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ORIGINAL ARTICLE

A diagnostic protocol for autism: an Italian experience

Maddalena Duca, Annio Posar, Antonia Parmeggiani

Abstract:

Background and aims: *The usefulness of a protocol for aetiologic diagnosis in pervasive developmental disorders (PDD) has been considered in literature and the results are heterogeneous. We addressed this topic, evaluating an Italian sample of patients with PDD.*

Material and methods: *We included in the study the patients referred to the Autism Centre of the University of Bologna from January 1999 to September 2009, affected by PDD according to DSM-IV-TR. All the subjects underwent anamnesis, clinical, laboratory and instrumental investigations for an aetiological diagnosis. First, we evaluated neurobiological findings in the whole sample. After, we divided the patients into two groups, respectively syndromal (with genetic or metabolic syndromes, cerebral lesions and/or microcrania/macrocrania) and non-syndromal (without genetic, metabolic diseases, cerebral lesions and/or microcrania/macrocrania), that we compared each other for several variables.*

Results: *We selected 90 subjects (mean age 11 years 1 month, range 2-36 years, mean follow-up 3 years 8 months), respectively with autistic disorder (55.5%), pervasive developmental disorder not otherwise specified (38.9%), Asperger disorder (4.4%), Rett disorder (1.1%). Male-to-female ratio was 3:1. Organic pre-, peri-, and postnatal antecedents were found in 27.8% of subjects. Neurological signs were present in 83.3%; brain imaging showed pathological findings in 33.3%. Genetic examinations disclosed abnormal karyotype in 7.8%, one patient had the Cowden syndrome, one the Rett disorder variant CDKL5, one the Wilson-Turner syndrome.*

The syndromal group consisted of 42 subjects (46.7%), the non-syndromal one of 48 patients (53.3%). Dysmorphisms prevailed significantly in the syndromal cases (30.9%), but they were present also in 10.4% of the non-syndromal ones. Severe/profound mental retardation recurred more often in syndromal cases respect to non-syndromal ones (42.9% vs 29.2%), but the difference was not significant. We found epilepsy in 30.0% of the whole sample without a significant group difference.

Conclusion: *The identification of specific pathologies underlying PDD could help to provide a medical therapy in tractable conditions, and to give a genetic counselling. In our sample genetic examinations, MRI and EEG recordings had the best diagnostic yield, while metabolic tests and other examinations were negative or mildly altered. Diagnostic evaluation should be performed choosing the examinations based on clinical suspicion with minimal resources and patient discomfort and with the best diagnostic yield.*

Keywords: *pervasive developmental disorders, autism, genetics, mental retardation, epilepsy, neurobiology*
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Introduction

Pervasive developmental disorders (PDD) are characterized by social and communication deficits, repetitive and stereotyped behaviours and onset before 3 years of age [1]. Prevalence of PDD has increased over the past 10 years and now it is ranging from 60 to 70 per 10.000 [2], with a male-to-female ratio of 3-4:1; these data do not include the Rett disorder. The dramatic increment of PDD has been attributed mostly to a broader diagnosis due to the improved public awareness and a higher degree of professional understanding of the disorders, although a biologic increase should also be considered [3,4].

The disorders most frequently associated with autism are epilepsy and mental retardation. Since the first description by Kanner in 1943 [5], the association between PDD and epilepsy has been observed in a percentage ranging from 4 to 42% of the patients; these values are largely higher than those in the general population [6]. Mental retardation is frequently present in subjects with PDD, in fact 70-90% of cases has an intelligence quotient (IQ) < 70 and 40% of the latter cases has an IQ < 50 [7].

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With regard to PDD aetiology, it is important to distinguish "syndromal" and "non-syndromal" autism. Syndromal autism is characterized by the presence of peculiar phenotypes or a known aetiology, such as, for example, Fragile X syndrome or Tuberous Sclerosis [8]. It is also characterized by the occurrence of cerebral lesions detected through MRI or microcrania and macrocrania. Non-syndromal autism, instead, is devoid of known aetiological factors [9], although a probable genetic inheritance has been hypothesized. In fact the reported recurrence rate of autism in siblings ranges from 2 to 8% and the concordance in monozygotic twins from 60 to 92%. Recent studies of the genome-wide association have noted the involvement of multiple rare genic copy number variants, an observation that seems to support a genetic basis for autism [10].

