

Supplementary Appendix

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

Supplement to: Jentink J, Loane MA, Dolk H, et al. Valproic acid monotherapy in pregnancy and major congenital malformations. *N Engl J Med* 2010;362:2185-93.

Supplementary appendix, section 1

Participating registries: Belgium (Antwerp and Hainaut), Croatia (Zagreb), Denmark (Odense), France (Paris and Strasbourg), Germany (Mainz and Saxony Anhalt), Ireland (Cork & Kerry), Italy (Emilia Romagna and Tuscany), Malta, Netherlands (northern part), Norway, Poland (Wielkopolska, Poland), Spain (Basque Country), Switzerland (Vaud) and UK (Wales)

Supplementary appendix, section 2

We searched PubMed, Web of Science and Embase for studies addressing VPA exposure in pregnancy and found over 1500 studies using the following search strategy: {"valproic acid"[Mesh] OR "epilepsy/drug therapy"[Majr] OR "antiepileptic drugs"[ti] OR "AED"[ti]} AND {"congenital abnormalities"[Majr] OR "pregnancy complications/drug therapy"[Majr] OR "birth defects"[ti]}. The criteria for selecting studies were: non-overlapping cohort studies that reported a case list with detailed information about malformations, reported the size of the VPA exposed cohort and reported the definition of the study population and the study period. Eight cohort studies met the criteria (Table 1, in de paper)[12-17, 23-24]. Authors of two studies that did not include sufficient details to categorize some malformations were contacted to obtain additional information [13,16].

In the 8 studies, malformations reported in offspring with first trimester VPA exposure were classified according to the EUROCAT congenital anomaly subgroups. The EUROCAT AED database was used as the reference group; the prevalences of various malformations in this database were calculated after excluding registrations with maternal AED exposure, maternal epilepsy, or chromosomal anomalies. Differences in prevalence between the published studies and EUROCAT reference group were compared using a chi square test with Yates' correction (Program used: S-PLUS7.0). All malformation subgroups for which the prevalence in studies of maternal VPA exposure was significantly higher than in the EUROCAT referent group (at p-value less than 0.05) were considered to be "signals" and were studied further in the case-control study (see table SA1 below).

Table SA1: Result of the review of the 8 cohort studies [12-17, 23, 24]^.

Malformation sub group	Literature N=1,565		EUROCAT N=3,869,947		p-value [#]
	number	prev./1000	number [*]	prev./1000	
spina bifida	22	14.1	1933	0.5	p < 0.001
microcephaly	2	1.3	745	0.2	p = 0.030
Ventricular septal defect	12	7.7	11896	3.1	p < 0.001
Atrial septal defect	11	7.0	8428	2.2	p < 0.001
tetralogy of Fallot	3	1.9	991	0.3	p = 0.001
pulmonary valve atresia	3	1.9	339	0.1	p < 0.001
hypoplastic right heart	1	0.6	99	0.03	p = 0.023
cleft palate	13	8.3	2338	0.6	p < 0.001
diaphragmatic hernia	4	2.6	766	0.2	p < 0.001
Gastroschisis	2	1.3	807	0.2	p = 0.041
Hypospadias	22	14.1	5418	1.4	p < 0.001
club foot	8	5.1	3847	1.0	p < 0.001
Polydactyly	8	5.1	3594	0.9	p < 0.001
craniosynostosis	5	3.2	551	0.1	p < 0.001

[^] All specific malformations found in the literature review were defined over 75 malformation sub groups; 41 sub groups were filled with at least one case and 14 of these sub groups showed a significant increased prevalence compared with the EUROCAT population.

[#] calculated with a chi square test with Yate's correction

^{*} excluding chromosomal and AED exposed registrations

Supplementary appendix, section 3

Table SA2: Unadjusted odds ratio's for VPA monotherapy compared with 'no AED' (first column) and 'other AED monotherapy' (second column) using non-chromosomal controls (C₁) and chromosomal controls (C₂). And in the last two columns the cases exposed to no AEDs and to other monotherapy.

malformation sub group		VPA monotherapy			No VPA	
		exposed	OR _{unadj} [95%CI] [#] vs. no AED	OR _{unadj} [95%CI] [#] vs. other mono	No AED exposure	Other mono exposed
nervous	spina bifida N=2,046	27	C ₁ 11.9 [7.0-19.5] C ₂ 12.2 [6.1-25.8]	5.1 [2.3-11.6] 3.0 [1.0-8.9]	1996	13
	microcephaly* N=696	2	C ₁ 2.5 [0.3-9.7] C ₂ 2.6 [0.3-11.6]	1.6 [0.1-14.7] 1.0 [0.1-9.8]	690	3
congenital heart disease	ventricular septal defect N=11,711	19	C ₁ 1.4 [0.8-2.5] C ₂ 1.5 [0.7-3.2]	2.0 [0.9-4.3] 1.2 [0.4-3.4]	11659	23
	atrial septal defect N=8,267	19	C ₁ 2.0 [1.1-3.5] C ₂ 2.1 [1.0-4.6]	3.1 [1.3-7.1] 1.9 [0.6-5.5]	8216	15
	tetralogy of Fallot N=960	3	C ₁ 2.8 [0.6-8.6] C ₂ 2.8 [0.5-10.4]	1.5 [0.2-7.9] 0.9 [0.1-5.5]	951	5
	pulmonary valve atresia N=311	1	C ₁ 2.8 [0.1-16.7] C ₂ 2.9 [0.1-19.5]	2.4 [0.0-193.6] 1.5 [0.0120.7]	309	1
	hypoplastic right heart N=85	0	C ₁ - C ₂ -	- -	84	1
	cleft palate N=2,244	13	C ₁ 5.1 [2.5-9.7] C ₂ 5.3 [2.3-12.4]	2.9 [1.1-7.7] 1.7 [0.5-5.8]	2215	11
	diaphragmatic hernia N=754	2	C ₁ 2.3 [0.3-9.0] C ₂ 2.4 [0.3-10.7]	1.2 [0.1-8.9] 0.7 [0.1-6.1]	747	4
	gastroschisis N=798	1	C ₁ 1.1 [0.0-6.5] C ₂ 1.1 [0.0-7.6]	1.2 [0.0-24.0] 0.7 [0.0-15.6]	794	2
	hypospadias, boys only N=5,395	32	C ₁ 4.5 [2.6-7.8] C ₂ 4.9 [2.1-13.1]	6.6 [2.8-16.6] 3.3 [0.8-13.4]	5343	11
limb	club foot N=3,676	6	C ₁ 1.4 [0.5-3.4] C ₂ 1.5 [0.5-4.2]	1.2 [0.4-3.8] 0.7 [0.2-2.8]	3651	12
	Polydactyly N=3,500	9	C ₁ 2.3 [1.0-4.7] C ₂ 2.3 [0.9-5.9]	5.5 [1.4-25.4] 3.3 [0.7-17.4]	3481	4
	craniosynostosis N=520	4	C ₁ 6.8 [1.8-18.8] C ₂ 7.0 [1.7-22.9]	4.9 [0.7-55.2] 2.9 [0.4-35.8]	513	2

The numbers in the table can not be summed to get the total because polytherapy is not shown (eg. spina bifida 27+1996+13=2036 plus 10 polytherapy exposed=2046)

[^] C₁: Control group 1: all non-chromosomal, non-monogenic registrations without any of the malformations under study

C₂: Control group 2: all chromosomal malformations (for exact definitions, see Methods)

* club foot and microcephaly without spina bifida