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7q11.23 microduplication syndrome: neurophysiological and neuroradiological insights into a rare chromosomal disorder

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Abstract

Background The phenotypical consequence of the heterozygous chromosome 7q11.23 interstitial microdeletion is the Williams–Beuren syndrome, a very well-known genetic multi-systemic disorder. Much less is known about the reverse condition, the heterozygous interstitial microduplication of 7q11.23 region. The first molecular cytogenetic description was published in 2005, and only after several years were the reported patients numerous enough to attempt a description of a common phenotype.
Method By using a broad multidisciplinary approach, we investigated 12 patients with this rare genetic anomaly. Ten of them harboured the duplication of the classical Williams–Beuren syndrome region and two a slightly larger duplication. Upon a detailed description of the clinical and psychological features, we used electroencephalography and magnetic resonance imaging to explore neurophysiological function and brain structures.
Results We analysed the clinical, psychological, neuroradiological and neurophysiological features of 12 yet-unpublished individuals affected by this rare genetic anomaly, focusing specifically on the last two aspects. Several structural abnormalities of the central nervous system were detected, like ventriculomegaly, hypotrophic cerebellum, hypotrophic corpus callosum and hypoplastic temporal lobes. Although only one of 12 individuals suffered from seizures during childhood, three others had abnormal electroencephalography findings prominent in the anterior brain regions, without any visible seizures to date.
Conclusion Taken together, we enlarged the yet-underrepresented cohort in the literature of patients affected by 7q11.23 microduplication syndrome and shed further light on neuroradiological and neurophysiological aspects of this rare genetic syndrome.

Keywords autism spectrum disorder, brain abnormalities, EEG abnormalities, epilepsy, 7q11.23 micro-duplication, severe speech delay

Introduction

Deletion and duplication of submicroscopic chromosomal regions have – among others – been

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associated with neurodevelopment delay affecting different aspects of neurological and psychological development (Battaglia *et al.* 2013; Gershon & Alliey-Rodriguez 2013). Williams–Beuren syndrome (WBS) results from a heterozygous interstitial deletion of sub-band q11.23 of chromosome 7 long arm and is characterised by global developmental and growth delay, weak performance of spatial skills, speech delay, overly social behavior, phobia to loud noises, infantile hypercalcaemia, congenital heart failures and different facial abnormalities (Pober 2010). The interstitial microduplication of the same region 7q11.23 was described much later than WBS as a syndromic condition, when Somerville *et al.* (2005) reported a male patient with global neurodevelopmental delay, very severe speech delay, mild intellectual development delay [intellectual disability (ID)] and facial abnormalities (short philtrum, thin lips, and high and broad nose). Since the first description in 2005, some patients suffering from severe speech delay, ID and/or autism spectrum disorder (ASD) in combination with various congenital malformations were reported to harbour a 7q11.23 microduplication. 7q11.23 microduplication was also found to be a risk factor for schizophrenia. In a large meta-analytic study comprising almost 15 000 schizophrenia adult cases, a 10-fold higher prevalence of 7q11.23 microduplication was reported than in the size-matched healthy control group (Mulle *et al.* 2014).

Several aspects of this yet-unnamed syndrome have been studied, and common clinical features were searched in order to facilitate a clinical diagnosis. The most severe clinical issue of 7q11.23 microduplication patients is the speech delay. It was described in every reported patient, and both language aspects (reception and expression) were involved (Somerville *et al.* 2005; Van der Aa *et al.* 2009; Morris *et al.* 2015; Earhart *et al.* 2017). The speech impairment of 7q11.23 microduplication patients is clearly different from the corresponding disturbance of WBS individuals: while the first ones have very poor verbal and non-verbal communication skills, the latter ones present overwhelming social interaction skills going together with a mild speech delay.

A complex psychological study on 91 7q11.23 microduplication patients (79 children and 12 adults) investigated in-depth their psychopathological features and revealed that affected individuals suffer from a heterogeneous spectrum of psychotic disturbances: the majority of the paediatric patients have a social phobia or selective mutism, one-third of the patients suffer from attention deficit hyperactivity disorder, one-fourth present an oppositional deficit disorder, three-fourths are referred because of a severe speech sound disorder and one-third are characterised by ASD (Mervis *et al.* 2015). Several studies analysing very large patient cohorts had demonstrated the occurrence of this chromosomal anomaly in ASD patients (Merla *et al.* 2010; Kaminsky *et al.* 2011; Levy *et al.* 2011; Sanders *et al.* 2011).

