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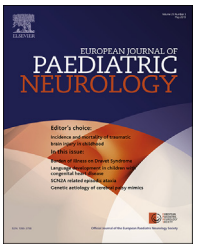

Original article

The genetic etiology in cerebral palsy mimics: The results from a Greek tertiary care center

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ABSTRACT

Objective: Non-progressive genetic disorders may present with motor dysfunction resembling cerebral palsy (CP). Such patients are often characterized as CP mimics. The purpose of this work was to delineate the clinical manifestations and molecular findings of CP mimic patients, with the ultimate goal to offer specific disease-modifying therapy and genetic counseling.
Methods: Retrospective study of 47 patients diagnosed with CP and no acquired etiology. Chart review of clinical, neuroradiological, biochemical and molecular data was performed.
Results: 31,91% of patients manifested with features resembling dyskinetic CP, 19,14% spastic CP, 10,63% ataxic CP and 38,30% mixed CP. In 23 patients molecular diagnosis was reached and included 5 hereditary spastic paraplegia genes (*SPG*) in spastic CP mimics; *HPRT1*, *TH*, *QDPR*, *DDC* in dystonic CP mimics; *ADCY5* and *NIKX2-1* in choreic CP mimics;

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Ataxia
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CANA1A in ataxic CP mimics; and *SPG*, *PDHA1*, *NIKX2-1*, *AT*, *SLC2A1* and *SPR* in mixed CP mimics. In 14 patients, the etiological diagnosis led to specific treatment.

Conclusions: CP mimics show a number of features that differ from classic CP and can be used as diagnostic clues, including presence of mixed motor features, minor dysmorphic features, oculogyric movements, multiple features of autonomic dysfunction, and acquired microcephaly. A more stringent use of the concept of CP focused on acquired lesions during the perinatal and infancy periods, and excluding disorders that could be of genetic origin, could contribute to a purer use of the term. Identification of a specific genetic cause for CP mimics may in certain cases lead to etiologic treatment.

1. Introduction

Cerebral palsy (CP) in an umbrella term that comprises a group of permanent disorders of development of movement and posture, causing activity limitation that is attributed to non progressive disturbances that occur in the developing fetal or infant brain.[1] Along with motor disabilities, children with CP have disturbances of sensation, perception, learning and behavior. CP imposes great demands on health, social, and educational services, as well as a large financial and emotional burden on families.[2]

The vulnerability of motor pathways during brain development is determinant for the motor manifestation in CP. The etiology of CP varies. The most frequent causes of CP include complications of prematurity, perinatal or postnatal hypoxic-ischemia, bilirubin exposure, infection or trauma.[2] However, in a number of patients with CP an etiology cannot be determined, particularly for those born at term and/or without a clear brain lesion identifiable by neuroimaging.[3] It is suspected that much of this unknown pathophysiology may be due to genetic or epigenetic factors. Indeed, current estimates indicate that as many as 30% of patients diagnosed with CP may be genetically determined.[3]

Yet, in practice there is variability in the use of the term CP among clinicians. While there is full agreement when the etiology is related to prematurity or other perinatal complications, the use of the term CP with other underlying etiologies is less consistent. For example, disorders with known genetic basis such as brain malformations and syndromic conditions with CNS involvement are regarded as CP. On the other hand, other non progressive genetic defects that manifest with motor dysfunction and abnormal neurodevelopment in infancy and early childhood have been classified as CP mimics or congenital idiopathic CP.[4–6] These different approaches in the classification of genetic conditions leading to clinical pictures suggestive of CP are a source of confusion in clinical practice. Moreover, in such genetic cases clinical course and prognoses may differ, and some of these conditions may be treatable and/or preventable. In the era of genomic medicine a reconsideration of the etiological categorization of CP is needed in order to better assess and manage these patients.

The genomic evaluation of children with neurodevelopmental conditions has become a regular diagnostic tool used by health providers in developed countries with a strong health care system. Chromosomal microarray and clinical exome sequencing are increasingly being used in the diagnostic evaluation of patients with CP when there is suspicion of a genetic etiology, while whole genome sequencing (WGS) is mostly used in a research setting.[7] In this report we present our experience with a group of patients who were assessed at our institution with an initial diagnosis of CP, but in whom the condition was not associated with known perinatal complications or with the brain lesions commonly related to CP. The main goal of this work was to delineate the clinical manifestations, laboratory data and molecular findings of patients who are regarded as CP mimics, so that in the future more targeted approaches to diagnosis and management can be developed, and genetic counseling can be provided to the families.

2. Methods

This represents a retrospective study of patients diagnosed with CP without apparent etiology, assessed in the First Department of Pediatrics of the National and Kapodistrian University of Athens at Agia Sofia Children's Hospital from 2011 to 2016.

These patients were classified as CP mimics
Inclusion criteria were:

- Non progressive disorder of development of movement and posture leading to activity limitation starting in infancy or early childhood
- Normal brain MRI or minor non specific findings.

