



FACULDADE DE MEDICINA DA UNIVERSIDADE DE COIMBRA

MESTRADO INTEGRADO EM MEDICINA – TRABALHO FINAL

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Risk for autism and parent's age and education level?

ARTIGO CIENTÍFICO

ÁREA CIENTÍFICA DE PEDIATRIA

Trabalho realizado sob a orientação de:
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MAIO/2016

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Parental profile in autism spectrum and other neurodevelopmental disorders

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Acknowledgements

I thank *Dr. Frederico Duque*, without which i would have never realized my study. I appreciate all the help you gave me. Thank you for your indispensable advice, availability and patience to clarify all the doubts that have arisen during this work.

I thank *Professora Doutora Guiomar Oliveira*, for your attention and availability.

I thank *Dr. Miguel Patrício* for the indispensable help in the statistical analysis and all the availability.

I thank my family and my friends for their support and encouragement, never allowing me to give up.

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Abstract

We investigated the influence of advanced parental age in Autism Spectrum Disorder (ASD) and other Neurodevelopmental Disorders (OND), also comparing with data of Portuguese population. Participated 1973 children and adolescents: ASD (N=1202) and OND (N=771). Parental ages were divided into four categories. Comparison of parental age between clinical groups and with population data was performed using t-Student tests. Odds ratios and confidence intervals were estimated using logistic regression analysis, adjusted for risk factors. We found statistically significant differences between maternal ages of ASD and OND children, but not between paternal ages. The impact of advanced maternal age was higher in ASD than OND, although influencing both. There is an increase in mean maternal age at birth over the years, with values higher than population data. Older maternal age and parental education was independently associated with increased odds of ASD.

Keywords

Autism spectrum disorder; Neurodevelopmental disorders; Parental age; Parental education

Abbreviations

ADI-R – Autism Diagnostic Interview-Revised

ADOS – Autism Diagnostic Observation Schedule

ASD – Autism Spectrum Disorder

CI – Confidence Interval

F – Female

FSIQ – Full-Scale Intelligence Quotient

GDD – Global Developmental Delay

GDQ – Global Developmental Quotient

DSM-5 – Diagnostic and Statistical Manual of Mental disorders fifth edition

ID – Intellectual Disability

ISCED – International Standard Classification of Education

LD – Learning Disorder

M – Male

OND – Other Neurodevelopmental Disorders

OR – Odds Ratio

SD – Standard Deviation

Introduction

Autism Spectrum Disorder (ASD) is an early-onset and life-long neurodevelopmental disorder (American Psychiatric Association 2013; Centers for Disease Control and Prevention 2014) with a high worldwide prevalence and a distribution of four males (M) to one female (F) (Fombonne 2003; Oliveira et al. 2007; Zablotsky et al. 2015). Deficits in social interaction and communication, as well as repetitive patterns of behaviour and interests are the core characteristics of ASD differently from other neurodevelopmental disorders (OND) (American Psychiatric Association 2013; Fombonne 2003).

Advanced parental age has currently attracted considerable attention as a potential risk factor for ASD, since the beginning of this trend of delayed reproduction, in many countries all over the world. According to data presented by the *Statistic Portugal* (Statistic Portugal 2015), the mean age of the mother at birth of a child, in 2010, was 28.6 years, reflecting a delay of two years in motherhood, as compared to 2000. In 2014, the average age of women at birth stood around 31.5 years, showing a consistent increase in maternal age at birth (Statistic Portugal 2015).

The increasing prevalence of autism over time is a well-known worldwide phenomenon without a clear explanation.

Studies have reported increased risk of ASD when both parents are of older ages (Croen et al. 2007; Durkin et al. 2008; Idring et al. 2014; Lampi et al. 2013; Parner et al. 2012; Quinlan et al. 2015; Rahbar et al. 2012; Sandin et al. 2015; Shelton et al. 2010) when there are older fathers but not older mothers (Balkom et al. 2012; Hultman et al. 2010; Onofrio et al. 2015; Reichenberg et al. 2006; Tsuchiya et al. 2008); when there are older mothers but not older fathers (Glasson et al. 2004) or, neither older fathers nor older mothers (Larsson et al. 2005). Furthermore, authors (Idring et al. 2014; Shelton et al. 2010) demonstrated that the

risk of offspring with ASD was greater for older mothers as compared with older fathers, namely, mothers over 40 years old had 51% and fathers over 40 years old had 46% higher odds of having a child with autism. However, Sandin et al. (2015) found that younger maternal age was also associated with increased risk for ASD (mothers with 20 years *versus* mothers between 20 and 29 years).

