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Clinical features and magnesium levels: Novel insights in 15q11.2 BP1–BP2 copy number variants

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Abstract

Background Investigating copy number variations (CNVs) such as microdeletions or microduplications can significantly contribute to discover the aetiology of neurodevelopmental disorders. 15q11.2 genomic region, including *NIPA1* and *NIPA2* genes, contains a recurrent but rare CNV, flanked by the break points BP1 and BP2. Both BP1–BP2 microdeletion and microduplication have been associated with intellectual disability (ID), neuropsychiatric/behavioural disturbances and mild clinical features, even if with incomplete penetrance and variable expressivity. The pathogenic role of this CNV is quite unclear though. Unknown variants in other DNA regions and parent-of-origin effect (POE) are some of the mechanisms that have been proposed as an explanation of the wide phenotypic variability. As *NIPA1* and *NIPA2* encode for proteins that mediate magnesium (Mg^{2+}) metabolism, it has been suggested that urinary Mg^{2+} levels could potentially represent informative and affordable biomarkers for a rapid screening of 15q11.2 duplications or deletions. Furthermore, magnesium supplementation has been proposed as possible therapeutic strategy.

Methods Thirty one children with ID and/or other neurodevelopmental disorders carrying either a duplication or a deletion in 15q11.2 BP1–BP2 region have been recruited. When available, blood samples from parents have been analysed to identify the CNV origin. All participants underwent family and medical data collection, physical examination and neuropsychiatric assessment. Electroencephalogram (EEG) and brain magnetic resonance imaging (MRI) scan were performed in 15 children. In addition, 11 families agreed to participate to the assessment of blood and urinary Mg^{2+} levels.

Results We observed a highly variable phenotypic spectrum of developmental issues encompassing ID in most subjects as well as a variety of behavioural disorders such as autism and attention-deficit disorder/attention-deficit hyperactivity disorder. Dysmorphic traits and malformations were detected only in a minority of the participants, and no clear association with growth anomalies was found. Abnormal brain MRI and/or EEG were reported respectively in 64% and 92% of the subjects. Inheritance assessment highlighted an excess of duplication

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of maternal origin, while cardiac alterations were detected only in children with 15q11.2 CNV inherited from the father. We found great variability in Mg^{2+} urinary values, without correlation with 15q11.2 copy numbers. However, the variance of urinary Mg^{2+} levels largely increases in individuals with 15q11.2 deletion/duplication.

Conclusions This study provides further evidence that 15q11.2 BP1–BP2 CNV is associated with a broad spectrum of neurodevelopmental disorders and POE might be an explanation for clinical variability. However, some issues may question the real impact of 15q11.2 CNV on the phenotype in the carriers: DNA sequencing could be useful to exclude other pathogenic gene mutations. Our results do not support the possibility that urinary Mg^{2+} levels can be used as biomarkers to screen children with neurodevelopmental disorders for 15q11.2 duplication/deletion. However, there are evidences of correlations between 15q11.2 BP1–BP2 CNV and Mg^{2+} metabolism and future studies may pave the way to new therapeutic options.

Keywords 15q11.2 BP1–BP2 CNV, Intellectual disability, Magnesium, Neurodevelopmental disorders, Parent-of-origin effect

Backgrounds

Copy number variations (CNVs) such as microdeletions or microduplications drive the pathogenesis of many neurodevelopmental disorders.

Non-allelic homologous recombination (NAHR) involving low copy repeats (LCRs) is one of the multiple mechanisms resulting in generation of CNVs (Locke *et al.*, 2004). In particular, the long arm of human chromosome 15 (15q11.2–13.3) contains five clusters of LCRs referred as breakpoints 1–5 (BP1–BP5), predisposing to several CNVs.

The breakpoints BP1 or BP2 and the breakpoint BP3 flank the well-known Prader–Willi/Angelman region. The larger typical type I deletion extends from BP1 to BP3, and it is approximately 6.6 Mb in size, while the smaller typical type 2 deletion extends from BP2 to BP3 and involves approximately 5.3 Mb.