Exclusion criteria were:

- Gestational age of 36 week gestation or less
- Perinatal complications: asphyxia, respiratory distress syndrome requiring mechanical ventilation, meningitis/encephalitis, non physiological jaundice.
- Presence of acquired and/or progressive lesions on brain MRI, such as ischemic lesions, hemorrhage, calcification.
- Presence of dysplastic lesions on brain MRI.
- Patients with an epileptic encephalopathy.
- Patient with major dysmorphic features
- Patients with multiple congenital anomalies.

Chart review was performed and the following were documented: prenatal and perinatal history, family history, developmental status, associated medical problems, general exam and neurological exam. Functional status was determined by the Gross Motor Function Classification System (GMFCS). Clinical features of disease progression were based on the thorough review of medical history and neurological exam in all patients, and ongoing follow up in 30 patients (patients 1, 3, 5, 10, 12–24, 26, 27, 29, 31, 34–36, 38, 40, 41, 45–47).

Diagnostic work up performed prior to the assessment at our institution was reviewed. Brain MRI were reviewed together with a neuroradiologist (I.N.). Patients underwent neurometabolic work up that was delineated according to clinical picture and previous diagnostic studies.

Parental informed consent was obtained. Blood samples from patients were collected and DNA was extracted from peripheral blood lymphocytes according to standard procedures. The following molecular studies were performed following standard procedures:

- Array CGH was performed in 6 patients (pat 10, 20, 27, 29, 31, 47).[8]
- Depending on the patients' phenotype, specific syndromes were suspected and genetic tests were performed as previously described.[9–11]
- These genetic tests included:
  - o Methylation Specific Polymerase Reaction (MS-PCR) for Prader Willi and Angelman syndromes and triplet specific PCR for Fragile X syndrome (patients 27, 28, 29, 35, 41, 44).[9,10]
  - o Screening for a founder mutation in exon 5 of the *TH* gene (c.707T > C) (patients 10, 21 and 47).[11]
  - o Sanger sequencing of specific genes that were performed either at our institution or at outside laboratories through institutional collaborations, or through commercial laboratories (patients 1, 2, 3, 6, 7,8, 9, 11, 16, 17, 18, 19, 20, 22, 23, 24, 27,28, 30, 37, 38, 42, 45, 46).
  - o Multiplex ligation dependent probe amplification (MLPA) analysis was performed when indicated (patients 27, 28)
- Next generation sequencing targeted at specific groups of genes was performed according to clinical presentation through institutional collaborations (pro bono) in 7 patients (patients 13, 14, 23, 26, 32, 39, 40); and clinical exome sequencing was performed in 4 patients through institutional collaborations (pro bono) (patients 25, 34, 35, 41)[12,13] and in 4 patients (patients 5, 29, 31, 37) in the Commercial diagnostic laboratory Genomedica S.A., Piraeus, Greece.

This study was conducted in accordance with GCP and ethical principles deriving from the declaration of Helsinki, and from regulations in force for observational studies.

3. Results

A total of 47 CP mimic patients meeting the above criteria were collected. Twenty patients (43%) were referred to our center for a first assessment, and 27 (57%) were referred for a second assessment after their local pediatrician or pediatric neurologist had performed a diagnostic work up that was non-contributory.

Twenty three patients were born via cesarean section, however in none of them was this performed under emergency procedure. Four patients had low birth weight ranging from 2150 to 2400 gr and 3 had a birth head circumference below the third percentile ranging from 30 to 31,5 cm. In 1/3 of cases mild pre- or perinatal problems were reported, however in none of them was there evidence of fetal distress based on low Apgar score, acidosis pH < 7.00, need for mechanical ventilation or need for resuscitative measures, and none showed evidence of encephalopathy and/or seizures at any time during the neonatal period. The main clinical findings are depicted in Tables 1–3. The main diagnostic procedures, including neuroimaging, biochemical-metabolic studies and molecular studies are included in Tables 4 and 5.

Mean age at first assessment at our institution was 4 years (range 6 months-to-19 years). Regarding onset of symptoms, we divided the patients in those with first symptoms during infancy (24 patients), and those with first symptoms during the toddler period (23 patients). The most frequent symptom at onset was motor delay followed by gait abnormalities and movement disorders (Table 2). None of the patients showed evidence of neurological progression. Thirty patients are currently followed up at our institution and their mean age is 9,9 ± 6,4 years. Seventeen patients continue their follow up with their local MD.

3.1. *Motor disorders and severity of CP mimics*

Fifteen patients manifested with features resembling dyskinetic CP (12 dystonic and 3 choreic), 9 resembled spastic CP, 5 ataxic CP and 18 mixed CP. Regarding the distribution of the motor disorder it was generalized in 31, paraplegia in 9, and diplegia in 7. Four of the patients resembling diplegic CP showed some degree of asymmetry on motor exam. GMFCS was variable with the dystonic CP mimics being the more severe ones (Table 2).