Epidemiologic studies indicate that environmental factors such as teratogens, prenatal infections such as rubella and cytomegalovirus, and perinatal insults account for few cases of autism [11].

Moreover a recent survey relates that among children with PDD and low genetic susceptibility, some maternal and obstetric factors may play an independent role in autism aetiology [12]. All these findings suggest that the pathogenesis underlying PDD is complex, and that genetic and environmental factors probably interact with each other interfering with the normal brain development [13].

The usefulness of a diagnostic protocol for PDD has been considered in literature and the results are heterogeneous. In the 90's the need for extensive medical examinations in all children with autism has been disputed because of their invasiveness, high cost and sometimes uselessness [14,15]. However, in recent years, several authors have stressed the importance of looking for various biological disorders associated with autism, even when scientific evidence is not immediately available [3,16].

The aim of this retrospective study is to evaluate, in a large sample of Italian patients with PDD, the application and the usefulness of a diagnostic protocol to identify the causes and the different pathologies that may be associated with PDD.

Material and Methods

Among the subjects referred to the Autism Centre of the University of Bologna from January 1999 to September 2009 we have considered retrospectively 106 patients with PDD according to DSM-IV-TR [1].

All subjects underwent anamnesis, clinical, laboratory and instrumental investigations for an aetiological diagnosis on the basis of their clinical picture [17]. We examined the following variables in the sample: the mean age at the last observation; gender; diagnostic type of PDD according to DSM-IV-TR [1], Childhood Autism Rating Scale (CARS), Autism Diagnostic Observation Schedule (ADOS), and Autism Diagnostic Interview-Revised (ADI-R); duration of follow-up; familial and personal antecedents; psychomotor development; autistic regression; early signs of autism; epileptic seizures; IQ level; neurological examination; screening for celiac disease (anti-gliadin, anti-endomysial and anti-tissue transglutaminase antibodies); muscle enzymes (creatine phosphokinase-CPK, lactate dehydrogenase-LDH); antibody levels to toxoplasma, rubella, cytomegalovirus, herpes simplex I, herpes simplex II (TORCH); blood and urine metabolic/immunologic tests (amino acid profile, lysosomal enzymes, urine oligosaccharides and mucopolysaccharides, organic acids); genetic examinations: high resolution karyotype, search for Fragile X, genome-wide subtelomere screening, array-comparative genomic hybridization (a-CGH), methyl CpG binding protein 2 (MeCP2), cyclin-dependent kinase-like 5 (CDKL5) and phosphatase and tensin homolog (PTEN) analysis; or other specific metabolic and genetic tests based on the clinical suspicion; prolonged awake/sleep (at least 1 whole a sleep cycle) EEG recordings; brain MRI; hearing assessment; and cognitive testing (Wechsler, Leiter-R and Stanford-Binet Scales).

Sixteen out of 106 patients were excluded because they did not undergo enough examinations due to non-compliance. Based on the results of the investigations before mentioned, we divided the sample of patients into two groups: non-syndromal (without genetic, metabolic diseases, cerebral lesions and/or microcrania/macrocrania) and syndromal (with genetic or metabolic syndromes, cerebral lesions and/or microcrania/macrocrania). Then, we compared the two groups for several variables such as gender; mean age at onset of the autistic symptoms; mean age at the last observation; PDD type according to DSM-IV-TR [1]; familial and organic pre-, peri- and postnatal antecedents; psychomotor development; neurological examination; celiac disease; partial deficiency of arylsulfatase A (ASA); EEG paroxysmal abnormalities (PA) without epileptic seizures; epilepsy or febrile convulsions; and cognitive assessment.

Statistics: Statistical evaluation was performed in order to compare the two groups, respectively non-syndromal and syndromal, with regard to the above mentioned variables. Particularly, Student T test was utilized to compare age of autism onset and age at the last observation, while Fisher exact test was used for all other variables. P values <0.05 were considered statistically significant. True Epistat software was utilized.

