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A new case of rare proximal 3q13 interstitial deletion

Case Report

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Abstract: We present the case of a 20-year-old man referred to the clinical geneticist because of mental retardation and dysmorphic features because of concerns about hereditability when his older, healthy brother was expecting a child. Deletion of proximal 3q arm was found with standard G-banding, and array comparative genomic hybridisation (array-CGH) was used to further locate the breakpoints. A unique interstitial deletion del 3q13.11q13.33 was confirmed. The first clinical symptoms in the 20-year-old were described at the age of 4 months when the pediatrician reported muscle hypertonia of the lower limbs, which later evolved into hypotonia. Later clinical observations revealed that the patient's psychomotor development was delayed: he exhibited craniofacial abnormalities, cryptorchidism, thoracic kyphosis, and tapering fingers. Interstitial deletions of the proximal long arm of chromosome 3 have rarely been reported:; there are only 12 previously reported cases. The breakpoints and sizes of described deletions vary greatly, which makes definite genotype-phenotype conclusions impossible at this time. Developmental delay is one of the common features described in the majority of reported cases. The BTB-zinc finger gene *ZBTB20* might be a potential candidate gene: it was shown in the mouse hippocampus to be expressed during the important period of neurogenesis of pyramidal neurons. Also, four of patients reported to date had agenesis of the corpus callosum and one, holoprosencephaly. We suggest that the *GAP43* gene is involved in the development of structural neurological abnormalities in patients with 3q deletion.

Keywords: *3q13.11-q13.33 • Array CGH • Interstitial deletion • Mental retardation • GAP-43 • ZBTB20*

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1. Introduction

For more than four decades, the search for chromosomal rearrangements in patients with developmental delay, different birth defects, and dysmorphisms has depended on standard chromosome analysis using GTG-banding. Analysis with the so-called conventional karyotyping has a limited resolution of 5 to 10 Mb. Nevertheless, many different syndromes have been successfully defined and diagnosed in this way.

There have also been many different case reports where only a few cases of similar chromosomal abnormalities have been described in the literature and no definite syndrome has been proposed; this includes 3q deletions. Deletion of the chromosome 3q proximal region is very rare. Only 12 cases have been described to date, with rearrangements varying greatly in size and in breakpoints [1-11]. As expected from variable rearrangements, the phenotypic characteristics of the reported cases were also diverse. Clinical manifestations that were present in more than two patients included developmental delay, facial dysmorphism, brain anomalies, muscle hypotonia, genitourinary and skeletal abnormalities, and cardiac defects. Three genes have been proposed to be responsible for these clinical manifestations: Shimojima *et al* [11] described the smallest deletion of the proximal 3q region and concluded that the deleted BOC gene might be responsible for muscle hypotonia. The same group also suggested that a deleted DRD3 gene is involved in neurological symptoms such as developmental delay and dysarthria. Simovich *et al* [10] described another relatively small deletion in the proximal 3q region and suggested that the deleted CBLB gene is responsible for the craniofacial phenotype in patients with proximal 3q deletion.

We report another case of small deletion encompassing 3q13.11-q13.31, which was initially identified using conventional karyotyping and further investigated with array-CGH.

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2. Case report

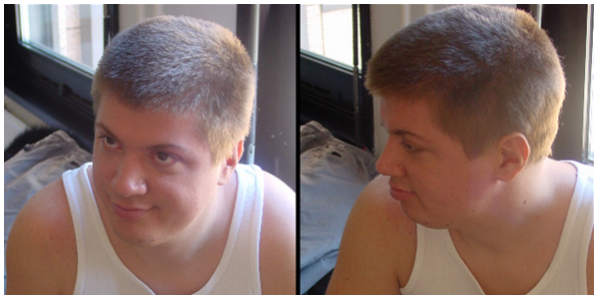
Our patient was a 20-year-old man who was referred to our Institute for the evaluation of dysmorphic features and developmental delay. He was born to a 26-year old gravida-2, para- 2 healthy mother and a 27-year-old healthy father. He had a healthy older brother. There was no family history of mental retardation, birth defects, infant death, stillbirth, recurrent miscarriages, or chromosomal rearrangements.

His prenatal history revealed no complications and no documented exposure to medications, alcohol, or smoking. The boy was born at term by normal vaginal delivery with birth weight 3350 g, birth length 50 cm, and Apgar score 9/10.