3.2. *Diagnostic studies*

All patients underwent neuroimaging and a comprehensive biochemical-metabolic work up except for 2 patients with a known family history of hereditary spastic paraplegia (SPG) (Table 4). In 14 patients, biochemical-metabolic analysis led to an etiological diagnosis, including 8 neurotransmitter defects (5 tyrosine hydroxylase deficiency (pat 15–19)), 1 aromatic amino acid decarboxylase deficiency (pat 14), 1 dihydropteridine reductase deficiency (pat 13), 1 dopamine metabolism defect (pat 38), 1 Lesch Nyhan disease (pat 11), 2 ataxia telangiectasia (pat 39, 43), 1 pyruvate dehydrogenase deficiency (pat 41) and 2 glucose transporter deficiency (pat 45, 46) (Table 5). Of these patients, molecular confirmation was reached in all of them except for 2 patients: the 1 patient with ataxia telangiectasia and the patient with a dopamine metabolism defect.

Results of molecular analysis are depicted in Table 5. Definite molecular diagnosis was reached in 23 patients:

- Spastic CP mimics: 5 hereditary spastic paraplegia genes 2 *SPG4* and 3 *SPG3A*.
- Dystonic CP mimics: 1 *HPRT1*, 5 *TH*, 1 *QDPR*, 1 *DDC*.

Table 1 – CP mimic: Perinatal and family history.

CP Mimics (N = 47)	Prenatal history	Birth weight (mean ± SD)	Microcephaly at birth/minor dysmorphic features[a]	Events in neonatal period	Family history
Spastic (N = 9)	1 Early contractions	3342.86 gr ± 412.78			4 Spastic paraplegia
Dystonic (N = 12)	1 Maternal Diabetes	2825 gr ± 324.61	2/6	1 Low temperature	1 Dopa responsive dystonia
	1 Maternal anticoagulation				
	1 Twin gestation				
	1 in vitro fertilization				
Choreic (N = 3)	1 Maternal Herpes virus infection	2909.67 gr ± 441.42		1 Respiratory distress	
Ataxic (N = 5)	1 in vitro fertilization	2977.5 gr ± 32.02	0/2	1 Respiratory distress and infection	
Spastic-dystonic (N = 7)	1 Maternal treatment with methimazole	3261.43 gr ± 507.62	1/2		
	1 Triplet gestation and in vitro fertilization				
Ataxic dyskinetic (N = 7)	1 Maternal ureaplasma infection	3145 gr ± 339.20	0/1	1 Icterus	1 Chorea and hypothyroidism
	1 Prolonged labor			1 Hepatitis	
	1 Polyhydramnios				
Ataxic-Spastic (N = 1)		3100 gr			
Ataxic-Spastic-Dyskinetic (N = 3)		3000 gr ± 804.67		1 Respiratory distress	1 Absence epilepsy and learning disability

ASD Autistic spectrum disorder.
[a] Minor dysmorphic features are defined as unusual morphologic features that are of no serious medial or cosmetic consequence to the patient.

- Choreic CP mimics: 1 *ADCY5*, 1 *NIKX2-1*.
- Ataxic CP mimics: 1 *CANA1A*.
- Mixed CP mimics:1 hereditary spastic paraplegia gene *SPG4*, 1 AT, 1 *PDHA1*, 1 *NIKX2-1*, 2 *SLC2A1*, 1 *SPR*.

In female patient 20 findings on array CGH disclosed duplication of Xq28 (132 kb), that resulted in partial duplication of the *L1CAM* gene [(NC_000023.10:g(?_153005532)_(153137814_?)dup, hg19]. Single nucleotide aberrations and deletions in male patients have been characterized as responsible for L1CAM syndrome,[14] however the pathogenicity in females depends on skewed X-inactivation.

In 3 patients molecular findings where not conclusive, but were thought to be partially related to the patients' condition:

- Patient 21 manifesting as dystonic CP-mimic and patient 35 manifesting as spastic-dystonic CP-mimic showed low levels of homovanilic acid on CSF (25 nmol/L (304–658) and 65 nmol/L (156–410) respectively) and responded dramatically to L-dopa therapy. They were both heterozygotes for the known Greek founder mutation in exon 5 of the *TH* gene (c.707T > C). A second mutation in the *TH* gene was not detected and the diagnosis of tyrosine hydroxylase deficiency could not be confirmed. The possibility of copy number variation on the *TH* gene or the existence of mutations in unknown regulatory regions could not be ruled out.
- Patient 37 manifesting as ataxic-dyskinetic CP-mimic was found to be a heterozygote for a mutation in the *SPTBN2* gene (c.1295G > A). This mutation was also found in the asymptomatic mother. Mutations in the *SPTBN2* gene can be inherited in an autosomal dominant or recessive fashion. When inherited in an autosomal dominant fashion they are believed to have incomplete penetrance. The possibility of copy number variation on the *SPTBN2* gene or the existence of mutations in unknown regulatory regions could not be ruled out.

3.3. *Clinical course*

As expected, as part of the inclusion criteria, none of the patients showed evidence of progressive neurological deterioration. However, a number of clinical features that may be indicative of an evolving aspect of their condition were noted:

- Patient 12 manifesting as a dystonic CP mimic and without an etiological diagnosis, developed 2 episodes of status dystonicus during late adolescence in the setting of severe constipation and ileus.
- Patient 24 manifesting as a choreic CP mimic due to *NIKX2-1* mutation had a history of respiratory distress and pneumothorax in the neonatal period that was managed with CPAP. At the age of 10 years she was diagnosed with hypothyroidism. She showed considerable improvement of her chorea with age, but it reappeared in the setting of treatment with antidepressants and low doses of atypical antipsychotics.

