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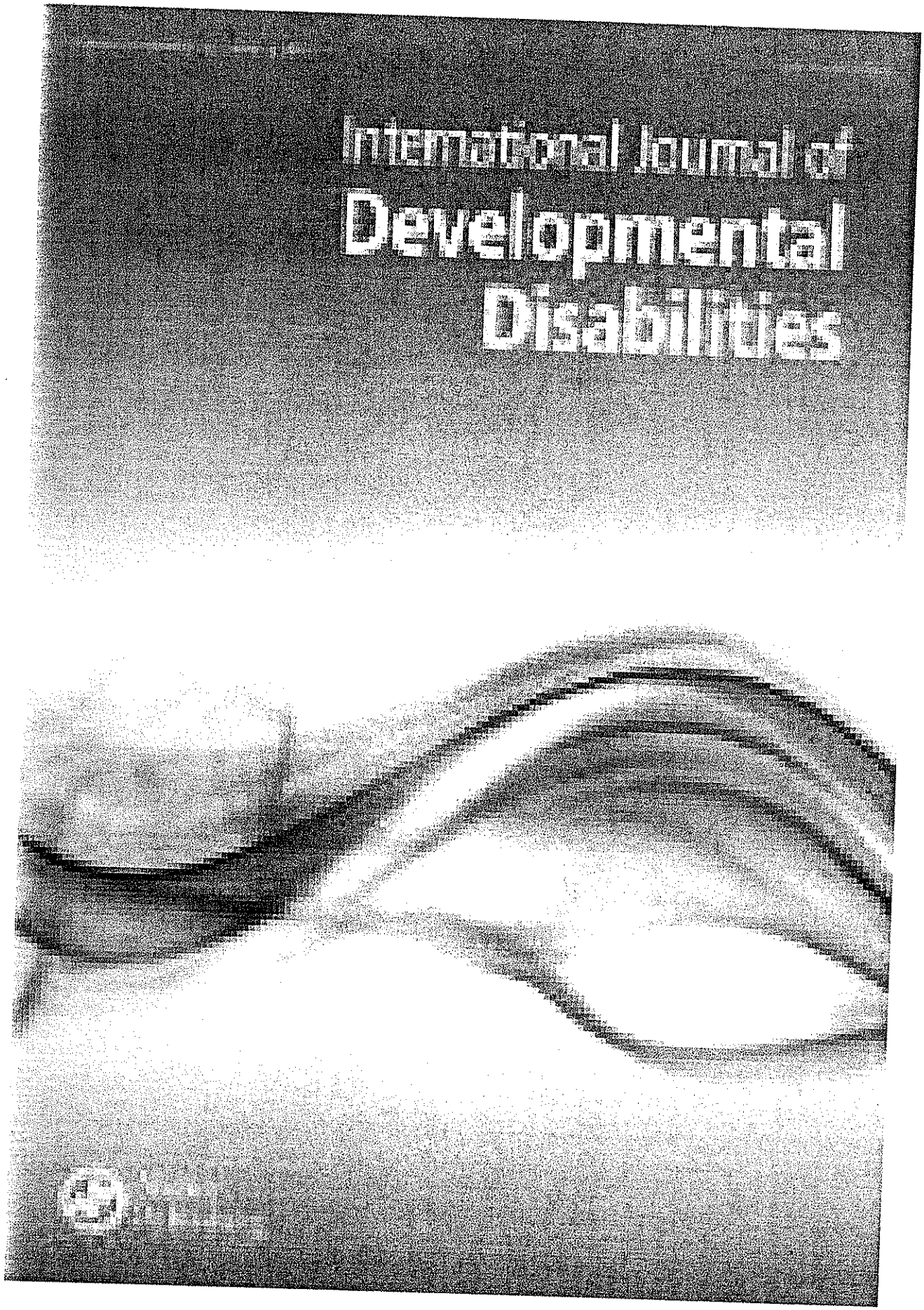
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Perinatal and parental risk factors in an epidemiological study of children with autism spectrum disorders

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Objectives: This study examines several perinatal and parental risk factors in an epidemiological study of children with autism spectrum disorders (ASD).
Methods: Based on a sample of 273 children with ASD who have been followed up at a General Hospital of North Greece, an additional sample of 273 healthy children, matched for age, is also recruited as a control group.
Results: The innovative results indicate significant correlation of ASD with three critical categories of factors: genetic, perinatal, and environmental. According to the empirical findings of multivariate logistic regression analysis, critical factors indicating higher risk for autism disorders include: male gender; gestational age (GA); multiple gestations; maternal age at delivery; and, maternal education.
Conclusions: The significant impact of perinatal and environmental factors can be indicative of their amplifying impact on genetic prone subjects.

