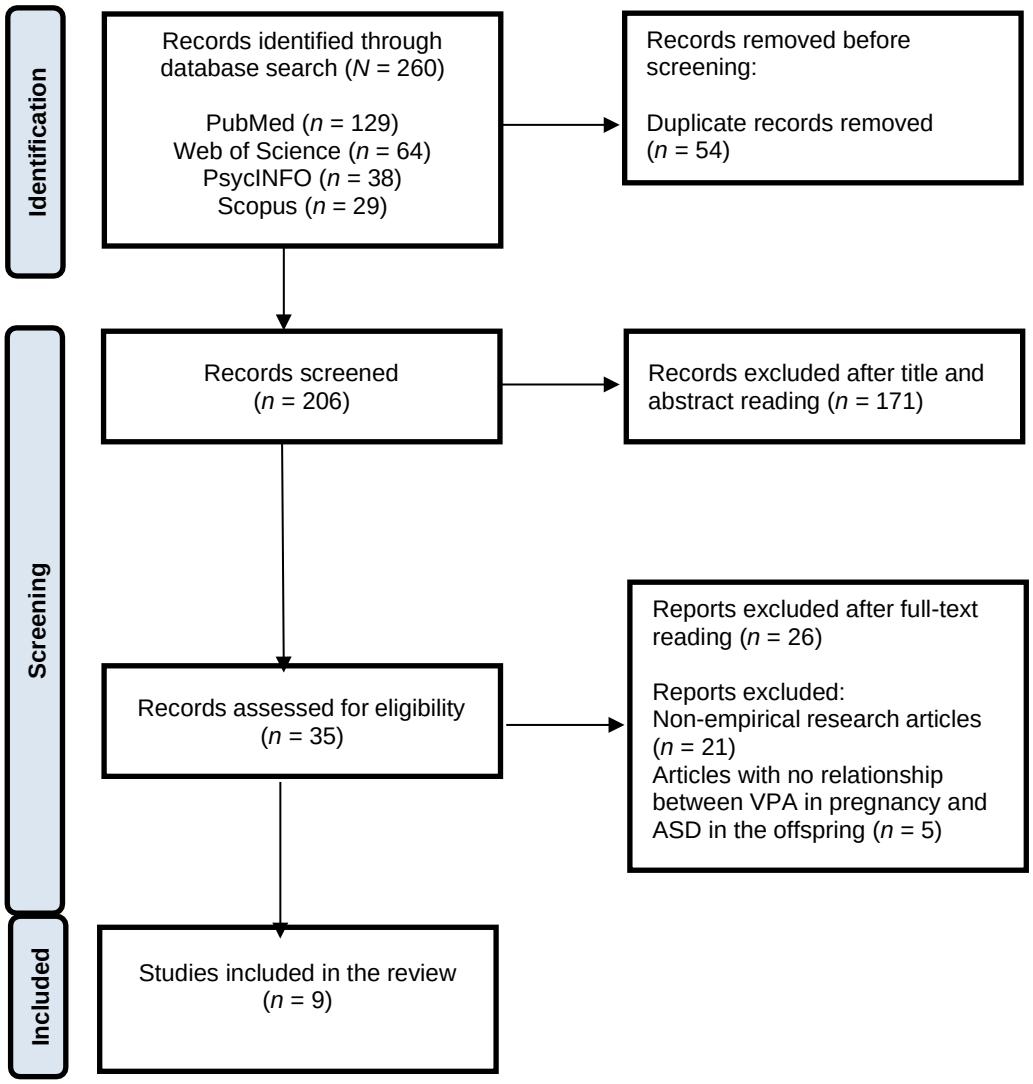

Figure 1. –PRISMA flow diagram of studies' selection process.

RESULTS

All nine articles included in this review were observational studies consisting of eight population-based cohort studies and one population-based case-control study. Seven included statistical models adjusted for potential confounders, and two reported ASD frequency rates [24,25]. The sample size of the included studies ranged from 105 [22] to 4 494 926 participants [23]. Six studies were conducted in Europe [23-28], two in Australia [22, 29], and one in Israel [30]. A summary of the characteristics of each study is provided in Table 1.

In all nine studies, children of mothers who were exposed to VPA during their pregnancy were recruited from the general community, to evaluate the occurrence of ASD, comparing to VPA-unexposed children. The age of the children included in the studies ranged between three and twelve years old.

The diagnosis of ASD was based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV), International Classification of Diseases, 9th Revision , of the World Health Organization (ICD-9), International Statistical Classification of Diseases and Related Health Problems, 10th Revision, of the World Health Organization (ICD-10), the Ages and Stages Questionnaire (ASQ), the Autism Spectrum Quotient - Children's Version (AQ-Child), the Early Screening of Autistic Traits (ESAT), the Modified Checklist for Autism in Toddlers (MCHAT), the Childhood Autism Rating Scale (CARS), the 40-item Social Communication Questionnaire, and data collected from the Scandinavian multi-registry study of antiepileptic drug teratogenicity (SCAN-AED).

Association between VPA exposure and ASD

Six articles provided evidence of an association between prenatal exposure to VPA and ASD [22-27]. Wood et al.'s [22] study predicted that children prenatally exposed to VPA would exhibit elevated rates of ASD, in comparison to the general population. Bjørk et al.'s [23] study also showed that, comparing with unexposed children, the adjusted hazard ratio (aHR) of ASD was 3.44 (95% CI 2.77- 4.28) in children from the total population prenatally exposed to VPA monotherapy. Likewise, Christensen et al. [26] observed an aHR of ASD of 3.0 (95% CI, 1.7-5.4) and an aHR of childhood autism of 4.9 (95% CI, 2.3-10.3) among the children exposed to VPA in monotherapy, as compared to unexposed children. Even when controlling for epilepsy and psychiatric illness, the risk was still elevated, suggesting that the effect of VPA on autism development is independent of the underlying condition. In Bromley et al.'s [27] study, 7.46% of the children exposed to VPA in monotherapy had neurodevelopmental disorders (ND), comparing with 1.87% of the AEDs-unexposed children. In the regression model, the authors estimated the risk of ND, but the most common diagnosis was ASD. In a previous article, Bromley et al. [24] had reported ASD frequency rates seven times greater for children prenatally exposed to VPA monotherapy, comparing with AEDs-unexposed children (6.3% and 0.9%, respectively). In the other study showing ASD frequency rates, Rasalam et al. [25] reported that the prevalence of ASD among children exposed to VPA alone was 8.9% (95% CI 1.3 -16.5), as compared to unexposed children.

No association between VPA exposure and ASD was found in three studies [28-30]. Veiby et al.'s [28] study in Norway showed that children exposed to VPA monotherapy, screened at 18 and 36 months of age, presented, for ASD, odds ratios (OR) of 1.0 (95% CI 0.2 - 4.5) and 3.7 (95% CI 0.5 - 28.4), respectively, when compared

to children of parents without epilepsy. In Australia, Honybun et al. [29] compared VPA-exposed children with those exposed to other AEDs and found no evidence of an effect of VPA exposure on parent-reported Autism Quotient (AQ) scores, β = -0.80 (95% CI -8.64 - 7.044). In Israel, Janecka et al. [30] inspected VPA exposure during pregnancy via the effects on its pharmacologic target (i.e., inhibiting GABA transaminase), and found no association with ASD owing to the small number of exposed children (N = 2) and statistically non-significant results for VPA after adjustments [aHR = 3.15 (95% CI 0.82 - 12.06; p = 0.09].