In a 2012 meta-analysis focused on advanced maternal age (Sandin et al. 2012) using data from over 25000 ASD cases and eight million controls, aggregated from ten studies, investigators found that mothers with ages equal or above 35 years had 1.5-fold increased odds of having a child with ASD compared to mothers between 25 and 29 years old. A similarly large 2010 advanced paternal age meta-analysis (Hultman et al. 2011) of data from 11 studies found that fathers aged 40 to 49 years old had a 1.8-fold increased risk of having a child with ASD, when compared to fathers with 29 years or less.

Studies (Idring et al. 2014; Parner et al. 2012; Shelton et al. 2010) examining effects of interactions of maternal and paternal age on ASD risk, found increasing risk for ASD with advancing maternal age regardless of the father's age, whereas increased risk with advancing paternal age was primarily observed among younger mothers, namely younger than 30 (Shelton et al. 2010) or 35 years (Idring et al. 2014; Parner et al. 2012). Idring et al. (2014) speculated that this finding could be related to a lower risk of pregnancy complications and other non-heritable risk factors in younger mothers, in whom the paternal age effect is consequently more evident.

Moreover, although poorly understood, it has been suggested that the association between socioeconomic status/parental education, especially maternal education (Durkin et al. 2008; Sandin et al. 2015) and ASD (Durkin et al. 2010; Larsson et al. 2005; Quinlan et al. 2015) can potentially effect these associations (Sun et al. 2014).

A number of mechanisms underlying the association between parental age and offspring with ASD have been proposed; however, mechanisms underlying maternal and paternal age effects are likely different, although those differences are difficult to disentangle due to high correlation between both ages (Idring et al. 2014; Lee and McGrath 2015; Shelton et al. 2010). So, it is still not clear whether paternal and maternal ages represent independent risk factors.

This study aims to characterize the role of advanced parental age at birth of a large Portuguese sample of children with ASD and compare with children with OND (Others Neurodevelopment Disorders). Furthermore, it is intended to compare with Portuguese population data published by *Pordata*© (*Database of Contemporary Portugal*; <http://www.pordata.pt/Portugal>).

Methods

Participants

Participants included 1973 children and adolescents, ranging in age from 2 years to 17 years and 11 months. They were divided into two clinical main groups: ASD (N=1202; 1009Males (M)/193Females (F) ratio M/F-5/1) *versus* OND (N=771; 551M/220F ratio M/F-2.5/1). Participants were seen as part of an outpatient clinic between 1995 and 2015. To be included in this study, all participants had to have both parental date of birth and also subjects date of birth.

ASD diagnosis was assigned on the basis of the gold standard instruments: parental or caregiver interview (Autism Diagnostic Interview– Revised, ADI-R) (Lord et al. 1994) direct structured proband assessment (Autism Diagnostic Observation Schedule, ADOS) (Lord et al. 1989) and clinical examination performed by experienced neurodevelopmental Paediatricians and a multidisciplinary team. The current diagnostic criteria for autism were revised according to the Diagnostic and Statistical Manual of Mental Disorders 5, DSM-5 (American Psychiatric Association 2013). All ASD patients had positive results in the ADI-R and ADOS for autism or ASD, and met the criteria for ASD from the DSM-5.

A comprehensive medical observation evaluated associated medical condition such as epilepsy, neurocutaneous or other genetic syndromes, or other usual comorbidity in ASD samples. All population in this study is routinely followed by this team in a clinical set at least two times per year.

In the OND group were included subjects diagnosed and followed in our outpatient clinic with Intellectual Disability (ID; N=191; 24.7%) (Full-Scale Intelligence Quotient – FSIQ \leq 70), Global Developmental Delay (GDD; N=152; 19.7%) (Global Developmental Quotient – GDQ \leq 70), Learning Disorders (LD; N=182; 23.6%) (FSIQ $>$ 70) or Language Development

Disorders. The parents of participants included in the OND group completed the Social Communication Questionnaire to exclude co-morbidity with ASD (Rutter et al. 2003).