Keywords: Autism, genetic, perinatal, environmental, risk factors

Introduction

Autism spectrum disorder (ASD) is considered to be a biologically determined behavioural syndrome, appearing prior to 36 months of a child's age and is characterized by qualitative impairment in reciprocal social interaction and communication, with restricted, repetitive, and stereotypical patterns of behaviour, interests, and activities (ICD-10, 2010; DSM-5; American Psychiatric Association 2013). The actiology of ASD is unknown. Genetic predisposition and environmental factors have been related to ASD's pathogenesis. Epidemiological ASD surveys are recorded in the UK and in many other countries since the mid-1960s (Lotter 1966). The majority of past epidemiological surveys have been based on a definite diagnosis of ASD, according to different criteria over time. The estimated prevalence of ASD in different countries and decades is seen to vary substantially, for instance, from 11.0 to 116.1 per 10,000 children population samples (Kielinen, *et al.* 2000; Chakrabarti and Fombonne 2001; Lingam *et al.* 2003; Gurney *et al.* 2003; Cederlund *et al.* 2008; Fombonne *et al.* 2006; Baird *et al.* 2000, 2006). Different rates of ASD incidences have been estimated, as, for instance, 10 incidences per 10,000 children (Gillberg and Wing 1999); 5 to 50 incidences per 10,000 children (Reichenberg *et al.* 2006); or, 60 incidences per 10,000 children (Constantino and Todd 2005). Males are affected more frequently than females, with a prevalence ratio of approximately 4:1 (Gillberg and Wing 1999; Constantino and Todd 2005).

During the last decades, several studies have examined perinatal and parental risk factors for ASD development in offsprings, compared with non-ASD population-based controls (Juul-Dam, Townsend and Courchesne 2001; Croen, Grether, Hoogstrate and Selvin 2002a, 2002b; Hallmayer *et al.* 2002; Hultman, Sparen and Cnattinius 2002; Wilkerson, Volpe, Dean and Titus 2002; Glasson *et al.* 2004; Larsson *et al.* 2005; Grether *et al.* 2009; Frans *et al.* 2013; Windham *et al.* 2011). Several studies focus on the perinatal period, searching for possible risk factors associated with the development of ASD. Mothers with previous unfavourable obstetric events had increased risk for offsprings with ASD (Hultman

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et al. 2002; Gialloreti, Benvenuto, Benassi and Curatolo 2014). Other perinatal factors that have been linked with the development of ASD include gestational age (GA), mode of delivery, congenital malformations, low birth weight (LBW), maternal use of drugs during pregnancy, and neonatal hyperbilirubinaemia (Croen *et al.* 2002a, 2002b, 2007, 2011; Hertz-Picciotto *et al.* 2006; Leavey, Zwaigenbaum, Heavner and Burstyn 2013). However, a large number of studies contradict the effect of perinatal factors in the development of ASD (Deykin and MacMahon 1980; Gillberg and Gillberg 1983; Bryson, Smith and Eastwood 1988; Bolton *et al.* 1997; Hultman *et al.* 2002). The advanced maternal age is another epidemiological factor that has been related with ASD development (Gillberg and Gillberg 1983; Reichenberg *et al.* 2006; Croen, Najjar, Fireman and Grether 2007).

Recent studies argue that there are significant differences in ascertainment between epidemiological studies performed several decades ago and more recently (Miller *et al.* 2013). There has been considerable variation in the results, with 38 risk factors to be associated with ASD in at least one study (Williams *et al.* 2008). However, in only a few studies do the authors indicate these factors as being critical for ASD. In summary of these findings, male gender increases the risk of ASD up to six times (Windham *et al.* 2011; Campbell, Chang and Chawarska 2014); low Apgar score two- to threefold (Hultman *et al.* 2002; Larsson *et al.* 2005); advanced maternal age (over 35 years) threefold (Hultman *et al.* 2002; Croen *et al.* 2007). Nevertheless, these findings have not been consistently confirmed by other studies (Larsson *et al.* 2005). Twin studies demonstrated conflicting results (Hultman *et al.* 2002; Larsson *et al.* 2005; Hallmayer *et al.* 2011). The presence of perinatal stress has been a popular field for scientists to investigate in relation to ASD development but relevant outcomes vary (Lobascher, Kingerlee and Gubbay 1970; Knobloch and Pasamanick 1975; Deykin and MacMahon 1980; Gillberg and Gillberg 1983; Bryson *et al.* 1988; Langridge *et al.* 2013).