Results

Clinical, laboratory and instrumental findings in the whole sample

At the last observation, the mean age of patients was 11 years and 1 month (range: 2-36 years); males were 68, females 22, male-to-female ratio was 3:1. Fifty cases (43 males; 7 females) presented autistic disorder (55.5%); 35 (21 males; 14 females) had pervasive developmental disorder not otherwise specified (38.9%); four males had Asperger disorder (4.4%); one female had Rett disorder (1.1%). The mean follow-up from the first to the last observation was 3 years and 8 months.

A family history of both psychiatric (pervasive developmental, personality, anxiety disorders; depression; or schizophrenia) and neurological disorders (speech and development delay; mental retardation; epilepsy and/or febrile convulsions) was present in 23/90 patients (25.5%), of only neurological disorders in 33/90 (36.7%), and of only psychiatric disorders in 7/90 (7.8%). Organic pre-, peri- and postnatal antecedents were found in 25/90 subjects (27.8%): the most frequent conditions were risk of miscarriage in 14, cord around neck in four and premature birth in three.

Psychomotor development was regular only in two patients (2.2%), regular with autistic regression in 18 (20.0%), delayed in 58 (64.4%), delayed with autistic regression in 12 (13.3%). Early signs of autism, during the first 12 months of life, were found in 62/90 subjects (68.9%); the most frequent signs were the gaze avoidance in 22/62 (35.5%) and the lack of anxiety around a stranger in 21/62 (33.6%). In the remaining 28 cases early signs had not been described by the parents before 1 year of age, and no pathological behaviours were detected based on the available home videos. Neurological examination showed pathologic signs, single or associated, in 75/90 cases (83.3%): the most frequent were hyperlaxity (70.7%), hypotonia (33.3%), dysmorphisms (24.0%), and macrocrania (12.0%).

The screening for celiac disease was performed in 79/90 patients: the result was negative in 78 of them (98.7%), whereas in one case (1.3%) the diagnosis of celiac disease was confirmed by the intestinal biopsy. In all patients muscle enzymes (CPK, LDH) were tested, and we found a mild increase in CPK in eight of them (8.9%); since no child had an apparent clinical muscle disorder, the distress at the moment of the blood sample could explain this finding.

TORCH antibodies were tested in 39/90 patients: three children were positive for anti-cytomegalovirus IgG, three for anti-rubella IgG, three for anti-herpes simplex I and anti-herpes simplex II IgG and one for anti-measles IgG. None, however, manifested signs or symptoms of a congenital infection.

Lysosomal enzymes (beta-esosaminidase A and B, ASA) were tested in 64 patients: 57 of them had normal values and seven cases had low levels of ASA (10.9%) suggesting a pseudodeficiency [18]. Serum and urinary amino acids were assayed in 81 patients; seven of them had slightly heterogeneous altered findings with no pathological significance. We have analyzed urine oligosaccharides, mucopolysaccharides and organic acids in 48 patients; none had pathological findings.

Genetic examinations disclosed abnormal karyotype in 7/90 cases (7.8%): trisomy 21 in two, ring chromosome 22 in one, interstitial deletion of chromosome 2 in one, balanced translocation t(6;10) associated with pericentric inversion of chromosome 9 in one, balanced translocation t(5;10) in one, and pericentric inversion of 7q21.2-7q31.2 in one case. Moreover, we diagnosed in one patient the Cowden syndrome with a de novo PTEN gene mutation, in one case the Rett disorder with CDKL5 mutation, and in one child the Wilson-Turner syndrome. A-CGH test was made in 6.7% of patients of our sample, and it was negative for all these.

Twenty-seven (30.0%) out of the 90 patients developed epilepsy or febrile convulsions: partial epilepsy in 16 (59.2%), generalized in five (18.5%) and epilepsy with subcontinuous spike-waves during slow sleep in one case without a clinical picture identifying a Landau-Kleffner syndrome or epilepsy with continuous spike-waves during slow sleep. Five patients (18.5%) had febrile convulsions. EEG PA without epileptic seizures occurred in 16 (17.8%) patients. Hearing assessment (audiogram and/or brainstem evoked potentials) was normal in 87/90 patients (96.7%) and with non-specific findings in the other three cases.

