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Maternal Influenza A(H1N1) Immunization During Pregnancy and Risk for Autism Spectrum Disorder in Offspring

A Cohort Study

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Background: There are concerns that influenza vaccine exposure during pregnancy may be associated with increased risk for autism spectrum disorder (ASD).

Objective: To examine the risk for ASD in offspring of mothers who were vaccinated against influenza A(H1N1)pdm09 ("swine flu") during pregnancy.

Design: Population-based cohort study using nationwide registers.

Setting: Seven health care regions in Sweden.

Participants: Live births between October 2009 and September 2010, with follow-up through December 2016. In total, 39 726 infants were prenatally exposed to H1N1 vaccine (13 845 during the first trimester) and 29 293 infants were unexposed.

Measurements: Cox regression was used to estimate hazard ratios (HRs) for the primary outcome, ASD, before and after adjustment for potential confounders. The secondary outcome was autistic disorder (AD).

Results: Mean follow-up was 6.7 years in both unexposed and exposed children. During follow-up, 394 (1.0%) vaccine-exposed and 330 (1.1%) unexposed children had a diagnosis of ASD. In adjusted analyses, prenatal exposure to H1N1 vaccination was not associated with a later diagnosis of ASD (adjusted HR [aHR], 0.95 [95% CI, 0.81 to 1.12]) or AD (aHR, 0.96 [CI, 0.80 to 1.16]). The 6-year standardized cumulative incidence difference between the unexposed and exposed children was 0.04% (CI, −0.09% to 0.17%) for ASD and 0.02% (CI, −0.09% to 0.14%) for AD. Restricting the analysis to vaccination in the first trimester of pregnancy did not influence risk estimates (aHR, 0.92 [CI, 0.74 to 1.16] for ASD and 0.91 [CI, 0.70 to 1.18] for AD).

Limitation: Data on H1N1 influenza infection are lacking.

Conclusion: This large cohort study found no association between maternal H1N1 vaccination during pregnancy and risk for ASD in the offspring.

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Autism spectrum disorder (ASD) is a severe neurodevelopmental childhood disorder characterized by impaired communication, lack of social skills, and repetitive behavior. This disorder is influenced by both hereditary and environmental factors (1, 2). Only a few specific genes and environmental risk factors have been robustly associated with ASD (3).

Influenza epidemics have been linked to increased mortality. Historical data suggest that pregnancy is a risk factor for influenza severity and, in retrospect, research studies suggest that pandemic influenza during pregnancy may increase risks for stillbirth (4) and preterm birth (5). Influenza vaccination during pregnancy has a protective effect on maternal health and benefits offspring health (6). Several studies have examined fetal and infant outcomes (7, 8) after maternal H1N1 vaccination during pregnancy, consistently finding no increased risks.

Although some studies indicate that influenza vaccination during pregnancy protects against morbidity in both the woman and her offspring (9), the long-term risks of H1N1 vaccination exposure during fetal life have not been examined in detail. A recent U.S. study using Kaiser Permanente data reported an increased risk for ASD in the offspring of women who received influenza vaccination during the first trimester (adjusted hazard ratio [aHR], 1.20 [95% CI, 1.04 to 1.39]) (10), whereas the aHR for ASD associated with influenza vaccination anytime during pregnancy was 1.10 (CI, 1.00 to 1.21). That study covered births between 2000 and 2010, thus including both seasonal and pandemic influenza vaccines. The proportion of H1N1 vaccine and associated risks were not reported. We are only aware of 1 study specifically examining H1N1 vaccination during pregnancy and risk for ASD in the offspring (11). In that study, analyses of ASD were restricted to second- and third-trimester vaccine exposure, for which the rate ratio was 1.22 (CI, 0.79 to 1.86). The study lacked power to conduct analyses for first-trimester exposure, the period when organ development takes place. It is possible that vaccination during early pregnancy could affect neurodevelopment and ASD risk. Influenza vaccination may induce a proinflammatory response in the host (12). In a case–control study (13), women giving birth to a child who later developed ASD demonstrated higher levels of proinflammatory markers during pregnancy.

See also:

Editorial comment 658

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Supplement

We sought to investigate whether maternal influenza A(H1N1)pdm09 vaccination during pregnancy was associated with increased risk for ASD in offspring. By linking Swedish national health registers and creating a large population-based cohort, we compared risks for ASD and autistic disorder (AD) in children with prenatal exposure to vaccination and those who were unexposed.

METHODS

Data Sources

During the 2009 influenza pandemic, Swedish authorities recommended vaccination against H1N1 influenza to the entire population and specifically encouraged certain risk groups, including pregnant women. Vaccinations with the AS03-adjuvanted influenza A/H1N1pdm09 vaccine (Pandemrix) were offered free of charge from October 2009 through December 2010. Pregnant women received 1 vaccine dose.