The mechanisms leading to the development of ASD or autistic phenotype remain still unknown. During the past 30 years, numerous investigations have attempted to identify a pattern or a causal pathway for ASD, starting from the perinatal period. Despite the numerous studies investigating the causal relationship between different factors and ASD development, the empirical findings are seen to be inconsistent and often contradictory. This outcome can be related to considerable variations in the methods of analysis, sample size, variable selection, data quality, and diversified characteristics of control groups.

In view of these contradictory findings, this study proceeds to examine potential relationships between ASD and risk factors, such as gender, birth weight (BW), GA, Apgar score, multiple gestation, parity, parental age at delivery, parental education, and social status. Furthermore, additional information, collected by means of semi-structured interviews that were conducted with children's parents, is also incorporated; although previous studies have relied predominantly on children's records and reports as sources for data collection.

In this background, the purpose of this study is to examine a variety of critical epidemiological factors in a study group of children with ASD in Greece and compare the empirical findings with a control group of healthy children, matched for age. The critical factors incorporated in this study (such as LBW and GA) have been also proposed in other major recent studies (such as Larsson *et al.* 2005; Kolevzon, Gross and Reichenberg 2007; Schendel and Karapurkar Bhasin 2008). Although other important factors, such as foetal distress or labour related ones, are of core interest, they can constitute potential interesting ideas for further research and lead to the expansion of this study at a subsequent stage. To the authors' knowledge, this is one of the few first international empirical studies but one of the pioneer ones in the Greek educational policy field focussing on the interrelation of critical factors on ASD aetiology. Moreover, the study attempts to provide a range of quantified outcomes on these critical issues, especially in the context of Greece.

It should be noted that this study uses the term autism only as in the earlier past studies that incorporate this specific terminology and does not proceed to any adjustment into ASD. The term ASD is used in more recent studies and this is what the current study is indeed dealing with.

Methods

Participants

Subject Recruitment

The study cohort group consists of 273 children with ASD and their parents. The diagnosis is pursued by specialist clinical doctor on children's development (I.T.)[1], examines, and follows up children and newborn children (I.T. stands for the name initials of the practitioner doctor in the Centre of Developmental Paediatrics). They are followed up at a General Hospital of North Greece, the largest Hospital in Northern Greece with specific department on Developmental Paediatrics that employs a wide range of

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personnel recruited on the assessment of developmental disorders and is the only specialized Hospital that serves the population in this region. Moreover, 273 healthy children with no history of ASD, who have been visiting the hospital for routine health check-ups, are recruited as controls.

The sample population of the study has been distributed between males and females in order to take into account convincing past empirical evidence that indicated ASD to be more common among males than females (Table 1).

The ages of the children is especially relevant as a critical input because the most severely affected children with ASD are more likely to be diagnosed at a younger age, whereas those with milder symptoms may not be diagnosed until later school age (Table 2).

The mean age of the ASD children sample at the time of the study stands at 6 years (SD: 1.8).

Autism spectrum disorder was not the only criterion considered for setting-up the control recruitment sample. Particular care has been placed upon critical factors that should be eliminated, such as cases including, in addition, other psychiatric illnesses that have close relation to ASD or risk factors, such as schizophrenia, Down's syndrome, or cerebral palsy.

Measures

Parental characteristics were obtained by semi-structured interviews. The questions of the interview included risk factors for ASD, like gender, BW, GA, Apgar score, multiple gestations, parity, parental age at delivery, parental education, and social status.

Procedures

Semi-structured interviews were carried out at a General Hospital of North Greece by one of the researchers (I.T.) in this study, who was following up children with ASD on a regular basis, from September 2008 until September 2009. Moreover, from 2008 to 2009, 273 consecutive healthy children with no history of ASD who visited the General Hospital for routine examinations were recruited as controls. They were frequency matched to patients, upon the basis of age (±2 years).