Epilepsy was present in about 15–20% of reported 7q11.23 microduplication patients. The age of onset varied among different reports, starting during infancy in some patients (Berg *et al.* 2007; Torniero *et al.* 2007), and adolescence or adulthood in others (Berg *et al.* 2007). In some patient cohorts, epilepsy was totally absent (Dixit *et al.* 2013). Antiepileptic treatments (e.g. topiramate and valproate, or carbamazepine or phenobarbital) were successful in stopping the seizures in almost all cases and could be terminated in many cases after a seizure-free condition was reached. Only in few cases were the seizures intractable (Van der Aa *et al.* 2009).

The neuroradiological evaluation of affected 7q11.23 microduplication individuals was reported in a few papers (Patil *et al.* 2015). A unique report based on functional magnetic resonance imaging (MRI) study of one ASD patient revealed that brain regions (amygdala, cingulum and orbital frontal cortex) assigned to social functions were switched off in comparison with those of an age-matched control (Prontera *et al.* 2014). Although these findings were based on a single patient, these results encourage further studies by functional MRI approaches in order to investigate social brain functions in this rare genetic disorder.

A number of other congenital abnormalities (heart defects, cryptorchidism, cleft palate, craniosynostosis and joint laxity) were described in few patients each (Van der Aa *et al.* 2009). Aortopathy seems to be among the most frequent non-neuropsychological anomalies in individuals harbouring 7q11.23 microduplication (Zarate *et al.* 2014; Parrott *et al.* 2015).

Only one case with 7q11.23 triplication was reported (Beunders *et al.* 2010). Because the affected patient presented with a phenotype at the most severe end of the pathological spectrum, the dosage dependency of one or more genes of 7q11.23 band was emphasised.

The aim of our multicentric retrospective study was to specifically analyse neurophysiological and neuroradiological features of 12 individuals (11

probands and one parent) affected by 7q11.23 microduplication and to broaden our insights into epileptological and neuroradiological aspects of this rare gene, dosage-dependent syndrome.

Methods and material

Patients

Each of the 12 patients was studied through karyotyping and molecular cytogenetics by geneticists after intellectual delay was suspected by developmental neurologists or paediatricians in five clinical centres. Ethylenediaminetetraacetic acid and/or heparinised blood samples of 12 patients and their parents were acquired upon informed consensus. DNA was extracted from ethylenediaminetetraacetic acid blood sample, and chromosomes were prepared from heparinised blood sample according to standard procedures.

Molecular karyotyping

Molecular karyotyping or array-based comparative genome hybridisation (aCGH) and data analysis were performed by using the Human Genome CGH Microarray chip 180K (patients 1, 8, 9, 10, 11 and 12) and 60K (patients 2, 3, 4, 5, 6 and 7) (Agilent Technologies, Santa Clara, CA; GRCh37/hg19), according to the manufacturer's instructions. The corresponding microarray chips were scanned by the Agilent Microarray Scanner (G2565BA), and data analysis was performed using Agilent FEATURE EXTRACTION software v 11.5 and GENOMIC WORKBENCH software v 7.0 (Agilent Technologies). Conspicuous regions were compared with known copy number variations, as provided by the Database of Genomic Variants (DGV) (http://projects.tcag.ca/variation/).

Cytogenetics and molecular cytogenetics

Chromosomes were analysed in all 12 patients by GTG banding; 15 metaphases were studied per case in banding cytogenetic analyses.

Molecular cytogenetics was performed to confirm presence of interstitial duplication in the index cases (patients 8 and 9) and in their parents (patient 10 and her partner) to find out about a potential parental origin of the microduplication. As probes for fluorescence *in situ* hybridisation (FISH), the commercially available probe combination LSI ELN (7q11.23)/LSI D7S486, D7S522 (7q31) (Abbott Molecular, Mannheim, Germany) was applied. Twenty-five to 108 metaphases and 15 to 25 interphase nuclei were analysed per case.

Magnetic resonance imaging

Magnetic resonance imaging examinations were performed in 9 subjects on 1.5-T systems. Qualitative assessment of MRI scans was performed on T1-weighted images, T2-weighted images, proton density images and 1- to 3-mm-thick coronal fluid-attenuated inversion recovery images.