The relationship between VPA dose and ASD was analyzed in five studies [22,23,26,27,29]. Two showed a VPA dose effect [22,23], whereas the other three showed non-significant dose effects [26,27,29]. Wood et al. [22] reported that there was a dose-response relationship between VPA and risk of autism (r = 0.61; p = 0.001), but only for children exposed to VPA alone (and not in polytherapy). In Bjørk et al.'s [23] study, for any ND, including ASD, the aHR was 2.27 (95%CI 1.86 - 2.77) in children exposed to VPA doses <750mg/day *versus* 5.64 (95%CI 4.65 - 6.84) in children exposed to VPA doses >750mg/day, suggesting that the risk of ASD increased with higher doses of VPA. In contrast, in Christensen et al.'s [26] study, the risk of ASD was similarly increased with both high (>750 mg/day) [aHR = 2.5 (95% CI 1.03 - 6.1)] and low doses (<750 mg/day) [aHR = 3.2 (95% CI 1.7 - 6.2)] of VPA. In Bromley et al.'s [27] study, the results indicated a dose effect for VPA monotherapy, but the association failed to reach statistical significance (p = 0.10), and Honybun et al.'s [29] study showed no association between VPA dosage and ASD symptoms in any of the pregnancy trimesters.

Among the studies that compared exposure to VPA monotherapy and to VPA in combination with other AEDs (polytherapy), two reported greater effects of VPA

polytherapy over VPA monotherapy exposure [22,27], one showed the opposite pattern [26], and one showed no difference between VPA monotherapy and VPA polytherapy exposure on ASD [29]. Wood et al. [22] reported that a greater proportion of children exposed to VPA polytherapy had autism, comparing with children exposed to VPA monotherapy (46.7% *vs.* 11.5%, respectively), with CARS scores registering significantly higher means in the VPA polytherapy group, comparing with all the other groups, including the VPA monotherapy and the non-VPA polytherapy groups (range of means differences = 7.55–9.42; $p < 0.001$). However, the authors noted that the dose of VPA was significantly higher in VPA polytherapy [mean dose of 1589 mg/day (*SD*= 986.66)], comparing with VPA monotherapy [mean dose of 961.78 mg/day (*SD* = 629.74)], suggesting the possibility of a dosage effect. Bromley et al.'s [27] study also showed that the likelihood of having a ND (mostly an ASD) diagnosis was six times higher in children exposed to VPA monotherapy (aOR 6.05; 95% CI 1.65-24.53; $p = 0.007$) and ten times higher in those exposed to VPA polytherapy (aOR 9.97; 95% CI 1.82-49.40; $p = 0.005$), comparing with AEDs-unexposed children. In contrast, Christensen et al. [26] found the opposite pattern. Although VPA monotherapy and VPA polytherapy were both also associated with an increased risk of ASD, the adjusted hazard ratios were greater for VPA monotherapy (aHR of ASD = 3.0; 95% CI, 1.7-5.4) than for VPA polytherapy (aHR of ASD = 1.9; 95% CI, 0.5-7.7), comparing to children unexposed to AEDs. Finally, Honybun et al.'s [29] study showed no difference between VPA monotherapy and VPA polytherapy exposure on overall AQ scores (mean difference = −4.70; 95% CI −15.85, 7.71).

The relationship between early *versus* late VPA exposure in pregnancy and risk of ASD was analyzed in two studies, which showed concordant results [23,26]. Bjørk et al.'s

[23] study showed that VPA exposure in the 2nd and 3rd trimesters only, without 1st trimester exposure, was still associated with an increased risk of ND, including ASD [aHR = 1.9 (95% CI 1.0, 3.6)], and Christensen et al.'s [26] study showed no difference in the risk of ASD between offspring of women who redeemed prescriptions for VPA early [aHR of ASD = 2.9 (95% CI 1.6, 5.1)], *versus* late [aHR of ASD = 3.1 (95% CI 0.8, 12.2)], in pregnancy.

Results on VPA exposure by sex were reported in three studies [25,26,29]. Christensen et al. [26] concluded that ASD and childhood autism were more frequent among boys than among girls who were exposed to VPA in utero [aHR of ASD = 2.9 (95% CI, 1.7-4.9) – 10 males and 4 females were diagnosed with ASD; aHR of childhood autism = 5.2 (95% CI, 2.7-10.0) – 5 males and 4 females were diagnosed with childhood autism]. In contrast, Honybun et al.'s [29] study showed no evidence of sex differences in VPA-exposed children (mean difference = 1.31; 95% CI = −10.38 to 12.99), and in Rasalam et al.'s [25] study, the male-to-female ratio was 2:3 for ASD in children exposed to VPA monotherapy, but no statistical test was provided.

Risk of bias in individual studies

After assessment of risk of bias in the studies included in the review, six studies [22,23,25,26,28,29] were determined to have a low risk of bias and three studies [24,27,30] had a moderate risk. The results are shown in Table 1.

Table 1: Characteristics of included studies measuring risk of ASD with VPA exposure in pregnancy.

Author, Year and Country	Study Design, Duration and Sample Size	Measures	Results	Conclusions	Quality Rating
Bjørk et al. 2022 [23] Denmark Finland Iceland Norway Sweden	Population-based cohort. From 1996 to 2017. VPA group: 2 421 children prenatally exposed. Comparison group: 4 463 879 children with no AED exposure. Median (IQR) age at end of follow-up: 8 (4.0-12.1) years.	• The diagnosis of ASD was based on collected data from SCAN-AED on health and social registers. • ASMs prescriptions were identified through NPR (ATC classification codes N03, N05BA09, and S01EC01). **Statistics:** • Cox regression adjusted for potential confounders. **Other information:** The exposure window was defined from the last menstrual period to the day of birth.	Among 2421 children from the total population prenatally exposed to VPA monotherapy: • aHR of ASD = 3.44 (95% CI, 2.77-4.28). • Incidence rate per 1000 person-years = 3.22 (95% CI, 2.59-4.00). • Cumulative risk of ASD by age 8 years = 2.5% (95% CI, 1.9-3.3). Among 1884 children of WWE prenatally exposed to VPA monotherapy: • aHR of ASD = 2.4 (95%CI, 1.7-3.3). • Incidence rate per 1000 person-years = 3.45 (95% CI, 2.72-4.39). • Cumulative risk of ASD by age 8 years = 2.7% (95% CI, 2.0-3.6). Among 1982 children exposed to VPA doses <750mg/day: • 97 (4.9%) had NDs, including ASD. • aHR = 2.3 (95%CI, 1.9-2.8) for any ND, including ASD. Among 945 children exposed to VPA doses >750mg/day: • 103 (10.9%) had NDs, including ASD. • aHR = 5.6 (95%CI, 4.7-6.8) for any ND, including ASD. Among 252 children exposed to VPA monotherapy in 2nd and 3rd trimester only: • aHR = 1.9 (95%CI, 1.0-3.6) for any ND, including ASD.	• Prenatal exposure to VPA was associated with greater risk of ASD, which increased with higher doses. • Exposure to VPA in the 2nd and 3rd trimester only, without 1st trimester exposure, was still associated with an increased risk of ASD.	Good *
Bromley et al. 2008[24] England	Population-based cohort. From 2000 to 2006. Total *N*: 633	• The diagnosis of ASD was based on criteria of the DSM-IV. **Statistics:**	Among 633 children of the total cohort: • 10 (1.6%) were diagnosed with ASD (6 with autism + 4 with AS).	• Exposure to VPA in utero may be associated with an increased risk of ASD.	Fair