The interview process was based on a semi-structured framework. This means that the basis of the field research framework was mainly guided and supported by the hospital written records run regularly by the respective paediatrics doctors with speciality on infant development. The validity of the results obtained from this process has been supported by the diversity of hospitals, doctors, and infant clinics and time-spans incorporated in this field research.

Classification of exposure variables

Classified variables have been selected as most important on the basis of recent empirical research on the topic under study (Juul-Dam *et al.* 2001; Croen *et al.* 2002a, 2002b; Glasson *et al.* 2004; Larsson *et al.* 2005; Reichenberg *et al.* 2006; Williams *et al.* 2008; Volkmar and Wiesner 2009). The relevant thresholds have been selected on the basis of the available sample population characteristics and the hospital existing data records. Birth weight was classified as <2500, 2501–3500, 3500 g (Table 3). Gestational age was classified as <36, 36–37 weeks, 38–42 weeks. Apgar score at 5 minutes was classified as <8 and 8–10. Maternal age at time of child's birth was classified as <25, 25–29, 30–34, and >35 years, while paternal age at time of child's birth was classified as <30, 30–34, 35–39, and >40 years. Parental education was classified as <6, 7–12, and >12 years. Parental social status, based on income, was classified as low, middle, and high. The individual economic basis categorization has been based on past relevant empirical practice and reference (e.g. Fombonne 2003).

The choices of categories and critical factors were selected on the basis of recent empirical research on the topic under study (e.g. Eaton, Mortensen, Thomsen and Frydenberg 2001; Juul-Dam *et al.* 2001; Croen *et al.* 2002a, 2002b; Hultman *et al.* 2002; Glasson *et al.* 2004; Larsson *et al.* 2005; Reichenberg *et al.* 2006; Williams *et al.* 2008). The relevant thresholds have selected on the basis of the available sample population characteristics and the hospital existing data records.

Table 1 Autism Spectrum Disorders (ASD) sample's gender

Gender	*N*	%
Male	214	78.38
Female	59	21.61
Total	273	100

Table 2 Dates of Autism Spectrum Disorders (ASD) children's birth

Year of birth	*N*	%
1995–1997	15	5.50
1997–2000	16	5.86
2000–2002	65	23.80
2002–2003	83	30.40
2003–2005	78	28.57
2005–2008	16	5.86
Total	273	100.0

Table 3 Autism Spectrum Disorder (ASD) children's birth weight

Birth weight	*N*	%
<2.500 g	29	10.62
2.501–3.500 g	140	51.3
>3.500 g	104	38.1
Total	273	100.0

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Statistical Analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS), version 19.0 (IBM). All sociodemographic and perinatal characteristics were categorical and they were expressed as frequencies (and percentages). The chi-square test was used to evaluate any potential association between the incidence of ASD and potential risk factors, while odd ratios (cOR) with their 95% confidence intervals (CI) were estimated as the measure of these associations. Parents' age was expressed also as mean ± standard deviation and was compared using Student's *t*-test. We run a *t*-test in order to only indicatively test the robustness of parental age as a risk factor and check its validity further. The methodology applied has followed past empirical practice. A multivariate stepwise logistic regression model was constructed to explore the independent effect of children's characteristics on the incidence of ASD. The multivariate regression was bidirectional. All tests were two-tailed and statistical significance was considered for P values <0.05.

Results

Children's birth characteristics

The study included 273 children diagnosed with ASD, born between 1995 and 2008. Table 4 presents the distribution of sociodemographic and perinatal variables, such as gender, BW, GA, Apgar score, multiple gestations, parity, parental age at delivery, parental education, and social status, among cases and control children.

Table 5 presents the association between ASD children's characteristics and the presence of ASD, expressed as odds ratios (cOR) with 95% CI and the crude cORs for the associations of each one of these variables with the incidence of autism.