Results

Clinical, neuropsychological and psychological features

The clinical scenario characterising twelve 7q11.23 microduplication patients, all of Caucasian ethnicity, was heterogeneous; however, the severe receptive and expressive speech delay was the common feature unifying the cohort (Table 1). The mean age at the enrolment in the study was 14 years (range 2–37 years), and the mean age at the execution of the genetic investigations was 11.8 years (range 1–34 years). Apart from patient 11, all probands had no consanguineous parents. Patient 10 was genetically investigated after the genetic diagnosis was performed in her two children (patients 8 and 9). Brachycephaly (in eight patients) and the prominent forehead (in four patients) were the most common facial abnormalities. Skull asymmetry and macrocephaly, which were previously described in other affected individuals, were not found in our cohort. Only one patient presented a short philtrum. None of the seven male patients had cryptorchidism, and diaphragmatic hernia was not present in any of the 11 patients investigated by ultrasound imaging. Eight patients were investigated by echocardiography because of the previous description of congenital heart failures in individuals harbouring heterozygous 7q11.23 microduplications. Among these eight patients, one presented an aortic valve insufficiency and aortic bulb dilatation (39 mm), one had a patent foramen ovale with left–right shunt and one had a mild hypertrophy of right atrium and left ventricle.

Table 1 Schematic summary of clinical, neurophysiological, neuroradiological, psychological and genetic features of the 12 studied patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12
Patient information												
(1) Gender	Female	Female	Male	Male	Male	Male	Male	Male	Female	Female	Male	Female
(2) Current age (years)	2	18	23	12	18	15	13	11	10	37	18	13
(3) Age at genetic investigation (years and months)	1	13	18	7	12	11	12	7	5	34	17	5
(4) Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
(5) Consanguinity of the parents	No	No	No	No	No	No	No	No	No	No	Yes	No
(6) Kinship								Son of patient 10 and brother of patient 9	Daughter of patient 10 and sister of patient 8	Mother of patients 8 and 9		
Facial dysmorphisms												
(1) Skull asymmetry	No	No	No	No	No	No	No	No	No	No	No	No
(2) Macrocephaly	No	No	No	No	No	No	No	No	No	No	No	No
(3) Brachycephaly	**Yes**	No	**Yes**	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	No	**Yes**
(4) Prominent forehead	**Yes**	No	No	**Yes**	No	No	No	No	No	No	No	**Yes**
(5) Short philtrum	No	**Yes**	No	No	No	No	No	No	No	No	No	No
Intellectual delay												
(1) IQ value (psychological test; age at performance)	ND	50 (WISC-III test; at 12 years 1 month)	65 (WISC-III test; at 15 years 5 months)	50 (WISC-III test; at 8 years 6 months)	64 (WISC-III test; at 8 years 7 months)	30 (WISC-III test; at 9 years 8 months)	56 (WISC-III test; at 9 years 5 months)	70 (K-ABC test; at 4 years)	73 (K-ABC test; at 6 years)	Intellectual disability (test results not available)	74 (CFT-20 R test; at 15 years)	53 (WISC-III test; at 6 years)
(2) Speech delay	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
(3) Autistic behaviour	No	No	No	No	No	**Yes**	No	**Yes**	**Yes**	**Yes**	No (but depressive mood and panic attacks)	No
Abnormal CNS structures at brain imaging								ND	ND	ND		
(1) Pachygyria/agyria/polymicrogyria	No	No	No	No	No	No	No				No	No
(2) Cortex and sulci	Normal	Normal	**Dilatation of subarachnoidal sulci at the left temporal**	Normal	Normal	**Hypotrophic temporal lobes**	**Mildly dilated parietal sulci**				Normal	Normal

Table 1. *(Continued)*

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12
			lobes, insula and bilateral parietal regions									
(3) Hypoplasia of hippocampus	No	No	No	No	No	No	No				No	No
(4) Cerebellum	**Global cerebellar hypotrophy**	**Mild cerebellar vermis hypotrophy**	Normal	Normal	Normal	**Hypotrophic cerebellar vermis**	Normal				Normal	Normal
(5) Corpus callosum	**Thin**	**Mildly hypoplastic**	Normal	**Thinning of posterior part**	Normal	Normal	Normal				Normal	**Thin**
(6) Ventricular system	**Dilatation of internal and lateral ventricles**	Normal	**Mild dilatation of cerebral ventricles**	**Asymmetry of lateral ventricles (left > right)**	**Asymmetry of lateral ventricles (left > right)**	Normal	Normal				**Dilatation of lateral ventricles**	**Symmetric dilatation of lateral ventricles**
Structural abnormalities of internal organs												
(1) Diaphragmatic hernia (by abdominal ultrasound)	No	No	No	No	No	No	No	No	No	No	ND	No
(2) Cryptorchidism			No	No	No	No	No	No			No	
(3) Congenital defects of heart and ascending aorta (by echocardiography)	No	No	**Aortic valve insufficiency and aortic bulb dilatation (39 mm)**	**Patent foramen ovale, left > right shunt**	No	No	No	ND	ND	ND	ND	**Mild atrial septal defect and mild hypertrophy of right atrium and left ventricle**
Epilepsy												
(1) Clinical features, frequency and duration of epileptic seizures	No	No	No	No	No	No	No	No	No	**Presumably generalised epilepsy; seizures started at 1 year**	No	No
(2) EEG features	Age-matched normal	Age-matched normal	**Diffuse sharp waves,**	Age-matched normal	**Sharp waves, synchronous**		Age-matched normal	ND	ND	Unknown results	Age-matched normal	**Sporadic asynchronous**