Table 2 – CP Mimics: Main motor Features.

CP Mimics (N = 47)	Onset		Presentation				Distribution of Motor Disorder			Gross motor function classification system
	Infancy	Toddler	Motor delay	Gait disturbance	Movement disorder	Combination of symptoms	Generalized	Diplegia	Paraplegia	
Spastic (N = 9)	0	9	1	5	0	3 MD and GD	0	0	9	2 (N = 8) 3 (N = 1)
Dystonic (N = 12)	9	3	5	1	0	6 MD and MVT	11	1	0	1 (N = 1) 3 (N = 3) 4 (N = 1) 5 (N = 7)
Choreic (N = 3)	2	1	2	0	0	1 MD and MVT	3	0	0	1 (N = 2) 2(N = 1)
Ataxic (N = 5)	4	1	2	0	0	2 MD and GD 1 MD and MVT	5	0	0	1(N = 1) 2(N = 3) 3(N = 1)
Spastic-dystonic (N = 7)	3	4	4	1	0	2 MD and MVT	1	6	0	2 (N = 3) 3 (N = 3) 5 (N = 1)
Ataxic dyskinetic (N = 7)	4	3	3	2	0	1 GD and MVT 1 MD, GD and MVT	7	0	0	1(N = 1) 2(N = 4) 3(N = 2)
Ataxic-spastic (N = 1)	1	0	1	0	0		1	0	0	4(N = 1)
Ataxi-spastic-dyskinetic (N = 3)	1	2	1	1	0	1 MD and GD	3	0	0	1(N = 2) 2(N = 1)

MD – Motor delay, GD – Gait disturbance, MVT – Movement disorder.

Table 3 – CP Mimics: Other clinical features and comorbidites.

CP Mimic (N = 47)	Minor dysmorphic features*	Speech delay	Epilepsy	Paroxysmal events	Autonomic dysfunction	Other Comorbidities
Spastic (N = 9)		1				
Dystonic (N = 12)	6	11		1 Paroxysmal retrocollis 7 Oculogyric episodes	8 Hyperhidrosis 5 Stuffed nose 4 Sleep disturbance 2 Constipation	2 Hypothyroidism 1 Optic atrophy, myopia 3 Feeding difficulties 3 Scoliosis 1 Hip dislocation
Choreic (N = 3)		1		1 Episodes of stiffening at night		1 Hypothyroidism
Ataxic (N = 5)	2	3 (1 ASD)		1 Hypoglycemia 1 Oculogyric episodes		1 Hearing loss
Spastic-dystonic (N = 7)	2	2	1		2 Hyperhidrosis 1 Heart arrhythmia	1 Peters anomaly 1 Feeding difficulties 1 Scoliosis 1 Hip dislocation
Ataxic dyskinetic (N = 7)	1	4 (1 ASD)		3 Oculogyric episodes 1 Episodes of stiffening at night	1 Hyperhidrosis 1 Poor temperature control	1 Hypothyroidism
Ataxic-spastic (N = 1)		1				
Ataxic-spastic-dyskinetic (N = 3)		2 (1 ASD)	2	1 Paresis 1 Migraine	1 Sleep disturbance	1 Hypothyroidism

- Patient 41 manifesting as an ataxic dyskinetic CP mimic due to pyruvate dehydrogenase deficiency, developed features of fatigue and diurnal fluctuation with parkinsonian features at the age of 10–11 years, without any regression in her mental or motor skills at baseline, and without any changes in her neuroimaging.
- In 12 patients head growth slowed down and 6 of them developed acquired microcephaly including: 1 patient with pyruvate dehydrogenase deficiency (pat 41), 2 patients with glucose 1 transporter deficiency (pat 45, 46), 1 patient with tyrosine hydroxylase deficiency (pat 19), 1 patient with a suspected dopamine synthesis defect (pat 38) and 1 patient with ataxic-spastic CP-mimicker and no etiological diagnosis (pat 44).

3.4. *Treatment*

In 14 patients, the etiological diagnosis led to specific treatment: allopurinol in 1 patient with Lesch Nyhan disease, dopaminergic medication in 10 patients with biogenic amine neurotransmitter disorders, and ketogenic diet in 2 patients with glucose 1 transporter deficiency and in 1 patient with pyruvate dehydrogenase deficiency. Except for the patient with Lesch Nyhan disease, in all other patients treatment led to a considerable improvement in their neurological status.