The proximal 15q11.2 genomic region, located between BP1 and BP2, contains a recurrent CNV of approximately 500 kb, found in 0.5% to 1% of the population. Microdeletions and microduplication of BP1–BP2 region have been proposed as susceptibility locus for neuropsychiatric/behavioural disturbances and mild clinical features, known as Burnside–Butler syndrome (Burnside *et al.*, 2011; Cox & Butler, 2015; Ho *et al.*, 2016; Butler, 2017). A recent review found a significant association of the 15q11.2 BP1–BP2 deletion with brain anomalies and cognitive impairment (van der Meer *et al.*, 2020).

The BP1–BP2 genomic region includes four highly conserved genes (*NIPA1*, *NIPA2*, *CYFIP1*, and *TUBGCP5*). Mutations in the four genes included in the region have been associated with several diseases, and their biological functions have been carefully studied (Rafi & Butler, 2020).

NIPA1 is highly expressed in the brain, where it potentially plays a role in nervous system development and maintenance. Mutations in *NIPA1* (non-imprinted in Prader–Willi/Angelman syndrome I) cause autosomal dominant hereditary spastic paraplegia (HSP) and postural disturbance. The length of the GCG repeat tract in *NIPA1* gene has been recently investigated as a possible modifying factor for C9orf72 mediated amyotrophic lateral sclerosis, with contrasting results (Rainier *et al.*, 2003; Chen *et al.*, 2005; Goytain *et al.*, 2007; Van Der Zwaag *et al.*, 2010; Tazelaar *et al.*, 2019). *NIPA1* acts as magnesium (Mg^{2+}) transporter (Goytain *et al.*, 2007). Altered Mg^{2+} concentration leads to redistribution of the protein: NIPA1 is recruited to the plasma membrane when Mg2 + is low whereas high Mg2 + level leads to accumulation of the protein in endosomes (Goytain *et al.*, 2007).

Similarly to NIPA1, NIPA2 (Non-Imprinted in Prader–Willi/Angelman syndrome II) encodes for a selective $Mg2^{+}$ transporter and plays a role in magnesium metabolism and regulation of renal conservation (Goytain *et al.*, 2007; Goytain *et al.*, 2008). By participating in the gating and the activation of channels and receptors such as *N*-methyl-D-aspartate receptors, magnesium plays a critical role in seizures (Xie *et al.*, 2014). *NIPA2* mutations decrease intracellular Mg^{2+} concentration causing childhood absence epilepsy (Xie *et al.*, 2014).

Focusing on the involvement of *NIPA1* and *NIPA2* in magnesium homeostasis, Picinelli *et al.* suggested that urinary Mg^{2+} levels could potentially represent informative and affordable biomarkers for a rapid screening of 15q11.2 duplications or deletions

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(Picinelli *et al.*, 2016). Butler reported that caregivers that administered magnesium supplements to the people with a 15q11.2 BP1–BP2 microdeletion observed improvements in behaviour and symptoms relieve (Butler, 2019). This experience suggests that magnesium supplementation could also be applied to behavioural problems seen in Prader–Willi syndrome (PWS) and Angelman syndrome (AS) caused by a larger 15q11-q13 type I deletion (Butler, 2019).

CYFIP1 (Cytoplasmic Fragile X mental retardation Interacting Protein 1) interacts with FMRP, the protein coded by the *FMR1* gene and associated with Fragile X syndrome (FXS), the most common cause of intellectual disabilities (IDs) in males (Ciaccio *et al.*, 2017). Moreover, reduced CYFIP1 expression leads to dysregulation of schizophrenia and epilepsy-associated gene networks (Hsiao *et al.*, 2016; Nebel *et al.*, 2016; Hagerman *et al.*, 2017; Reijnders *et al.*, 2017).

Finally, *TUBGCP5* (TUBulin Gamma Complex associated Protein 5) is essential for microtubule nucleation at the centrosome (Izumi *et al.*, 2008). Mutations in this gene are associated with attention-deficit hyperactivity disorder (ADHD) and obsessive–compulsive disorder (De Wolf *et al.*, 2013). Biallelic loss of this gene has been associated with primary microcephaly (Maver *et al.*, 2019).