Male gender was more frequent among patients in comparison to healthy controls [214 (78.4%) of 273 patients vs 140 (51.3%) of 273 control subjects, $P<0.001$; cOR 3.45]. The ASD sample cases were more likely to have shorter GA ($P=0.009$) and lower BW ($P=0.001$); the odds of developing ASD was significantly increased for GA <36 weeks [24 (8.8%) cases vs 10 (3.7%) control subjects; cOR 2.67]. This finding appears to be in line with other past studies (such as Bolton *et al.* 1997; Hultman *et al.* 2002; Madsen *et al.* 2002; Larsson *et al.* 2005). Past empirical evidence taken into account justifies this finding on the basis of GA of <35 or 37 weeks. Birth weight ≤2500 g [29 (10.6%) cases vs 8 (2.9%) control subjects; cOR, 4.25]. Apgar scores at 5 minutes were more often <8 (up to 7) in cases [19 (7.0%) cases vs 8 (2.9%) control subjects. $P=0.030$; cOR, 2.48]. Although more than half (55.7%) of the ASD children were first born, there was a tendency ($P=0.052$) towards higher frequencies of ASD children than healthy controls among the second [98 (35.9%) cases vs 83 (30.4%) control subjects] and third or more birth order [23 (8.4) cases vs 13 (4.8%) control subjects], resulting in an increased risk for ASD of 1.38 ($P=0.086$) and 2.06 ($P=0.047$) for children born second or third and beyond, respectively, compared to the ones that were born first. Twinning was considered as a risk factor for ASD, since twin pairs were significantly more common among patients than controls [18 (6.6%) cases vs 5 (1.8%) control subjects, $P=0.006$; cOR, 3.78].

Table 4 Presence of Autism Spectrum Disorders (ASD) in relation to children's characteristics

Key variables	Controls		ASD		*P* value
Gender					
Female	133	48.7	59	21.6	<0.001
Male	140	51.3	214	78.4	
Birth weight					
≤2500 g	8	2.9	29	10.6	0.001
2501–3500 g	164	60.1	140	51.3	
>3500 g	101	37.0	104	38.1	
Gestational age (weeks)					
<36	10	3.7	24	8.8	0.009
36–37	20	7.3	31	11.4	
38–42	243	89.0	218	79.8	
Apgar score at 5 minutes					
≤7	8	2.9	19	7.0	0.030
8–10	265	97.1	254	93.0	
Multiple gestation					
No	268	98.2	255	93.4	0.006
Yes	5	1.8	18	6.6	
Parity					
1	177	64.8	152	55.7	0.052
2	83	30.4	98	35.9	
≥3	13	4.8	23	8.4	
Maternal age at delivery					
<25	47	17.2	28	10.3	<0.001
25–29	85	31.2	56	20.5	
30–34	103	37.7	127	46.5	
≥35	38	13.9	62	22.7	
Paternal age at delivery					
<30	34	12.5	15	5.5	<0.001
30–34	86	31.5	62	22.7	
35–39	124	45.4	136	49.8	
≥40	29	10.6	60	22.0	
Maternal education					
≤6 years	104	38.1	15	5.5	<0.001
7–12 years	107	39.2	114	41.8	
>12 years	62	22.7	144	52.7	
Paternal education					
≤6 years	92	33.7	38	13.9	<0.001
7–12 years	111	40.7	113	41.4	
>12 years	70	25.6	122	44.7	
Social status					
Low	53	19.4	44	16.1	0.342
Middle	179	65.6	177	64.8	
High	41	15.0	52	19.0	

Parental Profile

The parental age of the ASD sample cases was significantly higher than the respective parental age of the control sample (maternal age: 31.71 ± 3.78 years vs 29.12 ± 3.31 years, $P<0.001$; paternal age: 36.33 ± 4.88 years vs 30.31 ± 4.11 years, $P<0.001$);

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Table 5 Association Between Autism Spectrum Disorders (ASD) children characteristics and the presence of ASD, expressed as Crude Odds Ratios (cOR) for Univariate Analysis and Adjusted Odds Ratios (aOR) for multivariate analysis with 95% confidence intervals (CI)