4. Discussion

In our study, out of 47 patients with a clinical picture that had been regarded as CP, we found the underlying genetic molecular diagnosis in 23 patients. Equal proportion of males and females was noted, which is in contrast with the higher and unexplained excess of males in classic CP.[15] None of the families reported consanguinity, and in 7 patients there was a positive family history consistent with an autosomal dominant inheritance (Table 4). The most frequent type of motor disorder was mixed (38,30%) followed by dyskinetic (31,91%), spastic (19,15%) and ataxic (10,64%). This is in contrast with classic CP where the spastic type accounts for 85–91% of cases followed by the dyskinetic (7–17%) and the ataxic (4–6%).[22] The onset of motor symptoms and subsequent diagnosis of CP in our series of CP mimics was variable: in the spastic group it was during the toddler stage and in the dystonic, the chorea and the ataxic groups it was during infancy.

The distribution of the motor disorder in patients with classic dyskinetic and ataxic CP is often generalized, and this was also the case of our dyskinetic and ataxic CP mimic patients. In the case of classic spastic CP, 38% of patients manifest with hemiplegia,[22] while in our series the majority of spastic CP mimics manifested with paraplegia and were diagnosed with hereditary spastic paraplegia. As a consequence, in children with spastic CP and bilateral lower limb involvement, the analysis of *SPG* genes should be considered early in the course of the disease, especially when there is lack of perinatal risk factors and abnormalities on neuroimaging. This analysis should be pursued even in the absence of family history, since in the pediatric population de novo occurrence of hereditary spastic paraplegia is not uncommon.[23]

Spastic diplegia, with the lower limbs more affected than the upper limbs, is the most frequent form of classic CP (37%).[22] It is often seen in the setting of periventricular leukomalacia as a complication of prematurity. Spasticity is the most characteristic motor disorder and features of dystonia are also recognized.[24] A number of patients in this series of CP mimics demonstrated diplegia and interestingly the majority were of the spastic-dystonic type. Despite extensive diagnostic studies, including clinical exome sequencing, they remained undiagnosed except for one case with SPG4. It has

Table 4 – CP Mimics: Neuroimaging and metabolic work up.

CP Mimics (N = 47)	Brain MRI Findings	Spine MRI findings	Metabolic work up	
			Blood and Urine findings	CSF findings
Spastic (N = 9)	7 Normal	6 Normal	6 Normal	2 Normal 2 Non specific NT abnormalities
Dystonic (N = 12)	10 Normal 2 Lateral ventricle dilatation		9 Normal 1 ↑ Phenylalanine in blood 1 ↑ Uric ac in blood, ↑ purines in urine	3 Normal 1 Non specific NT abnormalities 8 Specific NT deficits
Choreic (N = 3)	3 Normal		3 Normal	2
Ataxic (N = 5)	1 Normal 1 Posterior fossa arachnoid cyst	1 Non specific signal change in cervical cord	4 Normal 1 ↑ Non specific organic acids in urine	2 Normal 1 Non specific NT abnormalities 1 ↓ Glucose[a]
Spastic-dystonic (N = 7)	4 Normal 2 Delayed myelination 1 Occipital horn dilatation	5 Normal 1 Mild hydromyelia of conus medularis	7 Normal	7 Normal
Ataxic-dyskinetic (N = 7)	6 Normal 1 Subependimal cyst	1 Normal	4 Normal 3 ↑ α-fetoprotein	3 Normal 1 Non specific NT abnormalities 1 ↑ Lactic acid 1 ↓ Glucose[a]
Ataxic-spastic (N = 1)	1 Mild cerebellar atrophy and delayed myelination	1 Normal		1 ↓ Glucose[a]
Ataxi-spastic-dyskinetic (N = 3)	2 Normal 1 Mild thick corpus callosum		1 Normal 1 ↓ Glutamine in blood 1 Mild ↑ Hydroxyvaleric acid in urine	2 ↓ Glucose 1 Non specific NT abnormalities 1 Non specific amino acid abnormalities

NT – Metabolites of biogenic amine (dopamine, serotonin) neurotransmitters.

[a] Low glucose in CSF with no molecular confirmation on *SLC2A1* sequencing and MLPA analysis.

been proposed that the motor manifestation of classic diplegic CP is the result of reduced functional connectivity of thalamus, basal ganglia, cerebral cortex, brain stem, and cerebellum, related to the characteristic acquired ischemic brain injury of prematurity, periventricular leukomalacia.[24] In our series, given the clinical similarities and the normal brain MRI, we postulate that the group of undiagnosed CP mimics with spastic-dystonic features may represent a primary disorder of connectivity involving these areas.

Low birth weight and minor/major birth defects are well known risk factors for classic CP.[20] In our series of CP mimics low birth weight was noted in 4 patients and minor dysmorphic features were noted in 22,92% of patients, being especially frequent in patients with dopamine deficiency states (Table 1). These features included hypertelorism, large palpebral fissure, long philtrum, macrostomia with down turned mouth corner, maxillary hypoplasia, often with narrow or high arched palate, and irregular placement of teeth. Furthermore, their extremities showed small hands and feet, brachydactyly, broad thumbs and/or toe and nail dysplasia. None of them showed evidence of arthrogryposis that could have been indicative of fetal hypokinesia as the cause of these dysmorphic changes. All together these observations suggest that biogenic amines play a role in tissue morphogenesis and in particular the formation of ectodermal appendages.

