Acta Medica Mediterranea, 2013, 29: 241

16P11.2 MICRODELETION/MICRODUPLICATION SYNDROME: FURTHER CHARACTERIZATION OF A CRITICAL REGION FOR NEUROPSYCHIATRIC DEVELOPMENT

MARTINA BUSÈ, MARIO GIUFFRÈ, MANUELA MARTINES* , ETTORE PIRO, MARIA PICCIONE, GIOVANNI CORSELLO
Dipartimento di Scienze per la Promozione della Salute e Materno Infantile, Università degli Studi di Palermo - *CRR Malattie rare AOOR Villa Sofia-Cervello Palermo

[Sindrome da microdelezione/microduplicazione 16p11.2: ulteriori caratterizzazioni di una regione critica per lo sviluppo neuropsichiatrico]

ABSTRACT

Several studies have shown the importance of Copy Number Variations (CNVs) in the etiology of autism spectrum disorders (ASDs). In particular, a CNV of about 550 Kb in 16p11.2 has recently been described in up to 1% of autistic patients. Deletions and mutual duplications of this region have also been associated with neurocognitive abnormalities, impairment in social interaction, language delay, congenital anomalies, minor dysmorphisms, respectively micro- and macrocephaly.

We report 4 patients with 16p11.2 microdeletion and one patient with 16p11.2 microduplication. All patients have language delay and dysmorphic features, even if there is no common phenotype.

Even in our experience, the identification by array-CGH of cryptic chromosomal abnormalities and de novo pathological CNVs helped us to make a more accurate genotype-phenotype correlation and to establish individualized diagnostic and therapeutic strategies.

Key words: 16p11.2, a-CGH, language delay, dysmorphic features.

Received *February 25, 2013;* ***Accepted*** *March 19, 2013*

Introduction

In recent years, the introduction of array-CGH (comparative genomic hybridization) has greatly improved the ability to analyze the human genome and is quickly revolutionizing the definition of molecular diagnostics in patients with "chromosomal" phenotype (intellectual disability, dysmorphic features, congenital anomalies) and normal karyotype[1-4]. Array-CGH analysis has led to the discovery of several submicroscopic genomic variants (duplications and deletions), which are responsible for a lot of syndromes associated with global developmental delay and congenital malformations[5]. Today we are showing that many of those patients who, in the past, received a clinical diagnosis of sporadic autosomal recessive syndrome or de novo autosomal dominant syndrome, are actually carriers of cryptic chromosomal imbalance responsible for their condition[6-8]. Many studies report that about 15% of these patients have a cryptic chromosomal anomaly, detected by array-CGH with a resolution of 1Mb.

All these conditions play a very important role in determining morbidity and mortality rates, especially in neonatal age, when many other risk factors (prematurity, twinning, nosocomial infections, chemical mediators, …)[16-22]. May add their cumulative effect influencing short and long-term outcome. Array-CGH also offer the possibility to identify de novo pathological chromosomal aberrations), by copy number variations (CNVs) in excess or in defect, compared to a control population. These CNVs are associated not only with specific clinical features, but also with an increased susceptibility to the development of complex diseases such as schizophrenia, autism and autoim-

mune diseases, thus changing the perception of structural genomic variation and causes of Mendelian diseases.

Several studies show the importance of CNVs in the etiology of autism spectrum disorders (ASDs). In particular, a CNV of about 550 Kb in the 16p11.2 region has recently been described in numerous studies. 16p11.2 microdeletion was initially reported to occur in up to 1% of autistic patients[23,24]. Further studies associated 16p11.2 microdeletion syndrome with delayed language development (with particular involvement of the expressive sphere), intellectual disability, impairment in social interaction and facial dysmorphism. Furthermore, deletions of this region seem to be associated with a form of early-onset obesity, due to haploinsufficiency of the SH2B1 gene, which is involved in leptin and insulin signaling, and with congenital anomalies like minor cardiac malformations, hemivertebrae and syringomyelia.

We report 4 patients with 16p11.2 microdeletion and one patient with 16p11.2 microduplication. Our objective is to contribute to the definition of the phenotype associated with 16p11.2 CNVs.

Materials and methods

We have carried out testing of the peripheral blood of our patients and their parents, followed by DNA extraction using QIAamp DNA Blood Midi Kit (Qiagen). Array-CGH analysis was initially performed in the DNA of the proband, then we confirmed the alteration with FISH (fluorescence in situ hybridization) or Dye-swap. FISH was performed using bacterial artificial chromosome (BAC) probes, that contain inserts of about 200 Kb. After that, the rearrangement has been looked for in the parents' DNA by array-CGH, in order to define a *de novo* or inherited origin.

