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BRIEF REPORT

Brief Report: Functional MRI of a Patient with 7q11.23 Duplication Syndrome and Autism Spectrum Disorder

Paolo Prontera · Domenico Serino · Bernardo Caldini · Laura Scarponi · Giuseppe Merla · Giuseppe Testa · Marco Muti · Valerio Napolioni · Giovanni Mazzotta · Massimo Piccirilli · Emilio Donti

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Abstract The duplication of the Williams–Beuren syndrome (WBS) region (7q11.23) is a copy number variant associated with autism spectrum disorder (ASD). One of the most intriguing aspects is that the reciprocal microdeletion causes WBS, characterized by hypersociability, marked empathy, and a relative capacity in verbal short-term memory and language. Herein, we studied, by using functional morphological and volumetric magnetic resonance, a 17-year-old male patient who displays a de novo 7q11.23 duplication and ASD. The limbic system of the patient appeared hypo-functional, while the total brain volume was increased, thus contrasting, in an opposite and intriguing manner, with the global brain volume reduction reported in WBS. Even if these findings come from the analysis of a single patient and, therefore, have to be considered preliminary results, they encourage carrying on further functional and volumetric studies in patients with 7q11.23 duplication, to fully elucidate the role of this gene-dosage alteration on brain development and limbic system function.

Keywords Autism spectrum disorders · 7q11.23 Duplication · Amygdala · Williams–Beuren syndrome · Limbic system · Magnetic resonance · Functional magnetic resonance

Introduction

Genome-wide analyses of copy-number variants (CNVs) have demonstrated that the duplication of the 7q11.23 region is associated with autism spectrum disorder (ASD) (7dup-ASD, OMIM #609757) and with a highly variable phenotype, mainly characterized by severe expressive language delay, mild or no cognitive impairment, minor facial dysmorphisms, normal growth, joint laxity, hypotonia and seizures (Somerville et al. 2005; Van der Aa et al. 2009; Merla et al. 2010; Levy et al. 2011; Sanders et al. 2011; Welberg 2011). Brain magnetic resonance (MR), when performed in 7dup-ASD patients, showed variable abnormalities including: ventricular dilatation, pachygiria, cortical thickening

P. Prontera (✉) · E. Donti
Medical Genetics Unit, Hospital "S. M. della Misericordia", University of Perugia, Via E. dal Pozzo, 06123 Perugia, Italy
e-mail: pprontera@hotmail.com; paolo.prontera@ospedale.perugia.it

D. Serino
Neurology Division, Ospedale Pediatrico Bambino Gesù, Rome, Italy

B. Caldini · L. Scarponi · M. Piccirilli
Section of Psychiatry and Clinical Psychology, Terni School of Medicine, University of Perugia, Perugia, Italy

G. Merla
Medical Genetics Unit, IRCCS "Casa Sollievo della Sofferenza" Hospital, San Giovanni Rotondo, Italy

G. Testa
Department of Experimental Oncology, European Institute of Oncology, Milan, Italy

M. Muti
Department of Radiotherapy, Hospital "S.Maria", Terni, Italy

V. Napolioni
Innovation Pole for Genomics, Genetics and Biology, Perugia, Italy

G. Mazzotta
School of Specialization in Childhood Neurology and Psychiatry, University of Perugia, Perugia, Italy

and, at least in one case, simplified gyral pattern, cerebral atrophy and cortical dysplasia of the left temporal lobe was ascribed as the plausible cause of the speech delay (Torniero et al. 2007, 2008; Van der Aa et al. 2009).

The reciprocal 7q11.23 microdeletion causes Williams–Beuren syndrome (WBS) (OMIM#194050), that is characterized by hypersociability, marked empathy, predisposition to music and a relative capacity in verbal short-term memory and language (Williams et al. 1961; Meyer-Lindenberg et al. 2006; Capitão et al. 2011). Morphological and functional brain imaging studies of WBS patients disclosed that frontal lobes, amygdala and other structures participating in emotional processing are increased in volume in contrast with a global brain volume reduction (Meyer-Lindenberg et al. 2006; Capitão et al. 2011).

Therefore, since the limbic system is commonly impaired in ASD while it is oversized in WBS, we supposed that it should be impaired/underdeveloped in 7dup-ASD. To this aim, we studied an ASD subject carrying a duplication of the 7q11.23, using both neuropsychiatric evaluations and brain imaging analyses.

Materials and Methods

A local ethical commission has approved the research and all the subjects signed an informed consent and received exhaustive information about the aim of the study.