Measures

All measures (even the ones referred to in the previous point for clinical characterization) were administered by experienced psychologists and neurodevelopmental paediatricians, for diagnostic and treatment planning, during routine clinical multidisciplinary assessments in a neurodevelopmental Unit that is a National reference for ASD and other neurodevelopmental disorders in a Tertiary Paediatric Hospital.

Parental Age

The Portuguese legislation (Despacho n^o5411/97 de 6 de Agosto) establishes the advanced maternal age - equal or above 35 years at the expected date of delivery - as one of prenatal risk factors and indicator of achievement of prenatal diagnostic.

Thus, it was established a reference age group between 25 and 29 years and 35 years of age or older as advanced parental age. Paternal age was classified using the same reference groups as maternal age.

Consistent with previous research (Durkin et al. 2008; Quinlan et al. 2015; Shelton et al. 2010) and using the published criteria by *Pordata*© (Statistic Portugal 2015) parental age was categorized into the following age groups: < 25 years; 25 – 29 years (referent category), 30 –34 years and 35 years of age or older.

Covariates

We selected covariates for inclusion in this analysis that had been found to be associated with ASD in the literature (Hultman et al. 2011; Larsson et al. 2005; Quinlan et al. 2015;

Sandin et al. 2012). To enhance comparability with previous studies, the following covariates were included: child's gender (male, female); maternal and paternal education (as indicator of socioeconomic status); birth order (1, 2, and 3 or more births); gestational age in completed weeks (<37, 37–41, 42–44); Apgar score at 1 and 5 minute of life and pregnancy complications (as indicator of fetal distress).

Procedure

All data was collected from a clinical electronic register - FileMaker-Pro 5 database according to national policy on archival research (*Comissão Nacional de Proteção de Dados*). The group of participants included in this study represents a subset of patients, which information is usually collected for clinical and research characterization of the outpatient clinic. A total of 1973 records meeting the inclusion criteria were included in this study.

We obtained information about: gender and date of child's birth; perinatal conditions (birth order, gestational age at birth, Apgar score at 1 and 5 minute of life) and parental characteristics (date of birth and education level). We considered low Apgar score at 1 minute and 5 minute when less than or equal a 7 and preterm delivery was defined as a birth occurring before 37 weeks of gestation.

Parental education was categorized according to International Standard Classification of Education – ISCED (UNESCO 2013): with no educational qualification; primary educational (ISCED level 1); lower secondary educational (ISCED level 2); upper secondary education (ISCED level 3); post-secondary non tertiary educational (ISCED level 4); tertiary educational (ISCED level 5 to 8). These categories are corresponding to the published criteria by *Pordata* (Statistic Portugal 2016): no level of education (ISCED 0); basic education that includes first second and third cycle (ISCED 1 and 2); secondary (ISCED 3); post-secondary (ISCED 4) and higher education (ISCED 5 to 8).

Statistical analysis

The description of quantitative, ordinal and nominal variables is made using means and standard deviations, medians and 25th and 75th percentiles and absolute and relative frequencies, respectively. The normality of the variables was assessed resorting to Shapiro-Wilk tests and by means of graphical analysis. Comparisons of quantitative variables between groups were performed using two sample (independent or paired, accordingly) t-Student tests. Comparisons between quantitative variables and a reference value were performed with one sample t-Student test. Mann-Whitney U tests were used to compare ordinal variables between groups. Association between qualitative variables was assessed using Chi-square tests. Univariate logistic regressions were employed to assess how different variables (maternal age, paternal age, gender, maternal education, paternal education, birth order, gestational age, pregnancy complications, low Apgar Score at 1 minute and low Apgar Score at 5 minutes of life) relate to the status of ASD among any developmental disabilities as whole (ASD and OND groups). The corresponding odds ratios for ASD were computed. A multivariate logistic regression model taking all of the aforementioned variables into account was used to compute adjusted odds ratios and corresponding confidence intervals. Correlations between pairs of quantitative variables were assessed computing Pearson correlation coefficients ($p < 0.05$).

All statistical computations were performed resorting to IBM SPSS Statistics version 23. We considered the significance level (α) = 0.05 ($p < 0.05$).

Ethics Statement

This study and all the procedures were reviewed and conducted in accordance with the declaration of Helsinki. Informed consent was obtained from the parents/guardians of all younger participants. Children and adolescents also gave oral informed consent.