MRI showed heterogeneous cerebral abnormalities in 30/90 cases (33.3%) (Table 1). Cognitive impairment occurred in 86.7% of the whole sample; 12 cases had a normal/borderline IQ (13.3%), 46 mild/moderate (51.1%), and 32 severe/profound mental retardation (35.5%).

Clinical, laboratory and instrumental findings in the two groups (non-syndromal and syndromal): In the non-syndromal group there were 48 patients (53.3%): 36 males and 12 females, male-to-female ratio was 3:1; the syndromal group consisted of 42 subjects (46.7%): 32 males and 10 females, male-to-female ratio was 3.2:1 (Table 2).

Table 1. MRI pathological findings in 30/90 patients (33.3%)

Cerebral lesions	N (%)
Damages from hypoxic ischemic injury in pre-perinatal period	9 (30.0%)
Enlargement of lateral ventricles	4 (13.3%)
Asymmetry of lateral ventricles	3 (10.0%)
Incomplete maturation of cerebral white matter	3 (10.0%)
Hypoplasia of the cerebellar vermis	3 (10.0%)
Mesial temporal sclerosis	2 (6.7%)
Thinning of corpus callosum	1 (3.3%)
Enlargement of the sylvian fissure with moderate temporo-polar atrophy	1 (3.3%)
Polymicrogyria of right precentral gyrus	1 (3.3%)
Asymmetry of temporal horns (right > left)	1 (3.3%)
Asymmetry of frontal horns (right > left)	1 (3.3%)
Arnold-Chiari malformation type 1	1 (3.3%)

Table 2. Comparison between non-syndromal and syndromal cases (Fisher exact test)

	Non-syndromal (48)		Syndromal (42)		p
	N°	%	N°	%	
Males	36	75.0%	32	76.2%	>0.05
Females	12	25.0%	10	23.8%	>0.05
Autistic disorder	26	54.2%	24	57.1%	>0.05
Pervasive developmental disorder not otherwise specified	21	43.7%	14	33.3%	>0.05
Asperger disorder	1	2.1%	3	7.1%	>0.05
Rett disorder	0	0.0%	1	2.4%	>0.05
Neurologic familial antecedents	15	31.2%	18/41[a]	43.9%	>0.05
Psychiatric familial antecedents	5	10.4%	2/41[a]	4.9%	>0.05
Neurologic and psychiatric familial antecedents	16	33.3%	7/41[a]	17.1%	>0.05
No familial antecedents	12	25.0%	14/41[a]	34.1%	>0.05
Organic pre-, peri-, and postnatal antecedents	10	20.8%	15/41[a]	36.6%	>0.05
Delayed psychomotor development	32	66.7%	26	61.9%	>0.05
Regular psychomotor development with regression	9	18.7%	9	21.4%	>0.05
Delayed psychomotor development with regression	6	12.5%	6	14.3%	>0.05
Regular psychomotor development	1	2.1%	1	2.4%	>0.05

[a] = *For one syndromal patient familial and remote personal anamnestic data were not available*

PDD types occurred without a significant difference between the two groups; one syndromal patient had Rett disorder with CDKL5 mutation and early epileptic seizures.

With regard to gender and familial antecedents, we did not find any significant statistical difference between the two groups.

The family history was negative for neurological and/or psychiatric disorders in a higher percentage of syndromal patients. However in the syndromal group, the familial antecedents for neurological diseases appeared more frequently, while a family history for psychiatric diseases and for neurological and psychiatric disorders together affected more non-syndromal patients. Anyway, all these differences were not significant. Organic pre-, peri- and postnatal antecedents were found in 10 of the non-syndromal patients (20.8%) and in 15 of the syndromal ones (36.6%), without a significant difference between the two groups. Moreover, psychomotor development was associated with autistic regression in a higher percentage of syndromal patients, but without significant group differences.

The mean age at onset of autistic symptoms was 13 months for the non-syndromal subjects, and 12 months and 2 weeks for the syndromal ones without significant group differences, while the mean age at the last observation was significantly higher in syndromal (12 years and 11 months)

than in non-syndromal (9 years and 5 months) ($p<0.05$) (Student T test). We found a significant difference ($p<0.05$) between the two groups in neurological examination, in the syndromal group there was a higher percentage of positive signs. This was mostly due to the dysmorphisms that prevailed in the syndromal patients (30.9%) ($p<0.05$); however, not specific dysmorphic signs were found also in 10.4% of the non-syndromal cases (Table 3).

