Epidemiology of autism spectrum disorder in Portugal: prevalence, clinical characterization, and medical conditions

Guiomar Oliveira* MD PhD, Centro de Desenvolvimento da Criança, Hospital Pediátrico de Coimbra;
Assunção Ataíde BSc, Direcção Regional de Educação do Centro Coimbra;
Carla Marques MSc, Centro de Desenvolvimento da Criança, Hospital Pediátrico de Coimbra;
Teresa S Miguel BSc, Direcção Regional de Educação do Centro, Coimbra;
Ana Margarida Coutinho BSc, Instituto Gulbenkian de Ciência, Oeiras;
Luísa Mota-Vieira PhD, Unidade de Genética e Patologia moleculares, Hospital do Divino Espírito Santo, Ponta Delgada, Açores;
Esmeralda Gonçalves PhD;
Nazaré Mendes Lopes PhD, Faculdade de Ciências e Tecnologia, Universidade de Coimbra;
Vitor Rodrigues MD PhD;
Henrique Carmona da Mota MD PhD, Faculdade de Medicina, Universidade de Coimbra, Coimbra;
Astrid Moura Vicente PhD, Instituto Gulbenkian de Ciência, Oeiras, Portugal.

**Correspondence to first author at* Hospital Pediátrico de Coimbra, Av Bissaya Barreto, 3000-076 Coimbra, Portugal.
E-mail: guiomar@hpc.chc.min-saude.pt

The objective of this study was to estimate the prevalence of autistic spectrum disorder (ASD) and identify its clinical characterization, and medical conditions in a paediatric population in Portugal. A school survey was conducted in elementary schools, targeting 332 808 school-aged children in the mainland and 10 910 in the Azores islands. Referred children were directly assessed using the Diagnostic and Statistical Manual of Mental Disorders (4th edn), the Autism Diagnostic Interview–Revised, and the Childhood Autism Rating Scale. Clinical history and a laboratory investigation was performed. In parallel, a systematic multi-source search of children known to have autism was carried out in a restricted region. The global prevalence of ASD per 10 000 was 9.2 in mainland, and 15.6 in the Azores, with intriguing regional differences. A diversity of associated medical conditions was documented in 20%, with an unexpectedly high rate of mitochondrial respiratory chain disorders.

See end of paper for list of abbreviations.

Autism spectrum disorder (ASD) is a syndrome with a wide clinical phenotype, characterized by impairments in social interaction and reciprocal communication, and by patterns of stereotyped behaviours. The term ASD is used here to define a broad concept of autism, manifested as a spectrum of behavioural, cognitive, and linguistic problems that include autistic disorder, Asperger syndrome, and pervasive developmental disorder not otherwise specified (PDDNOS). ASD is a chronic and severe neurodevelopmental disorder with a significant social impact.[1]

Prevalence estimations for this pathology have increased over the last decade, with studies reporting values between 1.1 and 12 per 1000.[2–7] The reason for the increase in reported rates is not well understood. It may represent a true increase in prevalence, or it may be due to an increased awareness of the problem, in combination with the establishment in recent years of more inclusive diagnostic criteria.[8] Other variables could also be implicated in the variation in prevalence rates over the years, such as the methods of study or differences in the population sample (age range, mean cognitive level, frequency of associated medical disorders, or geographic location).[7,8]

Until the 1990s, ASD was seldom diagnosed in Portugal, and there was no specific policy on education or health provision for individuals with autism. Today, developmental outpatients' clinics in the main Portuguese hospitals use a set of formal instruments for ASD diagnosis, and there is governmental support for educational units in regular schools specifically for children diagnosed with autism. To our knowledge, the prevalence of ASD has not been estimated in Portugal or other southern European countries; such information is fundamental to the provision of adequate medical and educational resources for the affected children. The present study was a country-wide epidemiological study that aimed to survey a large target population and in which all children identified with ASD were directly observed using structured diagnostic instruments. It also investigated the population-based estimation of the frequency of medical conditions associated with autism and the clinical characterization of these paediatric patients.

Method

PARTICIPANTS

Portugal has a population of about 10 million, including 240 000 inhabitants in the Azores islands. The mainland is subdivided into five major geographic regions: Norte, Centro, Lisboa e Vale do Tejo (Lisboa VT), Alentejo, and Algarve.