Results

A summary of the characteristics of the sample is given in Table 1. The gender distribution included in ASD (5.2M:1F) and in OND (2.5M:1F). For all other characteristics, when we analyse the two main clinical groups (ASD *versus* OND) there were no statistically significant differences (Chi-square, $p > 0.05$; see Table 1 for details on exact p values).

Table 1. Characterization of the two main clinical groups (ASD vs OND) evaluated and followed up between 1995 and 2015

Characteristics	ASD	OND	p
Gender ^a (male : female)	1009 (83.9%) : 193 (16.1%)	551 (71.5%) : 220 (28.5%)	<0.001*
First-born ^a (yes : no)	637 (53.8%) : 546 (46.2%)	373 (49.5%) : 381 (50.5 %)	0.06
Preterm at birth ^a (yes : no)	102 (8.6 %) : 1082 (91.4%)	79 (10.6 %) : 663 (89.4 %)	0.137
Pregnancy Complications ^a (yes: no)	305 (25.6%) : 885 (74.4%)	200 (26.6 %) : 551 (73.4 %)	0.624
Low Apgar Score at 1 min ^a (yes : no)	128 (14.1%) : 780 (85.9%)	101 (15.1%) : 568 (84.9%)	0.577
Low Apgar Score at 5 min ^a (yes : no)	22 (2%) : 1088 (98%)	11 (1.6%) : 676 (98.4%)	0.559
Paternal education ^b	3 (1; 4)	2 (1; 3)	<0.001*
Maternal education ^b	3 (2; 6)	2 (1; 3)	<0.001*

Legend: Abbreviation: ASD - autism spectrum disorder; OND - other neurodevelopment disorders.

Data presented as ^a number of patients (percentage) or as ^b median (25th percentile; 75th percentile), where applicable. Chi-square (dichotomic variables); $p < 0.05$. Mann-Whitney U (ordinal variables); $p < 0.05$. All comparisons signalled * are significant.

A statistically significant difference between the two groups was found for parental education (Mann-Whitney U, $p < 0.001$) for ASD group, median parental education was level 3 and for OND group was level 2. In ASD subjects 75% of all fathers and mothers had a level of education equal or less than 4 and 6, respectively; and in OND group 75% of all fathers and mothers obtained lowest level of educational (level 3).

The mean age of mothers at the time of birth was 30.8 ± 5.2 years for ASD and 29.8 ± 5.7 for OND. The mean age of fathers was 33.2 ± 6.0 years and 32.7 ± 6.4 years among ASD and OND group, respectively (see Table 2). Statistically significant differences were found between the maternal ages of children with ASD and children with OND, $t(1533.486) = -3.927$, $p < 0.001$ but not between the corresponding paternal ages, $t(1562.170) = -1.840$, $p = 0.066$.

When compared with maternal age at birth of ASD child, on average, fathers were, approximately, 2 years older than mothers. In OND group, fathers were 3 years older, approximately (see Table 2). As for a comparison between maternal and paternal ages, statistically significant differences were found for both the ASD group and the OND group, $t(1203) = 17.292$, $p < 0.001$ and $t(771) = 15.951$, $p < 0.001$, respectively. Positive correlations were found between maternal and paternal ages in the ASD and OND groups, $r(1204) = 0.643$, $p < 0.001$ and $r(772) = 0.670$, $p < 0.001$. Table 2 also shows how mean parental ages differs across categories of parental education, birth order, gestational age, pregnancy complications and Apgar score at 1 and 5 minute.

Table 2. Mean Maternal and Paternal ages at birth of ASD subjects compared with OND cases, and in the sample as a whole (ASD and OND) stratified by covariate categories