Clinical Report

The boy is the first child of unrelated healthy parents. He was born in the 40th week after spontaneous delivery following a normal pregnancy. A slight delay in neuromotor development was reported, with independent walking reached at 17 months of age. The boy underwent speech therapy between the age of three and seven because of speech and language delay, but the lack of any significant clinical progress prompted for a new and more detailed diagnostic evaluation. Karyotype investigation, MRI scan, visual and acoustic evoked potentials, electroretinogram, electroneurography and electromyography were performed, all yielding normal results. Clinical and neurological evaluation revealed normal weight (25 kg, 25th-centile) and height (128 cm, 50th-centile), relative macrocephaly (55 cm, 98th-centile) and small mandible. Muscle trophism was globally diminished while muscle tone was slightly diminished in all districts.

The general cognitive ability assessed at 16 years with the LEITER-R test revealed a full-scale IQ of 73, with a poorer performance in Visualization (Figure Ground: 4, Form Completion: 6) and Reasoning tasks (Sequential Order: 7, Repeated Patterns: 4) versus Spatial tasks (Design Analogies: 8, Paper Folding: 9, Figure Rotation: 7). Psychiatric assessment was undertaken with the Childhood Autism Rating Scale (CARS), while adaptive behavior was evaluated through the Vineland Adaptive Behavior Scales

Table 1 (a) Volumetric analysis of amygdala and intracranial volume in the ASD subject and the seven controls; (b) statistical analysis of the volumetric results

Case	Sex	Ethnicity	Type	Age	IQ	Left A.V. (nun3)	Right A.V. (nun3)	Mean A.V. (mm3)	I.V. (cm3)	Mean A.V./ I.V.
(A) Volumetric analysis										
1	M	Caucasian	Patient	18	73	1,868	1,853	1,860.5	1,756	1.06
2	M	Caucasian	Control	24	95	1,723	1,713	1,718	1,395	1.23
3	M	Caucasian	Control	13	94	1,714	1,637	1,675	1,391	1.20
4	M	Caucasian	Control	28	110	2,095	2,037	2,066	1,700	1.22
5	M	Caucasian	Control	11	87	1,750	1,753	1,751.5	1,469	1.19
6	F	Caucasian	Control	11	89	1,235	1,520	1,377.5	1,192	1.16
7	F	Caucasian	Control	11	98	1,728	1,659	1,693.5	1,570	1.08
8	M	Caucasian	Control	9	99	1,660	1,644	1,652	1,346	1.23
			Mean ± SD	15	96±7	1,701 ± 250	1,709 ± 160	1,705 ± 200	1,438 ± 160	1.19 ± 0.05

	One mean test results			
	Patient	Controls (7)	*p* value	95 % CI for mean
(B) Statistical analysis				
Mean amygdala V.	1,861	1,705 ± 200	0.0874	1,519–1,891
Intracranic V.	1,756	1,438 ± 160	0.0021(*)	1,286–1,588
Mean A.V./I.V.	1.06	1.19 ± 0.05	0.0008(*)	1.14–1.24

A.V. amygdala volume, *I.V.* intracranial volume

Significant results are signed with (*)

(VABS). According to the DSM-IV-TR, the resulting clinical profile was a Pervasive Developmental Disorder-Not Otherwise Specified, (fully included in the "Autism Spectrum Disorder" category).

Genetic Testing

Array-Comparative Genomic Hybridization (CGH) was performed using commercially available arrays (CytoChip v3 BlueGnome, Cambridge, UK) on DNA extracted from peripheral blood (Nucleospin Blood Kit, Macherey–Nagel, Duren, Germany), as previously described (Prontera et al. 2013). CNVs detected by CGH analysis were validated by Fluorescent In Situ Hybridization (FISH) analysis using the BAC clone RP11-644H24, mapping to 7q11.23 region, and a centromeric probe for chromosome 7 (32 K Library; CHORIBAC/PAC resources), and Quantitative Real-Time PCR (qPCR) using protocols previously reported (Merla et al. 2010). FISH and PCR specific for 7q11.23 microsatellites were performed on parents to establish the origin of the CNV.