	Comparison group: children of 336 women without epilepsy who were not exposed to AEDs. Age distribution (years): 4.3% < 3, 27.9% between 4 and 5, and 67.8% ≥ 6.	• Frequency rates.	Among 249 children exposed to AEDs: • 7 (2.8%) were diagnosed with ASD (5 with autism + 2 with AS). Among 64 children prenatally exposed to VPA monotherapy: • 4 (6.3%) were diagnosed with ASD (3 with autism + 1 with AS). Among 51 children prenatally exposed to VPA+LTG (polytherapy): • 1 (2.0%) was diagnosed with ASD. Among 336 children of women without epilepsy who were not exposed to AEDs: • 3 (0.9%) were diagnosed with ASD (1 with autism and the other 2 with AS).	• Children exposed to VPA monotherapy had features of ASD seven times higher than the comparison group.	
Rasalam et al. 2005[25] Scotland	Population-based cohort. From April 1, 1981, to March 31, 2001. VPA group: 77 children prenatally exposed. Comparison group: children unexposed to AEDs in utero. Mean age of the children: 5y4m (SD 2y11m) at diagnosis and 9y10m (SD 3y10m) at the time of the study.	• The diagnosis of autistic disorder or AS was based on criteria of the DSM-IV and the ICD-10. **Statistics:** • Frequency rates. • Relative risks calculated through comparison with population prevalence studies.	Among 260 children exposed to AEDs: • Prevalence of ASD = 4.6% (95% CI, 2.0-7.2). • 12 children were diagnosed with ASD (6 boys; 5 girls with autistic disorder + 1 girl with AS). Among 56 children prenatally exposed to VPA monotherapy: • Prevalence of ASD = 8.9% (95% CI, 1.3-16.5). • 5 children were diagnosed with ASD (2 boys; 3 girls). Among 80 children prenatally exposed to CBZ monotherapy: • Prevalence of ASD = 2.5% (95% CI, 0.0-6.0). • 2 children were diagnosed with ASD (2 boys). Among 77 children exposed to VPA monotherapy or in combination with other AEDs: • Prevalence of ASD = 11.7% (95% CI, 4.4-19.0). • 9 children were diagnosed with ASD (3 boys; 6 girls). Among 110 children exposed to CZB monotherapy or in combination with other AEDs: • Prevalence of ASD = 4.5% (95% CI, 0.5-8.5).	• VPA is strongly implicated in increasing the risk of ASD in children exposed in utero.	Good

			• 5 children were diagnosed with ASD (4 boys; 1 girl).		
Christensen et al. 2013[26] Denmark	Population-based cohort. From January 1st, 1996, to December 31st, 2006. VPA group: 508 children prenatally exposed. Boys: 267 (52.5%) Girls: 241 (47.5%) Comparison group: children unexposed to VPA in utero. Mean age at end of follow-up: 8.84 (range, 4-14; median, 8.85) years.	• The diagnosis of ASD was based on ICD-10 codes F84.0, F84.1, F84.5, F84.8, and F84.9. **Statistics:** • Cox regression adjusted for potential confounders. **Other information:** The exposure window was defined from 30 days before the estimated day of conception to the day of birth.	Among 655 615 children of the total cohort: • 5437 were diagnosed with ASD (5423 unexposed to VPA + 12 exposed to VPA monotherapy + 2 exposed to VPA polytherapy), including 2067 with childhood autism (2058 unexposed to VPA + 7 exposed to VPA monotherapy + 2 exposed to VPA polytherapy). Among 508 children exposed to VPA (*vs*. not exposed): • AR of ASD = 4.42% (95% CI, 2.59%-7.46%). • aHR of ASD = 2.9 (95% CI, 1.7-4.9) – 14 children were diagnosed with ASD (10 boys; 4 girls). • AR of childhood autism = 2.50% (95% CI, 1.30%-4.81%). • aHR of childhood autism = 5.2 (95% CI, 2.7-10.0) – 9 children were diagnosed with childhood autism (5 boys; 4 girls). Among 432 children of WWE exposed to VPA: • AR of ASD = 4.15% (95% CI, 2.20%-7.81%). • aHR of ASD = 1.7 (95% CI, 0.9-3.2). • AR of childhood autism = 2.95% (95% CI, 1.42%-6.11%). • aHR of childhood autism = 2.9 (95% CI, 1.4-6.0). Among 6152 children of WWE not exposed to VPA: • AR of ASD = 2.44% (95% CI, 1.88%-3.16%). • AR of childhood autism = 1.02% (95% CI, 0.70%-1.49%). Among 441 children exposed to VPA whose mothers filled VPA prescriptions in the 1st trimester: • aHR of ASD = 2.9 (95% CI, 1.6-5.1). • aHR of childhood autism = 4.7 (95% CI, 2.2-9.9). Among 67 children exposed to VPA whose mothers only filled VPA prescriptions later in pregnancy: • aHR of ASD = 3.1 (95% CI, 0.8-12.2). • aHR of childhood autism = 8.3 (95% CI, 2.1-32.4).	• Maternal use of VPA during pregnancy was associated with a significantly increased risk of ASD in the offspring. • ASD and childhood autism are more frequent in boys than girls exposed to VPA in utero. • Risks of ASD were similarly increased with both high and low doses of VPA. • There was no difference in risk of ASD between offspring of women who redeemed prescriptions for VPA early *vs.* later in pregnancy, but the number of women who redeemed VPA only in the later stages of pregnancy was small. • Risks of ASD were increased for both VPA monotherapy	Good