	Number	Percentage within covariable	Maternal age mean (SD)	Paternal age mean (SD)
Group				
ASD	1202	60.9	30.8 (5.2)	33.2 (6.0)
OND	771	39.1	29.8 (5.7)	32.7 (6.4)
Gender				
Male	1560	79.1	30.6 (5.4)	33.1 (6.2)
Female	413	20.9	29.9 (5.5)	32.6 (6.2)
Maternal education (ISCED)				
1	428	21.7	30.7 (5.8)	33.6 (6.7)
2	380	19.2	28.4 (5.7)	31.1 (6.4)
3 or 4	572	28.9	29.9 (5.2)	32.7 (5.9)
5+	596	30.2	32.0 (4.7)	34.0 (5.6)
Paternal education (ISCED)				
1	612	31.0	29.7 (5.8)	33.2 (6.6)
2	436	22.1	29.3 (5.4)	31.4 (5.9)
3 or 4	484	24.5	30.9 (4.9)	32.9 (5.8)
5+	444	22.5	32.0 (5.1)	34.3 (5.9)
Birth order				
1	1010	51.1	28.7 (5.2)	31.1 (5.8)
2	698	35.3	31.6 (4.9)	34.3 (5.5)
3+	268	13.6	33.8 (5.2)	36.5 (6.7)
Gestational age (weeks)				
<37	181	9.4	31.0 (5.4)	33.4 (6.4)
>=37	1745	90.6	30.3 (5.4)	33.0 (6.2)
Pregnancy Complications				
No	1436	74.0	29.9 (5.2)	32.5 (5.9)
Yes	505	26.0	32.0 (5.8)	34.4 (6.8)
Apgar score at 1min				
<=7	229	14.5	30.1 (5.8)	32.6 (6.5)
>7	1348	85.5	30.7 (5.4)	33.2 (6.1)
Apgar score at 5min				
<=7	33	1.9	29.9 (5.2)	32.3 (5.5)
>7	1764	98.2	30.4 (5.4)	33.0 (6.1)

Legend: ASD - autism spectrum disorder; OND - other neurodevelopment disorders; SD - standard deviation; ISCED - international standard classification of education.

Maternal and Paternal age effects (Table 3)

With maternal and paternal age between 25 and 29 years as the reference group, the odds of being diagnosed with an ASD were reduced for maternal and paternal age below 25 years (OR 0.90; 95% CI 0.62-1.29 and OR 0.95; 95% CI 0.60-1.49, respectively), but not significantly in the adjusted analyses.

After we adjusted for the other parent's age and other covariates, the increase in ASD odds associated with maternal age of 35 years or older (relative to 25 - 29 years) was higher (OR 1.45; 95 % CI 1.02-2.06), comparing to the unadjusted analyses. However, the apparent increase in ASD odds associated with advanced paternal age, in the unadjusted analyses was no longer evident in the adjusted model.

In relation to paternal age, the odds of ASD were higher for paternal age from 30 to 39 years, but not reaching statistical significance (OR 1.20; 95 % CI 0.89-1.62). On other hand, children born to fathers with 35 years or older had lower odds of having ASD compared to fathers with age from 25 to 29 years.

Furthermore, the adjusted odds of ASD were two times higher in males than in females (OR 2.21; 95% CI 1.70-2.87). We also found that lower levels of parental education were associated with lowest odds of ASD, as compared to higher levels of parental education (ISCED level 5), whether in the adjusted as in the unadjusted analyses. However, with the exception of ISCED level 2 (OR 0.62; 95% CI 0.42-0.90), these results were not significant for other levels of maternal education, in the adjusted analysis.

We also observed that higher birth orders had lower odds of ASD, when compared to a firstborn child. These results were stronger in the adjusted than in the unadjusted analysis.

Having a preterm birth, pregnancy complications or low Apgar score at the first minute did not show higher odds of ASD, when compared to respective reference groups (term birth, without pregnancy complications or Apgar score above 7, respectively).

Table 3. Unadjusted and Adjusted Odds Ratios with 95% Confidence Intervals for ASD among children with diagnosis any developmental disabilities as a whole (ASD and OND)

