Article

Prenatal Environment and Perinatal Factors Associated with Autism Spectrum Disorder

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ABSTRACT

Background: Both genetic and epidemiological studies have indicated that environmental factors play an essential role in the development of autism spectrum disorder (ASD). We conducted this study to identify maternal exposure to environmental factors, in particular during the fetal development or perinatal period, associated with ASD.
Methods: Two independent samples of children with ASD and typical developed (TD) were from distinct regions in China. Multiple logistic regression analysis was performed to identify factors associated with ASD in each sample and then in the combined sample.
Results: Five factors were consistently associated with ASD in both samples. In the combined sample, maternal chemical exposure (odds ratio [OR] =4.50; 95% CI: 2.38-8.52), use of medication (OR = 3.19; 95% CI: 2.19-4.65), maternal infection (OR = 2.68; 95% CI: 1.99-3.61), threatened abortion (OR = 2.37; 95% CI: 1.61-3.50), and induced abortion before having the child (OR = 2.07; 95% CI: 1.65-2.60) showed strong associations with ASD; moreover, five factors explained 10-15% of the variation in the risk of ASD. A significant interaction between maternal infection and the use of medication during pregnancy was consistently detected in both independent and combined samples together.
Conclusion: Two novel risk factors of maternal chemical exposure and induced abortion may have important implications for understanding the etiology of ASD, particularly in China. Prospective studies are needed to validate these findings, and necessary interventions are recommended to reduce the risk of ASD.

KEYWORDS

Autism spectrum disorder, chemical exposure, induced abortion, maternal infection.

INTRODUCTION

Autism spectrum disorder (ASD) is a childhood-onset neurodevelopmental disorder and has lifelong and extensive effects on its victims and their families (1-3). The diagnosis of ASD is made more often in boys than in girls. While autism is heritable, only a limited number of common genetic variants have been associated with ASD (4-6). A

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recent population-based representative family study with whole-genome genotyped SNP data shows that 52% of the risk for autism is attributed to common variants (7). Moreover, a large number of *de novo* rare mutations or copy number of variants have been discovered in children with autism in samples from multiple populations, includ-ing China (8, 9).

Among the various environmental factors associated with ASD, maternal exposure to environmental factors during the prenatal period is considered a primary focus for investigation (10). Previous studies have associated prenatal stress, maternal infection, maternal conditions and perinatal characteristics, such as threatened abortion, an abnormal term of delivery, and cesarean delivery, with ASD (11-13). However, there are studies with inconsis-tencies. For example, a large-scale study did not provide consistent evidence for prenatal stress as a risk factor for autism (14). Also, the potential mechanism that maternal infection linked to ASD has been indicated. Prenatal viral infections have been associated with the risk of ASD (15); a meta-analysis of 15 studies that included more than 40,000 ASD cases also showed an association of prenatal infections with the risk of autism (11). Maternal infection that causes peripheral immune dysregulation can begin in fetal development and continue to adulthood (16), and it may cause ASD through maternal immune activation (MIA).

However, very few epidemiological studies of ASD have been conducted in China where a rapid social and economic transition has occurred in the past decades. The rapid increase in industrialization and urbanization, particularly the massive construction of housing and office buildings, began in the late 20th century and may have generated additional environmental risks for human diseases, including ASD. The new buildings and furnishings, with a lack of awareness of health risk from the construction, have raised concerns. Previous studies have indicated that prenatal exposure to outdoor air pollution or indoor renovation increased the risk of intrauterine inflammation (17) and early childhood ear infection (18). Moreover, induced abortion, which is likely associated with intra-uterine inflammation, has become a legal procedure commonly available in society due to the massive implementation of the family planning program in the 1980s.

We conducted this study to identify new environmental factors, in particular, maternal exposure during the fetal development and perinatal period, associated with ASD. The initial sample of patients with ASD used in this study was from the first national research program on the genetic study of autism(4), with a further interview and recuritment of healthy controls to collect the relevant history of maternal exposure and reproductive history. An independent sample of cases and controls were also collected for replication from a distinct region of China.