Case reports

Patients carrying a 16p11.2 deletion

Patient 1 is a 3-year old child. He is the fifth son of healthy, non-consanguineous parents, born at 37 weeks of gestation by cesarean section. He was reported to be small for gestational age (SGA), with a birth weight of 2120 g, a birth length of 45 cm and a head circumference of 34 cm.

He has mild dysmorphic features: short and down-slanted palpebral fissures, anteverted nares and a broad nasal bridge.

Neuropsychiatric evaluation revealed global developmental delay.

Cardiac and renal ultrasounds were reported normal.

He has a normal male karyotype (46, XY), but array-CGH analysis showed a 16p11.2 deletion of about 524 Kb (Figure 1). Array-CGH analysis showed normal copy numbers in both parents.

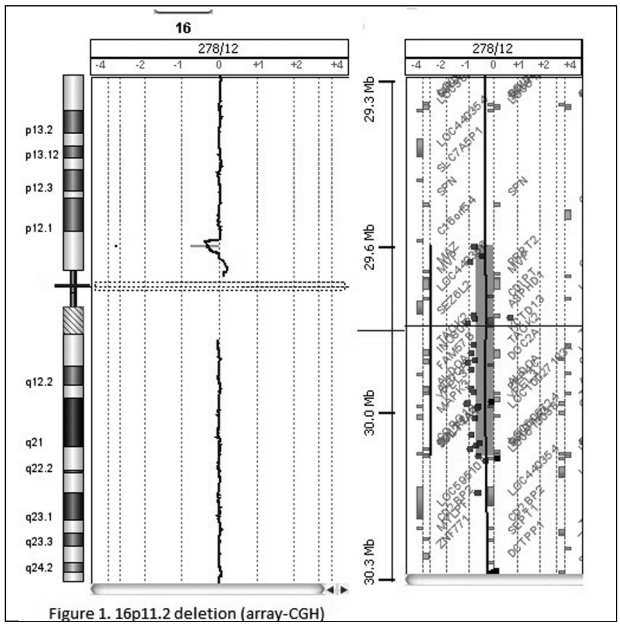

Figure 1. 16p11.2 deletion (array-CGH)

Patient 2 is a 4-year old boy, the first child of non-consanguineous parents. He was born at term by cesarean delivery, with a birth weight of 3450 g. He has a severe speech delay, behavioural disorder and obesity.

His family history is positive for speech retardation.

His karyotype was normal and array-CGH analysis showed a deletion of about 524 Kb in 16p11.2 region. Array-CGH in his parents confirmed a de novo deletion.

Patient 3 is a 4-year old child. He is the first child of non-consanguineous parents, born at 40 weeks of gestation after a normal pregnancy and delivery. His birth weight was 3870 g, birth length was 51 cm and head circumference 36 cm.

At birth he presented dysmorphic features, so a karyotype analysis (46, XY) was immediately performed.

He also had bilateral cryptorchidism, for which he underwent surgery at the age of 3 years. Neuropsychiatric evaluation revealed a severe expressive language disorder, while neuromotor development was normal.

Array-CGH analysis showed a 446 Kb 16p11.2 deletion, that was confirmed to be de novo.

Patient 4 is a 6-year old boy, the third child of non-consanguineous parents. He was born at term by cesarean section; during pregnancy intrauterine growth restriction (IUGR) was detected. At birth, he was reported to be small for gestational age, with a birth weight of 2530 g, a birth length of 44.8 cm and a head circumference of 31 cm (all measurements below the 3° centile). Despite this, his general condition was quite good. He also had cleft palate, for which he underwent surgery at the age of 1 year.

On examination at the age of 21 months, he had low weight (7.400 kg), short length (71 cm) and small head circumference (45.3 cm): these values confirmed his growth retardation.

His development was delayed and, in particular, a severe expressive language disorder was revealed.

Physical examination also showed dysmorphic features: short palpebral fissures, prominent forehead, sparse and thin hair, dysplastic ears, clinodactyly of the fifth finger and hyperlaxity. Cardiac, brain and abdominal ultrasounds were normal. Considering his growth retardation, screening for celiac disease, sweat testing for Cystic Fibrosis and x-ray of the hand were performed, but all results were negative.