Through a web-based vaccination register (14), we retrieved data on vaccinations from 7 Swedish health care regions (Kalmar, Östergötland, Stockholm, Värmland, Norrbotten, Västra Götaland, and Skåne), representing 61% of the Swedish population. Individual-level vaccination data were prospectively registered by using the personal identity number (15), which is assigned to all Swedish residents and can be used for register linkages.

We linked vaccination data to the Swedish Medical Birth Register (pregnancy and birth characteristics), the Cause of Death Register (mortality information), the Swedish National Patient Register (hospital-based inpatient and outpatient care, including outcome and covariate data, such as maternal comorbidity), and demographic and socioeconomic data from Statistics Sweden. All data were linked by the 2 government agencies Statistics Sweden and the National Board of Health and Welfare, which pseudonymized the data before delivery.

Study Participants

The Medical Birth Register was used to retrieve data on singleton live births with valid personal identity numbers in the 7 Swedish regions. Because very few vaccination doses were given after January 2010, we only included information about 69 455 infants born from 1 October 2009 to 30 September 2010. Infants delivered during this period were in utero at some point during the pandemic vaccination campaign. We excluded 10 infants with missing gestational age; 425 infants whose mothers had moved out of the health care region between 1 October 2009 and birth, because vaccination status could not be ascertained in these women; and 1 infant whose mother had an implausible vaccination date. Women had to live in the region at time of conception and at time of birth to be included in the study. The final cohort included 69 019 children to 69 002 mothers.

Exposure: H1N1 Vaccination During Pregnancy

"Exposure" was defined as H1N1 vaccination anytime during pregnancy as recorded in the Vaccination Register. All pregnancies without a record of vaccination during pregnancy were classified as unexposed. Considering that early pregnancy is crucial for fetal organ development and that the previous study linked first-trimester influenza vaccine exposure to ASD (10), we also classified whether vaccination occurred during the first trimester (first 14 weeks).

In 4 of the 7 health care regions, vaccination coverage was regarded as complete (cost reimbursements were linked to vaccination records). In 3 regions (Kalmar, Värmland, Norrbotten), which together accounted for 13% of the study participants in the cohort, exposure classification was not possible in 16% to 22% of the inhabitants.

Covariates

To adjust for potential confounding we included a set of covariates, all measured prospectively. We used the Medical Birth Register to access data on self-reported maternal smoking (0, 1 to 9, and ≥10 cigarettes per day) and self-reported height from first antenatal visit. In 90% of women, this visit takes place at the end of the first trimester (16). At this visit, the midwives also collect data on parity (here categorized as 0, 1, or ≥2) and maternal height and weight (allowing us to calculate body mass index [BMI]). Gestational age was based on ultrasonographic measurements when available; otherwise, it was calculated according to the first day of the last menstrual period. Data on maternal country of birth (Sweden vs. outside Sweden) and disposable annual income were retrieved from Statistics Sweden (17). We also included infant sex, maternal age at delivery, and health care region.

Data on the following maternal comorbid conditions were retrieved from the National Patient Register: cardiovascular disease, lung disease, diabetes, liver disease (including liver transplantation), renal disease (including dialysis and renal transplantation), inflammatory bowel disease, multiple sclerosis, and rheumatoid arthritis. Comorbid conditions were defined according to codes in the International Classification of Diseases, 10th revision (ICD-10) (**Supplement Table 1**, available at Annals.org). The National Patient Register covers both inpatient and hospital-based outpatient care and has a positive predictive value of 85% to 95% for most diseases (18).

ASD and Autistic Disorder

We defined ASD as ICD-10 codes F84.0, F84.1, F84.5, F84.8, and F84.9, as identified from primary and secondary diagnoses associated with outpatient visits and inpatient admissions. In a validation study of 177 ASD cases identified through register data, 96% were confirmed by medical record review (19). Our secondary outcome was autistic disorder (AD) (ICD-10 code F84.0), the most severe form of ASD. We are unaware of any validation studies of AD specifically. In a sensitivity analysis, the outcome definition was restricted to primary diagnoses alone.

Children were followed from the date of birth until first diagnosis of ASD, death, or the end of follow-up (31 December 2016), whichever came first.