	Unadjusted OR (95% CI)	Adjusted OR^a (95% CI)
Maternal age (years)		
<25	0.70 (0.54, 0.93)*	0.90 (0.62, 1.29)
25 to 29 ^b	1.00 (reference)	
30 to 34	1.25 (0.99, 1.57)	1.23 (0.92, 1.63)
≥ 35	1.16 (0.89, 1.50)	1.45 (1.02, 2.06)*
Paternal age (years)		
<25	0.68 (0.45, 0.97)*	0.95 (0.60, 1.49)
25 to 29 ^b	1.00 (reference)	
30 to 34	1.37 (1.07, 1.75)*	1.20 (0.89, 1.62)
≥ 35	1.09 (0.85, 1.38)	0.95 (0.68, 1.34)
Gender		
Male	0.48 (0.39, 0.60)*	2.21 (1.70, 2.87)*
Female ^b	1.00 (reference)	
Maternal education (ISCED)		
1	0.42 (0.32, 0.54)*	0.73 (0.49, 1.08)
2	0.35 (0.27, 0.46)*	0.62 (0.42, 0.90)*
3 or 4	0.50 (0.39, 0.65)*	0.77 (0.56, 1.06)
5 or more ^b	1.00 (reference)	
Paternal education (ISCED)		
1	0.36 (0.28, 0.47)*	0.55 (0.37, 0.82)*
2	0.33 (0.25, 0.45)*	0.46 (0.31, 0.68)*
3 or 4	0.55 (0.41, 0.74)*	0.62 (0.44, 0.89)*
5 or more ^b	1.00 (reference)	
Birth order		
1 ^b	1.00 (reference)	
2	0.89 (0.73, 1.09)	0.80 (0.61, 1.02)
3 or more	0.70 (0.53, 0.92)*	0.61 (0.42, 0.89)*
Gestational age (weeks)		
<37	0.79 (0.58, 1.08)	0.77 (0.53, 1.12)
≥37 ^b	1.00 (reference)	
Pregnancy Complications		
No ^b	1.00 (reference)	
Yes	0.95 (0.77, 1.17)	1.05 (0.82, 1.35)
Apgar score at 1 min		
≤ 7	0.92 (0.70, 1.22)	0.83 (0.61, 1.14)
>7 ^b	1.00 (reference)	
Apgar score at 5 min		
≤ 7	1.24 (0.60, 2.58)	1.80 (0.81, 3.98)
>7 ^b	1.00 (reference)	

Legend: ASD - autism spectrum disorder; OND - other neurodevelopment disorders; SD - standard deviation; ISCE - international standard classification of education; OR - odds ratio; CI - confidence intervals.

^aControlling for all covariates. (Odds ratio with confidence intervals that exclude 1.0)

^bReference group.

*Significantly different than reference group at $\alpha = 0.05$.

Comparison of the mean maternal age at birth in the present study vs Pordata© database

Statistically significant differences between the mean maternal ages at birth of the participants (ASD and OND) from the present study and the mean maternal age at birth of a child in Portugal, reported from *Pordata©* (Statistic Portugal 2015) were only seen for the year 2011. For all other years, statistical significance was not reached. The maternal mean age at birth of this study was higher than data from *Pordata©* (Statistic Portugal 2015), for all years (see Table 4).

Table 4. Mean maternal age at birth of participants study (ASD and OND) vs *Pordata* databases

Years	n	Mean Maternal age		p^b
		Present study	PORDATA	
1990	38	28.7 (6.1) ^a	27.1	0.118
2000	80	28.6 (5.6) ^a	28.6	0.997
2010	158	31.1 (5.8) ^a	30.6	0.300
2011	119	32.0 (5.5) ^a	30.9	0.040*
2012	91	31.5 (5.3) ^a	31.0	0.396
2013	35	32.3 (5.8) ^a	31.2	0.262

Legend:

^a SD - standard deviation.

^b The p -values included in the table were obtained from one sample t-Student tests.

* Statistically Significant.

Discussion

Up to date, several population based studies have examined parental age in relation to ASD, but to our knowledge, there are no studies that evaluate the association between parental ages with ASD risk with a control group of children and adolescents with OND diagnosis (autism excluded). This study allowed us to explore the role of parental age at birth in OND, other than ASD and reference population (Portuguese).

We found both mean maternal age (30.8 years) and mean paternal age (33.2 years) were higher for ASD than for OND subjects, but only with statistically significant differences between the maternal age and not between the corresponding paternal age.

When compared with maternal age at birth of ASD or OND child, on average, fathers were older than mothers, approximately 2 or 3 years, respectively. It is known that the reproductive age of men is higher than women, thus a father is viewed as having an advanced paternal age when equal or above 40 in some studies (Durkin et al. 2008; Idring et al. 2014; Shelton et al. 2010).