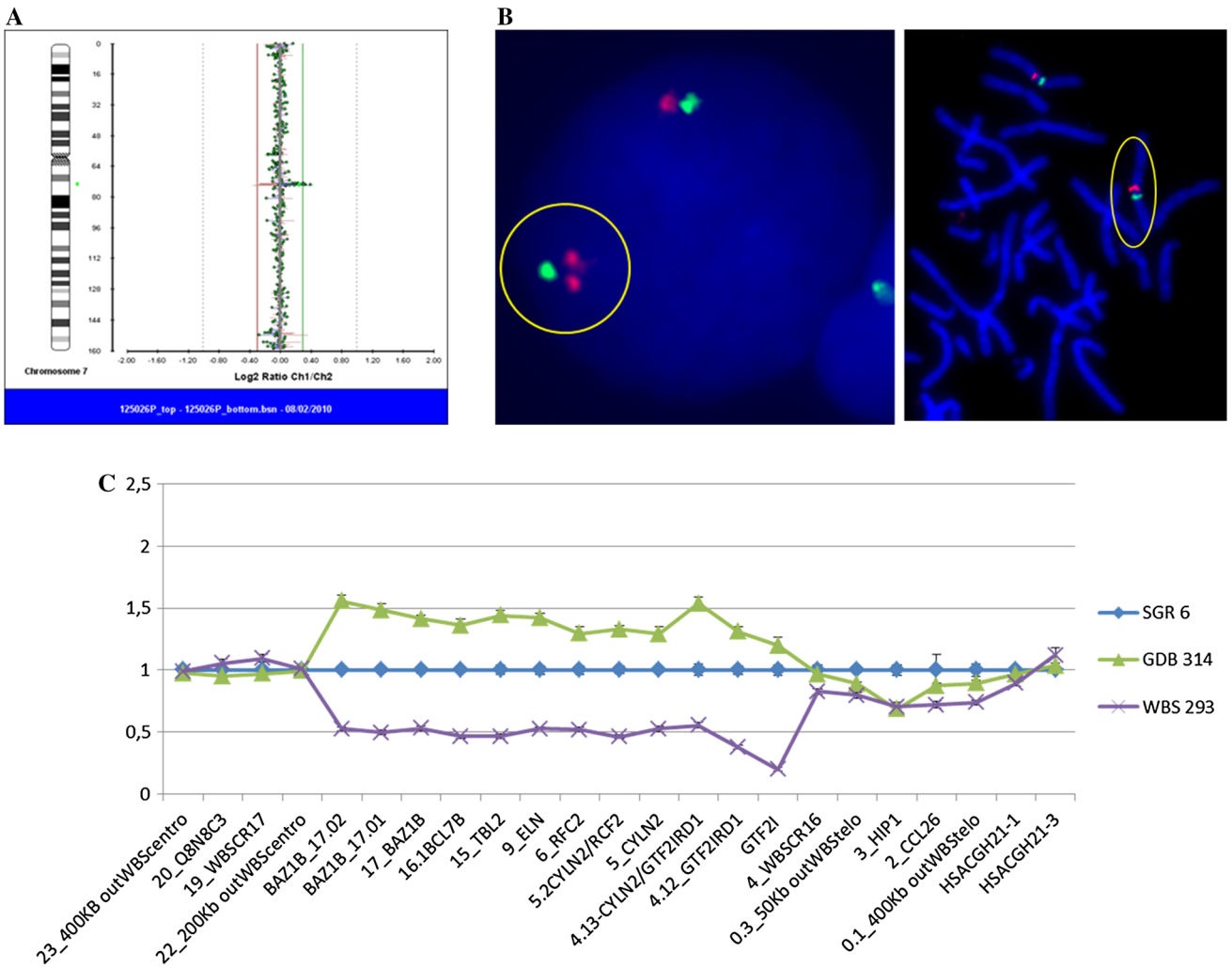

Fig. 1 **a** Array-CGH: profile of chromosome 7. The first clone duplicated in the centromeric region is RP11-101D2 while the last duplicated in the telomeric region is RP11-19F19. **b** Pictures show interphasic and metaphasic FISH experiments carried out using the BAC clone RP11-644H24 (*red spots*), mapping to 7q11.23 region, and a centromeric probe for chromosome 7 (*green spots*). Note the *double red signal* in interphase, that is also most pronounced in the metaphase (*yellow circles*). **c** Mapping Williams–Beuren region duplication syndrome in our patient. Relative DNA quantity was quantified for the proband (*green line* GDB314), a health control (*blue line* SGR6) and a patients with classical WBS deletion (*purple line* WBS293). The duplication spans from 72,873,625 to 74,114,443 Mb. The BAZ1B and GTF2I genes are located at the centromeric and telomeric ends of the region respectively (Color figure online)

Table 2 fMRI results.