In addition to magnesium transport regulation, these four genes are involved in biological processes that affect neurological development and function (Rafi & Butler, 2020). The alteration of these pathways can be an explanation for neurobehavioural disturbances and dysmorphic features in individuals with B1–B3 deletion (PWS/AS typical type I deletion) and BP1–BP2 CNV (Burnside–Butler syndrome).

However, BP1–BP2 CNVs are characterised by incomplete penetrance and variable expressivity (Cox & Butler, 2015). Data from population studies further indicate that BP1–BP2 CNV carriers unaffected by severe psychiatric or neurodevelopmental disorders have enhanced prevalence of dyslexia and dyscalculia (Stefansson *et al.*, 2008, 2014; Burnside *et al.*, 2011; Cox & Butler, 2015; Kendall *et al.*, 2017). Unknown variants in other DNA regions and complex interactions between several genes are just some of the mechanisms that have been proposed as an explanation of this wide phenotypic variability (Baldwin *et al.*, 2021). Although the four genes in BP1–BP2 region have been previously reported as non-imprinted, several studies described a methylated site within this region in human DNA samples and unequal gene expression in mice (Joshi *et al.*, 2016). Recently, a parent-of-origin effect (POE) of the 15q11.2 BP1–BP2 microdeletion has been hypothesised to explain different clinical phenotypes in children depending on the source of the parental CNV: When the deletion is maternal, there is a greater risk of macrocephaly, autism spectrum disorder (ASD) and epilepsy, while paternal deletion are associated with congenital heart disease (CHD) (Davis *et al.*, 2019).

All these observations underlie the functional role of the BP1–BP2 region in neurodevelopmental disorders and require more attentions from the medical community.

In the present study, we collected phenotypic data on 31 children, identified with 15q11.2 BP1–BP2 in two diagnostic centres in Milan.

We focused on the association between 15q11.2 BP1–BP2 CNVs and clinical features, brain magnetic resonance imaging (MRI), electroencephalogram (EEG) and biochemistry data.

This study has three main aims:

- to determine association of 15q11.2 CNV with neurological, psychological and cognitive performance, auxological data, brain MRI and EEG.
- to identify whether POE of the 15q11.2 BP1–BP2 CNV is associated with differences in clinical features in individual inheriting the alteration.
- to assess whether urinary Mg^{2+} levels represent a biomarker for a rapid screening of 15q11.2 CNVs. This biomarker could also be applied to people with PWS and AD with the larger 15q11-q13 type I deletions.

Methods

The Pediatric Genetic Unit of 'Fondazione IRCCS Ca' Granda Ospedale Maggiore' and the Department of Pediatric Neuroscience of 'Istituto Neurologico Carlo Besta' in Milan are reference centres for genetic evaluation of children with ID and other neurodevelopmental disorders, and patients are mostly referred by paediatricians and child neuropsychiatrists.

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Array comparative genomic hybridisation (array CGH) is proposed as first-line test for children with neurodevelopmental delay, ID or behavioural problems of unknown origin. If dysmorphisms or malformations suggest a specific diagnostic hypothesis, other targeted tests are performed [single gene test and next-generation sequencing (NGS) panels].

In the last 5 years, 818 array CGH tests have been performed and 31 children (ranged from 3 to 16 years old, mean age 8 years old) carrying either a duplication or a deletion in chromosome 15q11.2 between breakpoints BP1 and BP2 have been detected (3,79%). When available, blood samples from parents have been collected in order to distinguish de novo cases from familiar ones.

All children underwent clinical evaluation by an expert paediatric geneticist. Data were collected by medical record review regarding the following points: sex, age, family history, course of pregnancy and delivery, developmental delay, autism spectrum disorders and other behavioural problems, growth pattern, dysmorphic features, vision and hearing problems and other system involvement including cardiac, musculoskeletal, gastrointestinal and genitourinary abnormalities. All children underwent auxological measurements (height, weight and cranic circumference), physical examination and neuropsychiatric evaluation. International standardised scales have been used to assess the development (Griffiths, WPPSI-III and WISC-IV) and possible autism spectrum disorders. Neuropsychiatric diagnosis was made according to the Diagnostic and Statistical Manual of mental disorders (DSM V) criteria. Only 13 children performed an EEG and only 14 underwent a brain MRI scan due to different protocols in the two centres and the lack of consent of some families. No parent reported malformations or IDs or psychiatric illnesses. However, parental medical records about detailed assessments on general health, cognition and behaviour were not available.