	Autism					
	cOR	95%CI	*P* value	aOR	95%CI	*P* value
Gender						
Female	1			1		
Male	3.45	2.37–5.01	<0.001	3.71	2.55–5.40	<0.001
Birth weight						
≤2500 g	4.25	1.88–9.59	<0.001	n.s.		
2501–3500 g	1					
>3500 g	1.21	0.85–1.72	0.300			
Gestational age (weeks)						
<36	2.67	1.25–5.72	0.009	3.27	1.43–7.50	0.003
36–37	1.73	0.96–3.12	0.067	1.51	0.84–2.73	0.165
38–42	1			1		
Apgar score at 5 minutes						
≤7	2.48	1.07–5.76	0.035	n.s.		
8–10	1					
Multiple gestation						
No	1			1		
Yes	3.78	1.38–10.34	0.009	2.50	1.02–6.14	0.038
Parity						
1	1			n.s.		
2	1.38	0.96–1.98	0.086			
≥3	2.06	1.01–4.21	0.047			
Maternal age at delivery						
<25	0.90	0.51–1.61	0.732	0.93	0.53–1.66	0.825
25–29	1			1		
30–34	1.87	1.22–2.87	0.004	1.46	0.96–2.23	0.073
≥35	2.48	1.46–4.19	<0.001	3.04	1.80–5.13	<0.001
Paternal age at delivery						
<30	0.40	0.21–0.77	0.005	n.s.		
30–34	0.66	0.44–0.99	0.043			
35–39	1					
≥40	1.89	1.14–3.13	0.013			
Maternal education						
≤6 years	0.14	0.07–0.25	<0.001	0.48	0.30–0.75	0.001
7–12 years	1			1		
>12 years	2.18	1.46–3.24	<0.001	1.82	1.22–2.70	0.003
Paternal education						
≤6 years	0.41	0.26–0.64	<0.001	n.s.		
7–12 years	1					
>12 years	1.71	1.16–2.54	0.007			
Social status						
Low	0.84	0.54–1.32	0.447	–		
Middle	1					
High	1.28	0.81–2.03	0.287			

Note: The variables that exhibited a significant impact on the presence of ASD in the univariate analysis were, subsequently, entered into the multivariate logistic regression model. The variables marked with '–' were not finally qualified to be included; n.s., not significant; cOR, crude odds ratio; aOR, adjusted odds ratio.

CI are included in Table 5. Increased risk for autism was associated with maternal age between 30 and 34 years (127 [46.5%] cases vs 103 [37.7%] control subjects; (cOR, 1.87) and maternal age ≥35 years (62 [22.7%] cases vs 38 [13.9%] control subjects; (cOR 2.48), in relation to age group 25–29 years. Paternal age ≥40 years displayed increased risk for autism (60 [22.0%] cases vs 29 [10.6%] control subjects; (cOR, 1.89) in relation to age group 35–39 years; on the other hand, significant reduction in the risk of autism, by 60 and 34%, respectively, was observed, with paternal age <30 years (cOR, 0.40) and 30–34 years (cOR, 0.66).

As to educational characteristics, ASD sample parents had longer (>12 years) education than control sample parents ($P<0.001$ for maternal and paternal education). With duration of maternal education for 7–12 years (medium) as a reference group, the risk of developing ASD was significantly increased for longer maternal education (144 [52.7%] cases vs 62 [22.7%] for control subjects; (cOR, 2.18). A significant reduction in the risk was associated with shorter duration of maternal education (≤6 years) (15 [5.5%] cases vs 104 [38.1%] control subjects; (cOR 0.14). Similarly, shorter and longer paternal educational level yielded odds ratios for ASD of 0.41 ($P<0.001$) and 1.71 ($P=0.007$), respectively, compared to these with medium (7–12 years) paternal education. No difference in family's social status was observed between patients and control subjects

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(P=0.342). The parental education profile has been based on the overall sample population characteristics and not on specific educational conditions in the region. The educational system applies uniformly to all State regions. The factor of parental education years has been consistently incorporated in relevant past studies. For instance, Fombonne (2003) refers to 12 studies providing information on the social class of the families of ASD children. A number of studies (e.g. Lotter 1966; Brask 1970; Treffert 1970; Hoshino *et al.* 1982) indicate an association between ASD and social class or parental education. However, the data-collection period for these studies refers to prior to 1980. Nevertheless, subsequent studies conducted thereafter did not pro- 6 vide any evidence on this issue.

Multivariate logistic regression analysis was performed for all the variables that showed significant difference in univaried analysis (gender, BW GA, Apgar score, multiple gestation, maternal and paternal age at delivery, maternal and paternal education).

Multivariate logistic regression analysis revealed that the following independent determinants were significantly associated with higher risk for ASD: male gender [adjusted odds ratio (aOR)=3.71, $P<0.001$], GA <36 weeks (aOR=3.27, P=0.003), multiple gestations (aOR=2.50, P=0.038), maternal age at delivery over 35 years (aOR=3.04, $P<0.001$), and higher maternal education >12 years (aOR=1.82, P=0.003). On the contrary, low maternal education level (≤6 years) was significantly associated with lower risk for ASD (aOR=0.48, P=0.001).