Table 1. *(Continued)*

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12
			prominent over the anterior brain regions		**over the anterior brain regions**	Age-matched normal						**sharp waves over the temporal regions**
(3) Antiepileptic drugs	No	No	No	No	No	No	No	No	No	Drug unknown; therapy from 1st to 10th year of life	no	Valproate (as mood stabiliser)
Chromosomal anomalies										ND		
(1) CGH array Start and end points	7q11.23 (72,726,578-74,139,390) dn	7q11.23 (72,726,578-74,139,390)X3	7q11.23 (72,772,522-74,485,826)x3 dn, 13q14.3 (53,039,852-53,228,002)x3 dn	7q11.23 (72,726,578-74,139,390) x3 dn	7q11.23 (72,726,578-74,139,390) x3 dn	3q23 (142,368,749-142,548,625)x1 mat, 7q11.23(72,726,578-74,139,390)3 dn	7q11.23 (72,772,522-74,139,390)x3 dn, 20p12.1 (14,776,880-15,165,480)x1 dn	7q11.23q11.23(72,726,578-74,139,390)x3 mat	7q11. 23q11.23 (72,726,578-74,139,390) x3 mat		7q11.23 (72,745,047-74,339,045) x3 dn	7q11.23 (72,726,578-74,139,390)x3 dn
Length of duplicated region (minimal and maximal) (Mb)	1.41–1.94	1.41–1.97	1.71–2.31	1.41–1.97	1.41–1.97	1.41–1.97	1.95–2.18	1.41–1.94	1.41–1.94		1.59–2.94	1.40–2.0
(2) FISH analysis (used BAC sonds)	46,XX. ish dup(7)(q11.23q11.23)(ELN enh)[14]/ish7q11.23q11.23(ELNx2)[2]	ND	ND	ND	ND	ND	ND	46,XY. ishdup(7)(q11.23q11.23)(ELN+++)[20]/46,XY.ish7q11.23q11.23(ELNx2)[3]46,XY.nuc ishdup(7)(q11.23q11.23)(ELNx3)[14]/46,XY.nuc ish7q11.23q11.	46,XX. ishdup(7)(q11.23q11.23)(ELN+++)[22]/46,XX.ish7q11.23q11.23(ELNx2)[3]46,XX.nuc ishdup(7)(q11.23q11.23)(ELNx3)[14]/46,XX.nuc ish7q11.	46,XX. nuc ishdup(7)(q11.23q11.23)(ELNx3)[69]/7q11.23q11.23(ELNx2)[39]	ND	ND

Table 1. *(Continued)*

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12
Further remarks					The patient's brother has febrile seizures and speech delay	MRI: hypotrophic pons	Infantile psychosis	23(ELNx 2)[2] Febrile convulsions in first years of life	23q11.23 (ELNx2)[1] Febrile convulsions in first years of life			Psychotic phenotype, severe anxiety disorder and obsessive–compulsive disorder; sleep disorder; and migraine without aura

BAC, bacterial artificial chromosome; CGH, comparative genome hybridisation; CFT-20 R, Culture Fair Test 20 Revision; CNS, central nervous system; EEG, electroencephalography; FISH, fluorescence *in situ* hybridisation; IQ, intelligence quotient; K-ABC, Kaufman Assessment Battery for Children; MRI, magnetic resonance imaging; ND, no data; WISC-III, Wechsler Intelligence Scale for Children-Third Edition.