Statistical Analysis

To adjust for potential bias due to differences in length of follow-up, Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and associated 2-sided 95% Wald-type CIs. To address potential confounding, we controlled for maternal age at delivery, maternal BMI, infant sex, maternal parity, maternal smoking, maternal country of birth, maternal disposable income, health care region, maternal comorbidity, and prenatal study time (time since 1 October 2009 until birth). Maternal age at delivery, disposable income, and prenatal study time were modeled by using penalized regression splines (20, 21) to allow for smooth nonlinear relationships with the outcomes. In addition, disposable income was log-transformed owing to skewness. For each fitted model, regression standardization (22) was used to obtain the conditional (on the potential confounders) association between the cumulative incidence and the time to event in the unexposed and exposed children. If the measured confounders are sufficient for confounding control, the

Table 1. Maternal Characteristics of Live Births in Sweden (1 October 2009 to 30 September 2010), by Influenza A(H1N1)pdm09 Vaccine Exposure Status During Fetal Life

Characteristic	Unexposed Infants (*n* = 29 293)	Vaccine-Exposed Infants (*n* = 39 726)
Age at infant birth, *n* (%)		
<25 y	4722 (16.1)	4107 (10.3)
25–29 y	8463 (28.9)	10 503 (26.4)
30–34 y	9548 (32.6)	15 028 (37.8)
35–39 y	5369 (18.3)	8466 (21.3)
≥40 y	1191 (4.1)	1622 (4.1)
Body mass index, *n* (%)		
<18.5 kg/m^2	762 (2.8)	808 (2.2)
18.5–24.9 kg/m^2	16 470 (60.4)	23 465 (63.6)
25.0–29.9 kg/m^2	6643 (24.4)	8581 (23.2)
≥30 kg/m^2	3377 (12.4)	4061 (11.0)
Missing	2041	2811
Parity, *n* (%)		
0	13 566 (46.3)	16 985 (42.8)
1	10 372 (35.4)	15 869 (39.9)
≥2	5355 (18.3)	6872 (17.3)
Smoking, *n* (%)		
0 cigarettes/d	26 305 (92.2)	36 510 (94.5)
1–9 cigarettes/d	1722 (6.0)	1667 (4.3)
≥10 cigarettes/d	494 (1.7)	462 (1.2)
Missing	772	1087
Country of birth, *n* (%)		
Sweden	19 788 (67.6)	32 127 (80.9)
Other	9505 (32.4)	7599 (19.1)
Income, *n* (%)		
<200 000 SEK	9965 (34.2)	9891 (25.0)
≥200 000 SEK	19 154 (65.8)	29 679 (75.0)
Missing	174	156
Health care region, *n* (%)		
Stockholm	10 940 (37.3)	14 764 (37.2)
Östergötland	1801 (6.1)	2644 (6.7)
Skåne	6357 (21.7)	7485 (18.8)
Västra Götaland	6798 (23.2)	11 338 (28.5)
Kalmar	1208 (4.1)	940 (2.4)
Värmland	940 (3.2)	1633 (4.1)
Norrbotten	1249 (4.3)	922 (2.3)
Comorbidity, *n* (%)		
No	25 800 (88.1)	35 261 (88.8)
Yes	3493 (11.9)	4465 (11.2)
Infant sex, *n* (%)		
Male	14 982 (51.1)	20 534 (51.7)
Female	14 311 (48.9)	19 192 (48.3)

SEK = Swedish krona.

Table 2. Risk for Autism Spectrum Disorder and Autistic Disorder in Children According to Influenza A(H1N1)pdm09 Vaccine Exposure Status During Fetal Life

Outcome and Exposure	Unexposed*		Exposed		Hazard Ratio (95% CI)	
	Children, *n*	Events, *n* (%)	Children, *n*	Events, *n* (%)	Unadjusted	Adjusted†
Autism spectrum disorder						
Vaccination anytime during pregnancy	29 293	330 (1.1)	39 726	394 (1.0)	0.87 (0.75–1.00)	0.95 (0.81–1.12)
Vaccination during first trimester	55 174	609 (1.1)	13 845	115 (0.8)	0.81 (0.66–0.99)	0.92 (0.74–1.16)
Autistic disorder						
Vaccination anytime during pregnancy	29 293	253 (0.9)	39 726	295 (0.7)	0.85 (0.72–1.00)	0.96 (0.80–1.16)
Vaccination during first trimester	55 174	462 (0.8)	13 845	86 (0.6)	0.80 (0.64–1.01)	0.91 (0.70–1.18)

* In the analysis of first trimester, unexposed women were unexposed during the first trimester but may have undergone H1N1 vaccination later in pregnancy.
† Adjusted for maternal age at delivery, maternal body mass index, infant sex, maternal parity, maternal smoking, maternal country of birth, disposable income, maternal health care region, maternal comorbidity, and prenatal study time (time since 1 October 2009 until birth).

standardized cumulative incidences can be interpreted as the counterfactual cumulative incidences we would have observed, had everybody in the population been either unexposed or exposed. The average causal effect is then given by the difference between the standardized cumulative incidence functions for the unexposed and exposed children. The proportional hazards assumption was assessed graphically by using plots based on the scaled Schoenfeld residuals (23), revealing no violations.

In our main analysis, we examined the risk for ASD in offspring of women having an H1N1 vaccination during pregnancy with those unexposed to such vaccination. We also compared exposure to vaccination in the first trimester (first 14 weeks) with no exposure during this trimester. Individuals in whom exposure classification was not possible were regarded as unexposed in the main analyses; in sensitivity analyses, all individuals from Kalmar, Värmland, and Norrbotten were excluded. In another sensitivity analysis, we added maternal epilepsy, neurologic disease (other than multiple sclerosis and epilepsy), and psychiatric disease to our model (**Supplement Table 1** shows the ICD-10 codes).

