3q26.33-3q27.2 microdeletion: a new microdeletion syndrome?

3q26.33-3q27.2 MICRODELETION: A NEW MICRODELETION SYNDROME?

Giorgia Mandrile[a], Anna Dubois[b], Jodi D. Hoffman[c], Vera Uliana[a], Emilio Di Maria[a,d], Michela Malacarne[e], Domenico Coviello[e], Francesca Faravelli[a], Simon Zwolinski[b], Stephen Hellens[b], Michael Wright[b], Francesca Forzano[a]*.

[a] Division of Medical Genetics, Galliera Hospital, Genova, Italy

[b] Northern Genetics Service, Newcastle upon Tyne Hospitals, Newcastle upon Tyne, United Kingdom

[c] Division of Genetics, Floating Hospital for Children,Tufts Medical Center, Boston, MA

[d] Department of Health Sciences, University of Genova, Italy

[e] Laboratory of Human Genetics, Galliera Hospital, Genova, Italy

corresponding author

* Francesca Forzano, M.D.

Division of Medical Genetics, Galliera Hospital

Via Volta, 6 - 16128 Genova – Italy

e-mail: forzanof@galliera.it - phone +39 0105634367 - fax +39 01057481026

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ABSTRACT

We describe three unrelated patients of European descent carrying an overlapping 3q26.33-3q27.2 microdeletion who share common clinical features: neonatal hypotonia, severe feeding problems, specific facial features, abnormal dentition, recurrent upper airways infections, developmental delay and severe growth impairment. One of the patients carries a smaller deletion and presents a milder phenotype. We propose that 3q26.33-3q27.2 microdeletion may represent a novel condition caused by the haploinsufficiency of dosage sensitive genes, several of which are involved in brain development.

1. INTRODUCTION

Intellectual disability (ID), isolated or associated with congenital anomalies, is a significant clinical and social problem, involving 1-3% of the general population worldwide [1]. Chromosomal microarray techniques can identify a submicroscopic unbalanced chromosomal rearrangement in up to 15% of patients affected by ID [2], occasionally leading to the identification of 'new' microdeletion/duplication syndromes. International databases of chromosome imbalance such as DECIPHER (http://decipher.sanger.ac.uk) are extremely useful in matching patients carrying similar chromosomal rearrangements.

Here we describe three unrelated patients of European descent, 2 of them reported in DECIPHER data base, carrying an overlapping 3q26.33-3q27.2 microdeletion and sharing common clinical features. We therefore suggest that 3q26.33-3q27.2 microdeletion may represent a novel condition caused by the haploinsufficiency of dosage sensitive genes within this region.

2. PATIENT DATA

2.1 Patient 1

Patient 1 was the only child of healthy non consanguineous parents who presented at 2 years and 8 months of age. Intrauterine growth restriction (IUGR) was noted early in the pregnancy; biochemical screening for Down syndrome and serial ultrasound scan did not reveal placental or fetal abnormalities. The mother reported normal fetal movements.

Cesarean section was performed at 32 weeks gestation due to growth arrest: birth weight was 1380 g (10-25th centile, according to Hall growth curves [3]), birth length 39.5 cm (10-25th centile), head circumference (OFC) 29.5 cm (50th centile). In the neonatal period, he showed bronchopulmonary dysplasia of prematurity, marked hypotonia, muscular

hypotrophy, inguinal hernia and patent ductus arteriosus, which resolved after pharmacological treatment. Brain ultrasound, electroencephalography (EEG), routine blood analysis, plasma aminoacids and mucopolysaccharide screening were normal.

The baby had severe feeding problems and gavage feeding was used for the first 2 months of life. Feeding difficulties have only slightly improved. At 6 years of age, he is not yet able to chew and can only eat homogenized food. Oral aversion and lack of appetite are consistently reported. Gastroesophageal reflux and frequent vomiting require continued omeprazole treatment.

He also presented with recurrent upper airway infections, bladder diverticula, vesicoureteric reflux and retractile right testicle which required orchidopexy.

Results of Auditory Brainstem Response (ABR) were inconclusive due to the patient's poor cooperation, however the parents did not suspect hearing difficulties. Ophthalmological examination revealed mild myopia and astigmatism (wears corrective lenses) and normal fundus oculi.

He wears orthopedic insoles for pes planus.

Development was delayed: head control was achieved at 10 months of age, he sat at 17 months of age and babbled at 18 months of age. At 6 years of age, he has not developed speech and does not have bladder or bowel sphincter control. Teeth eruption was markedly delayed: at 6 years of age dentition is still incomplete, with the upper jaw more involved.

Growth parameters at 2 years 8 months and 6 years of age all plotted between -3 and -4 standard deviations (SD) (Table 1). Parental stature was at the 25^{th}-50^{th} centile (mother 160cm and father 173 cm).