The study targeted children born between 1990 and 1992, living in mainland Portugal or the Azores, and attending elementary school (age 6–9y) in the school-year 1999 to 2000. In Portugal, elementary school is mandatory and free and the percentage of children attending school in this age group is 100%. Children in this age range can attend three types of schools: public and private elementary schools, and special education schools. The survey was, therefore, directed at these three types of schools and was expected to include all children in the autism spectrum in this age group.

Given the large number of schools in mainland Portugal (Table I), we opted for surveying approximately 20% of mainland public and private elementary schools, randomly chosen and distributed by district and geographic region (see Appendix I), and all special education schools. In the Azores,

given the smaller number of schools, we surveyed all elementary schools; there are no special education schools in these islands. The schools were identified using a government database. The total target population of children attending all schools in the 1999 to 2000 school year, and born from 1990 to 1992 was 332 808 in the mainland and 10 910 in the Azores (Table I).

SURVEY METHOD

The survey took place in May 2000. The selected schools were sent a mailing that included a letter to the school Director introducing the study and its objectives, an information leaflet on the behavioural and developmental characteristics of children with ASD, and a checklist questionnaire (Appendix II). Teachers of children born between 1990 and 1992 were asked: (1) the total number of children in the specified age range attending their class; (2) whether there were any children in that age range in their class with the characteristics described in the information leaflet; and (3) to answer a 12-question checklist (Appendix II) for each child who exhibited any of the characteristics described in the information leaflet.

The questions in the checklist were based on the 12 clinical criteria for autistic disorder outlined in the *Diagnostic and Statistical Manual of Mental Disorders* (4th edn; DSM-IV),[1] generally covering the whole range of autistic manifestations. The questions were simplified to facilitate understanding. At the time of the survey, teachers had had extensive daily contact with the children for at least 8 months.

As a pilot test, the survey had been sent to eight mainland schools, selected on the knowledge that children with autism did or did not attend these schools. In total, 356 children in the defined age range attended these schools, six of whom had a previous diagnosis of ASD. All schools responded and all children with autism, but only the six children previously known to have autism were identified by their teachers.

To estimate the validity of the survey method, we conducted a simultaneous systematic search for all children born from 1990 to 1992 ($n=56\,325$) in the Centro region and registered in educational, social, and health services with a diagnosis of ASD. Centro comprises about 20% of the Portuguese population.

CASE DEFINITION, DIAGNOSIS OF ASD, AND COGNITIVE AND MEDICAL EVALUATION

Children referred by teachers were selected for evaluation when they scored more than three positive items on the 12-question checklist. To make sure that this cut-off was adequate, all children referred by the teachers in Centro were observed to establish whether any children with less than four positive answers could be diagnosed with ASD. Observation of the referred children took place within 1 year after the survey.

Diagnosis and assessment of the children followed an in-depth evaluation protocol by a clinical team with extensive experience in observation of children with autism, comprising a developmental paediatrician, two psychologists, one special education teacher, and a social worker. The paediatrician collected the clinical history and observed all the children, which entailed extensive interaction and semi-structured activities in a clinical setting. Children were diagnosed with ASD using the Autism Diagnostic Interview–Revised (ADI-R),[9] the Childhood Autism Rating Scale (CARS),[10] and following the criteria for autistic disorder of the DSM-IV. Diagnosis of ASD required that the child met the ADI-R cut-off for autism and/or fulfilled DSM-IV criteria for autistic disorder, and had a functional level equal to or above 12 months in performance or autonomic skills, which were assessed using the Griffiths Scales of Mental Development.[11] The paediatrician, who had obtained reliability on the ADI-R, directly observed all children and reviewed comprehensive clinical material, including the ADI-R, CARS, and DSM-IV criteria on every case. The remaining team members obtained reliability on the ADI-R with the paediatrician. A consensus clinical diagnosis, including the coding of DSM-IV and ADI-R items, was achieved among the clinical investigation team.

Clinical assessment included history-taking, medical examination, and functional evaluation. Developmental or intellectual quotients were determined using the Griffiths Mental Scale–II[11] for children with a cognitive age below their chronological age, or the Portuguese version of the Wechsler Intelligence Scale for Children (WISC)[12] for children with a cognitive age near their chronological age.

Socioeconomic status was evaluated using the Graffar scale and the results obtained for the population with autism were compared with the social class distribution obtained for a group of 110 school-age children with global developmental delay. This control group consisted of children observed for the first time at the Centro de Desenvolvimento do Hospital Pediátrico in the years 2001 and 2002, for which a social evaluation was routinely performed by the same team of social workers using the same method. There are no available statistics on social class distribution of children in Portugal so no comparison was attempted with children in the general population.