At the age of 4 months, first clinical symptoms were described when the pediatrician reported muscle hypertonia of the lower limbs, which later evolved into hypotonia.

The patient's motor and language development were delayed; he did not sit until 11 months and started walking only when he was 17 months old. At 24 months, he had about 25 words in his vocabulary. At 24 months of age, the social quotient (SQ) using The Vineland Social Maturity Scale and the developmental quotient (DQ) using Brunet-Lézine's psychomotor developmental test were calculated: the SQ was 0.54 and the DQ 0.71. Both receptive and expressive language skills were also severely delayed.

Figure 1. Craniofacial characteristics in patient with 3q13.11q13.33 deletion. Prominent forehead, low hairline, broad nasal bridge, long face, mild degree of hypertelorism with moderate synophrys and thin upper lip can be seen.



Cryptorchidism was present and surgically corrected. He was examined regularly, and endocrinological and/or neurological abnormalities were ruled out. He was first seen by a clinical geneticist at the age of 20 years. His psychomotor development was delayed, and he attended a special school. His head circumference was 61 cm; height, 190 cm (98th centile); and weight, 95 kg (98-99th centile). He exhibited craniofacial abnormalities including a prominent forehead, low hairline, broad nasal bridge, and long face (Figure 1). He had mild hypertelorism and moderate synophrys. The upper lip was thin. He had a high-arched palate and crumbled teeth. The external ears were simple. Musculoskeletal examination revealed thoracic kyphosis and tapering fingers. No other abnormalities were detected.

3. Materials and methods

3.1 Cytogenetic banding

Peripheral lymphocytes were cultured and harvested using standard techniques.

3.2 Array-CGH analysis

Genomic DNA was extracted from the peripheral blood sample using QIAamp DNA Blood kit (Qiagene, Hilden, Germany). 1.5µg of each genomic patient DNA and reference DNA was fragmented, cleaned, concentrated (Zymo Clean and Concentrator Kit), and labeled with Cy3 or Cy5 (Bioprime DNA Labeling System, Invitrogen; Cy3and Cy5, Amersham Biosciences, Buckinghamshire, UK). Labeled DNA was hybridised to high resolution CGH micro-array containing 3000 BAC clones with an average resolution of 1Mb (Array Genomics, Voisins Le Bretonneux, France), washed, scanned, and analysed according to the manufacturer's protocol. Colour reverse experiments were performed to validate the results.

4. Results

Chromosome studies using standard G-banding revealed an interstitial deletion of the proximal long arm of chromosome 3, located between 3q12-q13. Because of the resolution limits, we were unable to identify the precise breakpoints. Therefore, array-CGH was applied. The analysis of parental chromosomes showed normal karyotypes.

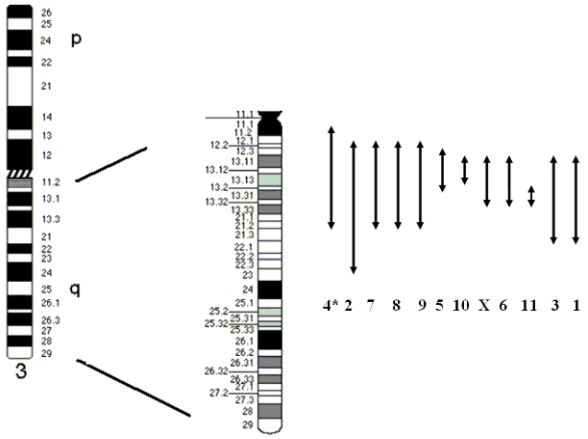
Using array CGH analysis, the deletion on chromosome 3 was mapped to 3q13.11-q13.31. The last proximally present clone was DBACA5ZA08V (position according to the UCSC Human Genome Browser, assembly February 2009 (GRCh37/hg19) is 101.843.998-102.031.801), and the first proximally missing clone was RP11-91B3 (position 105.304.420-105.483.270). The last distally missing clone was RP11-217N3 (position 115.284.915-115.439.893) and the first distally present clone was RP11-373C21 (position 117.180.037-117.347.710). This confirmed an approximately 10 Mb large deletion of the proximal long arm of chromosome 3 and mapped it more accurately.

According to the UCSC Human Genome Browser, 75 hypothetical or known genes map to the above mentioned deleted region. Gene products with known functions are involved in different cellular processes, for example, signal transduction (genes CBLB and CD47) and transcription (genes BBX and IFT57); also, some genes code for structural parts of different receptors (genes BOC, ATP6V1A). For majority of genes, the precise function in humans is still unknown.