METHODS

Study design

Two independent samples of ASD and typical developed (TD) children were from distinct regions of China. One from the south of China served as a discovery sample, and the other from the north of China served as a replication sample. The sample size required for an adequate power of 80% to detect a risk factor was calculated to guide the subject recruitment. The ethics committee of the Second Xiangya Hospital of the Central South University approved this study, which was conducted between January 2008 and December 2016. The guardians of all participants provided written informed consent before enrollment.

Ascertainment of cases and controls

Case-parent triad family samples were initially recruited for genetic studies through a multicenter clinical network for ASD, which comprised multiple public or private schools for special education and hospital outpatient clinic, mostly located in the urban areas of China (4, 19). The cases and controls of the south sample were from the same city in Changsha, Hunan. The north sample was mainly from Qingdao, Shandong. Children with ASD met a diagnosis of Autistic Disorder or Pervasive Developmental Disorder-not otherwise specified (PDD-NOS) at a local hospital, which was confirmed by a senior child psychiatrist of the Second Xiangya Hospital at schools or outpatient according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR). Subsequently, an independent child psychiatrist of the Second Xiangya Hospital confirmed the diagnosis with a self-design semi-structured interview, including a parent interview in which a psychiatrist reviewed the child's symptom list with parents and conducted an interview with the child (20). We excluded the children with an inconsistent diagnosis of Asperger in this study. Typical developed (TD) children without a family history of ASD were recruited from regular kindergarten classes as controls in the same or adjacent provinces and matched for age. TD children were those who performed well in a kindergarten or primary school without parents or teachers reporting severe learning, emotional, behavioral, or social problems. Head of the class teachers conducted the interviews with TD children according to the instruction and a list of criteria for TD defined for this study.

Instrument and data collection

A structured questionnaire was used to collect information on children and their parents. The information collected included the general conditions of children and parents maternal pregnant and prenatal conditions, and child development, history of diseases and medications. The data collection mainly focused on the history of pregnancy and prenatal exposure to adverse events, such as chemi-cals (e.g., paint, benzene, and lead), prenatal stress, history of abortions and diseases, particularly maternal infection and use of medication during pregnancy, birth or perinatal conditions. Teachers or research associates sent a self-reported structured questionnaire with detailed instructtions to the parents; a trained investigator sub-sequently contacted the parents to complete the ques-tionnaire and forms. Also, parental age and level of parental educations were used to control for potential confounding. Data were entered using the Portable Document Format Form and then exported into a database file.

Statistical analysis

The frequency of all single variables was examined to detect potential data errors, and recording was performed when necessary for analysis. A cross-tabulation of the single variable by ASD status was used to assess the statistical association using the Chi-square or Fisher's exact test. Stepwise logistic regression was performed to select variables that may be associated with ASD in each sample separately and the combined sample. The threshold p-value for a variable entering and removing from the model was 0.15. Nagelkerke's R^2 measured the variation in the risk of disease explained by risk factors in a logistic regression (21).

RESULTS

Prenatal environmental factors associated with ASD

Table 1 presents the frequency of individual variables by ASD and TD in both samples. There were 582 children with ASD and 414 TD children in the discovery sample and 821 ASD and 489 TD children in the replication sample. According to a power calculation, each sample was able to provide an adequate power of 80% to detect a risk factor with an intermediate effect size for an association (Figure S1). There was no significant difference in the mean age of the ASD and TD children or their parents (Table S1).