Table I: Number of schools and target population in mainland Portugal and the Azores in school-year 1999 to 2000[a]

	Type of school	*Nr of schools*	*Nr of children in schools*	*Nr of schools selected (%)*
Mainland Portugal	Public elementary	8774	299 329	1788 (20.4)
	Private elementary	524	32 400	127 (24.2)
	Special education	248	1079	248 (100.0)
	Total	9546	332 808	2163 (22.7)
Azores islands	Public elementary	249	10 260	249 (100)
	Private elementary	5	650	5 (100)
	Total	254	10 910	254 (100)

[a]Statistics taken from government registries.

PREVALENCE CALCULATION

The prevalence study in the mainland was conducted in three population clusters: a random sample with a target population of students attending public elementary schools (N1) with the number of students surveyed (n1); a random sample with a target population of students attending private elementary schools (N2), and the number of children surveyed (n2); and all children attending special education schools (N3) equal to the number of students studied (n3). For the estimation of prevalence of the total target population (P) we used the following formula:

$$P = (N1/N \times P1) + (N2/N \times P2) + (N3/N \times P3);$$

in which N represents the target number of children in the chosen age range attending all types of schools (N1+N2+N3). P1 represents the prevalence in a sample studied in public elementary schools (P1=number of children with autism in public elementary schools [n1]); P2 represents the prevalence in a sample studied in private elementary schools (P2=number of autistic children in private elementary schools [n2]); and P3 represents the prevalence in a sample studied in special education schools (P3=number of children with autism in special schools [n3]). For these prevalence rates, 95% confidence intervals (CI) for large samples were determined.[13]

SCREENING FOR ASSOCIATED MEDICAL CONDITIONS

Children with autism with no previous diagnosis of associated medical conditions underwent a broad laboratory investigation, including testing for fragile X mutations (FRAXA and FRAXE); chromosomal abnormalities (karyotype study); neurocutaneous syndromes (skin observation and computer-assisted tomography or nuclear magnetic resonance neuroimaging); endocrine (thyroid function screening); and metabolic disorders (analysis of plasma lactate and pyruvate, amino and organic acids, oligosaccharides and mucopolysaccharides, purine and pyrimidine metabolites, guanidinoacetate and creatine metabolism, and congenital glycosylation carbohydrate deficient transferrin). Children with high levels of plasma lactate were further tested, as previously described,[14] to assess the possibility of mitochondrial disorder.

The Ethical Committee at the Hospital Pediátrico de Coimbra approved the collection of data and biological specimens from patients for research purposes. All parents or their legal representatives gave their informed consent.

Results

PREVALENCE OF ASD IN MAINLAND PORTUGAL AND THE AZORES

In mainland Portugal, a response rate of 87.7% was obtained, which corresponds to 1898 responses from the 2163 elementary and special education schools surveyed. Thus, 19.9% (1898 out of 9546) of all registered schools were surveyed, corresponding to 59 478 surveyed children (17.9% of the target population). The schools that did not respond were evenly distributed throughout the country, without any regional or demographic clustering, indicating that they did not have different characteristics from the study sample. The number of children referred by the teachers (i.e. identified with at least one positive item in the checklist) was 226. In the Azores we had a response rate of 85% (216/254) with 8317 children surveyed (76.2% of the target population). The number of children referred by the teachers was 25. Table II and Table III show the number of children surveyed and the number of children referred by teachers, distributed by geographic region, and by school type respectively.

In the mainland, 198 out of 226 (87.6%) referred children were selected for observation, according to the previously determined criteria (Table II). The clinical team directly assessed 182 (91.9%) of the children selected (16 declined assessment) of whom 107 (58.8%) met criteria for ASD. In Centro, none of the five children with less than four positive items in the checklist fulfilled criteria for ASD. In the Azores, 24 children were selected for evaluation; one declined to further participate in the study, so the clinical team assessed 23 children (Table II). The criteria for ASD were met in 13 of 23 children (56.5%). In total (mainland and Azores), 120 out of 205 assessed children were diagnosed with ASD. In this sample of 120 children with ASD, 115 fulfilled DSM-IV criteria for autistic disorder (the remaining five met the cut-off for autism in the three domains of the ADI-R and fulfilled DSM-IV criteria for PDDNOS) and 111 met the cut-off for autism in the ADI-R (the remaining nine fulfilled DSM-IV criteria for autistic disorder).