5. Discussion

The rapid development of molecular cytogenetic technologies, such as array-based comparative genomic hybridization (array-CGH), is blurring the boundaries between cytogenetics and molecular genetics. The resolution limit is defined by number and spacing of clones or oligonucleotides on the microarray; therefore, one can expect essentially limitless resolution in the foreseeable future. In the cases of individuals with nonspecific symptoms and no clear suspicion of genetic syndrome/disease, the clinician can use the so-called "genotype-first" approach to define genotype-phenotype interactions in rare chromosomal imbalances [12]. Using this approach, one can define groups of individuals with similar chromosomal abnormalities and then later clinically examine the group to characterize correlated phenotypic features. In addition, the International Standard Cytogenomic Array (ISCA) Consortium recently reported a consensus statement on the use of array-CGH instead of standard G-banded karyotyping as the first-tier cytogenetic diagnostic test for patients with developmental delay/intellectual disability, autism spectrum disorders, or multiple congenital anomalies [13]. Therefore, we can expect that more and more reported chromosomal aberrations will be defined using array-CGH technologies.

Figure 2. Previously described deletions which included the region that was deleted in our patient. (* numbers of respective references, X – our case report)



We present a case with a unique interstitial deletion 3q13.11q13.33 that was thoroughly characterized using classical karyotyping and BAC array-CGH. One of the mechanisms for occurrence of unbalanced chromosomal rearrangements is non-allelic homologous recombination between different low copy number repeats (LCRs). In the deletion reported herein, there are two small LCRs at each proximal and distal breakpoint, but neither can explain the formation of this interstitial deletion.

To date, deletions involving the chromosome 3q proximal region are very rare; a defined clinical phenotype has not yet been established. Only 12 patients have been described [1-11], and all except two presented with bigger deletions than that we report in this case (Figure 2).

The group of 12 reported cases with proximal 3q deletion is very heterogeneous based on the breakpoints and size of the deletion, which makes definite genotype-phenotype conclusions impossible at present. In general, larger deletions are associated with more severe phenotypes. In addition, the majority of those cases were analysed with conventional karyotyping only, which does not define the deletion limits in sufficient detail to allow a decision on which genes are included. Besides our case, only two of the previously reported individuals with proximal 3q deletion have been assessed using a molecular cytogenetic technique [10,11].

However, the reported patients share some common features. Four of the previously reported patients had agenesis of the corpus callosum, and one had holoprosencephaly. It has already been suggested [3,6] that the deleted region includes a gene involved in neuronal migration and formation of the corpus callosum, but no candidate gene has been proposed. An interesting candidate gene involved in this processes might be *GAP43*, which has been termed a 'growth' or 'plasticity' protein because it is expressed at high levels in neuronal growth cones during development and axonal regeneration [14]. Moreover, it has been shown in a mouse model of *GAP43*-deficiency that *GAP43* is required for commissure formation [15]. None of the three communications between the telencephalic hemispheres, anterior commissure (AC), hippocampal commissure (HC), and corpus callosum (CC) formed in any of the *GAP43* (-/-) mice in vivo. Interestingly, in mice with one functional and one disrupted allele *GAP43* (+/-), AC was not altered, whereas both HC and CC were missing or reduced. In 39% of the mice, both CC and HC were totally absent, and in 61% both were significantly reduced. Two recently reported cases [10,11] with smaller deletion than in our case are in accordance with our suggestion that *GAP43* might be involved in neuronal migration