Area	Z	Coordinate	Side	Voxels
Control				
Occipital Inferior (BA 19)	7.76	29 : −91 : −10	R	
Fusiform gyrus	4.31	34 : −34 : −28	R	3,383
Occipital Inferior (BA 19)	7.32	−26 : −97 : −11	L	2,548
Amygdala	**5.08**	**24 : −5 : −11**	**R**	**1,418**
Amygdala	**5.36**	**−20 : −1 : −12**	**L**	**1,301**
Orbito-frontal cortex	**6**	**5 : 46 : −8**	**R**	**430**
Cingulum anterior	**3.84**	**2 : 40 : −6**	**R**	**182**
Patient				
Occipital inferior (BA 19)	6.44	44 : −68 : −14	R	
Fusiform gyrus	6.18	24 : −72 : −14	R	2,573
Occipital inferior (BA 19)	5.56	−44 : −78 : −12	L	1,808
(BA 18)	5.36	−20 : −1 : −12	L	

Note that the limbic system (orbito-frontal cortex, amygdala and cingulum) is activated in the control (bold values) while is completely inactive in the patient, and for this reason they don't appear in the table

Functional, Morphological and Volumetric Magnetic Resonance Imaging (MRI)

The morphological and volumetric evaluations were carried out by comparing the 7dup-ASD patient with seven controls, reporting also the same educational level, normal auxometric parameters and IQ level (LEITER-R test) (Table 1). The morphologic and volumetric explorations and the exact localization of brain anatomy were assessed using a T1-weighted high-resolution acquisition. The volumetric analysis was performed using a manual encircling method (ITC and the Master Plan softwares) supported by an anatomic 3D atlas (Freesurfer software, at http://surfer.nmr.mgh.harvard.edu/) (Buckner et al. 2004).

fMRIs were performed by comparing the patient with a sex-age matched control (Case 2, Table 1) using a protocol commonly adopted to study the function of the limbic system, through the visualization of facial expressions that activates the implicit elaboration of emotion (Minshew and Keller 2010; Malisza et al. 2011).

Results

Genetic Testing

Array-CGH analysis revealed the presence of a ~1.2 Mb duplication in the 7q11.23 region (Fig. 1a), confirmed both by FISH and qPCR which also allowed a better definition of the duplication boundaries, spanning from 72,873,625 to 74,114,443 Mb (GRCH37/hg19). The duplication included all the genes commonly deleted in WBS patients (Fig. 1b, c): BAZ1B, BCL7B, TBL2, MLXIPL, VPS37D, WBSCR22, DNAJC30, STX1A, ABHD11, CLDN3, CLDN4, WBSCR27, WBSCR28, ELN, LIMK1, EIF4H, LAT2, RFC2, CLIP2, GTF2IRD1, GTF2I. Analysis of parental DNA demonstrated the presence of a de novo rearrangement on paternal chromosome 7 (data not shown).

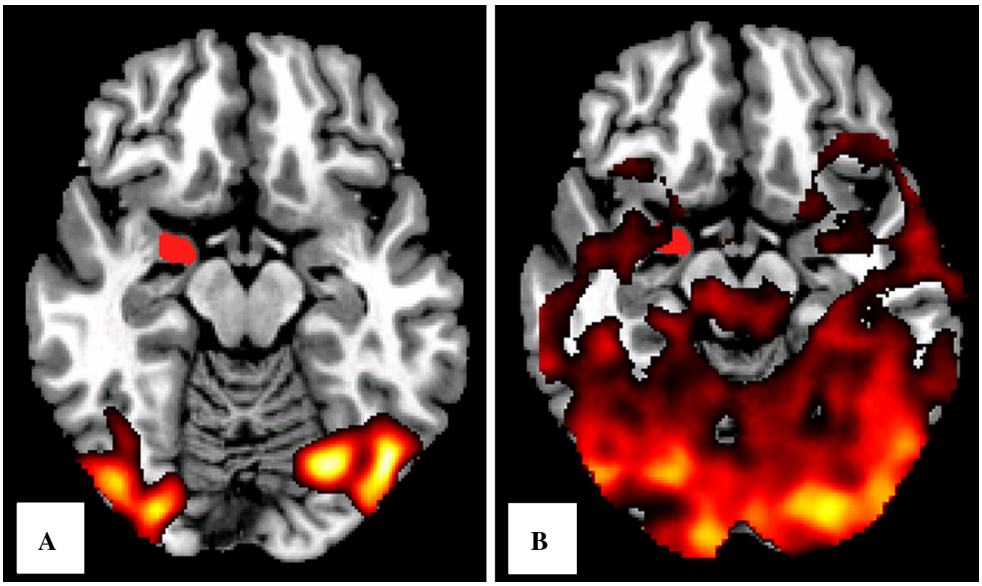

Fig. 2 fMRI partial results: note that during the same task the patient (**a**) activated the visual areas but not the amygdala (*red spot*) that, otherwise, is active in the control (**b**) (Color figure online)