Table II: Number of children in Portugal surveyed, referred by teachers, selected for assessment, observed, and diagnosed with ASD, and prevalence estimates per 10 000 children in target population for school-year 1999 to 2000 by geographic region

Geographic region	*Surveyed children, n*	*Referred children, n*	*Selected children, n (% referred)*	*Children observed, n (% selected)*	*Children diagnosed with ASD, n (% observed)*	*Prevalence of ASD per 10000 children (95% confidence interval)*
Norte	24 386	73	60 (82.2)	55 (91.7)	28 (50.9)	6.0 (5.0–7.5)
Centro	10 585	46	46 (100.0)	45 (97.8)	23 (51.1)	12.5 (9.6–15.0)
Lisboa e Vale do Tejo	19 359	90	78 (86.7)	70 (89.7)	50 (71.4)	12.3 (10.0–14.0)
Alentejo	2895	9	6 (66.7)	5 (83.3)	3 (60.0)	7.0 (3.0–11.0)
Algarve	2090	8	8 (100.0)	7 (87.5)	3 (42.9)	2.4 (0.3–5.0)
Unknown[a]	163	–	–	–	–	–
Mainland total	59 478	226	198 (87.6)	182 (91.9)	107 (58.8)	9.2 (8.1–10.0)
Azores	8317	25	24 (96)	23 (95.8)	13 (56.5)	15.6 (8.0–23.0)

[a]Unknown represents children surveyed but for whom information on school origin was missing in reply. None of these children were referred with four or more positive items in questionnaire.

The global prevalence of ASD in the target population from the mainland was 9.2 per 10 000 cases (95% CI 8.1–10.0). The distribution of prevalence in the target population by geographical regions and school type is shown in Tables II and III. Prevalence is higher in Lisboa VT and in Centro than in Norte, Alentejo, and the Algarve. In the Azores the global prevalence of ASD in the target population was 15.6 per 10 000 cases (95% CI 8.0–23.0), the highest in the country (Table II). The prevalence in Norte, the most populated region in Portugal (around 40%), is significantly lower ($p<0.001$) than in other less populated regions of Portugal where CIs obtained were still considered adequate for comparison (Lisboa VT, Centro, and the Azores).

The large majority of the children diagnosed with ASD were regularly followed in healthcare facilities (94.2%), although autism had not been diagnosed in a third of the children (34.2%). More than half the children (55%) attended special education schools exclusively. The distribution of the children with autism by social class (Graffar class I: 18%; class II: 6.7%; class III: 24.8%; class IV: 41%; class V: 9.5%) was different from that of a control group of children with global developmental delay (Graffar class I: 2%; class II: 6%; class III: 22%; class IV: 63%; class V: 7%); specifically, we found that children with autism were significantly more prevalent in the most affluent social class (Graffar class I), and less prevalent in the lower social class (Graffar class IV).

Sensitivity of the survey method and prevalence in registered cases in Centro

The systematic search for children with autism registered in the school-year 1999 to 2000 in educational, social, and health services in Centro identified 42 patients from 56 325 children born from 1990 to 1992. Nine children, identified and diagnosed with ASD through the school survey in Centro, were not included in health, social, or educational registries. The school survey in this region targeted 10 585 children in the specified age range. In 23 out of the 46 children referred by teachers, the diagnosis of ASD was confirmed (23 true positive cases). Only one child who attended a surveyed school was found in the registries, but had not been referred by the teachers in the survey, and was, therefore, the single false negative case for the school survey in Centro. The sensitivity rate was 95.8% (95% CI 87–100). The prevalence of ASD in that region, determined by the search of health, social, and educational registries, was 7.5 per 10 000 cases (95% CI 5.0–9.0). This value was lower than the 12.5 per 10 000 cases (95% CI 9.6–15.0) obtained by the school survey study in that region.

Clinical history and functional analysis of the 120 children diagnosed with ASD

In total, 120 children were diagnosed with ASD. The ratio of males to females was 2.9:1. The median age at observation was 9 years 11 months. Most children were healthy and presented no other problems. Epilepsy (at least two convulsions without fever) was documented in 19 children (15.8%).

Parents or caregivers reported developmental problems within the first year of their child's life in 55% of children, while problems were only apparent in the second year for 38%, and in the 3rd year in 7% of cases. Signs were noticed before 36 months in all children. In 11.7% of the children parents reported language regression (loss of at least five words that had been used regularly for 3 months before).