Discussion

Autism spectrum disorder is a behavioural syndrome of unknown aetiology. Like most behavioural syndromes, there is evidence that ASD is a heterogeneous disorder. In this study, several factors from different origins were related positively with ASD development. Subsequent to the multivariate statistical analysis, male gender, GA <36 weeks, multiple gestations, maternal age at delivery over 35 years, and maternal education ≥12 years were found to be persisting critical factors.

As regards the male gender, the empirical results are in agreement with relevant past empirical findings. Both the higher gender ratio and gender differences in the phenotypic profile for ASD support the hypothesis of gender differences in ASD aetiology, at least for some individuals (Gillberg and Wing 1999; Constantino and Todd 2003, 2005; Campbell *et al.* 2014). Different proposed explanations for gender differences contain genetic or epigenetic mechanisms that act independently of genetic liability, including ASD gene expression influenced by prenatal sex hormone exposure (Baron-Cohen and Hammer 1997; Szatmari, Jones, Zwaigenbaum and MacLean 1998; Pickles *et al.* 2000).

The ASD sample cases are found to have, more likely, shorter GA and lower BW; the odds of developing ASD are seen to increase significantly for GA <36 weeks and BW ≤2500 g, although this outcome does not remain statistically significant in the multivariate analysis. Studies focussing on single perinatal risk factors have reported a positive association for LBW (<2500 g) (Deykin and MacMahon 1980; Mann *et al.* 2010). In an earlier study, a gender stratification indicated increased ASD risk among boys (but not girls) of LBW (<2500 g) (Mason-Brothers *et al.* 1990). Schendel and Karapurkar Bhasin (2008) found that the prevalence of ASD in LBW or preterm children was markedly lower than that associated with other developmental disabilities; although, in multivariate analysis, BW of <2500 g and preterm birth at <33 weeks gestation were associated with an approximate twofold increase in ASD risk.

Bolton *et al.* (1997) concluded an increase in ASD risk associated with GA of <35 weeks, LBW children born term, and short GA. These findings are consistent with those of a large register-based study from Sweden (Hultman *et al.* 2002). In a recent study (Schrieken *et al.* 2013), prematurity/LBW were more prevalent in a group of ASD children compared to controls.

In this study, those cases that scored a low Apgar score (8 or less minutes) indicated a higher tendency (risk) to ASD, although the multivariate statistical analysis did not support statistical significance. The birth 7 order and being factor has been consistently incorporated in relevant past studies (e.g. Deykin and MacMahon 1980; Tsai and Stewart 1983; Piven *et al.* 1993; Bolton *et al.* 1997; Juul-Dam *et al.* 2001; Croen *et al.* 2002a, 2002b; Madsen *et al.* 2002; Glasson *et al.* 2004; Larsson *et al.* 2005). For instance, empirical evidence indicates that ASD participants may be less common in first- and fourth-born children compared with the general population. Hence, this factor appears to potentially have some interest in relation to ASD. Birth order is a factor found to differ significantly when compared against the control group. Autism spectrum disorder children are seen to have been born more often as second or third and beyond in the row, although statistical significance was not robust in the multivariate statistical analysis. According to several studies, birth order (especially first or last born) is an ASD risk factor (Deykin and MacMahon 1980; Glasson *et al.* 2004; Schrieken *et al.* 2013; Gialloreti *et al.* 2014). Piven *et al.* (1993) argue that differences in the number of unfavourable pre- or perinatal events between ASD children and siblings were attributable to difference in parity.

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Twinning was also observed more often in the group of ASD children. In past studies on twins and families, the concordance rate was over 36% for monozygotic twins and 3% for dizygotic twins (Lauritsen and Ewald 2001). Aronson, Hagberg and Gillberg (1997) believe that a concordance rate for monozygotic twins of <100% indicates that nongenetic factors also play a causal role. In a recent study (Hallmayer *et al.* 2011), the large proportion of ASD in twins is attributed mainly to shared environmental factors, in addition to moderate genetic heritability (Hallmayer *et al.* 2011).