			Among 388 children exposed to VPA monotherapy (*vs*. unexposed to AEDs): • aHR of ASD = 3.0 (95% CI, 1.7-5.4) – 12 children were diagnosed with ASD. • aHR of childhood autism = 4.9 (95% CI, 2.3-10.3) – 7 children were diagnosed with childhood autism. Among 120 children exposed to VPA polytherapy (*vs*. unexposed to AEDs): • aHR of ASD = 1.9 (95% CI, 0.5-7.7) – 2 children were diagnosed with ASD. • aHR of childhood autism = 5.2 (95% CI, 1.3-21.3) – 2 children were diagnosed with childhood autism. Among 325 children of women who used a low VPA dose (≤750 mg/day): • aHR of ASD = 3.2 (95% CI, 1.7-6.2). • aHR of childhood autism = 5.4 (95% CI, 2.4-12.1). Among 183 children of women who used a high VPA dose (>750 mg/day): • aHR of ASD = 2.5 (95% CI, 1.03-6.1). • aHR of childhood autism = 4.3(95% CI, 1.4- 13.4).	and VPA polytherapy, but the number of women in VPA polytherapy was small.	
Wood et al. 2015[22] Australia	Population-based cohort. From November 2007 to May 2010. VPA group: 41 children prenatally exposed. Comparison group: general population. Mean age of assessment (*SD*): 7.4 (0.6) years.	• The diagnosis of autism traits and autism was based on the CARS (scores ≥ 30 consistent with a diagnosis of autism). • "Autistic traits": any child with CARS scores > 27. **Statistics:** • Chi-square analysis for frequency data.	Linear regression: • Mean VPA dose significantly predicted CARS scores after controlling for variables (*B* = 0.002; *p* = 0.002). • In the analysis repeated with VPA exposure as a binary variable (i.e., not dose variable), the overall model and specific predictors remained significant (data N/A). Among 26 children exposed to VPA monotherapy: • 3 (11.5%) had autism (*n* = 2) or autistic traits (*n* = 1). • 1 scoring CARS > 30 had a daily dose of 3000 mg and 1 scoring CARS 27-29 had a daily dose of 1000-1500 mg. Among 15 children exposed to VPA polytherapy: • 7 (46.7%) had autistic traits (6 scored CARS > 30).	• Children exposed to VPA polytherapy had a higher risk of autism than did children exposed to VPA monotherapy (but VPA doses were significantly higher for polytherapy, comparing with monotherapy). • CARS scores were not elevated in	Good

		• Mann-Whitney test to compare those with and without elevated CARS scores. • Linear regression controlling for confounders.	• Mean dose of VPA was significantly higher (*M* = 1589.00, *SD* = 986.66) comparing with VPA monotherapy (*M* = 961.78, *SD* 629.74) exposure (*t*20.7 = - 2.22, *p* = 0.04). • CARS scores were significantly higher than for groups exposed to polytherapy without VPA (range of mean differences 7.55–9.42, *p* < 0.001). • The relationship between dose and CARS scores was significant in those exposed only to VPA (*r* = 0.61, *p*=0.001).	children exposed to polytherapy without VPA. • There was a positive dose-response relationship only between VPA monotherapy and autism risk.	
Bromley et al. 2013[27] England	Population-based cohort. From 2000 to 2004. VPA group: 70 children prenatally exposed. Boys: 34 in mono- and 10 in polytherapy Girls: 16 in mono- and 10 in polytherapy. Comparison group: 214 children born to women without epilepsy. Mean age of assessment: 6 years.	• Diagnosis of ASD: N/A. **Statistics:** • Multiple logistic regression analysis for the likelihood of ND in offspring of WWE exposed to AED treatments when compared with controls, adjusted for potential confounders.	Among 415 children included, 19 had a diagnosis of an ND. Of those 19 children, 12 (9 boys; 3 girls) were diagnosed with ASD. Among 214 children of non-WWE unexposed to AEDs (controls): • 4 (1.87%) were diagnosed with ASD. Among 50 children of WWE exposed to VPA monotherapy: • 6 (12.0%) were diagnosed with ND, 4 (8.0%) of whom with ASD (1 with daily doses of 600 mg and 3 with daily doses of 1000 mg or more). • Those children were six times (aOR 6.05, 95% CI 1.65 to 24.53, *p*=0.007) more likely to be diagnosed with an ND than controls (4/214; 1.87%). Among 20 children of WWE exposed to VPA polytherapy: • 3 (15%) were diagnosed with ND, 1 (5.0%) of whom with ASD (with daily doses of 2000 mg). • Those children were ten times (aOR 9.97, 95% CI 1.82 to 49.40, *p*=0.005) more likely to be diagnosed with an ND than controls. • There was a positive association between the prevalence of ND and the dose of VPA monotherapy, but it failed to attain significance (*p*=0.10).	• VPA exposure is associated with an increased risk of NDs and ASD was the most common diagnosis. • A dose effect for VPA monotherapy was indicated but the association failed to reach statistical significance.	Fair

Veiby et al. 2013[28] Norway	Population-based cohort. From mid-1999 to December 2008. VPA group: 25 children of WWE prenatally exposed (at 18 months) and 19 children of WWE prenatally exposed (at 36 months). Comparison group: children of parents without epilepsy.	The diagnosis of ASD was based on: • ASQ (motor and communication skills at 18 and 36 months). • 40-item Social Communication Questionnaire (autistic traits at 36 months). • MCHAT (to detect children with a greater range of autistic traits). • ESAT (severely affected behavior at 18 months). **Statistics:** Logistic regression (adjusted ORs), adjusted for confounders.	Screening tools at 18 months of age (61 351 children): • 184 children exposed to AEDs had increased risk of abnormal scores for autistic traits (3.5% *vs*. 0.9%; OR 2.7, 95% CI, 1.1–6.7), comparing to 60 583 children of parents without epilepsy. • Among 25 children of WWE exposed to VPA monotherapy, 2 (8.3%) had autism traits, OR 1 (95% CI 0.2–4.5). Screening tools at 36 months of age (44 147 children): • 139 exposed children to AEDs had increased risk of abnormal score for autistic traits (6.0% *vs*. 1.5%; OR 3.4, CI 1.6–7.0), comparing to 43 571 children of parents without epilepsy. • Among 19 children of WWE exposed to VPA monotherapy, 1 (5.6%) had autism traits OR 3.7 (95% CI 0.5–28.4).	• Prenatal exposure to AEDs was associated with more autistic traits at both 18 and 36 months, but not in children exposed to VPA monotherapy.	Good
Honybun et al. 2021[29] Australia	Population-based cohort. From 2007 to 2014. VPA group: 54 children prenatally exposed. Boys: 28 (52%) Girls: 26 (48%) Comparison group: children exposed to other AEDs. Mean age (SD): 7.08 (2.16) years.	• The diagnosis of ASD was based on the the parent-reported AQ-Child. **Statistics:** • GLMMs for the relationship between clinical-demographic variables and psychometric scores. • ANCOVA for the VPA by sex interaction, with α = 5% and β = 80%.	• From 121 children, 54 were prenatally exposed to VPA (mean dose ± *SD* = 644 ± 310 mg/day) and 67 were exposed to other AEDs. There was no evidence of an effect of VPA exposure on ASD (comparing with exposure to other AEDs), β = -0.80 (95% CI -8.64, 7.044). Among 54 children exposed to VPA (dosage information was available for 48): • Mean VPA dosage (*SD*) = 693 (304) mg/day in those exposed to VPA monotherapy (*n* = 30). • Mean VPA dosage (*SD*) = 561 (311) mg/day in those exposed to VPA polytherapy (*n* = 18).	• There was no impact of VPA mono- or polytherapy on overall AQ scores. • There was no association between VPA dosage and ASD symptoms (possibly related to the low VPA doses used in the study). • Prenatal VPA exposure seems to negate the usual male	Good