Table 3. Comparison between non-syndromal and syndromal cases (Fisher exact test)

	Non-syndromal (48)		Syndromal (42)		p
	N°	%	N°	%	
Positive neurological examination	36	75.0%	39	92.9%	**<0.05**
Dysmorphisms	5	10.4%	13	30.9%	**<0.05**
Hypotonia	12	25.0%	13	30.9%	>0.05
Hyperlaxity	27	56.2%	26	61.9%	>0.05
Partial deficiency of arylsulfatase A (ASA)	3/32[a]	9.4%	4/32[a]	12.5%	>0.05
Celiac disease	0/42[b]	0.0%	1/37[b]	2.7%	>0.05
EEG PA without epileptic seizures	4	8.3%	12	28.5%	**<0.05**
Epilepsy/FC	12	25.0%	15	35.0%	>0.05
Normal/borderline IQ	7	14.6%	5	11.9%	>0.05
Mild/moderate mental retardation	27	56.2%	19	45.2%	>0.05
Severe/profound mental retardation	14	29.2%	18	42.9%	>0.05

EEG PA = electroencephalogram paroxysmal abnormalities; FC = febrile convulsions; IQ = intelligence quotient.
[a] = 64/90 patients performed lysosomal enzyme analysis.
[b] = 79/90 patients performed screening for celiac disease.

The lysosomal enzyme analysis, performed on 64/90 patients, allowed us to find a partial deficiency of ASA in a greater number of syndromal patients without significant group differences; the screening for celiac disease, performed in 79/90 patients, disclosed only one syndromal patient with celiac disorder. No significant differences between the two groups were found in occurrence of epilepsy, while EEG PA without seizures were significantly more frequent ($p<0.05$) in the syndromal subjects. The neuropsychological assessment did not show any significant difference between the two groups. In particular severe/profound mental retardation prevailed in syndromal cases respect to non-syndromal ones, but also this difference was not significant. Three out of 14 non-syndromal cases with severe/profound mental retardation had epilepsy, one had not frequent EEG PA, and one presented dysmorphisms.

Discussion

In the literature, there is agreement that a prompt diagnosis, an early and intensive intervention on the children affected by PDD could improve the prognosis [19]. The identification of a specific condition underlying the PDD could be useful, for example, to provide medical treatment to children with unsuspected epilepsy, with a rare metabolic disease or with other tractable conditions, and to advise unaffected family members on the PDD recurrence risk. At last, families could benefit from having a medical diagnosis even if defining a specific aetiology has no immediate therapeutic or preventive implications [15,16].

Genetics

The genetic evaluation has the best diagnostic yield, and allows a defined diagnosis from 6 to 15% of the cases [4]. In our study, we found a specific genetic disorder in 11.1% of the patients, a higher percentage than the one reported by Abdul-Rahman and Hudgins (8.3%), and similar to the one observed by Herman et al. (10.0%) [20,21]. A positive result at genetic testing gives a defined diagnosis in some of the syndromal patients. Moreover, the exclusion of a clear genetic aetiology may help in the assessment of the recurrence risk also in the families of the non-syndromal cases. The percentage of chromosomal abnormalities and defects in single genes would likely be greater if more sophisticated techniques of genetic investigation such as a-CGH could be utilized routinely [21]. In fact, this examination gives a diagnostic yield of 21% in PDD children analyzed in a recent survey [22]. In our study, a-CGH has become more easily available only recently, and so it was made only in the 6.7% of the whole sample.

Cerebral lesions

Several authors have described different cerebral lesions associated with PDD [16,23]. Using cerebral MRI we have found heterogeneous abnormalities (33.3%) which confirm the data available in the literature: asymmetry of lateral ventricles, thinning of corpus callosum, vermis cerebellar hypoplasia, polymicrogyria, and incomplete maturation of the white matter. Even if none of these abnormalities is specifically related to autism, we suggest to examine through MRI patients with PDD, particularly those with a clinical suspicion of cerebral lesion, based on anamnestic data of early suffering, presence of epileptic seizures, positive neurological signs, etc.