The outcomes, the exposures, and 6 of the covariates had no missing observations, whereas there were missing values for maternal BMI (7.0%), maternal smoking (2.7%), and maternal income (0.5%) (**Supplement Table 2**, available at Annals.org). In total, 7.8% of the records in the study cohort were incomplete. To account for the incomplete data, assuming a missing-at-random mechanism, multiple imputation was used to generate 25 imputed data sets. The incomplete variables across the study cohort were imputed by using multiple imputation by chained equations (24, 25). Models were then fitted to the imputed data sets, and the corresponding estimates were combined into an overall estimate. The associated variance estimates were properly adjusted to account for the within- and between-imputation variability (26).

The potential effect of unmeasured confounding was addressed by using E-values (27, 28). We computed the non-null E-value for the upper limit of the CI of the exposure–outcome association: that is, the minimum amount of unmeasured confounding needed to shift the estimate to a specified hypothetical ("true") value that could be considered clinically meaningful. We also computed the usual (null) E-value for the lower limit of the CI of the exposure–outcome association to obtain the threshold needed for the CI to not include 1.0.

All statistical analyses were performed with R, version 3.6.0, using the add-on packages mice (version 2.47.1), survival (version 2.44-1), and stdReg (version 3.3.0). Additional details of the statistical methods are provided in the **Supplement** (available at Annals.org).

The study was approved by the Stockholm Ethics Review Board (number 2017/2440-32; 18 December 2017). The Vaccination Register used for this study required personal consent of individuals undergoing vaccination. Given that all registry data were pseudonymized before delivery to researchers, we were unable to contact any participants, and the review board waived informed consent (29).

Role of the Funding Source

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RESULTS

The cohort included 39 726 vaccine-exposed and 29 293 unexposed children. Compared with mothers of unexposed children, the mothers of vaccine-exposed children were older at delivery; had a higher disposable income; and less often had high BMI, were smokers, or were born outside Sweden (**Table 1**). Mean length of follow-up was 6.7 years (SD, 0.5). Characteristics according to H1N1 vaccine exposure during the first trimester are shown in **Supplement Table 3** (available at Annals.org), and **Supplement Table 4** (available at Annals.org) shows study participants according to offspring development of ASD. Some 10.5% of mothers of offspring with ASD were smokers, and 36.3% were born outside Sweden; among mothers of offspring

without ASD, 6.4% were smokers and 24.7% were born outside Sweden (**Supplement Table 4**).

Incidence rates of ASD and AD were similar throughout the study period (**Supplement Figure 1**, available at Annals.org) and between the study regions (**Supplement Figure 2**, available at Annals.org). Of the 39 726 vaccine-exposed children, 394 (cumulative incidence, 1.0%) had a diagnosis of ASD during follow-up compared with 330 (1.1%) among 29 293 unexposed children.

After adjustment for potential confounders, H1N1 vaccine exposure during fetal life was not associated with a later childhood diagnosis of ASD (aHR, 0.95 [CI, 0.81 to 1.12]) (**Table 2**). The **Figure** shows the standardized cumulative incidences of ASD according to H1N1 vaccine exposure status. Standardized cumulative incidence differences between the unexposed and exposed children obtained from the adjusted analyses are shown in **Supplement Figure 3** (available at Annals.org) and were 0.04% (CI, −0.09% to 0.17%) for ASD and 0.02% (CI, −0.09% to 0.14%) for AD at age 6 years. The Schoenfeld residuals did not indicate any change in ASD risk for different ages (**Supplement Figure 4**, available at Annals.org).

Restricting the analyses to vaccine exposure in the first trimester of pregnancy did not influence risk estimates for ASD (aHR, 0.92 [CI, 0.74 to 1.16]) (**Table 2**). We found no association between H1N1 vaccine exposure during pregnancy and the secondary outcome of AD (aHR, 0.96 [CI, 0.80 to 1.16]) (**Table 2**).