Current developmental or intellectual level was evaluated in 90.8% of the children. The remaining children either presented very low functional levels or behavioural disturbances that were not compatible with direct assessment. In such cases, adaptive behaviour was evaluated by interviewing parents or caregivers. Cognitive functioning was within the normal range (IQ≥70) in 20 children (17%), and the remaining 100 (83%) had learning disability* (IQ <35–69). The ratio of males to females was higher in the group with normal IQ (5.7:1) and lower in the group with learning disability (2.6:1).

From the 205 children assessed in-depth, 85 (41.5%) were not diagnosed with ASD. In this group, the male to female ratio was 2:1, and the percentage of learning disability, 38.8%, was much lower than in the ASD group (83.3%).

*North American usage: mental retardation.

Table III: Number of children surveyed, referred by teachers, selected for assessment, observed, and diagnosed with ASD, and prevalence estimates per 10 000 children for school-year 1999 to 2000 by school type in mainland Portugal and the Azores

	Public elementary schools	*Private elementary schools*	*Special education schools*
Mainland			
Children surveyed, *n*	51 109	7290	1079
Children referred, *n*	128	4	94
Children selected, *n* (% referred)	107 (83.6)	4 (100)	87 (92.6)
Children observed, *n* (% selected)	96 (89.7)	2 (50)	84 (96.6)
Children identified with ASD, *n* (% observed)	40 (41.7)	1 (50)	66 (78.6)
Prevalence of ASD per 10 000 (95% CI)	7.8 (5.4–10.3)	1.4 (1.3–4.1)	611.2 (468.2–754.0)
Azores			
Children surveyed, *n*	7686	631	0
Children referred, *n*	24	1	0
Children selected, *n* (% referred)	23 (95.8)	1 (100)	0
Children observed, *n* (% selected)	22 (95.6)	1 (100)	0
Children identified with ASD, *n* (% observed)	12 (54.5)	1 (100)	0
Prevalence of ASD per 10 000 (95% CI)	15.6 (6.8–24.4)	15.8 (15.0–46.0)	0

ASD, autism spectrum disorder; CI, confidence interval.

SCREENING FOR MEDICAL CONDITIONS ASSOCIATED WITH ASD: HIGH PREVALENCE OF MITOCHONDRIAL DYSFUNCTION IN PATIENTS WITH AUTISM

At the outset of the study, 15% of the patients (18/120) had a previously identified associated medical disorder (Fig. 1): chromosomal abnormalities in five patients (four cases of trisomy 21 and one of microdeletion on chromosome 15q11-q13); molecular mutations on the *FMR-1* gene in three patients; previous brain infections in four children (two prenatal); malformative syndromes in four patients; and septo-optic dysplasia and hypoxic–ischemic encephalopathy in one patient each. These patients did not undergo any further aetiological investigation.

For the remaining 102 children (with apparently idiopathic autism) we planned the laboratory investigation protocol previously mentioned. The full pre-defined investigation protocol could only be applied to 56 patients; in the remaining 46 children only some of the tests were or had previously been performed (the parents or caregivers declined to participate in the full aetiological investigation).

Laboratory test results were within the normal range in all patients except for the karyotype study, which was abnormal in one of the 82 tested children (1.2%), and for levels of plasma lactate, which were abnormal (≥2.5mmol/L) in 14 of the 69 tested children (20.3%; Fig. 1).

The abnormal karyotype was a deletion in the short arm of chromosome 9 (syndrome 9p-) in a male with normal IQ and no obvious dysmorphism. Fluorescent *in situ* hybridization detected a deletion (9p24→pter) in the specific region with the telomeric probe (PAC43N6).

The lactate/pyruvate ratio was measured in 11 of the 14 children with hyperlactacidemia, and was found to be elevated in nine patients. Mitochondrial respiratory chain (MRC) function in mitochondria isolated from deltoid muscle was studied in 11 patients. Deficiency of one or more respiratory chain complexes, most frequently of complexes I, IV, and V, was confirmed in six patients. None of the tested mitochondrial DNA (mtDNA) deletions or mutations was found in these children. According to established criteria for mitochondrial disorder, five of these 11 patients were classified as *definite* MRC disorder cases.[14]

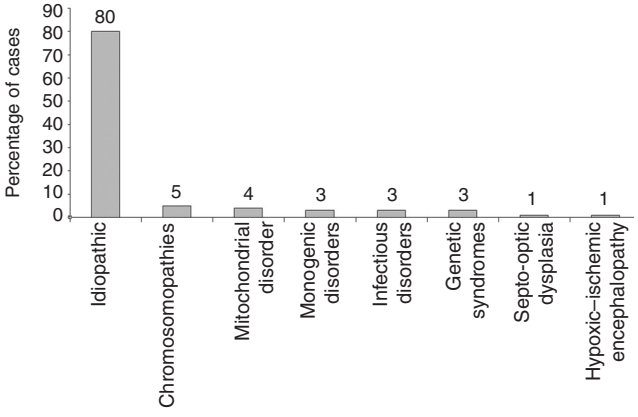

Figure 1: *Distribution of associated medical conditions in this population sample.*