The DNA of the children and their parents was isolated from the peripheral blood. Molecular karyotyping was performed through array-CGH using SurePrint G3 Human CGH Array Kit, 8x60K and 4x180K (Agilent Technologies, Santa Clara, CA, USA). Labelling and hybridisation were performed according to the manufacturer's protocol. Agilent Feature Extraction was used to quantify the fluorescence of the scanned images and Cytogenomics 2.7 software was used for data analysis. CNV call was performed using the ADAM-2 algorithm. Probe positions are referred to hg19/GRCh37.

We reproposed the study performed by Picinelli *et al.* (2016) with a larger sample. Eleven families agreed to participate to the evaluation of magnesium levels. The magnesium levels of 29 subjects were collected: 9 parents with two copies of the region BP1–BP2, 4 children and 3 parents with single copy of the region BP1–BP2, 7 children and 6 parents with three copies of the region BP1–BP2.

All participants were evaluated for serum creatinine levels to ensure proper kidney function. Twenty-one subjects dosed urinary magnesium levels through 24-h urine collection. Magnesium levels were determined using a colorimetric assay kit (Xylidyl Blue method). The normal range for magnesium levels was defined according to the CLSI/IFCC C28-A guideline (2008).

Ethics statement

All parents of the children involved in our study subscribed a written consent to perform genetic tests and to anonymously use the data collected. The investigations were carried out in accordance with the principles laid down in the 2013 revision of the Declaration of Helsinki. The study was approved by the *Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico* Ethical Committee and Scientific Board (N°734-2018).

All authors agreed to the submission of the manuscript to the journal.

Results

Array CGH identified 31 children, 13 females (42%) and 18 males (58%) from 3 to 16 years old, carrying a CNV in chromosome 15q11.2 between breakpoints BP1 and BP2. There were 17 deletions, 13 duplications and 1 triplication identified. All 31 CNVs involved the genes *TUBGPC5*, *CYFIPI*, *NIPA2* and *NIPA1*.

Twenty-nine children resulted as carriers only of a CNV in 15q11.2 region, but two of our subjects also had additional CNVs, described below.

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Clinical features

The clinical features of 28 children (17 males and 11 females) in our cohort with only 15q11.2 deletion (16) or duplication (12) are summarised in Table 1. Normal pregnancy and delivery were reported in all cases. Most of them showed normal growth parameters, but weight was under the 3rd centile in 12% of children (10% in deletion carriers and 14% in duplication carriers) and height was under the 3rd centile in 17% (18% in deletion carriers and 17% in duplication carriers). Weight was above the 97th

Table 1 Clinical features and neuroradiological data (electroencephalography and brain magnetic resonance imaging) of 28 children in our cohort with 15q11.2 deletion (16 children) or duplication (12 children)