On physical examination we noted thin skin with very little subcutaneous fat, mild pectus carinatum, mild kyphosis, joint laxity, clinodactyly of the fourth toes. The third toes overlap the fourth toes (also reported in the father).

2.2 Patient 2

Patient 2 was the second child born to healthy unrelated parents. He was first seen in clinic at one month of age. He is now eighteen years of age. He was noted to have IUGR and was born by emergency Cesarean section at 37 weeks gestation. He needed resuscitation at birth for poor respiratory effort. His birth weight was 1590 g (<3rd centile [3]) and his OFC at birth was 29.5 cm (<3rd centile [3]). He was hypotonic with a prominent nasal bridge, short philtrum, micrognathia and undescended testes. At three hours of age he had a tonic seizure with apnoea. He had several more seizures over the first 24 hours of life. He was started on phenobarbital and broad spectrum antibiotics and the seizures resolved. An EEG showed no background seizure activity, cranial ultrasound was unremarkable apart from a small cyst adjacent to the choroid plexus, and blood chemistries and metabolic screening were normal. G-banded karyotype was reported as 46,XY.

He was able to bottle feed initially, however, gastrostomy feeding was required from 2 to 13 years of age for poor weight gain. He had conductive hearing loss and required grommets.

He has micropenis and cryptorchidism and required testosterone therapy at puberty. He has pes planus and abnormal foot position for which he wears orthotics.

His development was delayed. By 4 years of age he was walking and had several single words. He progressed to being able to speak in short sentences. He has a learning disability and behavioural difficulties.

His teeth were slow to erupt and by the age of fifteen years he still had his primary dentition, despite his adult teeth erupting. He has bilateral keratoconus. At the age of 16.75 years, his height was 147.6 cm (-4SD) and his weight 30.8 kg (-3 to -4 SD).

2.3 Patient 3

Patient 2 was the second child of non-consanguineous healthy parents. Family history was non-contributory. She was evaluated at the genetics clinic at 11 years of age regarding a possible diagnosis of Williams-Beuren syndrome due to a history of failure to thrive, hypotonia, attention deficit disorder (ADHD) and an extremely friendly personality. The

pregnancy was complicated by chronic polyhydramnios and intrauterine growth restriction. A level 2 ultrasound had shown possible problem with the kidneys (ultrasound report unavailable). During the pregnancy, cigarette smoking was reported (5-10 cigarettes per day). Due to the risk of preterm labor, the mother was given cortisone at 32 weeks gestation. The child was born at 38 5/7 weeks gestational age via Cesarean section secondary to breech presentation; birth weight was 1975 g (<<3rd centile [3]), length 43.82 cm (<<3rd centile [3]), OFC 32 cm (10-25th centile [3]). APGAR scores were 8/9. At birth the following features were noted: cutis aplasia of scalp (2,5 x 3,8 cm), with underlying bony defect, hypotonia, edema of the genitals, and sacral dimple. She had poor suck and swallow with feeding issues resulting in projectile vomiting, often through the nose. She cried inconsolably, stiffened frequently and had gastroesophageal reflux requiring therapy with ranitidine and metoclopramide. She required multiple formula changes and required two to three hours to take two to three ounces of formula. For many years, she had difficulty growing, prompting cystic fibrosis screening which was negative. Her weight did not normalize until approximately nine to ten years of age and she has become overweight for height since that time.

Frequent episodes of abdominal pain, diarrhea and constipation were reported.

Primary teeth loss was delayed and teeth were small, pointed, and crowded; all but one required extraction. Moreover, salivary thickening was noted. She choked frequently when drinking.

The child has never had a seizure, but does stare for several minutes at a time. Brain MRI showed a 12 mm Chiari malformation, crowding of the foramen magnum, narrowing of the inferior fourth ventricle, limited flow of cerebrospinal fluid through the foramen magnum causing ventriculomegaly involving the third and lateral ventricles. Glasses were used because of astigmatism and myopia.

Frequent episodes of otitis media were reported, with intermittent decreased hearing

secondary to fluid. She is very sensitive to loud noises, becoming anxious and upset. She was recently noted to have acanthosis nigricans and “pre-diabetes”. She reportedly urinates frequently and drinks excessively. She has enuresis and frequent daytime incontinence. She has a history of hypermobility of the hips. She has a history of eczema.

Her development was delayed; she showed gross motor impairment with decreased hip strength and poor posture control with delays in fine motor skills. She walked at 20 month of age and language was slightly delayed. She had a learning disability and she required occupational, physical, speech therapy, and social skills training.

She excels in verbal areas, spelling, and reading, has average working memory, and has difficulty with maths and step-wise work. She learned to tie her shoes at age 11 years of age. She has inconsistent visual skills and poor writing skills. She was diagnosed with ADHD, is overly affectionate with those around her and has an extreme sense of empathy.