Another important finding in our study was the impact of advanced maternal age higher in ASD than in OND individuals, although influencing both of them, since the mean maternal age at birth was higher in ASD than in OND. This could be explained by the fact that previous studies found that children with ID, which represent 25% of our OND group, were usually born to younger mothers (Leonard et al. 2005), but not all (Myrskylä et al. 2013), since they generally are behaviourally immature (Kuntz and Lampert 2016; Morinis et al. 2013) and/or have lower socioeconomic status (Boden et al. 2008). One study (Pinborough-Zimmerman et al. 2011) examined sociodemographic risk factors associated with ASD and ID and found that ID (without ASD) cases were significantly less likely to have mother with over 13 years of education. This can be in accordance with our results, which found that 75%

OND children had parents with a level of education equal or less than 3 (according to ISCED, corresponds to 12 years of schooling in Portugal).

A Swedish study (Idring et al. 2014) demonstrated that advanced parental age was more strongly associated with ASD with intellectual disability, compared to ASD without intellectual disability. The control for IQ or exclusion of those individuals with ASD who have intellectual disability can be useful to a better enlightenment about these associations.

This analysis has also allowed us to assess the odds of being diagnosed with ASD. We found that only maternal age of 35 years and older was independently associated with an increased risk of ASD among children with diagnosis of any developmental disabilities as whole (ASD and OND groups). We found that the odds of ASD were 1.45 times higher for children born to mothers with 35 years of age or older, when compared to the reference age group. In terms of paternal age, our results suggest that the apparent association between ASD risk and advanced paternal age observed in the unadjusted analyses may be due to its association with maternal age.

As there is no studies that used OND as control group, to evaluate the association between parental ages with ASD risk, our study gives consistency. Furthermore, the ability to control for important confounders differs between studies. Although most were adjusted for co-parental age, gender, not all of them were adjusted for confounders such as birth order, socioeconomic status or parental education (Glasson et al. 2004) and perinatal condition (Croen et al. 2007; Reichenberg et al. 2006; Shelton et al. 2010).

Our results were similar to others (Glasson et al. 2004; Idring et al. 2014; Parner et al. 2012; Sandin et al. 2012; Shelton et al. 2010) that also found that the risk of ASD to be positively and independently associated with advanced maternal age. In 2012 meta-analysis (Sandin et al. 2012) investigators concluded that the association between advanced maternal age and risk of autism persisted after the effects of paternal age have been considered,

supporting an independent relation between higher maternal age and autism, as our study. However, in opposite of Shelton et al. (2010) and Idring et al. (2014), we did not find an increased risk with advancing paternal age, primarily observed among younger mothers.

This analysis differs from several studies that found only advanced paternal age (Balkom et al. 2012; Hultman et al. 2010; Onofrio et al. 2015; Reichenberg et al. 2006; Tsuchiya et al. 2008) or both parents of older ages to be associated with ASD (Croen et al. 2007; Durkin et al. 2008; Idring et al. 2014; Lampi et al. 2013; Parner et al. 2012; Quinlan et al. 2015; Rahbar et al. 2012; Sandin et al. 2015; Shelton et al. 2010).

Additionally, this analysis showed higher odds of ASD males than females in unadjusted and adjusted analysis (the adjusted odds of ASD were two times higher in males than in females). These gender findings are similar with those reported in some population based studies (Croen et al. 2002; Pinborough-Zimmerman et al. 2011). Two studies (Croen et al. 2007; Reichenberg et al. 2006) observed that the effects of paternal and maternal age on risk of autism varied as a function of the offspring gender: the association between advancing paternal age and autism was stronger in female offspring, whereas the association between advancing maternal age and autism was stronger in male offspring. In a 2012 meta-analysis focused on advanced age maternal (Sandin et al. 2012), investigators support this hypothesis, which may point to a possible sex-specific aetiology in autism.

Others studies (Croen et al. 2007; Durkin et al. 2008; Glasson et al. 2004; Shelton et al. 2010) found birth order increases with parental age. We also found a decline in ASD odds associated with increasing birth order, stronger in the adjusted analysis than in the unadjusted analysis. These results may be linked to recent trends in small family size and delay of parenting (Durkin et al. 2008), as seen In Portugal: in 2014, each woman has on average 1.23 children; in 2000, that average was 1.55 children (Statistic Portugal 2015). However, information available for the present study did not allow examination of these hypotheses.

Several explanations have been offered for the observed association between ASD and advanced parental age. One is that increasing likelihood of mutagenesis in the paternal germ line due to ageing (Neale et al. 2012), however our study failed to confirm the association between risk of ASD with advanced paternal age.