Although none of the 11 probands suffered from clinically manifest epileptic seizures, each of them was investigated by electroencephalography (EEG), and in three patients, abnormal waves were observed. Patients 3 and 5 presented sharp waves over both frontal regions and patient 12 sporadic waves over both temporal regions (Fig. 1). Patient 10 (mother of patients 8 and 9) suffered from generalised epilepsy in childhood and was treated with one antiepileptic drug. Unfortunately, neither EEG findings nor detailed information about their previous antiepileptic therapy could be reported in detail.

Ten patients were investigated by psychological tests in order to evaluate their cognitive abilities. Although different tests (Wechsler Intelligence Scale for Children-Third Edition, Kaufman Assessment Battery for Children and Culture Fair Test 20 Revision) were used and the testing was performed at different ages among the patients, all 10 psychologically investigated probands had an IQ below 75 (range 50–74). Patient 1 was not tested because of the very young age. Patient 10 (affected mother of patients 8 and 9) had obvious cognitive deficits but was not formally tested.

Neuroradiological features

Nine patients were investigated using brain MRI scan, and a heterogeneous picture was revealed. The most common finding was the symmetric or asymmetric dilatation of the cerebral ventricles, with six patients (1, 3, 4, 5, 11 and 12) showing this anomaly. Two patients (3 and 7) presented a mild dilatation of temporal and parietal sulci, and one (6) had mildly hypotrophic temporal lobes. The cerebellum was either partially (vermis) or globally hypotrophic in three patients (1, 2 and 6). The corpus callosum was shown to be thin or partially hypoplastic in four patients (1, 2, 4 and 12). None suffered from pachygyria or polymicrogyria, and the hippocampus was normally configured in all nine investigated patients. Taken together, a specific and common neuroradiological picture could not be described in these nine investigated patients (Fig. 2).

Genetic features

The skull abnormalities in several patients and the global neurodevelopmental delay with major

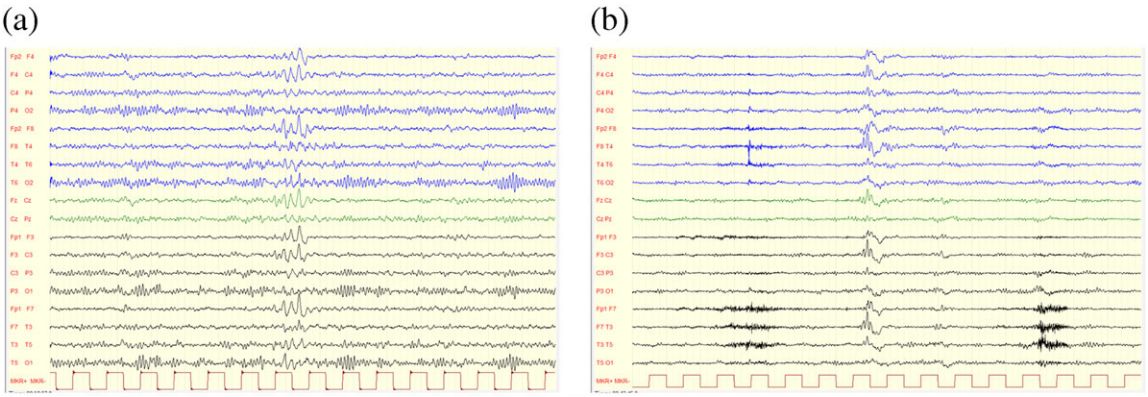

Figure 1 Electroencephalography abnormalities. Schematic representation of abnormal electroencephalography findings from patients 3 (a) and 5 (b). Both present generalised sharp waves, prominent over the anterior brain regions. [Colour figure can be viewed at wileyonlinelibrary.com]