Array-CGH analysis revealed a deletion of about 520 Kb in the 16p11.2 region. Array-CGH analysis showed normal copy numbers in both parents.

Patient carrying a 16p11.2 duplication

Patient 5 is a 14-year old boy, born as the second child of healthy, non-consanguineous parents. Neuropsychological examination at the age of 9 years indicated mild mental retardation and speech delay. His gross and fine motor development were delayed too.

On physical examination, we detected some dysmorphisms: elongated facies, protruding ears, prominent nose and hyperlaxity.

Cardiac ultrasounds and a brain MRI were normal. EEG showed some abnormalities without overt seizures.

Karyotype and Fragile X syndrome analysis were also normal. Array-CGH analysis showed a 16p11.2 duplication of about 520 Kb (Figure 2). Performing array-CGH in both parents, we revealed that the rearrangement was de novo.

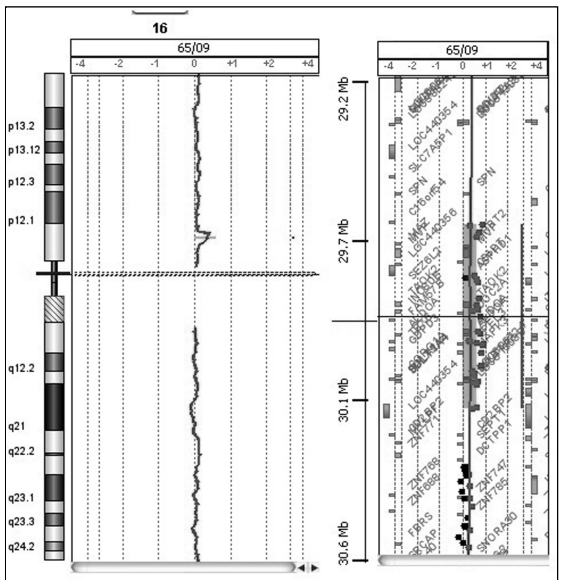

Figure 2. 16p11.2 duplication (array-CGH)

Discussion

16p11.2 deletion/duplication syndrome is a good example of the utility of array-CGH in the identification and definition of new rare syndromes. Thanks to the use of this technique in the study of cohorts of individuals with intellectual disability, speech delays and/or autism spectrum disorders, and with a normal karyotype, today we know the role of chromosome 16 CNVs in the etiology of these disorders. Analysis of our patients revealed that 4 children with the microdeletion and one child with the microduplication all have language delay and dysmorphic features, even if there is no common model. Two patients with deletion were reported to be small for gestational age (SGA), and one of them also had postnatal growth retardation. Only one patient presents behavioral problems and obesity. Furthermore, two patients have, respectively, bilateral cryptorchidism and cleft palate. In addition to these features, the only patient with microduplication has mild mental retardation, motor delay and EEG abnormalities (Table 1).

This study confirms the findings of the most recent data in the literature, namely that typical features of the syndrome are a variable degree of language delay and dysmorphisms. As seen, other clinical manifestations can be associated. However, in none of our patients did we find the congenital anomalies described in association with 16p11.2 deletion (cardiac malformations, hemivertebrae and syringomyelia).

Common phenotype	Patient	Patient	Patient	Patient	Patient
	1	2	3	4	5
Dysmorpich features	+	+	+	+	+
Language delay	+	+	+	+	+
Learning disability/ Intellectual disability	-	-	-	-	+
Social impairment	-	+	-	-	-
Seizures/EEG abnormalities	-	-	-	-	+
Obesity	-	+	-	-	-
Cardiac malformations	-	-	-	-	-
Hemivertebrae	-	-	-	-	-
Syringomyelia	-	-	-	-	-
Other features	SGA	-	Cryptorchidism	SGA, postnatal growth retardation, cleft palate	Motor delay

Table 1: Clinical features of 16p11.2 microdeletion/microduplication syndrome.

We have also focused on the molecular genetic pathogenesis of these rearrangements. 16p11.2 CNVs are mediated by recombination between flanking 147 Kb low-copy repeat sequences (LCR) with 99.5% sequence identity[18-22].