			• There was no evidence for a difference on ASD between VPA monotherapy and polytherapy (mean difference = −4.70, 95% CI −15.85, 7.71). • There was no evidence of ASD differences between boys and girls in the VPA-exposed group (mean difference = 1.31, 95% CI −10.38, 12.99). There was no evidence of a relationship between AQ scores and VPA dosage at: • 1st trimester (*b* = −0.004, 95% CI −0.02, 0.02). • 2nd trimester (*b* = −0.003, 95% CI −0.01, 0.02). • 3rd trimester (*b* = 0.003, 95% CI −0.01, 0.02).	sex-related predominance in the incidence of ASD.	
Janecka et al. 2018[30] Israel	Population-based case-control. From January 1, 1997, to December 31, 2007. Cases: 1405 children with ASD. Controls: 94 844 children without ASD. Mean (SD) age at the end of follow-up: 11.6 (3.1) years.	• The diagnosis of ASD was based on ICD-9 and ICD-10. **Statistics:** • HRs and 95% CIs of ASD risk associated with exposure to medication groups using Cox proportional hazards regression, adjusted for confounders. **Other information:** The exposure interval was defined as the pregnancy period - 280 days before birth.	Among 2 (6.9%) children exposed to GABA transaminase inhibitors (solely VPA): • aHR of ASD = 3.15 (95% CI, 0.82–12.06; *p* = 0.09). • The estimates of the risk of ASD diminished after successive adjustments. • Although the HRs remained high in both main and sensitivity analyses, they were not statistically significant after adjustments.	• Owing to the low number of exposed children and statistically non-significant results for VPA after adjustments, there was no association between VPA exposure during pregnancy and risk of ASD in offspring, via effects on its pharmacologic target (inhibiting GABA transaminase).	Fair

Abbreviations: AEDs, antiepileptic drugs; aHR, adjusted hazard ratio; ANCOVA, analysis of covariance; ANU, Australian National University; aOR, adjusted odds ratio; APR, Australian Pregnancy Register; AQ, Autism Spectrum Quotient; AQ-Child, Autism Spectrum Quotient-Children's Version; AR, absolute risk; AS, Asperger syndrome; ASD, autism spectrum disorder; ASQ, Ages and Stages Questionnaire; ATC, Anatomical Therapeutic Chemical; CARS, Childhood Autism Rating Scale; CBZ, carbamazepine; CI, confidence intervals; Diff, difference; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders (4th edition); ESAT, Early Screening of Autistic Traits; FVS, fetal valproate syndrome; GABA, gamma-aminobutyric acid;

GLMMs, general linear mixed-effects models; HR, hazard ratio; ICD-9, International Classification of Diseases (Ninth Revision) of the World Health Organization (WHO); ICD-10, International Statistical Classification of Diseases and Related Health Problems (Tenth Revision) of the World Health Organization (WHO); ID, intellectual disability; IQR, interquartile range; LTG, lamotrigine; M, mean; MCHAT, Modified Checklist for Autism in Toddlers; MoBa, Norwegian Mother, Father and Child Cohort Study; N/A, not available; ND, neurodevelopmental disorder (defined as any diagnosis of autism, intellectual disability, or global developmental delay); NICHQ, National Institute for Children's Health Quality; NPR, national patient registries; OR, odds ratio; SCAN-AED, Scandinavian multi-registry study of antiepileptic drug teratogenicity; SD, standard deviation; VPA, valproate/valproic acid; WWE, women with epilepsy.

* The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines and Reporting of Studies Conducted Using Observational Routinely Collected Data (RECORD).

DISCUSSION

This systematic review aimed to clarify the association between in-utero VPA exposure and development of ASD. Although many documents were retrieved initially (*N* = 260), only 9 eligible studies addressing that association were included.

Whereas six studies [22-27] indicated that prenatal exposure to VPA was associated with ASD development, this association was not found in three studies [28-30], possibly due to the parent-reported measures used to assess ASD (response aspects such social stigma, or overlooking children's features, might occur) [28,29], and the small number of children exposed to VPA [30].

The fact that an increased risk of ASD from VPA exposure was found in the offspring of mothers both with and without epilepsy [22-27], suggests that maternal epilepsy influence might not exist. This suggestion is consistent with the results of animal research [31-35].

The conflicting results observed as regards the association between VPA dose and ASD across studies [22,23,26,27,29] might be related to the dose ranges used in the different studies. In those that showed no association between VPA dosage and ASD, in particular [27,29], Honeybun et al. [29] observed that VPA doses were generally low in their study (mean < 750 mg/day). In contrast, Bromley et al.'s [27] lowest VPA dose interval included values already above 750 mg/day (100-900 mg/day), thus, mainly high doses were possibly used across their sample. Nevertheless, Christensen et al.'s [26] study showed that, although high VPA doses (>750 mg/day) present the highest ASD risk, lower VPA doses (<750 mg/day) may still be harmful to the offspring. Further research is thus warranted to clarify the association between VPA dose and ASD.

The studies that compared children exposed to VPA monotherapy and VPA polytherapy [22,26,27,29] had inconsistent results, possibly also confounded by different VPA doses used in monotherapy *versus* polytherapy [22]. More studies are necessary to clarify whether the risk of ASD in the offspring is higher when VPA is taken alone or in combination with other AEDs.

Only two studies examined the period when VPA exposure occurs (earlier *versus* later) in pregnancy (Christensen et al. [26] and Bjørk MH et al. [23]). Both presented strong evidence indicating that VPA exposure can be harmful to the children regardless of the pregnancy trimester when the exposure occurs. More research could be useful to test and eventually corroborate these results.

The three studies that inspected the association between in-utero VPA exposure and ASD by sex presented conflicting results [25,26,29]. Whereas Christensen et al. [26] reported that ASD is more frequent in male than in female children exposed to VPA in utero, Honybun et al. [29] found no difference in ASD between prenatally VPA-exposed girls and boys, and Rasalam et al. [25] found a male-to-female ratio of 2:3 for ASD in children exposed to VPA monotherapy. Results from meta-analyses indicate a male-to-female ratio of 3:1 for ASD, as well as sex differences in how the behavioral phenotype of ASD is expressed [36]. Female participants have been found to exhibit less stereotyped behaviors than their male counterparts [37], and the diagnosis can be delayed in female patients because of the heterogeneity in symptom manifestations [38]. Rasalam et al. [25] and Honybun et al.'s [29] results seem to go against the usual male sex-related predominance in the incidence of ASD, suggesting that girls might be more sensitive to VPA than do boys, thus becoming more similar to boys as regards ASD (with non-significant differences) when exposed to VPA. Further research is necessary

for a better understanding about the interaction of sex with VPA exposure and ASD development.

For some women with epilepsy, VPA may be the most effective treatment, which may continue during pregnancy because uncontrolled seizures can be harmful for the mother and the fetus [39]. Knowledge about the risks of ASD development in the offspring is thus important, namely, for informed decisions to be possible if VPA is the treatment of choice [40]. Given this review's results, there is a need for research focusing on early detection of, and intervention for neurodevelopmental delays and disorders in children who were prenatally exposed to VPA.

Strengths and limitations

In this systematic review, title and abstract screening, data extraction and quality assessment were carried out by the two authors independently to minimize human error, subjectivity, and the possibility of missing publications. Assessment of risk of bias in the studies included in the review followed the National Institutes of Health's Study Quality Assessment Tools, with six studies [22,23,25,26,28,29] classified as having a low risk of bias, and three studies [24,27,30] as having a moderate risk.