In this study, the parents of ASD cases were seen to be significantly older than controls cases' parents. Maternal age over 35 years remained a statistical significant factor for ASD development in the multivariate analysis. Patients' parents had higher education level than the control subject parents. No difference in family's social status was observed between patients and control subjects.

The association of ASD with paternal age has been evaluated in numerous studies over the last decades (Lotter 1966; Piven *et al.* 1993; Bolton *et al.* 1997; Lauritsen and Ewald 2001; Croen *et al.* 2002a, 2002b; Hultman *et al.* 2002; Glasson *et al.* 2004; Larsson *et al.* 2005; Reichenberg *et al.* 2006). Mutation and/or epigenetic alterations are associated with advancing paternal age (Frans *et al.* 2013). In an Australian population, Glasson *et al.* (2004) found that increased maternal age, but not paternal age, was significantly associated with ASD risk, independently of other perinatal factors. Lauritsen and Ewald (2001) found that ASD risk was associated with increasing paternal (but not maternal) age. Larsson *et al.* (2005) reported statistically insignificant association of ASD with parental age, except from severely affected children with ASD. Reichenberg *et al.* (2006) found that increased paternal (but not maternal) age was associated with ASD risk after adjustment for years of birth and socioeconomic status. More recently, in a US study of large scale (n:23,311), Grether *et al.* (2009) found that a 10 year increase in maternal and paternal age was associated with 38 and 22% increase in cOR for autism, respectively. Windham *et al.* (2011) concluded that ASD prevalence was double in children of older mothers, aged over 40 years (Windham *et al.* 2011). Finally, in a study across generations (Frans *et al.* 2013), the advanced grand-parental age and paternal age were associated with increased ASD risk, something that could potentially develop over generations. Maternal education has been related positively with autism (Croen *et al.* 2002a, 2002b), while lower paternal education in a protective manner for ASD (Windham *et al.* 2011).

The study draws attention to the potential issue of inherent covariation among different variables, such as women education, mothers' age or fertility support. As discussed, women who are more educated are more likely to have delayed childbearing so are older when they have kids. On the other hand, older women are more likely to use fertility treatments resulting in multiple births; multiple births of course often affect BW and GA, etc. There is also the difference between LBW (in general) and LBW for GA; in general, a child who is born at 40 weeks but is of lower than expected weight is much more likely to have significant problems than a child born at 32 weeks who is maybe LBW technically, but not low for GA. These issues can be potential ideas as a future direction for research.

In conclusion, as revealed in the findings of this study, the risk factors associated positively with ASD can be grouped into three categories:

- genetic factors, as expressed by male gender, multiple gestation, GA, maternal age at delivery;
- perinatal factors, as expressed by GA and maternal age at delivery;
- environmental factors, as expressed by maternal education.

Some of these factors could be simultaneously included in more than one category, as they can have interactive action and can be interrelated (Schrieken *et al.* 2013). For instance, maternal age at delivery can affect GA, while maternal education can be linked with the maternal age at delivery.

It appears that the ASD aetiology is multivariate (Juul-Dam *et al.* 2001; Hallmayer *et al.* 2002; Glasson *et al.* 2004). Nevertheless, the genetic factor seems to be the strongest and most consistent, affecting all three categories. Furthermore, as to the ASD pathogenesis, heritable genetic vulnerability may amplify adverse effects triggered by environmental exposures (Stamou, Streifel, Goines and Lein 2013). The higher maternal educational level could be indicative as to the limited time spent with their children for their outgrowing. This issue requires further investigation, as there is no plausible explanation available yet. Over the last decades, epidemiological studies performed have exhibited significant differences in ascertainment (Miller et al. 2013). This empirical study is original for the domestic field and the references incorporated have been only indicative in order to support the relevant academic background, as no similar relevant studies for domestic research have been traced out.

This study, however, is limited by certain constraints. For a start, sample size had to be cut down, in order to keep sample's cohort at a manageable level. The sample was focussed only on children with ASD diagnosis and all the other cases were eliminated. Moreover, further evaluation of the interview and the process should be ideally conducted. In order to better understand the ASD puzzle as a complicated disorder and enlighten

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its pathogenesis, further empirical studies would be useful, interesting, and timely, albeit organized on the basis of strict and clear-cut scientific criteria.

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Disclaimer Statements

Contributors

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Conflicts of interest

Ethics approval

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