Table 1. Frequency of variables by case and control in both discovery and replication samples

			Discovery (South)			Replication (North)		
	Code	Category	TD, *n* (%)	ASD, *n* (%)	P	TD, *n* (%)	ASD, *n* (%)	P
Demographics								
Residence	1	City	349 (84.30)	476 (81.79)	0.300	421 (86.09)	706 (85.99)	0.959
	2	County	65 (15.70)	106 (18.21)		68 (13.91)	115 (14.01)	
Gender	1	Boy	214 (51.69)	510 (87.63)	<0.001	274 (56.03)	696 (84.77)	<0.001
	2	Girl	200 (48.31)	72 (12.37)		215 (43.97)	125 (15.23)	
Father's education	1	< high school	21 (5.53)	98 (16.98)	<0.001	32 (6.54)	109 (13.33)	<0.001
	2	High school	57 (15.00)	95 (16.46)		89 (18.20)	151 (18.46)	
	3	>high school	302 (79.47)	384 (66.55)		368 (75.26)	558 (68.22)	
Mother's education	1	< high school	30 (7.94)	120 (20.73)	<0.001	55 (11.34)	134 (16.44)	0.002
	2	High school	70 (18.52)	128 (22.11)		92 (18.97)	190 (23.31)	
	3	> high school	278 (73.54)	331 (57.17)		338 (69.69)	491 (60.25)	
Abortion history								
Induced abortion	1	Yes	123 (29.71)	238 (40.89)	<0.001	107 (21.88)	352 (42.87)	<0.001
	2	No	291 (70.29)	344 (59.11)		382 (78.12)	469 (57.13)	
Spontaneous abortion	1	Yes	22 (5.31)	52 (8.93)	0.032	23 (4.70)	74 (9.01)	0.004
	2	No	392 (94.69)	530 (91.07)		466 (95.3)	747 (90.99)	
Adverse events								
Chemical exposure	1	Yes	12 (2.90)	48 (8.25)	<0.001	3 (0.62)	71 (8.65)	<0.001
	2	No	402 (97.10)	534 (91.75)		481 (99.38)	750 (91.35)	
Maternal infection	1	Yes	62 (14.98)	188 (32.3)	<0.001	29 (5.98)	255 (31.06)	<0.001
	2	No	352 (85.02)	394 (67.7)		456 (94.02)	566 (68.94)	
Other diseases	1	Yes	25 (6.04)	59 (10.14)	0.022	29 (5.93)	61 (7.43)	0.299
	2	No	389 (93.96)	523 (89.86)		460 (94.07)	760 (92.57)	
Use of medications*	1	Yes	25 (6.04)	136 (23.37)	<0.001	30 (6.13)	186 (22.66)	<0.001
	2	No	389 (93.96)	446 (76.63)		459 (93.87)	635 (77.34)	
Threatened abortion	1	Yes	28 (6.76)	98 (16.84)	<0.001	23 (4.71)	115 (14.01)	<0.001
	2	No	386 (93.24)	484 (83.16)		465 (95.29)	706 (85.99)	
Psychosocial stress								
Mild emotional problem	1	Yes	50 (12.08)	120 (20.62)	<0.001	34 (6.95)	191 (23.26)	<0.001
	2	No	364 (87.92)	462 (79.38)		455 (93.05)	630 (76.74)	
Severe emotional problem	1	Yes	41 (9.90)	77 (13.23)	0.109	6 (1.24)	131 (15.96)	<0.001
	2	No	373 (90.10)	505 (86.77)		477 (98.76)	690 (84.04)	
Traumatic event	1	Yes	13 (3.14)	68 (11.68)	<0.001	28 (5.73)	100 (12.18)	0.001
	2	No	401 (96.86)	514 (88.32)			721 (87.82)	
Perinatal condition								
Term of delivery	0	NA	29 (7.00)	14 (2.41)	0.012	8 (1.64)	16 (1.95)	<0.001
	1	Premature	17 (4.11)	46 (7.90)		17 (3.48)	75 (9.14)	
	2	Post-term	11 (2.66)	30 (5.15)		13 (2.66)	54 (6.58)	
	3	Full-term	357 (86.23)	492 (84.54)		451 (92.23)	676 (82.34)	
Method of delivery	1	Natural	170 (41.06)	196 (33.68)	<0.001	199 (40.70)	281 (34.23)	<0.001
	2	Cesarean	183 (44.20)	327 (56.19)		206 (42.13)	447 (54.45)	
	3	Assisted	61 (14.73)	59 (10.14)		83 (16.97)	93 (11.33)	
First delivery	1	Yes	390 (94.20)	545 (93.64)	0.716	375 (81.52)	774 (94.28)	<0.001
	2	No	24 (5.80)	37 (6.36)		85 (18.48)	47 (5.72)	