Comparison between non-syndromal and syndromal cases

The comparison between non-syndromal and syndromal cases has produced interesting data, which we discuss in what follows. Mean age at the last observation was higher in the syndromal group ($p<0.05$); this finding could be explained by the greater complexity of the disease in these patients, and by their need for longer medical checks in a highly specialized centre.

No significant differences were found between the two groups for the neuropsychological evaluation; interestingly, a relatively high percentage of cases with severe/profound mental retardation belonged to the non-syndromal group. At present, some authors have supported the need to include all the patients with severe/profound mental retardation in the group of syndromal autism [24]; we believe, on the contrary, that the degree of the mental retardation should not be considered a specific marker of neither group studied; in fact, it has been reported that also severe mental retardation may be "idiopathic" [25].

In our sample most of non-syndromal cases with severe/profound mental retardation (9/14) had no epilepsy, EEG PA, and dysmorphisms.

We remark the importance of neuropsychological assessment that is essential because the level of cognitive impairment affects the prognosis.

Positive neurological examination (hypotonia, hyperlaxity, dysmorphic features) prevailed ($p<0.05$) in the syndromal group. This result is mostly related to the greater occurrence of dysmorphisms in the syndromal cases (30.9%). However, not specific dysmorphisms were present also in the non-syndromal cases (10.4%). Their presence suggests the need for a diagnostic protocol for all patients with PDD, as we have adopted.

According to the literature [18], the epilepsy was found in 30.0% of the whole sample without a significant difference between the two groups, while EEG PA without seizures were significantly higher in the syndromal cases ($p<0.05$). Nevertheless, the more intensive monitoring due to the longer follow-up in these patients may have influenced this result. The current understanding of the association between epilepsy and autism is still limited at the aetiological level but an underlying neurobiological pathological process could be the origin of different manifestations of cerebral dysfunction, including epilepsy, EEG PA and autism [26]. It is important to note that epilepsy could add additional problems in development, learning, quality of life of patients affected by PDD, and that this association should be routinely investigated also to decide for an appropriate medical treatment [27].

Final suggestions

Our study, describing a large sample of Italian patients with PDD evaluated in a systematic way, is one of the few studies that compare non-syndromal and syndromal cases [28]. The high occurrence of the latter ones may be partly related to our specialized domain in a University centre.

In our sample genetic examinations, MRI and EEG recordings had the best diagnostic yield, while metabolic tests and other examinations were negative or mildly altered. For this reason, laboratory and instrumental investigations should be selected in an accurate way by specialized clinicians. This means that the evaluation of children with autism should be performed by choosing the examinations based on clinical observation, minimal resources and patient discomfort, and with the best diagnostic yield. Our purpose of diagnostic protocol is represented in Table 4.

Table 4. Diagnostic scheme suggested by the authors

Anamnesis	**Familial antecedents:** Neurological and/or psychiatric disorders, genetic diseases. **Personal antecedents:** Pre-, peri-, postnatal distress, delayed development and/or autistic regression, early signs, febrile convulsions or epilepsy, etc.
Physical/neurological examination	
Diagnosis of PDD	According to DSM-IV-TR, CARS, ADOS, ADI-R.
Neuropsychological assessment	Wechsler, Leiter-R and Stanford-Binet Scales.
Auditory screening	Audiogram, brainstem evoked potentials.
EEG	Prolonged awake/sleep recordings.
Standard metabolic screening	Complete blood count, glycemia, azotemia, electrolytes, CPK, LDH, serum lactate and pyruvate, uric acid, ammonia, serum ceruloplasmin and cupric ion levels, thyroid function, screening for celiac disease, TORCH.
Metabolic screening (only in selected cases)	Amino acid profile, lysosomal enzymes, urine oligo and mucopolysaccharides, organic and very long chain fatty acids, purine/pyrimidine metabolism.
High resolution karyotype	
Fragile X mutation	
Genome-wide subtelomere screening	
A-CGH	
MeCP2 gene testing	All females with regression and suspicion of Rett disorder.
CDKL5 gene testing	Cases with early onset epilepsy and Rett features.
FOXG1 gene testing	Cases with suspicion of congenital Rett disorder.
PTEN gene testing	Head circumference >3 SD.
Genetic counselling (including other genetic testing based on clinical suspicion)	
Neuroimaging	MRI, when necessary CT scan.
Specific protocols (some rare conditions)	for example: Tuberous Sclerosis: dermatological examination with Woods lamp evaluation, abdominal ultrasound, echocardiography, dental examination, fundus oculi and TSC1, TSC2 genetic testing.