Strengths of this review include the prospective design of some studies [22,24,26-28], that provided better quality of data on VPA exposure and on confounding variables, sample sizes that were large enough to allow the inspection of the associations of prenatal VPA exposure with ASD, and analyses that were adjusted for many potentially confounding risk factors for ASD. The studies in this systematic review also had some limitations, including poor information on potentially intervening factors (e.g., folate

supplementation and use of alcohol or illicit drugs during pregnancy, no adjustments for other prescribed drugs or family history of ASD, specification of the type of disorder for which VPA was taken, and possible missed psychiatric diagnoses). It is also possible that the process of selecting live births may mask fetal deaths caused by toxic effects, which might alter the studies' conclusions. Moreover, in all the studies, there were no information about measures of blood levels of VPA.

CONCLUSION

The results from this systematic review suggest that the use of VPA during any pregnancy trimester, alone or combined with other AEDs, can be associated with a significantly greater risk of ASD developing in the offspring, comparing with VPA-unexposed children. This risk needs to be carefully weighed against the treatment benefits because epilepsy and uncontrolled seizures during pregnancy involve threats for both the mother and the fetus. There is much less information on how the dose of VPA taken during pregnancy relates to the risk of a child having neurological and developmental disorders, on the relative risks of taking VPA in mono- *versus* polytherapy, and on possible sex differences in VPA susceptibility as regards ASD development. Considering the limitations of the studies reported in this systematic review, further research is needed for a clear overview about the association between in-utero VPA exposure and risk of ASD.

Declarations of interest

None of the authors has any conflict of interest to declare.

REFERENCES

1. Macfarlane A, Greenhalgh T. Sodium valproate in pregnancy: what are the risks, and should we use a shared decision-making approach? *BMC Pregnancy Childbirth* 18.1 (2018): 1-11.
2. Mole TB, Appleton R, Marson A. Withholding the choice of sodium valproate to young women with generalised epilepsy: Are we causing more harm than good? *Seizure* 24 (2015):127-30.
3. Ogawa, Tetsuo, et al. Valproate-induced developmental neurotoxicity is affected by maternal conditions including shipping stress and environmental change during early pregnancy. *Toxicology Letters* 174.1-3 (2007): 18-24.
4. Vajda FJ, Hitchcock A, Graham J, et al. The Australian Register of antiepileptic drugs in pregnancy: the first 1002 pregnancies. *Australian and New Zealand Journal of Obstetrics and Gynaecology* 47.6 (2007): 468-474.
5. Meador KJ, Baker GA, Browning N, et al. Cognitive function at 3 years of age after fetal exposure to antiepileptic drugs. *New England Journal of Medicine* 360.16 (2009): 1597-1605.
6. Chomiak, T., Turner, N., Hu, B. What we have learned about autism spectrum disorder from valproic acid. *Pathology Research International*, 2013.
7. Williams, Gail, et al. Fetal valproate syndrome and autism: additional evidence of an association. *Developmental Medicine & Child Neurology* 43.3 (2001): 202-206.
8. Moore SJ, Turnpenny P, Quinn A, et al. A clinical study of 57 children with fetal anticonvulsant syndromes. *Journal of Medical Genetics* 37.7 (2000): 489-497.

9. Elsabbagh, Mayada, et al. Global prevalence of autism and other pervasive developmental disorders. *Autism Research* 5.3 (2012): 160-179.
10. Lord, C., Elsabbagh, M., Baird, G., Veenstra-Vanderweele, J., 2018. Autism spectrum disorder. *The Lancet* 392.10146 (2018): 508-520.
11. Zablotsky, B., Black, L.I., Maenner, M.J., Schieve, L.A. and Blumberg, S.J., 2015. Estimated prevalence of autism and other developmental disabilities following questionnaire changes in the 2014. *National Health Interview Survey* 87 (2015): 1-20.
12. Gardener H, Spiegelman D, Buka SL. Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. *Pediatrics* 128.2 (2011): 344-355.
13. Nicolini, C., Fahnestock, M. The valproic acid-induced rodent model of autism. *Experimental Neurology* 299 (2018): 217-227.
14. Cummings C, Stewart M, Stevenson M, Morrow J, Nelson J. Neurodevelopment of children exposed in utero to lamotrigine, sodium valproate and carbamazepine. *Arch Dis Child* 96.7 (2011): 643-647.
15. Eriksson, Kai, et al. Children exposed to valproate in utero—population-based evaluation of risks and confounding factors for long-term neurocognitive development. *Epilepsy Research* 65.3 (2005): 189-200.
16. Maenner, Matthew J., et al. Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2016. *MMWR Surveillance Summaries* 69.4 (2020): 1.
17. Baxter, Amanda J., et al. The epidemiology and global burden of autism spectrum disorders. *Psychological Medicine* 45.3 (2015): 601-613.

18. Cotariu D, Zaidman JL. Developmental toxicity of valproic acid. *Life Sciences* 48.14 (1991): 1341-1350.
19. Taleb, Abdoh, et al. Emerging mechanisms of valproic acid-induced neurotoxic events in autism and its implications for pharmacological treatment. *Biomedicine & Pharmacotherapy* 137 (2021): 111322.
20. Bromley, Rebecca, et al. Treatment for epilepsy in pregnancy: neurodevelopmental outcomes in the child. *Cochrane Database of Systematic Reviews* 10 (2014).
21. Banach, Ryan, et al. Long-term developmental outcome of children of women with epilepsy, unexposed or exposed prenatally to antiepileptic drugs: a meta-analysis of cohort studies. *Drug Safety* 33 (2010): 73-79.
22. Wood, Amanda G., et al. Prospective assessment of autism traits in children exposed to antiepileptic drugs during pregnancy. *Epilepsia* 56.7 (2015): 1047-1055.
23. Bjørk, Marte-Helene, et al. Association of prenatal exposure to antiseizure medication with risk of autism and intellectual disability. *JAMA Neurology* 79.7 (2022): 672-681.
24. Bromley, R. L., et al. Autism spectrum disorders following in utero exposure to antiepileptic drugs. *Neurology* 71.23 (2008): 1923-1924.
25. Rasalam, A. D., et al. "Characteristics of fetal anticonvulsant syndrome associated autistic disorder." *Developmental Medicine and Child Neurology* 47.8 (2005): 551-555.
26. Christensen, Jakob, et al. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. *JAMA* 309.16 (2013): 1696-1703.