Advancing maternal age has been associated with increased risk for obstetric and perinatal complications (Johnson et al. 2012) and these conditions have been associated with increased risk for autism (Fall et al. 2015; Larsson et al. 2005; Sandin et al. 2012). The effect of maternal age may point to age-related chromosome changes, pregnancy complications or environmental exposures during pregnancy (Blaschke et al. 2009; Burstyn et al. 2011; Kalkbrenner et al. 2012). However, in the present study we found that the preterm birth, pregnancy complications and low Apgar score at the first minute were not important predictors of ASD.

It is important to recognize that the potential causes of delayed reproductive age at birth of a child, such as higher educational levels, may also influence risk of ASD (Croen et al. 2002; Lee and McGrath 2015). The notion that children with autism tend to come from higher social classes dates back to the earliest description of the disorder by Kanner (1943) who characterized the high-risk families as *highly intelligent and educated*. Since then, there have been published some population-based studies (Croen et al. 2002; Durkin et al. 2010) that examine parental occupation, educational attainment, and level of intelligence, many of which support Kanner's initial observations (Sun et al. 2014). We found that lower levels of parental education were associated with lowest odds of ASD, as compared to higher levels of parental education (classification according to ISCED).

Some authors (Oliveira et al. 2006; Onofrio et al. 2015) suggested that older parents have higher levels of educational achievements, more stable family environment and economic position than other parents, improving knowledge and acceptance of autism as well as a

better and earlier access to health institutions (Croen et al. 2002; Sun et al. 2014), which may also contribute to increased awareness and expansion of the diagnosis of ASD.

We used parental education level as indicator of socioeconomic status, however it was not possible to examine whether a higher level of education reflects directly a higher socioeconomic status and greater access to educational and health services. In the same manner, we cannot evaluate with the data available, is that, compared with younger parents, older parents may be more aware of developmental abnormalities and better able to access diagnostic and special educational services.

This study is an actual and pertinent evaluation, since advanced parental age is not a rare event and has become ever more common in recent years, thus the actual percentage of risk of ASD in the general population attributable to exposure to advanced paternal age may be high. This analysis also has other important strengths. It focused on comparing children with ASD with children with OND. The diagnosis of both ASD and OND was assigned on the basis of the gold standard instruments and clinical examination performed by experienced neurodevelopmental Paediatricians. Other studies exploring parental age in individuals with ASD have relied upon a registered diagnosis without the use of such interviews.

However, it would also be of great interest to study the relation between ASD with a healthy control group. A good way to walk past this was by comparing maternal mean ages at birth over several years (1990, 2000, 2010 to 2013) from our databases, which had sizeable sample, allowing us to compare them with *Pordata*© (Statistic Portugal 2015). There were only found statistically significant differences for the year of 2011. However, the sample's size in each year had a critical role on the obtained *p*-values. Therefore, it would be interesting to sign that the maternal mean age at birth of this study participants was always higher than data from *Pordata*© (Statistic Portugal 2015), for all years considered. We also found that there was an increase in mean maternal age over the years. Therefore, our results

were similar to others (Durkin et al. 2008) that found ASD cases had older mothers, compared with the birth cohort as whole. This increase of mean maternal age at birth over the years is consistent with the increase in ASD prevalence (Oliveira et al. 2007; Zablotzky et al. 2015), what can indicate that there is a relation between them.

Our analysis had limitations as being a retrospective study. It was not possible to address the hypothesis those parents of autistic individuals delay paternity because of traits associated with the autism phenotype, an unmeasured factor in the present study potentially associated with both advanced parental age and ASD risk in offspring is parental history of psychiatric illness (Larsson et al. 2005; Puleo et al. 2008) or behavioural traits of parents, that may result in both delayed parenthood and genetic susceptibility to autism in offspring (Hultman et al. 2011; Larsson et al. 2005; Puleo et al. 2008; Sandin et al. 2015).

In conclusion, this study gives a major contribution with additional evidence from a distinctive view (comparing ASD *versus* OND) to the literature on the relationship between advanced maternal age and risk of ASD, and it emphasizes the importance of studying the implication of parental age at birth in neurodevelopmental outcomes, other than ASD, in children and adolescents.

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