*mainly antibiotics and traditional Chinese medicine for anti-flu; Premature delivery, <37 weeks, post-term delivery, ≥42 weeks)

We noted a significant difference in the frequency of multiple factors between the ASD and TD children. In the discovery sample, the mothers of the ASD children had more likelihood than the TD mothers of experiencing an induced abortion before having the child (40.9% vs. 29.7%), maternal chemical exposures (8.3% vs. 2.9%), maternal infection (32.3% vs. 15.0%), use of medication mainly including antibiotics and traditional antiviral Chinese medicine (23.4% vs. 6.0%), a threatened abortion (16.8% vs. 6.8%), a traumatic event (11.7% vs. 3.1%, respectively), an abnormal

term of delivery, such as premature or post-term (13.1% vs. 6.8%), cesarean delivery (56.2% vs. 44.2%), and suffering from a mild emotional problem (20.6% vs. 12.1%, respectively) or a severe emot-ional problem (13.2% vs. 9.9%). These factors were signi-ficantly associated with ASD (p<0.05); moreover, they were consistent in the replication sample.

Multiple logistic regression analysis showed that five factors were associated with ASD in both samples. In the discovery sample, we identified through stepwise logistic regression analysis that maternal chemical exposure, use of medication, threatened abortion, maternal infection, induced abortion before having the child, and suffering from a traumatic event were significantly associated with ASD (p<0.05); moreover, most of these factors showed stronger associations in the replication sample (Table 2). The association of suffering from a traumatic event during pregnancy with ASD in the discovery sample (odds ratio [OR] =2.63; 95% CI: 1.32-5.24) was not consistently associated with ASD in the replication sample; however, we noted that another measure, suffering from a severe emotional problem, was significantly associated with ASD in the replication sample (OR = 14.89; 95% CI: 5.66-39.13), with a large effect size. Both a traumatic event and severe emotional problem measured maternal psychological stress, and they were correlated with each other in both the discovery (OR =2.63; p=0.013) and replication (OR=3.10; p<0.001) samples in our data. Furthermore, the first delivery appears associated with the risk of ASD in the replication sample (OR=4.63; 95% CI: 2.84-7.54). These five factors consistently associated with ASD in both samples, account-ted for 9.60% and 15.02%, respectively, of the variation in the risk of ASD, according to the measure of Nagelkerke's R^2.

In the combined sample, all five factors showed a stronger association with the risk of ASD, and this was expected as the sample size increased. The frequency of a single variable was summarized for the combined sample (Table S2). We showed through a stepwise logistic regression analysis that maternal chemical exposure was strongly associated with the risk of ASD (OR = 4.50; 95% CI: 2.38-8.52) (Table 2); moderate effect sizes were also observed with the use of medication during pregnancy (OR=3.19; 95% CI: 2.19-4.65), maternal infection (OR=2.68; 95% CI:1.99-3.61), threatened abortion (OR = 2.37; 95% CI: 1.61-3.50), and induced abortion (OR=2.07; 95% CI: 1.65-2.60) for association with ASD. Five factors explained approximately 16% of the variation in the risk of ASD in the combined sample. Additional factors, such as suffering from a severe emotional problem, and perinatal conditions, such as the term of delivery, the method of delivery and first delivery, were significantly associated with ASD (P<0.001) (Table S3).