27. Bromley, Rebecca Louise, et al. The prevalence of neurodevelopmental disorders in children prenatally exposed to antiepileptic drugs. *Journal of Neurology, Neurosurgery & Psychiatry* 84.6 (2013): 637-643.
28. Veiby, Gyri, et al. Exposure to antiepileptic drugs in utero and child development: A prospective population-based study. *Epilepsia* 54.8 (2013): 1462-1472.
29. Honybun, Eliza, et al. Prenatal valproate exposure and adverse neurodevelopmental outcomes: Does sex matter? *Epilepsia* 62.3 (2021): 709-719.
30. Janecka, Magdalena, et al. Association of autism spectrum disorder with prenatal exposure to medication affecting neurotransmitter systems. *JAMA Psychiatry* 75.12 (2018): 1217-1224.
31. Miyazaki K, Narita N, Narita M. Maternal administration of thalidomide or valproic acid causes abnormal serotonergic neurons in the the offspring: implications for pathogenesis of autism. *International Journal of Developmental Neuroscience* 23.2-3 (2005): 287-297.
32. Ingram JL, Peckham SM, Tisdale B, et al. Prenatal exposure of rats to valproic acid reproduces the cerebellar anomalies associated with autism. *Neurotoxicology and Teratology* 22.3 (2000): 319-324.
33. Arndt T, Stodgell C, Rodier P. The teratology of autism. *International Journal of Developmental Neuroscience* 23.2-3 (2005): 189-199.
34. Rodier PM, Hyman S. Early environmental factors in autism. *Mental Retardation and Developmental Disabilities Research Reviews* 4.2 (1998): 121-128.
35. Schneider T, Przewlocki R. Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. *Neuropsychopharmacology* 30.1 (2005): 80-89.

36. Loomes R, Hull L, Mandy WPL. What is the male-to-female ratio in autism spectrum disorder? A systematic review and meta-analysis*. Journal of the American Academy of Child & Adolescent Psychiatry* 56.6 (2017): 466-474.
37. Rivet TT, Matson JL. Review of gender differences in core symptomatology in autism spectrum disorders. *Research in Autism Spectrum Disorders* 5.3 (2011): 957-976.
38. Begeer, Sander, et al. Sex differences in the timing of identification among children and adults with autism spectrum disorders. *Journal of Autism and Developmental Disorders* 43 (2013): 1151-1156.
39. Tomson T, Marson A, Boon P, et al. Valproate in the treatment of epilepsy in girls and women of childbearing potential. *Epilepsia* 56.7 (2015): 1006-1019.
40. Dawson G. Early behavioral intervention, brain plasticity, and the prevention of autism spectrum disorder. *Development and Psychopathology* 20.3 (2008): 775-803.

ANNEX 1 – *PRISMA 2020* FOR ABSTRACTS CHECKLIST

PRISMA 2020 for Abstracts Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Prenatal exposure to valproate and the risk of autism spectrum disorder: A systematic review.	Yes
BACKGROUND			
Objectives	2	To determine whether children exposed prenatally to valproate (VPA) have increased risk of autism spectrum disorders (ASD).	Yes
METHODS			
Eligibility criteria	3	The review included research studies reporting results on the use of VPA in pregnant women and its relationship with ASD in their offspring. Animal studies, non-empirical research articles and studies written in languages other than English were excluded.	Yes
Information sources	4	The databases were searched until October 2022: PubMed, Scopus, Web of Science and PsycINFO, without any publication year restrictions.	Yes
Risk of bias	5	The National Institutes of Health's Study Quality Assessment Tools was used for assessing risk of bias of individual studies, by two independent authors and disagreements were resolved through discussion.	Yes
Synthesis of results	6	Not applicable because this systematic review did not include a meta-analysis.	No
RESULTS			
Included studies	7	The nine studies included consisted of eight population-based cohort studies and one population-based case-control study. The sample size of the included studies ranged from 105 to 4 494 926. Six studies were conducted in Europe, two in Australia, and one in Israel.	Yes
Synthesis of results	8	Prenatal exposure to VPA was associated with ASD development in six studies, whereas three reported no association between VPA exposure and ASD, possibly due to sample size and ASD assessment method. Among five studies examining VPA dose effects, two reported a dose-response relationship with ASD, whereas one reported that the risk of ASD was similarly increased with both high and low VPA doses, and another two showed no association, possibly due to a limited range of doses in the samples. No difference was found between exposure to VPA monotherapy and polytherapy on ASD in one study, whereas another three reported differences, but in opposite directions. Two studies were concordant in that there was no difference in the risk of ASD between offspring of women who took VPA early and later in pregnancy. Results by sex (three studies) were inconsistent, with one study concluding that ASD was more frequent in VPA-exposed boys than girls, one showing no evidence of sex differences, and the other showing that ASD is more frequent in females than in males VPA-exposed children.	Yes

Section and Topic	Item #	Checklist item	Reported (Yes/No)
DISCUSSION			
Limitations of evidence	9	Limitations in this systematic review include poor information on potentially intervening factors (e.g., folate supplementation and use of alcohol or illicit drugs during pregnancy, no adjustments for other prescribed drugs or family history of ASD, specification of the type of disorder for which VPA was taken, and possible missed psychiatric diagnoses). It is also possible that the process of selecting live births may mask fetal deaths caused by toxic effects, which might alter the studies' conclusions. Moreover, in all the studies, there were no information about measures of blood levels of VPA.	Yes
Interpretation	10	This systematic review suggests that the use of VPA during any pregnancy trimester, alone or in combination with other antiepileptic drug, can be associated with a significantly greater risk of ASD in the offspring, comparing with unexposed children.	Yes
OTHER			
Funding	11	This research was not funded.	No
Registration	12	There is no review and no registration number.	No

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

For more information, visit: http://www.prisma-statement.org/

ANNEX 2 – *PRISMA 2020* CHECKLIST

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Prenatal exposure to valproate and the risk of autism spectrum disorder: A systematic review.	1
ABSTRACT			
Abstract	2	See the *PRISMA* 2020 for Abstracts checklist (Annex 1).	40
INTRODUCTION			
Rationale	3	VPA has been used increasingly to manage conditions other than epilepsy, particularly bipolar and other affective disorders, and, as a result, more women of childbearing age are likely to be exposed to this drug. VPA is generally not indicated for fertile women but, because it is the most effective therapy in some epilepsy syndromes, the patient has the right to choose VPA therapy, thus undertaking the elevated risk of developmental abnormalities appearing in the offspring, for greater safety regarding seizures. Becoming pregnant poses a challenging balance between optimizing seizure control and minimizing the potentially harmful effects of antiepileptic drugs (AEDs) on the fetus. Among the AEDs, VPA appears to induce developmental neurotoxicity in children's central nervous system, cognitive impairment and congenital malformations in the offspring. ASD refers to neurological and developmental disorders that can occur to different degrees and in a variety of forms. It can include childhood autism (autistic disorder), Asperger syndrome, atypical autism, and other or unspecified pervasive developmental disorders. A variety of environmental factors may increase the occurrence of ASD, and prenatal VPA exposure would be a modifiable environmental factor. In animals, prenatal exposure to VPA is known to cause ASD-like symptoms. The prevalence of ASD in children prenatally exposed to VPA has been suggested to range from 8% to 15%, as opposed to a general population prevalence of 0.6%–1%. In recent years, research on the pathophysiological mechanisms of ASD after VPA exposure has attracted extensive attention, and a significant number of research efforts have been attempted to define the contributory role of VPA to the impairment of normal prenatal growth and development. Related studies have found that VPA can change genes transcription levels, causing abnormal signaling pathways, synaptic dysfunction, and neurogenesis deficits, which can lead to ASD in the offspring. Previous systematic reviews have examined the association between in-utero VPA exposure and neurodevelopment, reporting a significant association of children's VPA exposure with lower IQ scores and poorer overall neurodevelopmental outcomes. However, the role of VPA exposure in the development of ASD is lacking in previous systematic reviews.	9-10