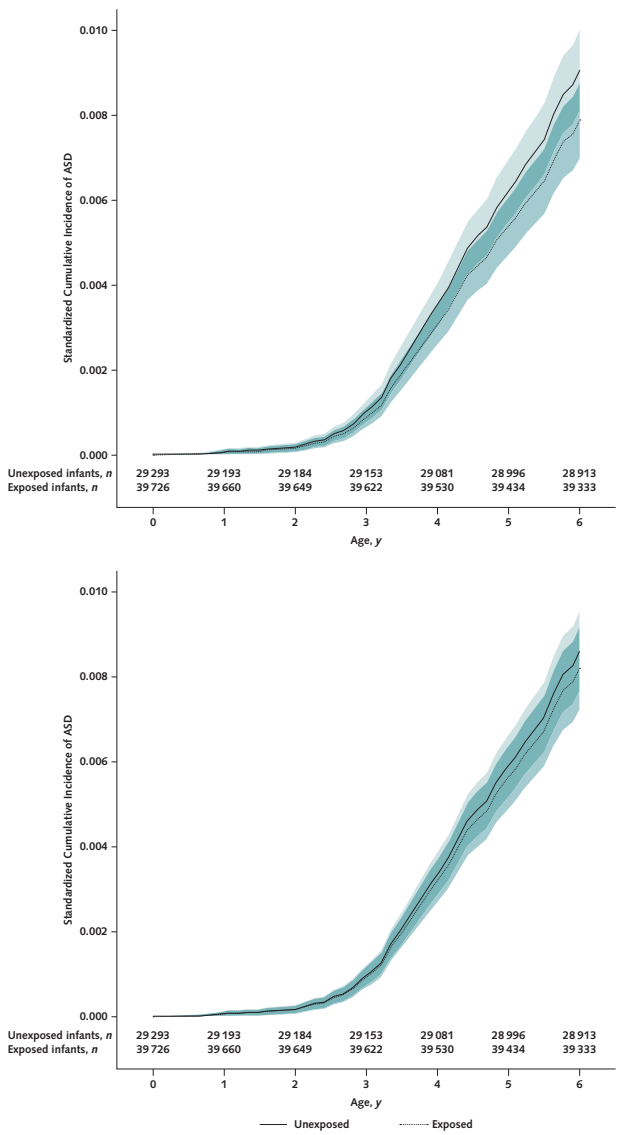
We performed 4 sensitivity analyses for ASD (**Table 3**). After exclusion of individuals in the 3 counties where vaccination data were incomplete, the aHR was 0.95 (CI, 0.80 to 1.12). When the outcome definition was restricted to primary diagnoses alone, the aHR was 0.94 (CI, 0.79 to 1.12). After follow-up was truncated at age 6 years, the aHR was 0.97 (CI, 0.81 to 1.16). In a post hoc analysis restricted to offspring of mothers born in Sweden, the aHR was 0.89 (CI, 0.72 to 1.09). The HRs were similar for AD (**Supplement Table 5**, available at Annals.org). Adding maternal epilepsy, neurologic disease (other than multiple sclerosis and epilepsy), and psychiatric disease to our model did not change our risk estimates (aHR, 0.95 [CI, 0.81 to 1.12] for ASD and 0.96 [CI, 0.80 to 1.16] for AD).

Although the analyses adjusted for potential confounders, the estimated associations may still be biased owing to unmeasured confounding. For this reason, we investigated the strength of unmeasured confounding needed to no longer be able to rule out an association between H1N1 vaccine exposure during fetal life and a later childhood diagnosis of ASD. We did this by computing the non-null E-value for the 95% upper limit of the CI and the (null) E-value for the 95% lower limit of the CI. Because the outcome is relatively rare (<15% at the end of follow-up), the risk ratio is approximately equal to the HR. Assuming that a 95% upper limit of the CI of 1.5 would be considered clinically relevant, shifting the observed 95% upper limit of the CI from 1.12 to 1.5 would require unmeasured confounding to have a 2.02-fold association with each of H1N1 vaccine exposure and later childhood diagnosis of ASD. The (null) E-value for the observed 95% lower limit of the CI was 1.77, indicating that unmeasured confounding must have a more than 1.77-fold association with each of H1N1 vaccine exposure and later childhood diagnosis of ASD for the 95% CI to exclude 1.0.

DISCUSSION

In this large population-based cohort study, we found no association between vaccination of mothers

Figure. Unadjusted (*top*) and adjusted (*bottom*) associations between age and standardized cumulative incidence of ASD, according to influenza A(H1N1)pdm09 vaccine exposure status during fetal life.



Shaded areas indicate 95% CIs, with the darker area showing the region in which the CIs overlap. The numbers at risk are shown on the *x*-axes. The graphs are truncated at 6 y of follow-up owing to the limited number of study participants followed beyond then. ASD = autism spectrum disorder.