Low-copy repeat elements, or duplicons, are blocks of a few repeated sequences, with very high mutual homology (from 90% to 100%) and large hundreds of kilobases (200-400 Kb). They are throughout the genome, representing up to 5% of the human genome, and are located in subtelomeric and pericentromeric regions. A high degree of homology, extension, orientation and mutual distance between two or more duplicons make the region in which they are present unstable. So they can lead to misalignment of chromosomes or chromatids and mediate nonallelic homologous recombination (NAHR) that can result in unequal crossing-over[23]. This will result in various genomic rearrangements, including inversions, translocations, duplications or deletions, as in the case of 16p11.2 microdeletion/microduplication.

In 16p11.2 region 27 genes are included, 8 of which have been identified as possible candidates in the genesis of the syndrome. Despite this, how deletion or duplication of these genes results in the clinical symptoms of the syndrome is unknown. In particular, the genes studied are:

• ALDOA: aldolase A, fructose-bisphosphate;

• DOC2A: coding for proteins of the synaptic vesicles;

• HIRIP3: HIRA-interacting protein 3, involved in gene transcription control;

• MAPK3: mitogen-activated protein kinase 3, a cell cycle regulator (this gene in not included in 524 Kb and 446 Kb regions identified in our patients);

• MAZ: MYC-associated zinc finger protein;

• PPP4C: protein phosphatase 4, catalytic subunit;

• SEZ6L2: seizure related 6 homolog (mouse)-like 2;

• TAOK2: TAO kinase 2, involved in cellular signaling and apoptosis.

The most studied gene was SEZ6L2, variations in which were originally associated with autism[24]. Kumar and, subsequently, Konyukh[25] came to the conclusion that there is insufficient evidence to establish the role of this gene in the onset of autism spectrum disorders or related disorders. Further studies are needed to understand how the loss or the duplication of this critical region can cause this particular phenotype.

Conclusions

Even in our experience, the identification of cryptic chromosomal abnormalities helped us to

make a more accurate genotype-phenotype correlation (linked not only to the type of chromosome involved in altering, but also to the extension of the region). We are also able to plan an appropriate global evaluation of the patient and organize a targeted follow-up, in order to prevent all possible manifestations. It is clear that, the early we make diagnosis, the better the patient outcome.

The identification of the cryptic anomaly responsible for the phenotype allows us to carry out the study of parents to detect possible cryptic balanced chromosomal translocations. It is possible, therefore, to perform genetic counseling, indicating a low risk of recurrence in de novo cases (possible gonadal mosaicism) and a higher risk in familial forms. Having also confirmed all cryptic chromosomal abnormalities by FISH allows us to offer the possibility of molecular cytogenetics prenatal diagnosis.