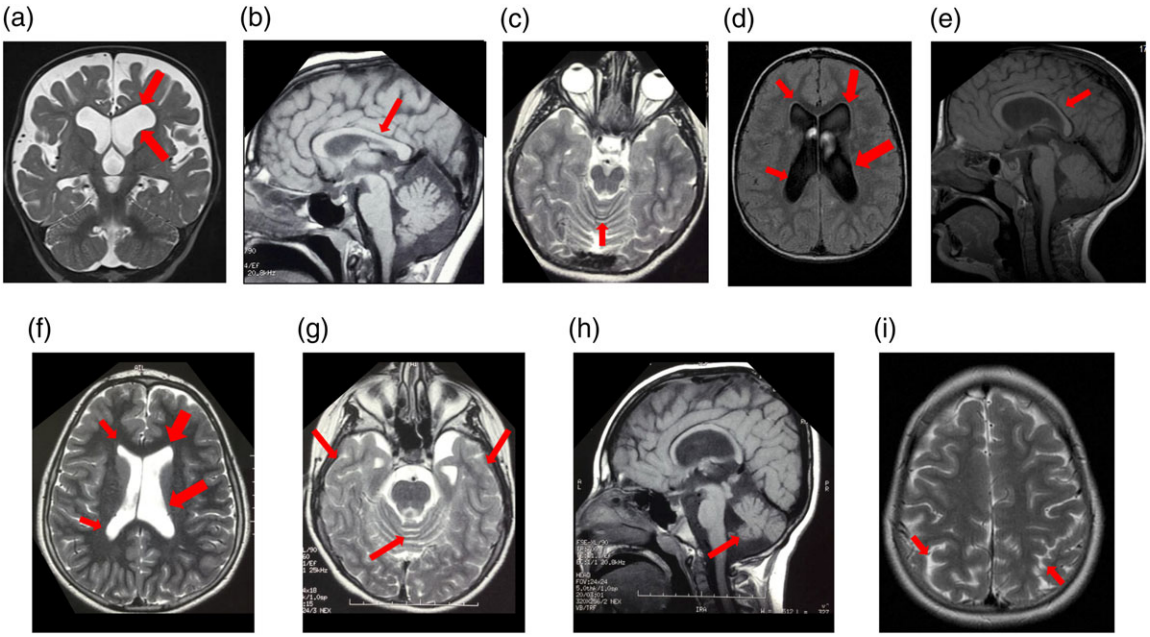

Figure 2 Brain magnetic resonance imaging (MRI) scans. Different brain anomalies were revealed by MRI scan in the patient cohort. Thinning of corpus callosum was found in patients 2 (b) and 4 (e). Hypotrophy of cerebellar vermis was described in patients 2 (c) and 6 (g and h). Asymmetric dilatation of lateral ventricles was revealed in patients 1 (a), 4 (d) and 5 (f). Mild dilatation of parietal sulci was shown in patient 7 (i). [Colour figure can be viewed at wileyonlinelibrary.com]

receptive and expressive speech impairment in all patients provided the indication for cytogenetic studies. The karyotype was analysed in all 12 individuals and found to be normal. Eleven patients were investigated by aCGH. Only patient 10 (mother of patients 8 and 9) was not studied by aCGH but by targeted FISH analysis, after aCGH array showed the 7q11.23 microduplication in her two children (patients 8 and 9).

The 7q11.23 microduplication detected by aCGH was confirmed to be present in practically all studied cells of patients 8 and 9 by FISH. Interestingly, the

microduplication was present in the mother (patient 10) but not in all of her cells: in only 69/108 (~64%) metaphases and 17/25 (68%) interphases was the interstitial 7q11.23 microduplication detected.

Patients 1, 2, 4, 5, 8, 9, 11 and 12 showed an isolated microduplication of sub-band 7q11.23. Patients 8 and 9 inherited the chromosomal anomaly from their mother, while patients 1, 3, 4, 5, 6, 7, 11 and 12 were demonstrated to have a *de novo* microduplication (their parents were negative for the anomaly in aCGH analysis). Origin of microduplication in patient 2 could not be investigated because of the missing compliance of her parents. The sizes of the duplicated regions were heterogeneous within the studied cohort. Eight patients (1, 2, 4, 5, 6, 8, 9 and 12) had an average duplication length of 1.69 Mb, with minimal and maximal extension measuring 1.4 and 2.0 Mb, respectively. The extension of the 7q11.23 microduplication was larger in patient 3 (average length of 2.01 Mb, with minimal and maximal length of 1.71 and 2.31 Mb, respectively), patient 7 (average length of 2.06 Mb, with minimal and maximal length of 1.95 and 2.18 Mb, respectively) and patient 11 (average length of 2.26 Mb, with minimal and maximal length of 1.59 and 2.94 Mb, respectively) (Fig. 3).

Patients 3, 6 and 7 harboured additional chromosomal anomalies. Patient 3 had a microduplication in sub-band 13q14.3, patient 6 presented a microdeletion in sub-band 3q23 and patient 7 showed a microdeletion in sub-band 20p12.1.

Discussion

The broad clinical use of molecular cytogenetic techniques for the etiological investigation of neurodevelopmental delay opened novel views on the clinical consequences of duplication or deletion of dosage-dependent genetic regions (Battaglia *et al.* 2013; Coci *et al.* 2016; Gonzales *et al.* 2016). Our study focused on a less-investigated syndrome – heterozygous interstitial 7q11.23 microduplication – and specifically on its neurophysiological and neuroradiological features.