Table 3. Sensitivity Analyses of Risk for Autism Spectrum Disorder in Children According to Influenza A(H1N1)pdm09 Vaccine Exposure Status During Fetal Life

Analysis	Exposure		Unexposed*		Hazard Ratio (95% CI)	
	Children, *n*	Events, *n* (%)	Children, *n*	Events, *n* (%)	Unadjusted	Adjusted†
Excluding women from Kalmar, Värmland, and Norrbotten						
Vaccination anytime during pregnancy	25 896	302 (1.2)	36 231	365 (1.0)	0.85 (0.73–0.99)	0.95 (0.80–1.12)
Vaccination during first trimester	49 597	564 (1.1)	12 530	103 (0.8)	0.77 (0.63–0.95)	0.89 (0.71–1.13)
Primary diagnosis only						
Vaccination anytime during pregnancy	29 293	296 (1.0)	39 726	343 (0.9)	0.84 (0.72–0.99)	0.94 (0.79–1.12)
Vaccination during first trimester	55 174	537 (1.0)	13 845	102 (0.7)	0.81 (0.66–1.01)	0.94 (0.74–1.19)
Truncated follow-up‡						
Vaccination anytime during pregnancy	29 293	265 (0.9)	39 726	313 (0.8)	0.87 (0.74–1.02)	0.97 (0.81–1.16)
Vaccination during first trimester	55 174	478 (0.9)	13 845	100 (0.7)	0.83 (0.67–1.03)	0.94 (0.74–1.20)
Mothers born in Sweden						
Vaccination anytime during pregnancy	19 788	190 (1.0)	32 127	271 (0.8)	0.87 (0.72–1.05)	0.89 (0.72–1.09)
Vaccination during first trimester	40 449	376 (0.9)	11 466	85 (0.7)	0.85 (0.67–1.08)	0.94 (0.72–1.24)

* In the analysis of first trimester, unexposed women were unexposed during the first trimester but may have undergone H1N1 vaccination later in pregnancy.
† Adjusted for maternal age at delivery, maternal body mass index, infant sex, maternal parity, maternal smoking, maternal country of birth, disposable income, maternal health care region, maternal comorbidity, and prenatal study time (time since 1 October 2009 until birth).
‡ Truncated at 6 years of follow-up.

against H1N1 influenza during pregnancy and childhood ASD in the offspring. Of note, the study had substantial precision, with the upper limit of the 95% CI consistent with no more than a 12% relative increase in risk for ASD and no more than a 6-year standardized cumulative incidence risk difference between the unexposed and exposed children of 0.04% for ASD and 0.02% for AD, with the upper 95% CI attaining 0.17% and 0.14%, respectively. Thus, a clinically meaningful increase in risk associated with H1N1 vaccine exposure in pregnancy seems unlikely. Furthermore, no association was found for vaccine exposure in the first trimester and ASD or the secondary outcome, AD.

Studies have explored the potential association between H1N1 vaccination and fetal outcomes (30), but few have examined outcomes beyond the perinatal period (31). Our null results for ASD contrast with those of Zerbo and colleagues (10), who reported aHRs of 1.20 (CI, 1.04 to 1.39) for influenza vaccine exposure in the first trimester and 1.10 (CI, 1.00 to 1.21) for exposure anytime during pregnancy. We cannot rule out that the differences in results are due to vaccine-specific differences, because we studied an adjuvanted A(H1N1)pdm09 vaccine whereas Zerbo and colleagues examined both seasonal and pandemic influenza vaccines over an 11-year period. Also, their 20% increased risk associated with vaccine exposure in the first trimester may be a spurious finding; the study did not provide any detailed adjustment for confounding, and the positive association vanished when they corrected for multiple comparisons. Another study (31) found no association between offspring ASD and H1N1 vaccine exposure during pregnancy, but it had limited follow-up for ASD (mean, 4.6 years) and limited statistical precision (6311 vaccine-exposed children vs. >39 000 in the current study) and was unable to analyze first-trimester data.

Among the strengths of our study is its large sample size and population-based data, including prospectively ascertained vaccination. Overall, the study included more than 39 000 live-born infants exposed to H1N1 vaccine during pregnancy. We included more than 13 000 exposed to H1N1 vaccine in the first trimester, which is important, if only early-pregnancy vaccination would affect the risk for ASD. Blastogenesis takes place in the first 2 months of pregnancy (32), which is when vaccination might have its most profound effect on fetal neurodevelopment. The prospective nature of the vaccination data collection eliminates recall bias. Of note, we had access to a rich set of potential confounders informative for maternal demographic and socioeconomic characteristics and health, including maternal psychiatric history before conception. Maternal health has been linked to ASD (33), and comorbidity was an important predictor of H1N1 vaccination in Sweden overall (14). We adjusted for disposable income as a measure of socioeconomic status because ASD incidence has been found to vary by socioeconomic status (34). It is also important that Swedish prenatal and childhood health care are free of charge (35) and that we had data on maternal country of birth, which may influence both the likelihood of having a vaccination and seeking health care for a child with abnormal behavior and potential ASD. The incidence rates of ASD were robust across birth years and health regions and counties.

Our study has limitations. First, we did not have data on H1N1 influenza infection in the pregnant mothers. If both vaccination and influenza (the latter being less common in persons undergoing vaccination) were risk factors for ASD in the offspring, we may have failed to detect an adverse effect of vaccination.