At 12 years 1 month of age, her height was at the 3rd centile, OCF at 10th centile, and weight at 50th centile. Paternal stature was 178 cm (50-75th centile [3]) and maternal stature was 178 cm (>95th centile [3]).

On physical examination we noted frontal bossing and mild temporal narrowing. Ears were mildly simple and somewhat thickened, measuring 5.2 cm (-2SD). Eyes had mildly narrow horizontal openings with normal slant. She had fused lower incisors, mildly flat and short philtrum, and mild retrognathia. Her labia minora were hypoplastic with a hypoplastic pubic pad. Fingers were mildly tapered with mild flattening of the ulnar border. She had multiple freckles of the left forearm and café au lait spot of left lower flank with mildly decreased tone.

3. METHODS AND RESULTS

3.1 Patient 1

Array-CGH performed using Human Genome CGH Microarray Kit 4 x 44K (Agilent)

detected a de novo 4.14 Mb microdeletion from 181.648.378 (3q26.33) to 185.786.898 (3q27.2). This result was confirmed through FISH (BAC – Sanger Center- RP11-63M3 3q27.1); the same FISH has been performed to analyze parents' samples. The deletion involves 43 genes (Genome assembly Feb. 2009 – UCSC hg19) (Fig. 1).

3.2 Patient 2

Array-CGH performed using a Sanger 1Mb BAC array (Build 36) detected a de novo 4.28 Mb microdeletion from 181.692.255 (3q26.33) to 185.969.168 (3q27.2). This result was confirmed by re analysis of the G-banded Karyotype which demonstrated that in hindsight the deletion was visible microscopically. The deletion involves 44 genes (Genome assembly Feb. 2009 – UCSC hg19) (Fig. 1). Parental samples were analyzed by. G-banded karyotype and these showed no evidence of the microdeletion. This confirmed that the deletion had arisen as a de novo event.

3.3 Patient 3

Array-CGH performed using Affymetrix 6.0SNP array kit detected a 2.09 Mb microdeletion from 183.047.473 (3q27.1) to 185.140.522 (3q27.2). This result was confirmed through FISH. The deletion involves 36 genes (Genome assembly Feb. 2009 – UCSC hg19) (Fig. 1). Maternal testing via FISH was normal (BAC-RP11-63G1 3q27.1-q27). Father unable to be tested to date.

4. DISCUSSION

Three patients are described with an overlapping 3q26.33-3q27.2 microdeletion, sharing specific features that could make this phenotype clinically recognizable (Fig. 2; Table 1). Patient 3 carries a smaller deletion, entirely embedded within the region deleted in the other two patients, and presents a milder phenotype, mainly with respect to intellectual disability and growth.

This disorder is possibly secondary to contiguous gene deletion. At least 40 genes are

located within this region (Fig. 1). At least six genes may be involved in brain development and function, particularly: *DCUN1D1*, for which the mouse ortholog is expressed in proliferating neuroblasts [4]; *DVL3*, a regulator of cell proliferation and neuroblast specification [5]; several genes belonging to the serotonin receptor family (*HTR3C, HTR3D, HTR3E*); *KLHL24*, involved in functional regulation of glutamate receptors [6]; *MAP6D1*, a neuronal protein that binds microtubules and plays a role in synaptic function in neuritis [7]. Several of the genes included in the deletion have already been associated with human pathologies, with both autosomal recessive and dominant transmission. Our patients did not show any symptoms of autosomal recessive diseases associated with mutations in the genes mapping to this region (e.g. *ALG3*, *MCCC1, EIF2B5, LIPH*) and therefore we did not sequence the contralateral alleles.

Within the common deleted region is the gene *THPO*. Gain of function mutations in this gene have been shown to be responsible for hereditary thrombocythaemia [8,9].

Patient 1 showed reduced platelet concentration ($127x10^3$/ul, n.v. 150-400) and patient 3 had platelet concentration at the low end of normal ($168x10^3$/ul, n.v. 140-400). Further assessment of the possible role of deletion of THPO in thrombocytopenia may be appropriate.

*CLCN*2, a gene for which the mice knock-out model of its homolog demonstrates leukoencephalopathy [10] and degeneration of retinal and testicular cells, leading to male infertility is present in the deleted region [11]. The *CLCN2* rat homolog is expressed in fetal lung and its expression rapidly decreases after birth; therefore its deficiency may be related to the neonatal respiratory distress [12] and testicular anomalies observed in our patients.

Several genes related to body growth are present in the deleted region. In all three patients *PARL* is deleted: the knock-out mouse (Parl -/-) shows growth retardation, cachexia and muscle atrophy. In patients 1 and 2 the *ETV5* gene is deleted. These patients present with more severe growth impairment than patient 3. ETV5 is a member of the ETS transcription

factors, ubiquitously expressed during development and adulthood. *ETV5* homolog knock-out mice show a progressive loss of spermatogonial stem cells and reduced body weight [13].