Global developmental delay with major speech impairment, learning difficulties and behavioural

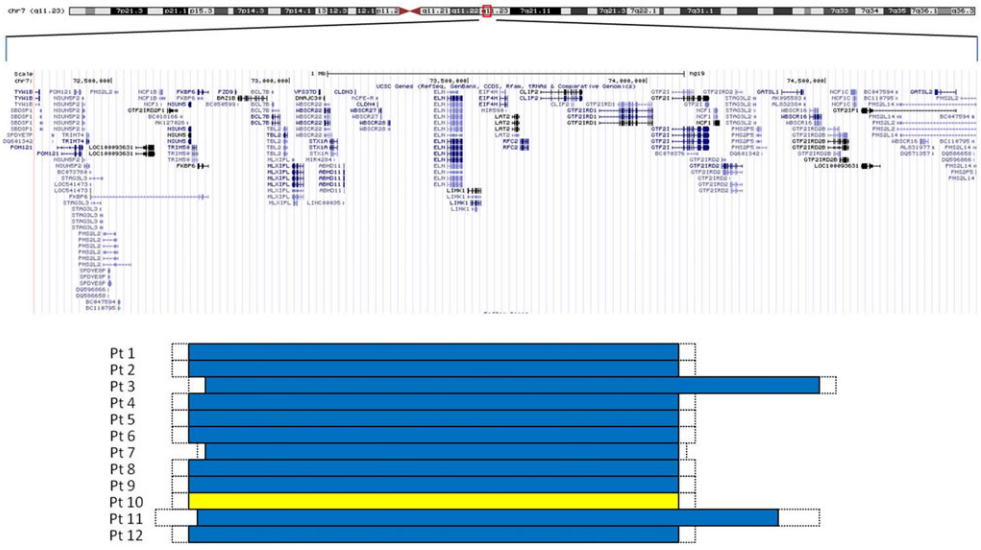

Figure 3 Schematic representation of the duplication lengths revealed in the 12 patients of the study cohort. Eleven probands (blue bars) were tested by comparative genome hybridisation (CGH) array. Of them, nine had an average duplication length of about 1.6 Mb and two of about 2 Mb. Patient 10 (yellow bar) was not tested by CGH array, and the length of her duplication was estimated to be as long as that of her children (patients 8 and 9). For all 12 patients, the coloured bars indicate the minimal duplication length, and the dashed extensions of the bars indicate the maximal duplication length (scheme based on University of California, Santa Cruz, genomic bioinformatics, GRCh37/hg19). [Colour figure can be viewed at wileyonlinelibrary.com]

abnormalities were described in almost all patients harbouring 7q11.23 microduplication. Only one case was reported in association with normal age-matched development and without learning difficulties. In this regard, our study is in line with the former reports, with all 12 patients presenting a delay in expressive and receptive speech development.

From 12 patients, only patient 10 (mother of probands 8 and 9) suffered from epileptic seizures during childhood, but unfortunately, we were not able to provide a detailed description of her epileptic features. Although statistical calculations are difficult because of the small cohort size, 8% of seizure-positive patients are less than the average prevalence (15–20%) reported in several previous studies. Moreover, other studies only reported if the patients had seizures or not but did not reveal any EEG features from the seizure-free patients. In our series instead, we reported the discovered EEG abnormalities. Three out of nine patients had diffuse sharp waves prominent over the anterior and temporal brain regions, although they did not have any epileptic seizures. Because of the small patient number, it is difficult to draw conclusions about the prevalence of the different clinical features in our cohort and to compare them with other clinical studies.

Within the literature, only few studies reported in detail the figurative description of their MRI findings (Depienne *et al.* 2007; Torniero *et al.* 2007; Torniero *et al.* 2008; Prontera *et al.* 2014; Morris *et al.* 2015; Patil *et al.* 2015). Among the nine patients investigated by MRI in our study, a common neuroradiological feature could not be revealed, like in the former 7q11.23 microduplication syndrome studies. Nevertheless, according to our findings in the patients, the symmetric or asymmetric dilatation of cerebral ventricles is the most common structural abnormality of the brain, and hypoplasia of subependymal tissues follows as the second most common one. Hypoplasia of the cerebellum and corpus callosum is less frequent than ventricle dilatation also in our patient cohort. No patients had hippocampal hypoplasia, while it was sporadically revealed in former reports (Torniero *et al.* 2008). Structural abnormalities of the cortex (pachygyria, agyria and polymicrogyria) were absent, while simplified gyral pattern was previously reported only by Torniero *et al.* (2008). The reason why the heterozygous duplication of the WBS region causes hypoplasia of different central nervous system zones remains, to date, not completely understood. Because 7q11.23 band harbours different DNA methylation-controlling genes (like *GTF2I* and *GTF2IRD1*), it can be speculated that the overexpression of one or more genes of this region causes the methylation-dependent under-regulation of other genes involved in brain tissue development (Strong *et al.* 2015).