Congenital microcephaly is the most common birth defect reported in CP, and in approximately half the cases patients have a recognizable cause including monogenetic disorders, trisomies, imprinting disorders, neurometabolic diseases, and a range of acquired conditions such as infections, teratogens or stroke.[21] In our series of CP mimics 3 patients had microcephaly at birth (Table 1). One of them was diagnosed with Lesch Nyhan disease, while in the others no etiological diagnosis was reached. Furthermore, despite the static clinical course and absence of neurological deterioration, 25,53% of patients in our series showed deceleration of head growth and half of them developed acquired microcephaly. These patients included treatable neurometabolic disorders characterized by energy deficit and defects of biogenic amine synthesis associated with congenital dopamine deficiency. It is well known that stagnation of brain growth and maturation is a common consequence of conditions associated with energy deficiency. In congenital dopamine deficiency states it is less often reported, but, when it occurs, it may be related to the known role of dopamine in the postnatal development of brain circuits during synaptic maturation and refinement.[25] Altogether, variable mechanisms of brain growth deceleration are implicated in this group of patients. This differs from acquired brain lesions in early life where brain growth slows down during the acute phase of an insult and subsequently regains growth leading or not to acquired microcephaly.

As in classic CP, our CP mimic patients suffered from variable comorbidities (Table 3). Interestingly, epilepsy has been reported to occur less often in CP mimics (6.3%) than in classic CP (41-to-67%).[26] In our series, no patient suffered from epileptic seizures. Also of interest is the frequency of non epileptic paroxysmal events in CP mimics (Table 3), and especially the case of paroxysmal oculogyric movements that is characteristic of dopamine deficiency states and may be associated with dystonic posturing of the limbs and/or the trunk. The presence of these paroxysms is very characteristic and is considered a diagnostic clue in these patients. Features of autonomic dysfunction such as excessive diaphoresis, temperature instability, profuse nasal and oropharyngeal secretions, gastrointestinal dysmotility and sleep disturbances were seen in 31.3% of patients. They were especially frequent in the dystonia group, particularly in patients with neurotransmitter defects, who tended to suffer from more than one feature of autonomic dysfunction compared with patients with other diagnoses (Table 3).

In this series of 47 CP mimics, an etiological diagnosis was reached in 27 patients, and confirmed molecularly in 23. Given the fact that more than half of the patients had been referred to our institution as a second and even third opinion, and in many of them a diagnostic work up had already been done, the diagnostic algorithm performed was heterogeneous. In addition, the capability to perform molecular studies was very variable from patient to patient. While in some cases such analysis was performed pro bono in institutions abroad, in others, families were able to afford the cost of a clinical exome sequencing in commercial laboratories in Greece. Despite these difficulties, the diagnostic yield is relatively high, and is likely to increase as the results of the studies that are still in progress become available. Recently, Takezawa et al. reported the genomic analysis of 17 masqueraders of full-term CP with normal or nonspecific findings on brain MRI. They found a similar rate (53%) of causative genetic variants in this group of patients. Similar to our study, SPG genes were found in patients masquerading as spastic CP, while in one patient masquerading as ataxic-CP a mutation in the *CACNA1* gene was detected. Differing from our case series, in this report only 2 patients presented with dyskinetic features, and in none of them could a final molecular diagnosis be reached.[27] It is possible that a proportion of undiagnosed patients mimicking CP don't actually have a genetic disorder. The future use of whole genome sequencing in clinical practice will help to further address this issue.

Our experience allowed us to provide a precision medicine approach. Thus, following the diagnosis, 14 patients were placed on specific treatment, and those with disorders of dopamine metabolism and glucose 1 transporter deficiency showed clear improvement of their condition. Furthermore, in all the patients with confirmed molecular diagnosis genetic counseling was provided to the families.

In recent years, high-throughput sequencing and comparative genomic hybridization *techniques* have become efficient strategies to find rare disease-causing mutations in children with neurodevelopmental disorders. While this practice is leading to an increase in diagnostic yield and a reduction in the burden of diagnostic tests, the need to perform good phenotyping remains essential to plan a targeted gene search and to interpret the results. In our current report, the intrinsic phenotypic characteristic of the patients that led to the suspicion of a genetic basis was the presence of a motor disorder reminiscent of CP, with lack of perinatal complications and abnormalities on neuroimaging. Furthermore we found that dystonia, presence of oculogyric movements, characteristic dysmorphic features and multiple features of autonomic dysfunction are diagnostic clues for neurotransmitter disorders; spasticity with involvement of the lower limbs and onset after infancy, regardless of family history, are diagnostic clues for hereditary spastic paraplegias;

Table 5 – CP Mimics: Molecular studies.