Clinical Features	All (28)		Del15q11.2 (16)		Dup15q11.2 (12)	
Sex						
Male	17/28	61%	7/16	44%	10/12	83%
Female	11/28	39%	9/16	56%	2/12	17%
Pregnancy and delivery						
Normal	28/28	100%	16/16	100%	12/12	100%
Growth parameters						
Weight						
<3°	2/17	12%	1/10	10%	1/7	14%
3°–10°	2/17	12%	1/10	10%	1/7	14%
10–90°	10/17	59%	6/10	60%	4/7	57%
90°–97°	2/17	12%	1/10	10%	1/7	14%
97°	1/17	6%	1/10	10%	0/7	0%
Height						
<3°	3/18	17%	2/11	18%	1/7	17%
3°–10°	5/18	28%	4/11	36%	1/7	17%
10–90°	7/18	39%	5/11	45%	2/7	17%
90°–97°	3/18	17%	0/11	0%	3/7	50%
97°	0/18	0%	0/11	0%	0/7	0%
Head circumference						
<3°	4/20	20%	1/12	8%	3/8	38%
3°–10°	2/20	10%	2/12	17%	0/8	0%
10–90°	8/20	40%	5/12	42%	3/8	38%
90°–97°	5/20	25%	3/12	25%	2/8	25%
97°	1/20	5%	1/12	8%	0/8	0%
Dysmorphic features	6/28	21%	3/16	19%	3/12	25%
Facial dysmorphisms	4/28	14%	2/16	13%	2/12	17%
Non-facial dysmorphisms	2/28	7%	1/16	6%	1/12	8%
Malformations	4/28	14%	1/16	6%	3/12	25%
Heart	2/28	7%	1/16	6%	1/12	8%
Kidney	1/28	4%	0/16	0%	1/12	8%
Eyes	2/28	7%	0/16	0%	2/12	17%
Clef palate	1/28	4%	1/16	6%	0/12	0%
Neurodevelopmental disorders	28/28	100%	16/16	100%	12/12	100%
Intellectual disability	22/28	79%	12/16	75%	10/12	83%
Learning disorders	3/28	11%	3/16	19%	0/12	0%
Autism/autistic features	8/28	29%	4/16	25%	4/12	33%
ADHD	7/28	25%	3/16	19%	4/12	33%
Brain MRI						
Abnormal brain MRI	9/14	64%	2/5	40%	7/9	78%
EEG						
Abnormal EEG	12/13	92%	5/6	83%	7/7	100%
Seizures	1/28	4%	1/16	6%	0/12	0%

ADHD, attention-deficit hyperactivity disorder; EEG, electroencephalography; MRI, magnetic resonance imaging.

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centile only in one deletion carrier, and none of the children showed height above the 97th centile. Microcephaly was found in 20% of children (1 deletion carrier and 3 duplication carriers), while macrocephaly only in 1 deletion carrier. In 6 out of 28 children (19% in deletion carriers and 25% in duplication carriers), unspecific and very variable dysmorphic features have been detected, including broad forehead, hypertelorism, dysmorphic ears, pectus excavatum, although without a recurrent pattern.

A few subjects (14%) had malformations: in particular, congenital heart defect in 1 deletion carrier (aortic coarctation) and 1 duplication carrier (patent foramen ovalis with shunt), unilateral hypoplastic kidney in 1 duplication carrier, cleft palate in 1 deletion carrier (the same with aortic coarctation), congenital cataract in 1 duplication carrier and strabismus in 1 deletion carrier.

All children presented with neurodevelopmental disorders, as it was a requirement to be a candidate for array CGH. Mild or moderate neurodevelopmental delay/ID was reported in 79%, learning difficulties in 11%, autism spectrum disorders (ASD) in 29% and ADHD in 15%.

The two children with additional genetic variants and the child with triplication are here discussed separately.

A 3-year-old girl showed neurodevelopmental delay, atrial septal defect and strabismus. The auxological measures were in range for her age. She was carrier of the recurrent 15q11.2 deletion, inherited from her father, but array CGH also identified a de novo deletion of 10.2 Mb in 1q42 region, responsible for cognitive impairment. We could assume a role of concause of 15q11.2 deletion, but the main culprit was likely the 10.2-Mb deletion.

Array CGH identified in a 17-year-old boy with ID a maternal duplication in 15q11.2 region and a de novo duplication of 784 kb in 16p13.11 region. As both these recurrent CNV are described as susceptibility factors for neurological manifestation, it was difficult to attribute the impact of each CNV on the phenotype.

In the case of a 5-year-old child with neurodevelopmental delay, malformation screening identified only agenesis of the corpus callosum at MRI of the brain. Array CGH detected a 591-kb triplication in 15q11.2 region. Curiously, the mother was carrier of a duplication of the same region, although asymptomatically.

Brain magnetic resonance imaging and electroencephalogram abnormalities

Among the 14 children who performed brain MRI scan, 2 deletion carriers (40%) and 7 duplications carriers (78%) presented abnormalities: 5 cases showed isolated malformations such as hypogenesis/agenesis of the corpus callosum (3 subjects), impaired rotation of the hippocampi and bilateral parietal polymicrogyria; 4 children presented alterations of the periventricular white matter, probably expression of perinatal hypoxic damage.