Table 2. Multiple logistic regression estimates of risk factors in both discovery and replication samples

	Discovery			Replication			Combined		
	OR	**95%CI**	**P**	**OR**	**95% CI**	**P**	**OR**	**95%CI**	**P**
Demographics									
Gender (Boy)	7.11	(5.01,10.07)	<0.001	4.66	(3.37, 6.46)	<0.001	5.58	(4.41, 7.07)	<0.010
Father's education									
Less than HS	2.75	(1.59,4.78)	0.003	3.71	(2.12, 6.48)	<0.001	3.00	(2.04, 4.42)	<0.001
High school	1.37	(0.90,2.07)	0.420	1.23	(0.83, 1.83)	0.041	1.28	(0.97, 1.70)	0.062
More than HS (Ref)									
Abortion history									
Induced abortion	1.57	(1.14,2.17)	0.006	2.59	(1.89, 3.56)	<0.001	2.07	(1.65, 2.60)	<0.001
Adverse events									
Chemical exposure	2.77	(1.34,5.75)	0.006	8.47	(2.46,29.14)	<0.001	4.50	(2.38, 8.52)	<0.001
Maternal infection	1.72	(1.18,2.51)	0.005	4.17	(2.56, 6.81)	<0.001	2.68	(1.99, 3.61)	<0.001
Use of medication	2.93	(1.74,4.91)	<0.001	3.37	(1.98, 5.75)	<0.001	3.19	(2.19, 4.65)	<0.001
Threatened abortion	2.20	(1.32,3.69)	0.004	2.66	(1.48, 4.79)	0.001	2.37	(1.61, 3.50)	<0.001
Psychosocial stress									
Severe emotional				14.9	(5.66, 39.13)	<0.001	2.58	(1.70, 3.91)	<0.001
Traumatic event	2.63	(1.32, 5.24)	0.006				1.52	(0.97, 2.37)	0.066
Term of delivery									
Premature				2.87	(1.45, 5.71)	0.127	2.55	(1.57, 4.13)	0.065
Post-term				2.49	(1.17, 5.32)	0.358	2.34	(1.37, 4.00)	0.193
Full-term (Ref)									
Methods of delivery									
Natural							1.40	(1.00, 1.95)	0.464
Cesarean							1.64	(1.19, 2.25)	0.004
Assisted (Ref)									
First delivery				4.63			3.27	(2.24, 4.80)	<0.001

Sex bias in environmental effect on ASD

In the sample of boys, all five factors except maternal infection showed a stronger association with ASD (Table 3). Moreover, maternal chemical exposure exhibited a strong association with ASD in the discovery sample (OR = 3.67; 95% CI: 1.27-10.62), the replication sample (OR=14.15; 95% CI: 1.89-106.19), and the combined sample (OR = 6.20; 95% CI: 2.43-15.81); however, similar effect sizes for the association with ASD were observed with the use of medication, threatened abortion, induced abortion and maternal infection in the combined sample (Table 3). Two different variables measured maternal psychological stress. It is interesting to note that experiencing a traumatic event was the second most influential factor associated with ASD in the discovery sample (OR=2.81; 95% CI: 1.16-6.82), whereas experiencing a severe emotional problem was the second

most influential factor in the replication sample (OR=11.79; 95% CI: 3.49-39.84). However, only experiencing a severe emotional problem was associated with ASD in the combined sample (OR=2.18; 95% CI: 1.32-3.60). We also observed that additional perinatal conditions, such as the term of delivery, the method of delivery, and whether a first delivery, were associated with ASD in the combined sample (p<0.05) (Table S3).

Table 3. Multiple logistic regression estimates of risk factors in the boys of both discovery and replication samples*