References

1) Corsello G, Giuffrè M. *Congenital malformations*. J Matern Fetal Neonatal Med 2012; 25 Suppl 1: *25-29*.
2) Ghirri P, Scaramuzzo RT, Bertelloni S, Pardi D, Celandroni A, Cocchi G, Danieli R, De Santis L, Di Stefano MC, Gerola O, Giuffrè M, Gragnani GS, Magnani C, Meossi C, Merusi I, Sabatino G, Tumini S, Corsello G, Boldrini A. *Prevalence of hypospadias in Italy according to severity, gestational age and birthweight: an epidemiological study*. Ital J Pediatr 2009; 35: *18*.
3) Giuffrè M, Schierz IM, La Placa S. *Newborn with prenatal diagnosis of CAM*. Area Pediatrica 2011; 12 (1): *25-26*.
4) Schierz IAM, Giuffrè M, Piro E, Ortolano R, Siracusa F, Pinello G, La Placa S, Corsello G. *Predictive factors of abdominal compartment syndrome in neonatal age*. Am J Perinatol 2013; DOI: 10.1055/s-0033-1334447.
5) Piccione M, Piro E, Serraino F, Cavani S, Ciccone R, Malacarne M, Pierluigi M, Vitaloni M, Zuffardi O, Corsello G *Interstitial deletion of chromosome 2p15-16.1: report of two patients and critical review of current genotype-phenotype correlation*. Eur J Med Genet. 2012 Apr; 55(4): *238-44*.
6) Viaggi CD, Cavani S, Pierluigi M, Antona V, Piro E, Corsello G, Mogni M, Piccione M, Malacarne M. *Characterization of a complex rearrangement involving chromosomes 1, 4 and 8 by FISH and array-CGH*. J Appl Genet. 2012; 3(3): *285-8*.
7) Piccione M, Serra G, Sanfilippo C, Andreucci E, Sani I, Corsello G. *A new mutation in EDA gene in X-linked hypohidrotic ectodermal dysplasia associated with keratoconus*. Minerva Pediatr. 2012; 64(1): *59-64*.
8) Piccione M, Niceta M, Antona V, Di Fiore A, Cariola F, Gentile M, Corsello G. *Identification of two new mutations in TRPS 1 gene leading to the tricho-rhino-phalangeal syndrome type I and III*. Am J Med Genet A. 2009;149A(8): *1837-41*.
9) Vissers LE, de Vries BB, Osoegawa K, Janssen IM, Feuth T, Choy CO, Straatman H, van der Vliet W, Huys EH, van Rijk A, Smeets D, van Ravenswaaij-Arts CM, Knoers NV, van der Burgt I, de Jong PJ, Brunner HG, van Kessel AG, Schoenmakers EF, Veltman JA (2003) *Array-based comparative genomic hybridization for the genomewide detection of submicroscopic chromosomal*.
10) Shaw-Smith C, et al. *Microarray based comparative genomic hybridisation (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features*. J Med Genet. 2004; 41: *241-8*.
11) Gribble SM, Prigmore E, Burford DC, Porter KM, Ng BL, Douglas EJ, Fiegler H, Carr P, Kalaitzopoulos D, Clegg S, Sandstrom R, Temple IK, Youings SA, Thomas NS, Dennis NR, Jacobs PA, Crolla JA, Carter NP. (2005). *The complex nature of constitutional de novo apparently balanced translocations in patients presenting with abnormal phenotypes*. J. Med. Genet. 42, *8-16*.
12) Piccione M, Vecchio D, Cavani S, Malacarne M, Pierluigi M, Corsello G. *The first case of myoclonic epilepsy in a child with a de novo 22q11.2 microduplication*. Am J Med Genet A. 2011 Dec; 155A(12): *3054-9*.
13) Striano P, Coppola A, Paravidino R, Malacarne M, Gimelli S, Robbiano A, Traverso M, Pezzella M, Belcastro V, Bianchi A, Elia M, Falace A, Gazzerro E, Ferlazzo E, Freri E, Galasso R, Gobbi G, Molinatto C, Cavani S, Zuffardi O, Striano S, Ferrero GB, Silengo M, Cavaliere ML, Benelli M, Magi A,Piccione M, Dagna Bricarelli F, Coviello DA, Fichera M, Minetti C, Zara F *Clinical significance of rare copy number variations in epilepsy: a case-control survey using microarray-based comparative genomic hybridization*. Arch Neurol. 2012 Mar; 69(3): *322-30*.
14) Piccione M, Antona R, Salzano E, Cavani S, Malacarne M, Morreale Bubella R, Pierluigi M, Viaggi CD, Corsello G. *Array-CGH and clinical characterization in a patient with subtelomeric 6p deletion without ocular dysgenesis*. Am J Med Genet A. 2012 Jan; 158A(1): *150-4*.
15) Piccione M, Serra G, Consiglio V, Di Fiore A, Cavani S, Grasso M, Malacarne M, Pierluigi M, Viaggi C, Corsello G. *14q13.1-21.1 deletion encompassing the HPE8 locus in an adolescent with intellectual disability and bilateral microphthalmia, but without holoprosencephaly*. Am J Med Genet A. 2012 Jun; 158A(6): *1427-33*.
16) Isidor B, Bourdeaut F, Lafon D, Plessis G, Lacaze E, Kannengiesser C, Rossignol S, Pichon O, Briand A, Martin-Coignard D, Piccione M, David A, Delattre O, Jeanpierre C, Sévenet N, Le Caignec C *Wilms' tumor in patients with 9q22.3 microdeletion syndrome suggests a role for PTCH1 in nephroblastomas*. Eur J Hum Genet. 2012 Nov 21 doi: 10.1038/ejhg.2012.252
17) Giuffrè M, Piro E, Corsello G. P*rematurity and twinning*. J Matern Fetal Neonatal Med 2012; 25 Suppl 3: *6-10*.
18) Giuffrè M, Cipolla D, Bonura C, Geraci DM, Aleo A, Di Noto S, Nociforo F, Corsello G, Mammina C. Epidemic spread of ST1-MRSA-IVa in a neonatal intensive care unit, Italy. BMC Pediatr 2012; 12: 64.
19) Cipolla D, Giuffrè M, Mammina C, Corsello G. *Prevention of nosocomial infections and surveillance of emerging resistances in NICU*. J Matern Fetal Neonatal Med 2011; 24 Suppl 1: *23-6*.
20) Lo Iacono M, Anzalone R, Corrao S, Giuffrè M, Di Stefano A, Giannuzzi P, Cappello F, Farina F, La Rocca G. *Perinatal and wharton's jelly-derived mesenchymal*

stem cells in cartilage regenerative medicine and tissue engineering strategies. Open Tissue Engineering and Regenerative Medicine Journal 2011; 4 (SPEC. ISSUE 1): *72-81*.