Almost all EEG showed anomalies (83% in deletion carriers and 100% in duplication carriers): the majority presented with diffuse epileptic abnormalities (seven subjects); two children had focal epileptic abnormalities in the temporal and occipital regions, three children had abnormalities in the organisation of the background rhythm due to excessive diffuse rapid activity in both wakefulness and sleep. Nevertheless, only one deletion carrier had seizures.

Hereditariness

We found that the CNV was inherited from a parent in 21 (84%) out the 25 families for which both parents were available for testing. Fourteen CNVs were inherited from the mother (67%) and only 7 CNVs (33%) from the father. Ten duplications had maternal origin (91%), while only one duplication had paternal origin. Four deletions (40%) were inherited from the mother, and six deletions (60%) were inherited from the father. Therefore, an excess of duplication of maternal origin was found with statistical significance (Fisher's exact test, $P < 0.05$).

Magnesium levels

Possible influences on magnesium levels were excluded because no patients used magnesium supplements. 27 (93%) out of 29 subjects, either children or parents, with one, two or three copies of BP1–BP2 region, presented normal serum magnesium levels (Figure 1). Only a child carrying the BP1–BP2 duplication and a normal father had blood magnesium values above average.

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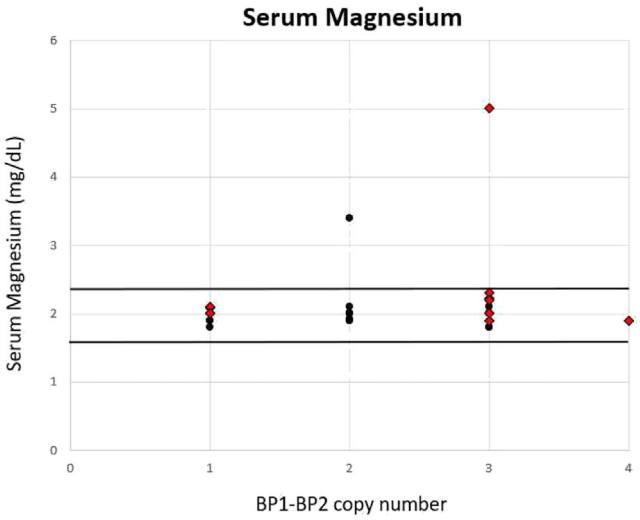

Figure 1. Serum magnesium levels in affected children (red rhombuses) and healthy parents (black circles). Horizontal lines delimit the upper and the lower limit of the normal range (1.7–2.2 mg/dL).

On the contrary, several subjects had urinary magnesium levels outside the normal range, regardless of the allele copy number: 4 (67%) normal parents had urinary magnesium levels below normal range; among the children carrying BP1–BP2 deletion, 3 (43%) children had low urinary magnesium levels, while 2 (29%) of them had high urinary magnesium levels; among the participants carrying the BP1–BP2 duplication, 3 (37%) children had urinary magnesium level below normal range, and 2 (25%) of them had urinary magnesium level over the normal range. The three groups had overlapping average values (64 mg/24 h in wild-type individuals, 87 mg/24 h in deletion carriers and 83 mg/24 h in duplication carriers). No differences between affected children and asymptomatic BP1–BP2 CNV carrier parents have been detected. However, an interesting phenomenon has been observed: the variance of urinary magnesium levels greatly increases in individuals with a CNV, as shown in Figure 2. Standard deviation of urinary magnesium levels was 45 mg/24 h and 49 mg/24 h in individuals with 15q11.2 deletion and duplication, respectively, while it was only 14 mg/24 h in wild-type parents.

The child with BP1–BP2 triplication had normal serum magnesium level, but urinary sample has not been collected.