	Discovery			Replication			Combined		
	OR	**95%CI**	**P**	**OR**	**95% CI**	**P**	**OR**	**95% CI**	**P**
Demographics									
Father's education									
Less than HS	2.51	(1.36, 4.64)	0.029	4.85	(2.42, 9.73)	<0.001	3.31	(2.08, 5.26)	<0.001
High school	1.54	(0.92, 2.55)	0.913	1.71	(1.06, 2.75)	0.340	1.64	(1.16, 2.32)	0.595
> HS (Ref)									
Abortion history									
Induced abortion	1.66	(1.13, 2.46)	0.010	2.89	(1.97, 4.24)	<0.001	2.31	(1.76, 3.05)	<0.001
Adverse events									
Chemical exposure	3.67	(1.27, 10.62)	0.016	14.2	(1.89, 106.19)	0.010	6.20	(2.43, 15.81)	<0.001
Maternal infection	1.56	(1.01, 2.40)	0.046	3.15	(1.76, 5.64)	<0.001	2.21	(1.56, 3.13)	<0.001
Use of medication	2.51	(1.35, 4.66)	0.004	4.14	(2.09, 8.17)	<0.001	3.34	(2.09, 5.33)	<0.001
Threatened abortion	2.79	(1.46, 5.35)	0.016	3.98	(1.80, 8.82)	<0.001	3.24	(1.94, 5.42)	<0.001
Psychosocial stress									
Severe emotional				11.8	(3.49, 39.84)	<0.001	2.18	(1.32, 3.60)	0.002
Traumatic event	2.81	(1.16, 6.82)	0.022				1.68	(0.96, 2.95)	0.070
Term of delivery									
Premature				2.70	(1.26, 5.79)	0.400	2.49	(1.43, 4.35)	0.159
Post-term				3.33	(1.18, 9.45)	0.210	2.49	(1.28, 4.85)	0.210
Full-term (Ref)									
Method of delivery									
Natural							1.56	(1.05, 2.31)	0.248
Cesarean							1.75	(1.19, 2.56)	0.013
Assisted (Ref)									
First delivery				4.67	(2.69, 8.12)	<0.001	3.40	(2.22, 5.22)	<0.001

* Estimates were obtained from the model with interaction; Ref, reference category; HS, High school.

Interaction between maternal infection and the use of medication

We observed a significant interaction between maternal infection and the use of medication on the risk of ASD in both samples. In the discovery sample, we identified a significant interaction between maternal infection and use of medication (p=0.016); maternal infection was strongly associated with ASD among mothers who also used medications during pregnancy (OR=5.48; 95% CI: 1.97-15.28), but it was not significantly associated with ASD among those who did not use medication (OR=1.40; 95% CI: 0.93-2.10). Similarly, in the replication sample, a significant interaction was observed (p=0.036); moreover, maternal infection was associated with ASD regardless of the use of medications, although the effect size was notably different between the mothers who used medication during pregnancy (OR =30.81; 95% CI: 3.92-242.50) and those who did not use medication (OR = 3.17; 95% CI: 1.89-5.31) (Table 4). The interaction was very significant in the combined sample ($p=9.37x10^{-04}$) (Table S3).

In the boy-only sample, the interaction between maternal infection and use of medication became more significant even when the sample size was reduced in the discovery sample (p=0.003), but less significant in the replication sample (p=0.117). It is interesting to note that we found a similarly significant effect size for the association of maternal infection with ASD when mothers used medication during pregnancy in the boys of both the discovery (OR= 13.81; 95% CI: 2.85-66.84) and replication (OR=14.64; 95% CI: 1.77-120.75) samples (Table 4). The interaction was also very significant in the boys of the combined sample ($p=1.26x10^{-03}$) (Table S3).

DISCUSSION

In this study, we identified through two independent samples that maternal chemical exposure, use of medication, maternal infection, threatened abortion, induced abortion, and maternal psychological stress during pregnancy was associated with ASD. The first five factors collectively explained 10-15% of the variation in the risk of ASD in both samples and 16% in the combined sample. A significant interaction between maternal infec-tion and the use of medication was found in the individual sample and combined. While some of these associated factors were consistent with previous studies (22, 23), our study provides novel insights into the environmental etiology of ASD that involves three different types of risk factors: chemical, infection and reproductive tract injury-induced causes.