21) David S, Bucchieri F, Corrao S, Czarnecka AM, Campanella C, Farina F, Peri G, Tomasello G, Sciumè C, Modica G, La Rocca G, Anzalone R, Giuffre M, Conway de Macario E, Macario AJL, Cappello F, Zummo G. *Hsp10: anatomic distribution, functions, and involvement in human disease*. Front Biosci (Elite Ed) 2013; E5: *768-778*.

22) Schierz IAM, La Placa S, Giuffrè M, Montalbano G, Lenzo M, Corsello G. T*ransient hepatic nodular lesions associated with patent ductus venosus in preterm infants*. Am J Perinatol 2011; 28 (3): *177-180*.

23) Kumar RA, Karamohamed S, Sudi J, Conrad DF, Brune C, Badner JA, Gilliam TC, Nowak NJ, Cook EH, Dobyns WB, Christian SL. *Recurrent 16p11.2 microdeletions in autism*. Hum Mol Genet.2008; 17: *628-38*.

24) Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, Saemundsen E, Stefansson H, Ferreira MA, Green T, Platt OS, Ruderfer DM, Walsh CA, Altshuler D, Chakravarti A, Tanzi RE, Stefansson K, Santangelo SL, Gusella JF, Sklar P, Wu BL, Daly MJ. *Autism Consortium; Association between microdeletion and microduplication at 16p11.2 and autism*. N Engl J Med. 2008; 358: *667-75*.

25) Ghebranious N, Giampietro PF, Wesbrook FP, Rezkalla SH. *A novel microdeletion at 16p11.2 harbors candidate genes for aortic valve development, seizure disorder, and mild mental retardation*. Am J Med Genet A. 2007; 143A: *1462-71*.

26) Sebat J., Lakshmi B., Malhotra D., Troge J., Lese-Martin C., Walsh T., Yamrom B., Yoon S., Krasnitz A., Kendall J. *Strong association of de novo copy number mutations with autism*. Science. 2007; 316: *445-449*.

27) Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, Shago M, Moessner R, Pinto D, Ren Y, Thiruvahindrapduram B, Fiebig A, Schreiber S, Friedman J, Ketelaars CE, Vos YJ, Ficicioglu C, Kirkpatrick S, Nicolson R, Sloman L, Summers A, Gibbons CA, Teebi A, Chitayat D, Weksberg R, Thompson A, Vardy C, Crosbie V, Luscombe S, Baatjes R, Zwaigenbaum L, Roberts W, Fernandez B, Szatmari P, Scherer SW. *Structural variation of chromosomes in autism spectrum disorder*. Am J Hum Genet. 2008; 82: *477-88*.

28) Stankiewicz P, Lupski JR. *Structural variation in the human genome and its role in disease*. Annu Rev Med. 2010; 61: *437-455*.

29) Kumar RA, Marshall CR, Badner JA, Babatz TD, Mukamel Z, Aldinger KA, Sudi J, Brune CW, Goh G, Karamohamed S, Sutcliffe JS, Cook EH, Geschwind DH, Dobyns WB, Scherer SW, Christian SL. Association and mutation analyses of 16p11.2 autism candidate genes. PLoS One. 2009; 4: *e4582*.

30) Konyukh M, Delorme R, Chaste P, Leblond C, Lemiere N, Nygren G, Anckarsater H, Rastam M, Stahlberg O, Amsellem F, Gillberg IC,Mouren-Simeoni MC, Herbrecht E, Fauchereau F, Toro R, Gillberg C, Leboyer M, Bourgeron T. 2011. *Variations of the candidate SEZ6L2 gene on chromosome 16p11.2 in patients with autism spectrum disorders and in human populations*. PLoS ONE 6: *e17289*.

Request reprints from:
Dott. ssa MARTINA BUSÈ
Via A. Giordano, 3
90127 Palermo
(Italy)