Second, we cannot rule out residual confounding. Of concern here would be factors associated with both

receipt of vaccine and lower risk for ASD, obscuring a true increased risk. One such factor might be higher maternal health consciousness. Women who are more concerned about their health may have chosen to participate to a higher degree, which could have introduced a "healthy-vaccine effect." However, because the entire Swedish population, and pregnant women in particular, were encouraged by the Swedish health authorities to participate for free, this risk was probably minimal.

Third, we cannot rule out confounding due to absence of paternal characteristics. Genetic causes explain most of the variation in ASD risk (3), and if a similar genetic set-up is associated also with vaccine exposure, this might influence our results.

Fourth, we did not have access to data on seasonal influenza vaccinations or other non-H1N1 vaccinations during or beyond the study period. In addition, if women in the unexposed group had H1N1 vaccination but were not registered in the vaccination register, risk estimates would have been driven toward the null.

Finally, we cannot rule out vaccine-specific effects. The H1N1 vaccine investigated in this study (Pandemrix) is an adjuvanted vaccine that is no longer in use, and results may not be applicable to nonadjuvanted influenza vaccines.

In conclusion, in this large cohort study, we found no association between maternal H1N1 vaccination during pregnancy and risk for ASD in the offspring.

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Reproducible Research Statement: *Study protocol:* Available from Dr. Ludvigsson (e-mail, jonasludvigsson@yahoo.com). *Statistical code:* Available from Dr. Winell (e-mail, henric.winell@ki.se). *Data set:* Not available.

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References

1. **Sandin S, Lichtenstein P, Kuja-Halkola R, et al.** The familial risk of autism. JAMA. 2014;311:1770-7. [PMID: 24794370] doi:10.1001/jama.2014.4144
2. **Sandin S, Schendel D, Magnusson P, et al.** Autism risk associated with parental age and with increasing difference in age between the parents. Mol Psychiatry. 2016;21:693-700. [PMID: 26055426] doi:10.1038/mp.2015.70
3. **Bai D, Yip BHK, Windham GC, et al.** Association of genetic and environmental factors with autism in a 5-country cohort. JAMA Psychiatry. 2019. [PMID: 31314057] doi:10.1001/jamapsychiatry.2019.1411
4. **Håberg SE, Trogstad L, Gunnes N, et al.** Risk of fetal death after pandemic influenza virus infection or vaccination. N Engl J Med. 2013;368:333-40. [PMID: 23323868] doi:10.1056/NEJMoa1207210
5. **Pierce M, Kurinczuk JJ, Spark P, et al; UKOSS.** Perinatal outcomes after maternal 2009/H1N1 infection: national cohort study. BMJ. 2011;342:d3214. [PMID: 21672992] doi:10.1136/bmj.d3214
6. **Madhi SA, Cutland CL, Kuwanda L, et al; Maternal Flu Trial (Matflu) Team.** Influenza vaccination of pregnant women and protection of their infants. N Engl J Med. 2014;371:918-31. [PMID: 25184864] doi:10.1056/NEJMoa1401480
7. **Zaman K, Roy E, Arifeen SE, et al.** Effectiveness of maternal influenza immunization in mothers and infants. N Engl J Med. 2008;359:1555-64. [PMID: 18799552] doi:10.1056/NEJMoa0708630
8. **Walsh LK, Donelle J, Dodds L, et al.** Health outcomes of young children born to mothers who received 2009 pandemic H1N1 influenza vaccination during pregnancy: retrospective cohort study. BMJ. 2019;366:l4151. [PMID: 31292120] doi:10.1136/bmj.l4151
9. **Fell DB, Sprague AE, Liu N, et al; Better Outcomes Registry & Network (BORN) Ontario.** H1N1 influenza vaccination during pregnancy and fetal and neonatal outcomes. Am J Public Health. 2012;102:e33-40. [PMID: 22515877] doi:10.2105/AJPH.2011.300606
10. **Zerbo O, Qian Y, Yoshida C, et al.** Association between influenza infection and vaccination during pregnancy and risk of autism spectrum disorder. JAMA Pediatr. 2017;171:e163609. [PMID: 27893896] doi:10.1001/jamapediatrics.2016.3609
11. **Hviid A, Svanström H, Mølgaard-Nielsen D, et al.** Association between pandemic influenza A(H1N1) vaccination in pregnancy and early childhood morbidity in offspring. JAMA Pediatr. 2017;171:239-248. [PMID: 27893898] doi:10.1001/jamapediatrics.2016.4023
12. **Christian LM, Porter K, Karlsson E, et al.** Proinflammatory cytokine responses correspond with subjective side effects after influenza virus vaccination. Vaccine. 2015;33:3360-6. [PMID: 26027906] doi:10.1016/j.vaccine.2015.05.008
13. **Goines PE, Croen LA, Braunschweig D, et al.** Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: a case-control study. Mol Autism. 2011;2:13. [PMID: 21810230] doi:10.1186/2040-2392-2-13
14. **Persson I, Granath F, Askling J, et al.** Risks of neurological and immune-related diseases, including narcolepsy, after vaccination with Pandemrix: a population- and registry-based cohort study with over 2 years of follow-up. J Intern Med. 2014;275:172-90. [PMID: 24134219] doi:10.1111/joim.12150
15. **Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, et al.** The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. Eur J Epidemiol. 2009;24:659-67. [PMID: 19504049] doi:10.1007/s10654-009-9350-y
16. **Stephansson O, Petersson K, Björk C, et al.** The Swedish Pregnancy Register – for quality of care improvement and research. Acta Obstet Gynecol Scand. 2018;97:466-476. [PMID: 29172245] doi:10.1111/aogs.13266