First, individual maternal exposure to chemicals was a novel and strong factor significantly associated with the risk of ASD in Chinese populations. This evidence was consistent in two independent samples separately and the combined sample. Both epidemiological and animal studies have suggested that air pollution affects the central nervous system (CNS) and contributes to the development of CNS disorders (24) directly through the olfactory pathway (25) or a mechanism that involves oxidative stress and mitochondrial damage (26, 27) or oxidative-mediated neurotoxicity (28). Traffic-related nitrogen dioxide and partiulate matter less than 2.5 or 10 micrometers in diameter ($PM_{2.5}$ or PM_{10}) during gestation and the first year of life (1, 29, 30) and air pollution, including the levels of

ozone (O3), carbon monoxide (CO), nitrogen dioxide (NO2), and sulfur dioxide (SO2) in the air, have been associated with the risk of ASD in multiple studies (31), although other studies fail to find consistent evidence after adjustment for socioeconomic status and other potential confoundders (32). To date, few studies have reported an association of ASD with exposure to benzene or paint, which individuals may have more likely faced, particularly young couples, in moving into new housing due to the massive urbanization and rapid development over the previous decades in China.

Table 4. Multiple logistic regression estimates of maternal infection stratified by use of medication during pregnancy

	Medication	Maternal infection	N	OR	95% CI	P	P*
Overall							
South	Yes (n=161)	Infection	91	5.48	(1.97, 15.28)	0.001	0.016
		No infection (ref)	70	1.00			
	None (n=835)	Infection	159	1.40	(0.93, 2.10)	0.106	
		No infection (ref)	676	1.00			
North†	Yes (n=215)	Infection	116	30.81	(3.92, 242.50)	0.001	0.036
		No infection (ref)	99	1.00			
	None (n=1091)	Infection	168	3.17	(1.89, 5.31)	<0.001	
		No infection (ref)	923	1.00			
Combined	Yes (n=376)	Infection	207	10.76	(4.26, 27.17)	<0.001	<0.001
		No infection (ref)	169	1.00			
	None (n=1926)	Infection	327	2.07	(1.51, 2.84)	<0.001	
		No infection (ref)	1,599	1.00			
Boys only							
South	Yes (n=131)	Infection	75	13.81	(2.85, 66.84)	0.001	0.003
		No infection (ref)	56	1.00			
	None(n=593)	Infection	128	1.17	(0.74, 1.83)	0.509	
		No infection (ref)	465	1.00			
North	Yes (n=177)	Infection	100	14.64	(1.77, 120.75)		
		No infection (ref)	77	1.00			
	None (n=789)	Infection	136	2.53	(1.38, 4.65)	0.003	
		No infection (ref)	653	1.00			
Combined	Yes (n=308)	Infection	175	20.78	(4.7, 91.79)	<0.001	0.001
		No infection (ref)	133	1.00			
	None (n=1382)	Infection	264	1.66	(1.16, 2.38)	0.007	
		No infection (ref)	1,118	1.00			

*p-value for testing of interaction; † Four individuals were missing for the use of medication or maternal infection; ref, reference category.

Our study provides novel evidence that maternal infection, use of medication, and their interaction are associated with ASD. A birth cohort study has shown that maternal infections such as rubella, influenza, and toxoplasmosis during pregnancy are risk factors for schizophrenia (33). We observed that maternal infection increased the risk of ASD, which is consistent with a study that showed serologically documented influenza exposure during early to mid-gestation is associated with a 3-fold increase in the risk of schizophrenia (34). Although inconsistent replications have been indicated in other human studies (35), a meta-analysis of 15 studies with 40,000 ASD cases provided consistent but weak supporting evidence (OR=1.13, 95% CI; 1.03-1.23) that maternal infection is associated with ASD (11). Nevertheless, animal studies have provided strong evidence for a mechanism of maternal immune activation (MIA) that is difficult to obtain from humans (16, 36). The use of medication, mostly antibiotics and traditional Chinese medicine, for antiviral treatments, such as cold or flu, which increases the risk of ASD, might provide evidence for the paradoxical hypothesis that amino-glycoside antibiotics could trigger the autistic syndrome in susceptible infants by causing the stop codon read-through. In contrast, other antibiotics could improve the symptoms of ASD (37). Interestingly, we observed a significant synergistic interaction between maternal infection and use of medication on the risk of ASD consistently in both samples; the interaction effect was more consistent and stronger in boys regarding the risk of ASD.