Table 1. **Clinical features in reported cases with proximal 3q deletion.**

	Jenkins et al. (1985)	Fujita et al. (1992)	Mackie Ogilvie et al. (1998), c1	Mackie Ogilvie et al. (1998), c2	McMorrow et al. (1986)	Okada et al. (1987)	Kosaki et al. (2005)	Simovich et al. (2008)	Our case	Lawson-Yuen et al. (2006)	Shimojima et al. (2009)	Arai et al. (1982)	Genuardi et al. (1994)
Deletion	3q11q21	3q12q23	3q12q21	3q12q21	3q12q21	3q12q21	3q12.2q13.2	3q13.11q13.12	3q13.11q13.33	3q13.1q13.3	3q13.2q13.31	3q13q21	3q13.2q21.3
Gender, Age at report	F, 8y	M, 6y	F, 4y	M, 17mo	M, Infant	F, 8y	M, at birth	M, 20mo	M, 20y	F, 4y	F, 3y 2mo	M, neonate	M, 5mo
Developmental delay	+	+	+	+	NA	+		-	+	+	+	NA	
CNS		-	-	ACC	ACC, Ventriculo megaly			-	-	ACC	-	Holoprosen cephaly	ACC
Craniofacial													
Microcephaly	-	+	-	-		+			-	-	-	+	-
Prominent forehead			-	+				+	+	-	-		
Hypertelorism	-		-	+		+		+	+	-	-		+
Epicanthal folds	+		-	+		+		+	-	-	+		+
Broad/flat nasal root		+		+		+		+	+	+	+		+
Small nose/ anteverted nares								+	-	+	+		+
Ears		Large		Over-folded			Low-set	Large	Simple	NL	NL		Low-set
Palate			High arched	High arched		High arched	High arched	NL	High arched	NL	NL	Cleft	
Genitourinary													
Cryptorchidism	NA	-	NA	-	+	NA		+	+	NA	NA	+	+
Hypoplastic penis	NA	-	NA	-	-	NA		-	+	NA	NA	+	+
Scoliosis	+	+	-	+		+		-	+	-	-		
Hypotonia	+	+	+	+		+		-	+	+	+		+
Other	Pterygium, webbed neck, puffy hands and feet, Ptosis	Talipes equinovarus, Joint contractures, Cataract, Exotropia, Ptosis	Myopia Unusual palmar crease on one hand	Lordosis, Kyphosis, hypoplastic pelvis	Hydronephro-sis, Talipes equinovarus, Joint contrac-tures	Talipes equinovarus Joint contractures Duplicated colleting renal system	OEIS, Midface hypoplasia, Pointed chin	Tetralogy of Fallot, Brachydactyly, Broad great toes	Tapering fingers	Strabismus	Joint hyperlaxity, valgus feet	Cleft lip, PDA	Talipes equinovarus, Joint contractures, puffy feet, low-set ears

** M-male, F-female, y-years, mo-months, NA-not applicable, NL-normal, blank space-no data available, ACC-agenesis corpus callosum, OEIS-Omphalocele-extrophy-imperforated anus defect*

and regulation of formation of corpus callosum. Neither of these cases has agenesis of the corpus callosum and the *GAP43* gene was not deleted in the patients; whereas in reported cases with larger deletions including deletion of *GAP43*, there are four cases with agenesis of the corpus callosum.

Developmental delay is another common feature described in 8 of the 12 patients, and it was present in our case as well. Among the remaining 4 cases, three were investigated as neonates /infants, a point at which it is not feasible to decide on developmental potential. Only one case is described that did not have a noticeable developmental delay until 20 months of age [10]. An interesting candidate gene involved in developmental delay might be the BTB-zinc finger *ZBTB20*, which is highly expressed in the brain. Hippocampal formation is composed of neurons that process information essential for cognitive functions such as memory and learning, and the *ZBTB20* gene has been shown to be expressed during the important period of neurogenesis of pyramidal neurons in the mouse hippocampus. Another gene has been correlated with developmental delay: Shimojima *et al* [11] have suggested that deleted *DRD3* is responsible for neurological symptoms, including developmental delay. Neither *ZBTB20* nor *DRD3* were deleted in the patient that was without noticeable developmental delay [10]. Thus, additional functional studies are needed to clarify the correlation of deleted genes to developmental delay.

Dysmorphic facial features, such as prominent forehead, hypertelorism, epicanthal folds, flat and broad nasal bridge, and anteverted nares are also common in individuals with proximal 3q deletion. Simovich *et al* [10] reported the deletion encompassing 3q13.11-q13.12 where only two genes were deleted; they have proposed that the *CBLB* gene deletion is correlated with the distinct facial features described here. The *CBLB* gene deletion was detected in our case as well, and among other abnormalities, the patient has a prominent forehead, broad nasal bridge, and mild hypertelorism, which is in accordance with the suggested genotype-phenotype correlation.

Other characteristic phenotype features include high arched palate, genitourinary abnormalities (most frequently cryptorchidism and small penis), and skeletal abnormalities. Still, all the reported features do not present a clearly defined phenotype. For further delineation of causative genes, additional knowledge on deleted gene functions and more cases of proximal 3q deletion are needed to be analysed.

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