17. **Ludvigsson JF, Svedberg P, Olén O, et al.** The Longitudinal Integrated Database for Health Insurance and Labour Market Studies (LISA) and its use in medical research. Eur J Epidemiol. 2019;34:423-437. [PMID: 30929112] doi:10.1007/s10654-019-00511-8
18. **Ludvigsson JF, Andersson E, Ekbom A, et al.** External review and validation of the Swedish National Inpatient Register. BMC Public Health. 2011;11:450. [PMID: 21658213] doi:10.1186/1471-2458-11-450
19. **Idring S, Rai D, Dal H, et al.** Autism spectrum disorders in the Stockholm Youth Cohort: design, prevalence and validity. PLoSOne.2012;7:e41280.[PMID:22911770]doi:10.1371/journal.pone.0041280
20. **Eilers PHC, Marx BD.** Flexible smoothing with B-splines and penalties. Stat Sci. 1996;11:89-121. doi:10.1214/ss/1038425655
21. **Therneau TM, Grambsch P.** Modeling Survival Data: Extending the Cox Model. Springer-Verlag; 2000.
22. **Sjölander A.** Regression standardization with the R package stdReg. Eur J Epidemiol. 2016;31:563-74. [PMID: 27179798] doi:10.1007/s10654-016-0157-3
23. **Grambsch PM, Therneau TM.** Proportional hazards tests and diagnostics based on weighted residuals. Biometrika. 1994;81:515-26. doi:10.1093/biomet/81.3.515
24. **van Buuren S, Groothius-Oudshoorn K.** mice: multivariate imputation by chained equations in R. J Stat Softw. 2011;45. doi:10.18637/jss.v045.i03
25. **Doove LL, van Buuren S, Dusseldorp E.** Recursive partitioning for missing data imputation in the presence of interaction effects. Comput Stat Data Anal. 2014;72:92-104. doi:10.1016/j.csda.2013.10.025
26. **Rubin DB.** Multiple Imputation for Nonresponse in Surveys. J Wiley; 1987.
27. **Ding P, VanderWeele TJ.** Sensitivity analysis without assumptions. Epidemiology. 2016;27:368-77. [PMID: 26841057] doi:10.1097/EDE.0000000000000457
28. **VanderWeele TJ, Ding P.** Sensitivity analysis in observational research: introducing the E-value. Ann Intern Med. 2017;167:268-274. [PMID: 28693043] doi:10.7326/M16-2607
29. **Ludvigsson JF, Håberg SE, Knudsen GP, et al.** Ethical aspects of registry-based research in the Nordic countries. Clin Epidemiol. 2015;7:491-508. [PMID: 26648756] doi:10.2147/CLEP.S90589
30. **Fell DB, Platt RW, Lanes A, et al.** Fetal death and preterm birth associated with maternal influenza vaccination: systematic review. BJOG. 2015;122:17-26. [PMID: 25040307] doi:10.1111/1471-0528.12977
31. **Ludvigsson JF, Ström P, Lundholm C, et al.** Maternal vaccination against H1N1 influenza and offspring mortality: population based cohort study and sibling design. BMJ. 2015;351:h5585. [PMID: 26572546] doi:10.1136/bmj.h5585
32. **Carlson BM.** Human Embryology and Developmental Biology. 5th ed. Saunders; 2013.
33. **Janecka M, Kodesh A, Levine SZ, et al.** Association of autism spectrum disorder with prenatal exposure to medication affecting neurotransmitter systems. JAMA Psychiatry. 2018;75:1217-1224. [PMID: 30383108] doi:10.1001/jamapsychiatry.2018.2728
34. **Durkin MS, Maenner MJ, Baio J, et al.** Autism spectrum disorder among US children (2002-2010): socioeconomic, racial, and ethnic disparities. Am J Public Health. 2017;107:1818-1826. [PMID: 28933930] doi:10.2105/AJPH.2017.304032
35. **Wettergren B, Blennow M, Hjern A, et al.** Child health systems in Sweden. J Pediatr. 2016;177S:S187-S202. [PMID: 27666267] doi:10.1016/j.jpeds.2016.04.055

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