Discussion

Microdeletion and microduplication in BP1–BP2 15q11.2 genomic region have been associated with ID,

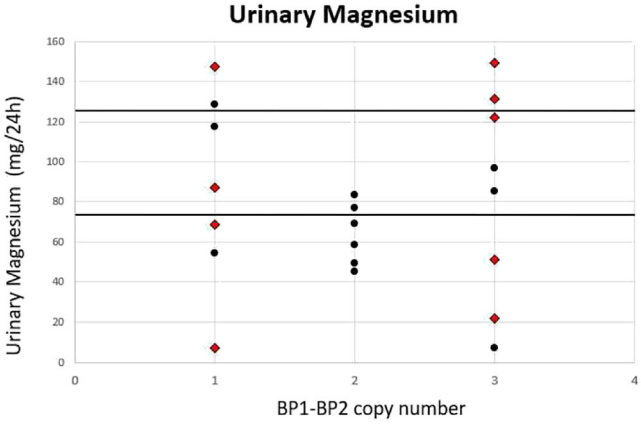

Figure 2. Urinary magnesium levels in affected children (red rhombuses) and healthy parents (black circles). Horizontal lines delimit the upper and the lower limit of the normal range (75–125 mg/24 h).

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neuropsychiatric/behavioural disturbances and mild clinical features.

BP1–BP2 region is spared in the smaller Prader–Willi/Angelman typical type II deletion (Burnside *et al.*, 2011; Cox & Butler, 2015; Butler, 2017). Several studies have shown a more severe neurodevelopmental phenotype in type I compared with type II deletion (Butler *et al.*, 2004; Bittel & Butler, 2005). In particular, individuals with PWS and type I deletion show more compulsive and self-injurious behaviour, lower cognitive performance and delayed acquired speech compared with PWS with type II deletion (Butler *et al.*, 2004; Hartley *et al.*, 2005; Varela *et al.*, 2005). Similarly, more seizures, more severe behavioural and cognitive impairment and higher likelihood of features of autism spectrum disorder are reported in AS with type I deletion (Sahoo *et al.*, 2007; Valente *et al.*, 2013). This evidence suggests that BP1–BP2 region could play an important role in neurodevelopment and cognitive functions.

Our study confirms the wide clinical spectrum of the 15q11.2 CNV, particularly on neurological, psychological and cognitive performance. All children presented with neurodevelopmental disorders as it was one of the inclusion criteria in the study. On the other hand, no parent reported IDs or psychiatric illnesses, even if parental medical records were not available. These are important biases that could hide the real impact of BP1–BP2 CNV in the general population. However, the phenotypic spectrum of developmental issues is highly variable encompassing ID in most children as well as a variety of behavioural disorders such as ASD and attention-deficit disorder/ADHD. One child had only learning disorders. No significant differences in neurological phenotype between deletion and duplication carries have been observed. Prevalence of neurological features in our cohort is similar to previous reports in literature: 79% for ID (73% in literature), 29% for ASD (27% in literature), 25% for ADHD (35% in literature). Abnormal brain MRI and EEG are found, respectively, in 64% and 92% of the individuals; however, only 1 child had seizures. In literature epilepsy is reported up to 26% in BP1–BP2 deletion carriers: in our genetic services children whose seizures are cardinal symptoms perform NGS epilepsy panel as first-line test. This could be an explanation for this difference.

Dysmorphic traits were detected only in a minority of children (21%), and no specific traits that could be used as diagnostic handles were identified, as previously reported. No clear association with growth anomalies was found, even if height, weight and head circumference were below the 3° centile respectively in 17%, 12% and 20% of participants and above the 97° centile only in 0%, 6% and 5%. There was not a different trend among deletion carriers compared with duplication ones.

This phenotypic spectrum in BP1–BP2 carriers correlates with strong evidence that all four genes are variously implicated in axonal growth and neural connectivity. However, some issues may question the real impact of 15q11.2 CNV on the phenotype in the carriers, in particular incomplete penetrance and variable expressivity.

We found that the CNV was inherited from asymptomatic parents in 21 (84%) out of the 25 for which both parents were available for testing. In literature, de novo frequency has been similarly estimated at 5% to 22%. Low parental penetrance may be related to incomplete information about the family or to subclinical manifestations of neuropsychiatric/behavioural problems. We did not evaluate parents with standardised tests, but only collecting a detailed family history.