Our cohort includes 10 patients with a duplication of the 'classic' WBS region. Few studies on 7q11.23 duplication syndrome tried to address which genes mapping in this band have a role in causing the pathological phenotype. Duplication of *CLIP2*, *FZD9*, *LIMK1* and *STX1A* has been hypothesised to be responsible for social behaviour anomalies, because their encoded proteins are involved in synapse formation and maintenance (Merla *et al.* 2010; Kaminsky *et al.* 2011; Levy *et al.* 2011; Sanders *et al.* 2011). In order to assess the functional consequences that dosage misbalances of the previously mentioned genes have on human behaviour, different mouse models harbouring either one or three copies of the single genes mentioned previously were established by different groups (Hoogenraad *et al.* 2002; Meng *et al.* 2002; Zhao & Pleasure 2005; McRory *et al.* 2008). *ELN* appears to be also sensitive to dosage changes. In fact, its haploinsufficiency (in WBS) was often associated with supravalvular aortic stenosis, and its duplication was revealed to be in association with aortic valve insufficiency. Because *GTF2IRD1* is highly expressed in craniofacial tissues, it has been claimed that its underexpression (in WBS) or overexpression (in 7q11.23 microduplication) could be involved in causing craniofacial abnormalities (e.g. tooth anomalies, brachycephaly and skull asymmetry) (Ohzama & Sharpe 2007). *GTF2I* maps at the telomeric end of 'classic' WBS region, and at least the first part is included within the duplication of 'classic' WBS region. By using a *gtf2i*$^{+/dupl}$ mouse model, a clear correlation was demonstrated between gene copy number and anxiety grade of the pups after separation from their mother (Mervis *et al.* 2012), resembling the anxiety behaviour of the affected children.

Apart from 10 patients with a 'classic' WBS region duplication, patients 3 and 11 had larger duplications, with *GFT2IRD2*, *STAG3L2*, *PMS2P5*, *GATSL1* and *WBSCR16* in patient 3 and *GFT2IRD2*, *STAG3L2* and *PMS2P5* in patient 11. Comparing the entire

clinical features of the 10 patients with 'classic' WBS region duplication with those of the two patients with 'atypical' duplication, we cannot claim that specific findings appear exclusively in combination with the larger duplication.

Interestingly, patient 10 had a mosaic condition in her peripheral blood cells; that is, only ~64–68% of her blood cells harboured the 7q11.23 microduplication. This can be explained in two ways: either the microduplication developed as a post-zygotic event and thus is not present in all her body cells, or the microduplication was present already in the zygote. In the latter case, a post-zygotic reversing mutation (e.g. by either loss of derivative chromosome 7 or monosomic rescue) would have to be postulated. As her phenotype is not less severe than that of other comparable cases – including her own children – she did not profit from her mosaic status detectable in her peripheral blood.

In patients 3, 6 and 7, the anomaly affecting chromosome band 7q11.23 was accompanied by one additional chromosomal abnormality, which we searched in the international data bank for human genomic structural variation DGV. Because the clinical pathophysiology of 13q14.3 microduplication, 3q23 microdeletion and 20p12.1 microdeletion syndromes has not been described in DGV, it is not possible to discuss about their pathogenic influence in the clinical syndrome of the patients mentioned previously.

Conclusions

Taken together, our study underlines the overall importance of performing genetic studies in patients affected by neuropsychological delay, because the results can be advantageous for understanding the patient's prognosis as well as for supporting the parents in the further family planning. The heterozygous duplication of 7q11.23 band is a rare chromosomal anomaly, which causes a syndrome characterised by some features common to every affected individual (severe speech delay, neurodevelopment delay and intellectual delay) and other heterogeneous findings (facial anomalies, seizures, congenital cardiac defects and urogenital anomalies). Our study investigated 10 'classical' and two 'atypical' cases and emphasised their neuroradiological and neurophysiological features.

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