PDD = Pervasive developmental disorders; EEG = Electroencephalogram; CARS = Childhood Autism Rating Scale; ADOS = Autism Diagnostic Observation Schedule; ADI-R = Autism Diagnostic Interview-Revised; CPK = Creatine phosphokinase; LDH = Lactate dehydrogenase; TORCH = Antibody levels to toxoplasma, rubella, cytomegalovirus, herpes simplex I, herpes simplex II; a-CGH = Array-comparative genomic hybridization; MeCP2 = Methyl CpG binding protein 2; CDKL5 = Cyclin-dependent kinase-like 5; FOXG1 = Forkhead box protein G1; PTEN = Phosphatase and tensin homolog; CT = computed tomography; TSC1 and TSC2 = Tuberous Sclerosis 1 and 2.

We suggest starting with a detailed anamnesis concerning prenatal history and with questions about the potential exposure to theratogenic factors. Familial antecedents and pre-, peri- and postnatal distress must be looked for; early signs of autism should be always investigated with the support of home videos.

The evaluation of the autistic symptoms and the neuropsychological assessment are decisive: while the accuracy of the diagnosis underlies the remainder of the evaluation, it is important that autistic-like symptoms of severe cognitive impairments be not confused with a primary disorder of socialization and communication.

An accurate neurological examination together with a detailed anamnesis is important to decide which tests should be performed in the diagnostic evaluation.

All patients diagnosed with autism should undergo a hearing assessment before further investigations; it is mandatory that an audiogram and eventually brain stem evoked potentials confirm a normal hearing before setting upon a diagnosis of PDD.

Standard metabolic screening are suggested to search for possible medical conditions associated with autism.

Selective metabolic testing should be performed only in the presence of suggestive clinical findings, including lethargy, cyclic vomiting, early seizures, dysmorphic features, severe/profound mental retardation, immune deficiency or unexplained haemolytic anemia, hyper- or hypotonia and muscle weakness [29].

Regarding the genetic evaluation, in agreement with some authors [3,4] we suggest to make the high resolution karyotype, search for Fragile X, genome-wide subtelomere screening, a-CGH in all patients diagnosed with PDD. The MeCP2 gene study is indicated in females with a history of regression and autistic signs complying with Rett syndrome, the CDKL5 gene study in those with early onset epileptic seizures, and the forkhead box protein G1 (FOXG1) gene in congenital Rett syndrome. Finally, the PTEN analysis is appropriate in all children with autism and extreme macrocephaly (>3 SD) [30].

Needless to say, the use of genetic methods should always be up-to-date since these are linked to the development of increasingly sophisticated research techniques as a-CGH [22]. Moreover, genetic counseling should always be performed to give information on recurrence risks in subsequent pregnancies.

When such rare conditions as Tuberous Sclerosis, associated with autism, appear, it is indicated to proceed with specific protocols [31].

In our experience, the high occurrence of epilepsy and EEG PA both in the non-syndromal and in the syndromal patients suggests the need for a prolonged awake/sleep EEG recording in all subjects with autism. Similarly, the presence of pathological findings detected by MRI in our sample supports the use and usefulness of imaging studies in the diagnostic investigation of PDD.

In conclusion, the application of a diagnostic protocol in our PDD sample has proved very helpful because it has allowed us to identify a high percentage of syndromal cases (46.7%). The distinction between non-syndromal and syndromal cases is essential to provide useful information about the recurrence, the prognosis and the treatment both of the behavioural problem and of the diseases associated with PDD. It is important to continue in this direction, carefully analyzing every patient, to expand the diagnostic yield.

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