POE is another factor that can contribute to phenotypic variability. Our study adds further clinical evidence that 15q11.2 BP1–BP2 microduplication or microdeletion may exhibit POEs, as the only paternally inherited CNV was associated with congenital heart disease.

In the study, an excess of amplification of maternal origin was found. The finding of several asymptomatic mothers suggests that females could be less vulnerable to the effects of variants. Girirajan *et al.* proposed that males are more prone to develop signs and/or symptoms as a consequence of a weak mutation on the X chromosome (Girirajan *et al.*, 2012). Thus, a male individual will require fewer mutational events to cross the threshold to disease. Future studies should deepen our knowledge of the role of these inherited variants in the pathogenesis of the disorder, helping genetic counselling and prognosis definition in families identified with 15qBP1–BP2 CNV in order to further expand the clinical phenotype of this emerging syndrome.

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Finally, a possible bias for the interpretation of our results, shared by previous study, is due to genetic heterogeneity and blended phenotypes; BP1–BP2 carries may have other congenital point mutations, which cannot be found via CGH array. DNA sequencing could be useful to exclude other pathogenic gene mutations.

Because *NIPA2* encodes for a Mg^{2+} transporter highly expressed in the renal tubule, Picinelli *et al*. in 2016 proposed that urinary Mg^{2+} levels could represent a biomarker for a rapid screening for 15q11.2 CNV. They investigated Mg^{2+} levels in a cohort of four subjects with BP1–BP2 CNV (one deletion and three duplications) and their asymptomatic parents carrying the same CNV, and they found that urinary Mg^{2+} levels were negatively correlated with the number of 15q11.2 alleles, even if a child carrying the duplication and his mother displayed urinary Mg^{2+} levels within the normal range. We replicated the study in a larger cohort of 11 children and their parents when available, for a total of 29 subjects. Serum Mg^{2+} levels were found within the normal range in most individuals, as reported in Picinelli's study. Magnesium transport regulation occurs through a balance between absorption and excretion and recent study found that urine magnesium excretion displays diurnal variation, which is likely related to increased uptake of magnesium during meals, and thus helping to maintain a stable concentration of magnesium in the blood (Jacobsen *et al.*, 2021). This could be an explanation for the observed normal serum magnesium level in our cohort. On the contrary, several subjects, either carrier or familiar non-carriers, had urinary magnesium levels outside the normal range, without correlation with 15q11.2 copy numbers, but the variance of urinary magnesium levels greatly increased in individuals with a CNV. The increase in variance of urinary Mg^{2+} levels in CNV carriers could be consistent with a possible interference of *NIPA2* alterations in physiological magnesium metabolism and a consequent impact on neurodevelopmental and cognitive performance in BP1–BP1 CNV carriers. Indeed, magnesium is involved in multiple processes including brain development and functioning and multiple studies have assessed Mg^{2+} status in neurodevelopmental disorder, although with conflicting results at times (Cao *et al.*, 2019; Yamanaka *et al.*, 2019). In a recent commentary, Butler highlighted that low levels of magnesium are found in people with seizures, depression and acute or chronic brain disease (Butler, 2019). However, lack of correlation with 15q11.2 copy numbers and urinary magnesium level was found. Seven other genes score higher than *NIPA1* and *NIPA2* for magnesium transport function among the 3472 genes relating to magnesium transport function in the genome (www.GeneCards.Org; Alpern *et al.*, 2008). Therefore, it is predictable that deletions/duplications of *NIPA1* and *NIPA2* alone might not be significant enough to effectively alter the magnesium levels in plasma or urine. However, given magnesium's key role in neuronal maturation and neuropathology, the particular loss of *NIPA1* and *NIPA2*-magnesium transporter functions might affect neuronal maturation.

In conclusion, our results do not support the use of urinary Mg^{2+} levels as informative and affordable biomarkers to screen children with neurodevelopmental disorders for 15q11.2 duplication/deletion, but we do not rule out that future studies may identify new correlations between this CNV and magnesium metabolism, paving the way to new therapeutic strategies.

Informed Consent Statement

All study participants or their legal guardian provided informed written consent prior to study enrolment.

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Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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