Moreover, we provide consistent replications of various factors, including psychological stress, threatened abortion, an abnormal term of delivery, and cesarean delivery, which have been shown as risk factors for ASD in the previous studies (13, 38). However, our finding that induced abortion was associated with the risk of ASD is interesting. Induced abortion is more prevalent in China (39, 40), mostly due to unintended pregnancies that occur in young women. This finding may have an important implication for public health. A case-control study with a small sample size conducted in the US in 1999 indicated that having a previous termination of pregnancy is associated with the risk of ASD (41). Potential explanations could be that a prior maternal induced abortion might increase the likely-

hood of having a preterm birth; extremely preterm births have a significant risk of autism comp-ared to full-term delivery (42).

Furthermore, preterm birth was identified as a risk factor for ASD in the combined sample of our study. Induced abortion is a traumatic experience or stressful event, at least for some young women; however, its impact on women's mental health may vary with personal experience and the context of the social culture. Induced abortion before 25 years has been associated with increased risks of mental health problems, although it is in a debate in Western society (43). We propose that induced abortion modifies the maternal reproductive tract immune status, which could span an extended period and thereby influence one or later pregnancies. The persistence of this change in local immune-inflammatory regulation could be due to a change in resident populations of key progenitor cell populations or the epigenetic profile and regulation of those relevant immune-inflammatory cell populations.

Our findings may have important implications for policy-makers from different sectors, clinicians, or health professionals. Although the mechanism of the associated factors has yet been elucidated, most of these factors are preventable. Improving the accessibility of young women to contraceptive services can reduce unintended pregnancy; pregnant women can reduce chemical exposure or maternal infections through maternal health care or reproductive health services. As a speculative hypothesis has been proposed on the child use of antibiotics and the onset of autism (37), one must be careful in treating maternal infection during pregnancy in clinical practice.

Limitations

While our study provided substantial evidence of novel risk factors for ASD, there are unanswered questions. Both epidemiological and animal studies have suggested that air pollution affects the central nervous system (CNS) and contributes to the development of CNS disorders (24). A dose-response relationship of air pollution or chemical exposure with ASD risk needs to be established through a more rigorous study design. Since the recruitment is in the form of active registration rather than a random sampling survey, the response rate was not available; most children with ASD were recruited from urban public or private schools for special education and hospital outpatient clinics. Therefore, these might limit the representativeness of the sample and then affect the generalizability of the study results.

Additionally, all cases were diagnosed according to clinical diagnostic criteria (DSM-IV-TR), autism diagnostic interview-revised (ADI-R), Autism Diagnostic Observation Schedule (ADOS) and some autism rating scales were not used for the diagnosis of ASD in the study. Although the controls were recruited from regular kindergarten classes in the same or adjacent provinces and were matched on age, convenience sampling might introduce selection biases. The interview-based measurements may be subject to recall bias. The environmental exposure was based on retrospective recall whether or not the exposure occurred during the specific time-window of the pregnancy. We did not measure the dose of exposure; this might be a rough measure for a study in environmental epidemiology. While detailed information on the medications and type of maternal infections was collected, our sample size was too limited to examine specific infections or medications.

CONCLUSION

Multiple environmental factors, in particular, new evidence of chemical exposure during pregnancy and induced abortion before pregnancy, are associated with an increased risk of ASD in offspring. Most of these risk factors are common and preventable by reducing environmental exposure, improving reproductive health, or increasing accessibility to contraceptive use or knowledge education in younger women.

Supplementary Online

CONFLICTS OF INTERESTS

The authors declare no conflict of interests regarding the publication of this paper.

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