Functional, Morphological, Volumetric MRI

Morphological and Volumetric MRI

The patient's brain showed cortical thickening and ventricular dilatation, but not pachygiria, neither cortical dysplasia of the left temporal lobe or simplified gyral pattern.

The results of volumetric analysis, carried out comparing the patient with the other seven healthy controls, are reported in Table 1A. The total intracranial volume (ICV) of the patient was 1,756 ml, about 25 and 17 % larger than that of the controls and the average value of the normal population (1,494 ± 117 ml), respectively (Woon and Hedges 2009; Reite et al. 2010). About 70 % of this difference was due to the cortical gray and white matter volume, while the remaining 30 % was attributable to the ventricles and subarachnoidal spaces. Comparison of the regional brain volumes between the patient versus control was carried out by normalizing the values (ml) of the brain parts of our interest (i.e. those presumably involved in ASD pathogenesis (amygdala) for the ICV, as suggested by Buckner et al. (2004). The results, depicted in Table 1B, indicated that the amygdala volume did not significantly differ between patient and controls, while; the amygdala/intracranial volume ratio of the patient was lower than the controls ($p < 0.1$).

Functional MRI (fMRI)

Both the patient and the sex-age matched control showed bilateral activation of secondary visual areas (BA 18 and 19) and of the right fusiform gyrus that is specifically involved in face recognition. However, contrary to what occurred in the control, as well as in normal population (Minshew and Keller 2010; Malisza et al. 2011) the patient showed no activation of the emotion-processing areas (i.e. amygdala, cingulum and orbital frontal cortex) (Table 2; Fig. 2).

Discussion

We carried out a detailed characterization of a subject featuring the duplication of the WBS interval and hallmark features of ASD.

The genetic characterization uncovered a duplication spanning the genes commonly involved in both 7q11.23 deletion and duplication syndromes, making this case genetically comparable to the majority of 7dup-ASD cases reported to date (Somerville et al. 2005; Van der Aa et al. 2009; Merla et al. 2010; Levy et al. 2011; Sanders et al. 2011; Welberg 2011). Neuropsychiatric and neuropsychological assessments diagnosed a form of ASD associated with a severe speech impairment that contrasts with the relatively good cognitive testing results (IQ = 73). These contrasting performances, between intellectual and speech abilities, have been already reported in many other 7dup-ASD patients (Van der Aa et al. 2009).

The fMRI study showed that the amygdala, the cingulum and the orbital frontal cortex (the so-called "social brain") of our patient appeared silent; while the volumetric evaluations disclosed that the total intracranial volume was higher than controls. This last finding is particularly significant vis a vis the opposite defect reported in WBS, in which several studies demonstrated that intracranial volume is lower than normal (Meyer-Lindenberg et al. 2006; Capitão et al. 2011). On the other hand, the absolute amygdala volume of the patient was similar to those of the controls (Table 1), but, if normalized for the total brain volume, it appeared relatively smaller ($p < 0.01$). This data suggests that there is not a co-linear increment of total brain and amygdala volumes, however, it is challenging to establish whether this discrepancy can account for the functional amygdala defect or whether it is an irrelevant finding.

Thus, despite the fact that our study is based only on a single case, and therefore further research are warranted, these preliminary findings suggest that genes within the 7q11.23 region may regulate the brain volume and the activity of the limbic structures. While halving of dosage (as in WBS) impacts negatively on brain volume and positively on amygdala and other limbic system structures (with a clinical correlate of moderate intellectual disability and emphatic behavior), a 1.5 fold increase in dosage may lead, in an intriguingly opposite fashion, to an increased brain volume and to limbic system dysfunction (with mild or no intellectual disabilities but severe social impairment). Thus, taken together with previous studies on WBS, the present preliminary finding suggests that the opposite neurobehavioral phenotypes characterizing these two syndromes could be traced to opposite genetic lesions through the newly defined symmetry in brain volume and in the activity of limbic structures.

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