Discussion

In this study, the prevalence of ASD in a southern European country was estimated for the first time through the survey of a large target population and direct observation of children, applying formal diagnostic instruments. In this population-based study, the frequency of medical conditions associated with ASD was determined, uncovering a high frequency of mitochondrial dysfunction in the children with autism.

The prevalence obtained for ASD in children in Portugal was close to 10 per 10 000, which is lower than values obtained for the most recent regional studies.[3–7,15] The narrow confidence interval (95% CI 8.1–10.0) indicates that the sample studied allowed the prevalence estimation in this target population with a good degree of precision. Various hypotheses can be put forward to explain the discrepancies in prevalence estimation among studies which include biases in survey methodology, differences in diagnostic criteria, or characteristics inherent to the population studied, and are discussed below.

For this prevalence study, surveying the school system was judged preferable to a multisource search of previously known ASD cases in health, educational, and social registries. First, because there is no efficient central health or social registry in Portugal, and second, because formal diagnostic instruments were not at the time employed by the majority of health professionals, leading to heterogeneous diagnosis criteria. As a control, however, a comprehensive search of previously diagnosed ASD cases in educational, social, and health registries in Centro, which represents approximately 20% of the country's population, was carried out. The prevalence estimation by the school survey was higher than the value estimated by the search of registered cases, indicating that the school survey had a better sensitivity than the multisource registry search. These results are in agreement with previous studies showing that prevalence estimations based on population surveys are more reliable than those based on the search of registered cases, which may lead to prevalence underestimation, particularly if good quality registries are not available.[16] We opted to survey school-aged children as opposed to preschool children or adolescents because clinical diagnosis at this age is known to be more reliable.[6,16,17] In addition, in Portugal virtually all children in this age range are enrolled in the school system, whereas the enrolling rates for earlier or later levels of schooling are lower. The choice of age range may explain the lower prevalence results to a certain extent, as studies in younger cohorts tend to report a higher prevalence of autism.[7,8,15]

The response rate to the survey was very high, demonstrating that teachers were aware of the importance of the problem. One possible question arising from this survey method is that teachers might not identify the children with milder problems and with better cognitive levels. Learning disability was indeed found in a very high percentage of cases (83%), contradicting recent reports of a trend towards decreased rates of learning disability in groups of children in the autistic spectrum.[3,5,15] However, while we acknowledge that a bias towards referral of more severe cases could not be controlled for, 41.5% of the children referred by the teachers were not diagnosed with ASD after assessment, and over half (61.2%) of these children without autism had a normal cognitive level. These observations indicate that teachers tended to refer most cases, even if only slight problems were apparent. We believe, therefore, that

such a bias would not significantly affect prevalence estimation. The finding of a higher prevalence in the school survey than that estimated by the search of registered cases further assured us that the identification by teachers was inclusive and thorough.

The participation rate in the assessment phase of this study was 92.3%. Even though some children identified by the schools could not be observed and, therefore, were not accounted for, this was a relatively small percentage (7.7%) and could not significantly change the prevalence. All patients were directly assessed, which is considered the best methodology in prevalence studies.[6] To our knowledge, few epidemiological studies have been carried out with direct observation of such a large number of children. The pre-defined protocol for diagnosis included extensive individual interaction with the children by an experienced clinical team, and subsequent fulfillment of the CARS and of DSM-IV criteria, the ADI-R structured diagnostic interview with the parents, and formal functional evaluation of the children. The diagnosis criteria were narrow, as they required that at least either the ADI-R or DSM-IV criteria were positive for autism or autistic disorder respectively. The most recent studies reporting much higher prevalence rates sometimes include cases that do not fully meet the cut-off of any standard diagnostic instrument, but for which ASD diagnosis relies on assessments by several experienced experts and consensus clinical diagnosis.[15] In the present study, however, it is striking that the diagnosis of ASD was excluded in 41.5% of the assessed children, and that in this group the ratio of males to females was 2:1, even though more than half of these children had a normal cognitive level. This observation suggests that in the non-autistic group we did not overlook a significant number of cases with ASD, because, generally, in the sub-groups of ASD in children with normal intelligence, the ratio of males to females is still higher. The prevalence obtained is within the range of previous studies in which stricter diagnosis criteria were applied.[18,19]