CP Mimics	Pat.	Study of single genes and specific syndromes	Array CGH	NGS	Findings	Molecular diagnosis
Spastic	1	*SPG4*			*SPG4*: c.1636G > A, p.G546R	Hereditary spastic paraplegia SPG4
	2	*SPG3A*			*SPG3A*: c.715C > T, p.R239C	Hereditary spastic paraplegia SPG3
	3	*SPG3A*, *SPG4*, *REEP1*, *CYP7B1*				Gene negative Hereditary spastic paraplegia
	5			+	*SPG3A*: c.1065C > A, p.N355K	Hereditary spastic paraplegia SPG3
	6	*SPG3A*, *SPG4*, *REEP1*, *CYP7B1*				
	7	*SPG3A*, *SPG4*, *REEP1*, *CYP7B1*				
	8	*SPG3A*			*SPG3A*: c.715C > T, p.R239C	Hereditary spastic paraplegia SPG3
	9	*SPG3A*, *SPG4*, *REEP1*, *CYP7B1*			*SPG4*: 1245+5G > A	Hereditary spastic paraplegia SPG4
Dystonic	10	Greek *TH* mutation	+			
	11	*HPRT1*			*HPRT1*: exon 5 deletion	Lesh Nyhan disease
	13			+	*QDPR*: c.466G > A, p.A156T c.609dupA	Dihydropteridine reductase deficiency
	14			+	*DDC*: c.799T > C, p.W267R homozygous	L- Amino acid decarboxylase deficiency
	15	Greek *TH* mutation			*TH*: c.707T > C, p.L236P homozygous	Tyrosine hydroxylase deficiency
	16	*TH*			*TH*: c.707T > C, p.L236P, homozygous	Tyrosine hydroxylase deficiency
	17	*TH*			*TH*: c.707T > C, p.L236P homozygous	Tyrosine hydroxylase deficiency
	18	*TH*			*TH*: c.707T > C, p.L236P homozygous	Tyrosine hydroxylase deficiency
	19	*TH*			*TH*: c.707T > C, p.L236P homozygous	Tyrosine hydroxylase deficiency
	20	*NIKX2-1* Prader Willi syndrome	+		Dup Xq28 (132 kb)	
	21	Greek *TH* mutation			*TH*: c.707T > C, p.L236P	
Choreic	22	*ADCY5*, *NIKX2-1*			*ADCY5*: c.1252C > T, p.R418W	ADCY5-related dyskinesia
	23	*NIKX2-1*		+		
	24	*NIKX2-1*			*NIKX2-1*: c.254_255insG, p.Y86L	Benign hereditary chorea
Ataxic	25			+		
	26		+	+	46XX9QH (benign)	
	27	Angelman syndrome Rett syndrome *SLC2A1*	+			
	28	Angelman syndrome *SLC2A1* *ATP6* mtDNA			*MT-ATP6*: A8411C homoplasmic	
	29	Prader Willi	+	+	*CACNA1A*: c.4526T > C, p.F1509S	CACNA1A Hereditary ataxia
Spastic dystonic	30	*SPG3A*,*SPG4*,*SPG11*,			*SPG4*: c.1496G > A, p.R499H	Hereditary spastic paraplegia SPG4
	31		+	+		
	32			+	CYP7B1: c.608G > A, p.C203Y MTPAP: c.1313-3C > T LYST: c.1686G > C, p.Q562H	
	33	Greek *TH* mutation				
	34			+		
	35	Prader Willi syndrome Greek *TH* mutation		+	*TH*: c.707T > C, p.L236P *LXR*: c.2610G > C, p.Q870H	

(*continued on next page*)

Table 5 – (continued)

CP Mimics	Pat.	Study of single genes and specific syndromes	Array CGH	NGS	Findings	Molecular diagnosis
Ataxic dyskinetic	37	SGCE, SLC2A1, SCAs		+	SPTBN2: c.1295G > A, p.R432H	
	38	NIKX2-1				
	39			+	AT: c.1215delT, p.N405fs c.1654delC, p.P552fs	Ataxia telangiectasia
	40			+	SPR: c.448A > G, p.R150G	Sepiapterin reductase deficiency
	41	Fragile X		+	PDHA1: c.802G > A, p.G268R	Pyruvate dehydrogenase deficiency
	42	NIKX2-1			NIKX2-1: 1597_insG	Benign hereditary chorea
Ataxic spastic	44	Prader Willi syndrome				
Ataxic spastic dyskinetic	45	SLC2A1			SLC2A1: c.1216_1217ins82	Glucose 1 transporter deficiency
	46	SLC2A1			SLC2A1: c.874T > C, p.Y292H	Glucose 1 transporter deficiency
	47	Greek TH mutation		+		

NGS Next generation sequencing.

and in patients with mixed motor disorder, deceleration of head growth and fluctuation of symptoms are clues for energy metabolism defects such as glucose 1 transporter deficiency or pyruvate dehydrogenase deficiency (Tables 1–5).

In summary, CP mimics show a number of features that differ from classic CP and can be used as diagnostic clues that should prompt the performance of further etiological investigations. It has to be noted that our study has several limitations, including its retrospective nature, the relatively small number of patients and the heterogeneity in the diagnostic studies performed. However, it reflects real life clinical practice in a large tertiary referral center.