Several segmental duplications have been reported close to the deleted regions' breakpoints. The three deletions present in our patients did not share common breakpoints, and cannot be considered recurrent CNVs, but it could be postulated that the whole region might be susceptible to non-homologous recombinations leading to chromosomal rearrangements.

In conclusion, based on our observations, we propose that 3q26.33-3q27.2 microdeletion constitutes a new clinically recognizable syndrome characterized by growth deficiency, severe feeding problems, hypotonia, muscular hypotrophy, dysmorphic features, and intellectual disability.

Clinical and molecular characterization of additional patients with a similar chromosomal deletion are needed to further delineate the phenotype and refine the minimal critical region.

CONFLICT OF INTEREST

Authors have no conflict of interest.

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LEGENDS OF FIGURES

Figure 1: schematic representation of 3q26.33-3q27.1 region with the minimal overlapping microdeletion and genes involved. Segmental duplications are indicated in pale grey when the level of similarity is 93 - 95.99%, and dark grey when 90 - 92.99%.

Figure 2: Patient 1 (A and B: age 6 years), Patient 2 (C and D: age 8, E and F: age 11), Patient 3 (G and H: age 1 years). Note common dysmorphisms: flat facial profile, medially sparse eyebrows, epicanthal folds, flat nasal bridge and tip, mild shortening of the philtrum Patient 1 and 2 share also downturned corners of mouth and small and spaced teeth at the lower jaw. In Patient 1, note also anteverted nares.

TABLE

Table 1: comparison of patients' clinical features

Phenotypic characteristic	Patient 1	Patient 2	Patient 3
DECIPHER ID	257773	1495	-
Sex	Male	Male	Female
Age at last examination	6 years	17 years	12 years
Size of the deletion	4.14 Mb	4.28 Mb	2.09 Mb
IUGR	Yes	Yes	Yes
Birth parameters			
Gestational age	32 w.g.	37 w.g.	38+5 w.g.
Birth weight	1380 g (-1.22 SD)	1590 g (-3.03 SD)	1975 g (-3.53 SD)
Birth length	39,5 cm (-1.21 SD)		43,82 cm (-4.12 SD)
OCF at birth	29,5 cm (-0.28 SD)	29,5 cm(-3.16 SD)	32 cm (-3.20 SD)
Feeding problems	Yes	Yes	Yes
Slender habitus/little subcutaneous fat	Yes	Yes	Until age 10 years
Height (at last examination)	98 cm (-3.68 SD)	147.6 cm (-4 SD)	136.2 cm (-2 SD)
Weight (at last examination)	13 Kg (-3.62 SD)	30.8 kg (-3/-4 SD)	40.8 kg (0 SD)
OCF (at last	46 cm (-4.44 SD)		51.2 cm (-1.15 SD)

examination)			
dysmorphic features			
flat facial profile	Yes	Yes	Yes
medially sparse eyebrows	Yes	Yes	Yes
epicanthal folds	Yes	Yes	Yes
flat nasal bridge and tip	Yes	Yes	Yes
anteverted nares	Yes	No	No
short philtrum	Yes	Yes	Mild
downturned corners of mouth	Yes	Yes	No
Eyes	Myopia/astigmatism	Bilateral keratoconus	Myopia/astigmatism
Teeth	at 6 years dentition is incomplete (upper jaw more compromised)	at 15 years still had primary dentition, despite adult teeth erupting	pointed, delayed falling out, wide spaced, fused lower incisors
Ears	Preauricular pit		mildly simple and thickened
Thin skin	Yes	Yes	No
Hand	Clinodactyly IV digit		Mildly tapered fingers/flattening of the ulnar border
Foot	Pes planus	Pes planus	Mild pes planus

Skeletal	Mild kyphosis Mild pectus carenatum		hypermobility of the hips
Genitalia	Retractable lift testicle	Undescended testes Micropenis	Hypoplastic labia minora and pubic pad
Psychomotor development			
Delayed milestones	Yes	Yes	Yes
Speech	Not acquired	Speaks in sentences	Good
Walking	Not acquired	4 years	20 months
Mental retardation	Severe	Severe	Learning disability, borderline IQ
Behaviour	Normal	Hyperactivity	ADHD
Neurology	Hypotonia	Hypotonia Tonic seizure at birth	Hypotonia Chiari malformation, Ventricular malformations
Other abnormalities	Recurrent upper airway infections Patent ductus arteriosus Gastroesophageal reflux Inguinal hernia Bladder diverticula	Recurrent otitis media Mild hearing impairment (grommets) Delayed puberty (testosterone therapy at puberty)	Recurrent otitis media Gastroesophageal reflux