Section and Topic	Item #	Checklist item	Location where item is reported
Objectives	4	The aim of this systematic review was to synthetize the most relevant research on this theme to provide an understanding on whether prenatal exposure to VPA is an important risk factor for the development of ASD in the offspring. VPA doses, uses (alone or in polytherapy), time (early *versus* later) in pregnancy, and sex differences in VPA-exposed children are also examined.	11
METHODS			
Eligibility criteria	5	The review included research studies reporting results on the use of valproate in pregnant women and its relationship with the increased occurrence of autism spectrum disorder in their offspring. Animal studies, non-empirical research articles and studies written in languages other than English were excluded.	12
Information sources	6	The databases were search until October 2022, including PubMed, Scopus, Web of Science and PsycINFO.	12
Search strategy	7	The databases were search without any publication year restrictions.	12
Selection process	8	The process of search, article screening, and data extraction was performed manually by the two authors independently, and questions arising in this process were discussed and resolved through consensus.	13
Data collection process	9	The process of data extraction was performed manually by the two authors independently on all the records included in the review, and questions arising in this process were discussed and resolved through consensus.	13
Data items	10a	Data were sought for the following details, which were extracted from all eligible studies: author's name, year of publication, study's country, research design, study's duration and sample size, population characteristics, measures to assess ASD, and the association (statistical test, including whether models were adjusted for confounders) between valproate exposure and ASD. Where results were not compatible with an outcome domain, the authors' reported results were specified.	13
	10b	Not applicable.	-
Study risk of bias assessment	11	The National Institutes of Health's Study Quality Assessment Tools was used for assessing risk of bias of individual studies, by two independent authors and disagreements were resolved through discussion.	30
Effect measures	12	The effect measure of the association between VPA exposure and ASD was the measure that the authors provided (Table 1).	20-26

Synthesis methods	13a	Not applicable because this systematic review did not include a meta-analysis.	-
	13b	Not applicable because this systematic review did not include a meta-analysis.	-
	13c	Not applicable because this systematic review did not include a meta-analysis.	-
	13d	Not applicable because this systematic review did not include a meta-analysis.	-
	13e	Not applicable because this systematic review did not include a meta-analysis.	-
	13f	Not applicable because this systematic review did not include a meta-analysis.	-
Reporting bias assessment	14	Not applicable because this systematic review did not include a meta-analysis.	-
Certainty assessment	15	Confidence intervals and *p*-values were consulted, along with presence of adjustments for confounders in the models, inspection of the sample in relation to its population, and study design.	20-26
RESULTS			
Study selection	16a	The search retrieved a total of 260 articles from across the four databases. After removal of 54 duplicate articles, 206 were screened by title and abstract reading, leading to the exclusion of 171 documents. The remaining 35 articles were read in full, and 26 were excluded.	12
	16b	Not applicable.	-
Study characteristics	17	All nine articles included in this review were observational studies consisting of eight population-based cohort studies and one population-based case-control study. Seven included statistical models adjusted for potential confounders, and two reported ASD frequency rates. The sample size of the included studies ranged from 105 to 4 494 926 participants. Six studies were conducted in Europe, two in Australia, and one in Israel. Bjørk et al. study was conducted in Scandinavia, published in 2022 and consists of a population-based cohort from 1996 to 2017; Bromley et al. study was conducted in England, published in 2008 and consists of a prospective population-based cohort from 2000 to 2006; Rasalam et al. study was conducted in Scotland, published in 2005 and consists of a population-based cohort from April 1, 1981 to March 31, 2001; Christensen et al. study was conducted in Denmark, published in 2003 and consists of a prospective population-based cohort from January 1st, 1996, to December 31st, 2006; Wood et al. study was conducted in Australia, published in 2015 and consists of a prospective population based cohort from January 1st, 1996, to December 31st, 2006; From November 2007 to May 2010; Bromley et al. study was conducted in England, published in 2013 and consists of a population based cohort from 2000 to 2004; Veiby et al. study was conducted in Norway, published in 2013 and consists of a prospective population based cohort from mid-1999 to December 2008; Honybun et al. study was conducted in Australia, published in 2021 and consists of a population based cohort from 2007 to 2014; Janecka et al. study was conducted in Israel, published in 2018 and consists of a population based case-control from January 1, 1997, to December 31, 2007.	15; 20-26 .

Risk of bias in studies	18	After assessment of risk of bias in the studies included in the review, six studies were determined to have a low risk of bias and three studies had a moderate risk.	19
Results of individual studies	19	Not applicable because this systematic review did not include a meta-analysis.	-
Results of syntheses	20a	Not applicable because this systematic review did not include a meta-analysis.	-
	20b	Not applicable because this systematic review did not include a meta-analysis.	-
	20c	Not applicable because this systematic review did not include a meta-analysis.	-
	20d	Not applicable because this systematic review did not include a meta-analysis.	-
Reporting biases	21	Not applicable because this systematic review did not include a meta-analysis.	-
Certainty of evidence	22	Not applicable because this systematic review did not include a meta-analysis.	-
DISCUSSION			
Discussion	23a	Prenatal exposure to VPA was associated with ASD development in six studies, whereas three reported no association between VPA exposure and ASD, possibly due to sample size and ASD assessment method. Among five studies examining VPA dose effects, two reported a dose-response relationship with ASD, whereas one reported that the risk of ASD was similarly increased with both high and low VPA doses, and another two showed no association, possibly due to a limited range of doses in the samples. No difference was found between exposure to VPA monotherapy and polytherapy on ASD in one study, whereas another three reported differences, but in opposite directions. Two studies were concordant in that there was no difference in the risk of ASD between offspring of women who took VPA early and later in pregnancy. Results by sex (three studies) were inconsistent, with one study concluding that ASD was more frequent in VPA-exposed boys than girls, one showing no evidence of sex differences, and the other showing that ASD is more frequent in females than in males VPA-exposed children.	15-19
	23b	Limitations in this systematic review include poor information on potentially intervening factors (e.g., folate supplementation and use of alcohol or illicit drugs during pregnancy, no adjustments for other prescribed drugs or family history of ASD, specification of the type of disorder for which VPA was taken, and possible missed psychiatric diagnoses). It is also possible that the process of selecting live births may mask fetal deaths caused by toxic effects, which might alter the studies' conclusions. Moreover, in all the studies, there were no information about measures of blood levels of VPA.	30-31
	23c	Not applicable.	-

	23d	Further research is needed for a clear overview about the association between in-utero VPA exposure and risk of ASD, for a better understanding about the interaction of VPA doses, time (early *versus* later) in pregnancy and ASD development, sex differences in VPA-exposed children with ASD, and also to clarify whether the risk of ASD in the offspring is higher when VPA is taken alone or in combination with other AEDs	28-32
OTHER INFORMATION			
Registration and protocol	24a	There is no review and no registration number.	-
	24b	There is no review and no registration number.	-
	24c	There is no review and no registration number.	-
Support	25	This research was not funded.	-
Competing interests	26	The authors declare that they have no conflicts of interest.	32
Availability of data, code and other materials	27	Not applicable.	-

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71