In conclusion, patients with CP due to hypoxic-ischemic events early in life and patients with neurogenetic and/or neurometabolic disorders may share similar clinical manifestations; however the ethiopathogenesis, prognosis, management and recurrence risk differ. A more stringent use of the concept of CP focused on acquired lesions during the perinatal and infancy periods, and excluding any disorder that could be of genetic origin, either neurometabolic, neurodevelopmental or related to brain malformations could contribute to a purer use of the term. Arriving at an etiological diagnosis in such patients has practical consequences, as it leads to more accurate prognosis, the provision of genetic counseling and, in a substantial minority, an improved clinical outcome.

Authors declaration

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Conflict of interest

None.

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REFERENCES

1. Rosenbaum P, Paneth N, Leviton A, Goldstein M, Bax M, Damiano D, et al. A report: the definition and classification of cerebral palsy April 2006. *Dev Med Child Neurol Suppl* 2007;**109**:8–14.

2. Colver A, Fairhurst C, Pharoah PO. Cerebral palsy. *Lancet* 2014;**383**(9924):1240–9.
3. Fahey MC, Maclennan AH, Kretzschmar D, Gecz J, Kruer MC. The genetic basis of cerebral palsy. *Dev Med Child Neurol* 2017;**59**(5):462–9.
4. Gupta R, Appleton RE. Cerebral palsy: not always what it seems. *Arch Dis Child* 2001;**85**(5):356–60.
5. Leach EL, Shevell M, Bowden K, Stockler-Ipsiroglu S, van Karnebeek CD. Treatable inborn errors of metabolism presenting as cerebral palsy mimics: systematic literature review. *Orphanet J Rare Dis* 2014;**9**:197.
6. Zarrinkalam R, Russo RN, Gibson CS, et al. CP or not CP? A review of diagnoses in a cerebral palsy register. *Pediatr Neurol* 2010;**42**(3):177–80.
7. Lee RW, Poretti A, Cohen JS, et al. A diagnostic approach for cerebral palsy in the genomic era. *Neuromolecular Med* 2014;**16**(4):821–44.
8. Oikonomakis V, Kosma K, Mitrakos A, et al. Recurrent copy number variations as risk factors for autism spectrum disorders: analysis of the clinical implications. *Clin Genet* 2016;**89**(6):708–18.
9. Tzagkaraki E, Sofocleous C, Fryssira-Kanioura H, et al. Screening of UBE3A gene in patients referred for Angelman Syndrome. *Eur J Paediatr Neurol* 2013;**17**(4):366–73.
10. Chonchaiya Weerasak, Schneider Andrea, Hagerman Randi J. Fragile X: a family of disorders. *Adv Pediatr* 2009;**56**:165–86.
11. Pons R, Kekou K, Antonellou R, et al. Analysis of a founder mutation in the TH gene in a cohort of Greek patients with Parkinson's disease. *Mov Disord* 2016;**31**(11):1753–4.
12. Yubero D, Brandi N, Ormazabal A, et al. Targeted next generation sequencing in patients with inborn errors of metabolism. *PLoS One* 2016;**11**(5):e0156359.
13. Tessa A, Battini R, Rubegni A, et al. Identification of mutations in AP4S1/SPG52 through next generation sequencing in three families. *Eur J Neurol* 2016;**23**(10):1580–7.
14. Vos YJ, Hofstra RM. An updated and upgraded L1CAM mutation database. *Hum Mutat* 2010;**31**(1):E1102–9.
15. Chounti A, Hägglund G, Wagner P, Westbom L. Sex differences in cerebral palsy incidence and functional ability: a total population study. *Acta Paediatr* 2013;**102**(7):712–7.
20. McIntyre S, Taitz D, Keogh J, et al. A systematic review of risk factors for cerebral palsy in children born at term in developed countries. *Dev Med Child Neurol* 2013;**55**(6):499–508.
21. Nelson KB, Blair E. Prenatal factors in singletons with cerebral palsy born at or near term. *N Engl J Med* 2015;**373**(10):946–53.
22. Novak I, Morgan C, Adde L, et al. Early, accurate diagnosis and early intervention in cerebral palsy: advances in diagnosis and treatment. *JAMA Pediatr* 2017;**171**(9):897–907.
23. Polymeris AA, Tessa A, Anagnostopoulou K, et al. A series of Greek children with pure hereditary spastic paraplegia: clinical features and genetic findings. *J Neurol* 2016;**263**(8):1604–11.
24. Pons R, Vanezis A, Skouteli H, et al. Upper limb function, kinematic analysis, and dystonia assessment in children with spastic diplegic cerebral palsy and periventricular leukomalacia. *J Child Neurol* 2017;**32**(11):936–41.
25. Frederick AL, Stanwood GD. Drugs, biogenic amine targets and the developing brain. *Dev Neurosci* 2009;**31**(1–2):7–22.
26. Christensen D, Van Naarden Braun K, Doernberg NS, et al. Prevalence of cerebral palsy, co-occurring autism spectrum disorders, and motor functioning - autism and Developmental Disabilities Monitoring Network, USA, 2008. *Dev Med Child Neurol* 2014;**56**(1):59–65.
27. Takezawa Y, Kikuchi A, Haginoya K, et al. Genomic analysis identifies masqueraders of full-term cerebral palsy. *Ann Clin Transl Neurol* 2018;**5**(5):538–51.