Determination of the prevalence per region of children with autism is essential to the provision of educational and health resources for them. However, we found some intriguing differences in regional prevalence rates: they were significantly lower in the Norte region compared with Centro, Lisboa VT, and the Azores. These discrepancies in prevalence estimations, obtained with the same methodology of study, raise the question of whether differences in the frequency of autism in diverse populations are a real phenomenon which may have a genetic basis. Support for this hypothesis comes from a recent report showing the absence of autism cases in the Inuit population of Northern Quebec.[20] In the Portuguese population, Y chromosome, mtDNA, and mutation prevalence studies suggest that there are different genetic influences in the northern, southern, and Azorean populations.[21,22] It is, for instance, known that in mainland Portugal the prevalence of the main mutation for hereditary hemochromatosis shows a north-to-south gradient compatible with the origin of this mutation in the Celtic/Nordic populations which colonized the northern areas, while Arabic populations, which occupied the central and southern regions of Portugal for many centuries, have a very low frequency of this disease.[23] In the Azores, consanguinity levels are very high, explaining why many recessive genetic disorders are more common in these islands. It is plausible, therefore, that population genetic specificities are involved in the regional variation of ASD prevalence within Portugal.

The distribution of children with ASD by social class shows an intriguing higher prevalence of children with autism in the most affluent social class. This is unexpected, given the social class distribution of a control group of children with global developmental delay recruited through the same central paediatric hospital and assessed during the same time period using the same social scale. Although it is not possible to compare these results directly with the social class distribution in Portuguese children in general, due to the unavailability of such demographic data, we know that a high prevalence of autism in the most affluent social class is far from the reality of the general population in this country. This control group also had the added benefit of allowing us to assess the specificity of this distribution for autism among the neurodevelopmental pathologies. The observation indicates that poverty-related factors do not have an impact in disease aetiology, which is compatible with the lower prevalence of autism in the poorest region of the country, the Norte region. Conversely, though, it may suggest an impact of wealth-related factors. Given that this distribution was obtained in a population-based survey which covered all social classes, awareness of the problem from families with a higher educational level cannot significantly bias the result. This observation is worthy of further analysis.

Neurodevelopmental symptoms in this group of patients were noticed at an early age: over half of the parents were worried in the first year and one-third in the second year. This is in agreement with the literature which reports that in 75 to 88% of cases, onset occurs up to the age of 2 years.[24] Regression of language or social interaction in the second year of life was found in 11.6% of the children, as reported in other studies.[5,24]

A defined medical condition associated with ASD was found in 20% of the cases, a rate superior to that reported in most epidemiological studies, in which, however, a systematic laboratorial investigation was not carried out.[5] The most frequent medical disorders associated with ASD were chromosomopathies, in a similar percentage to that found by others.[24] Among these, a partial deletion in the short arm of chromosome 9 was found in a male without obvious dysmorphism and with normal intelligence. The 9p- syndrome, with genetic variability of chromosomal region in monosomy, has previously been described and characterized by learning disability and craniofacial abnormalities, without reference to ASD.[25] However, a previous report has described a male patient with ASD, an IQ of 60, and a deletion in the short arm of chromosome 9,[26] a phenotype similar to our patient.

Unexpectedly, five cases of definite mitochondrial disease were identified and definite mitochondrial respiratory chain deficits were the second most frequent disorder associated with ASD (4.2%). This rate may be higher, as plasma lactate and pyruvate levels were determined in only 69 out of 120 children, and three patients with increased lactate levels did not undergo MRC functional study.[14]

Conclusion

The prevalence estimation of ASD for mainland Portugal and the Azores was close to 10 per 10 000 children. It is intriguing that there is a regional variation in prevalence, which could not be fully explained by methodological differences. This observation suggests that genetic population characteristics or regional environmental factors may contribute to the discrepancies found in prevalence estimations of ASD across studies. Repeating this study in the near future, to assess

whether there is a trend for prevalence increase in Portugal over time, would be very interesting. This population-based work further shows the heterogeneity of medical conditions associated with ASD and the utility of an exhaustive laboratorial investigation for the identification of non-idiopathic cases; this is particularly important for genetic studies. Finally, the original finding of a frequent diagnosis of MRC disease associated with ASD opens new perspectives for the aetiological investigation of this disorder.

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List of abbreviations

ADR-I	Autism Diagnostic Interview–Revised
ASD	Autism spectrum disorder
CARS	The Child Autism Rating Scale
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
MRC	Mitochondrial respiratory chain

Appendix I: Distribution of schools and children (total and surveyed) by geographic district throughout mainland Portugal

Mainland districts	*Total number of regular (public and private) schools*	*Total number of children in schools*	*Regular schools randomly selected n (% of total)*	*Regular schools surveyed n (% of total)*	*Children surveyed n (% of total)*
Aveiro	661	25850	142 (21.5)	125 (18.9)	4740 (18.3)
Beja	241	5367	42 (17.4)	36 (14.9)	686 (12.8)
Braga	788	33655	176 (22.3)	151 (19.2)	5765 (17.1)
Bragança	440	4250	82 (18.6)	67 (15.2)	815 (19.2)
C Branco	259	5873	46 (17.8)	43 (16.6)	766 (13.0)
Coimbra	592	12758	119 (20.2)	106 (17.9)	2394 (18.8)
Évora	154	5147	30 (19.4)	27 (17.5)	883 (17.2)
Faro	288	12759	64 (22.2)	50 (17.4)	2080 (16.3)
Guarda	435	5264	89 (20.4)	78 (17.9)	771 (14.6)
Leiria	599	15043	124 (20.7)	116 (19.4)	2616 (17.4)
Lisboa	1024	68846	204 (19.9)	172 (16.8)	11669 (16.9)
Portalegre	127	3734	24 (18.9)	21 (16.5)	648 (17.4)
Porto	1014	65937	203 (20.0)	175 (17.3)	11460 (17.4)
Santarem	543	13262	110 (20.2)	92 (16.9)	2806 (21.2)
Setubal	338	24889	73 (21.6)	63 (18.6)	4188 (16.8)
V Castelo	353	7894	74 (20.9)	58 (16.4)	1450 (18.4)
V Real	627	7472	135 (21.5)	119 (19.0)	1687 (22.6)
Viseu	815	13729	178 (21.8)	161 (19.8)	2812 (20.5)
Unknown[a]	–	–	–	11	163
Total Mainland	**9298**	**331729**	**1915 (20.6)**	**1671 (18.0)**	**58399 (17.6)**

Data shows that 20% of regular schools randomly selected are evenly distributed throughout the country, and that the percentage of children surveyed are approximately proportional to the percentage of schools surveyed. [a]Unknown represents schools and corresponding children that were surveyed but for which information on the school origin was missing in the reply.

Appendix II: 12-item checklist questionnaire

The child:	*Yes*	*No*
1. Shows marked impairment in the use of multiple non-verbal behaviours normally required for human communication and interaction (e.g. eye-to-eye gaze, facial expression and body expression are impaired or unusual), or does not even interact with other people	☐	☐
2. Does not develop peer relationships appropriate to developmental level (e.g. does not play with other children, prefers the company of adults)	☐	☐
3. Does not spontaneously seek to share enjoyment, interests or achievements with other people (e.g. does not show or call attention to what brings her joy)	☐	☐
4. Shows an inadequate response to the emotional cues from other people (e.g. may be indifferent to the arrival or departing of relatives, does not share the joy or sadness of others)	☐	☐
5. Has a delay or total lack of spoken language, and does not attempt to compensate through alternative modes of communication such as gesture or mime (e.g. pointing at what she wants or showing it through gesture)	☐	☐
6. Speaks, but is not capable of initiating or sustaining an adequate conversation with others (e.g. spoken language is focused on herself and not on the other person)	☐	☐
7. Speaks, but language is idiosyncratic (e.g. repeats what she heard, reverts pronouns and refers to herself in the 2nd or 3rd person, phrases are stereotyped and/or out of context, or uses a peculiar tone or rhythm, like a robot)	☐	☐
8. Lacks make-believe play or a poor imitative play not appropriate to developmental level	☐	☐
9. Shows abnormal interests with particular objects or activities, peculiar or too intense (e.g. excessive interest for traffic signs, car license plates or brands, numbers, movies)	☐	☐
10. Needs to maintain specific routines or rituals that are not functional (e.g. the door has to be open, the toy cars have to be aligned, likes to spin objects)	☐	☐
11. Shows abnormal stereotyped and repetitive movements (e.g. hand flapping, grimaces, jumping, mannerisms)	☐	☐
12. Is preoccupied with parts of objects but does not explore them functionally (e.g. rotates a car wheel or looks at an object from a specific angle)	☐	☐