Case Report

Phenotype Heterogeneity in 3q29 Microduplication Syndrome

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ABSTRACT: 3q29 microduplication syndrome is characterized by widely variable clinical presentation, but generally mild features. Developmental delay, particularly speech, and intellectual disability, eye abnormalities and heart defects are more frequently seen in affected individuals, although it is difficult to delineate a recognisable pattern. We describe a clinical case with a 1.65Mb duplication at 3q29 (chr3:195,979,518-197,638,922, GRCh37) identified by aCGH. The uncharacteristically late onset of the 34 years-old woman is marked by mild intellectual disability, progressive cortical atrophy and recurrent mucosal infections with *Candida albicans*. The gene content of the duplicated region-29 genes, including *PAK2, DLG1, BDH1, FBXO45* and *TFRC*-seems closely linked to neuronal development and synaptic function, explaining brain and eye development related findings. We speculate on the possible involvement of genes like *RNF168* in the aetiology of immunodeficiency. In-depth studies are needed to understand the pathophysiological mechanisms leading to the traits seen in this very rare syndrome.

KEYWORDS: *Immunodeficiency, 3q29 microduplication, aCGH, fronto-temporal dementia, RNF168 gene.*

Introduction

Copy number variations (CNVs) are genetic anomalies found in both apparently healthy individuals and clinically severe syndromes. Below the resolution of conventional cytogenetic examination, molecular methods provide valuable insight. aCGH is still first-tier clinical diagnostic test for the assessment of postnatal cases with developmental delay, intellectual disability and dysmorphic features, allowing detection of sub-microscopic genetic variation [1,2].

Adding SNP-array data, the technique can fine-map the breakpoints [2,3].

A notable limitation is the lack of detection of balanced rearrangements.

Previously reported features of 3q29 microduplication syndrome (OMIM#611936, Q92.3, ORPHA251038) include mild to moderate cognitive impairment, cerebral palsy, speech delay, autistic traits, seizures, obesity in childhood through adulthood. Microcephaly, and, perhaps less commonly, craniofacial dysmorphisms are present as well. In the initially reported cases, a long-narrow face, short philtrum, downturned corners of the mouth, high nasal bridge, bushy eyebrows, long eyelashes, up-slanting palpebral fissures or low-set ears were described [4-11].

The phenotype spectrum of this syndrome may also include ocular defects such as microphthalmia or aniridia, congenital heart malformations (ventricular septal defect), palate, renal and structural brain anomalies, musculoskeletal anomalies (chest-wall and fingers deformities) [5-8,12,13].

The psychiatric presentation has been shown to be more nuanced and may include attention-deficit/hyperactivity disorders, elimination disorders, and autism spectrum disorders, with unusual psychiatric presentation requiring case-by-case evaluation [14].

Despite having the largest cohorts of 3q29 patients to date, internet-based registries (https://3q29.com [15]; http://genome.emory.edu/3q29/ [16]), are mostly underpowered to draw unambiguous conclusions. Nonetheless, the use of standardized instruments and evidence-based approaches are valuable, as, overall, the clinical presentation as 3q29dup is widely varying, although being generally milder than the microduplication counterpart [17].

We report a postnatal diagnosis and aCGH characterization of a 3q29 duplication of 1.65Mb in a patient with severe intellectual disability, abnormal brain MRI findings, and recurrent mucosal *Candida* infections.

Case Report

The proband, 34-year old woman, was admitted for evaluation to the Medical Genetics Outpatient Clinic, Emergency Clinical Hospital of Craiova, Romania. The patient is the second child of healthy, non-consanguineous parents. She has negative family history for congenital anomalies. Following an unremarkable pregnancy, without any known teratogenic

exposures, she was born at term by spontaneous vaginal delivery. Weight at birth was 3450g (68th centile). The patient had a normal cognitive and motor development until the age of 10, when the onset of attention deficit, hyperkinesia, gait difficulties and abnormal coordination occurred. Physical and neurological examination at the time revealed spastic limbs, abnormal muscle tonus, toe walking and balance disorders. During the next years of life, the patient showed severe psychomotor delay with learning difficulties, behaviour problems, speech impairment, dysphagia, chronic pain and sleep disorder. At the age of 17 years, results of brain MRI, cerebral fluid analyses, EEG, abdominal ultrasound and urine/plasma metabolic screens returned normal. Microbiological analysis of throat swab identified the presence of *Klebsiella*, Methicillin-resistant *Staphylococcus aureus* (MRSA), and *Candida albicans*. Upper gastrointestinal endoscopy revealed inflammation of the oesophagus and presence of gastroesophageal reflux. Microbiological tests of the pharyngeal exudate revealed the exclusive presence of *C. albicans*.

At 34 years old, the patient has swallowing difficulties, severe balance problems, and walking is not possible. Her verbal communication is difficult, but she can understand and perform simple tasks. No vision or hearing problems are present. Physical examination reveals facial dysmorphism, dystonic position of the head, mildly stiff neck, and limbs spasticity. She has mildly dysmorphic features like downturned corners of the mouth, depressed nasal bridge, small bulbous nose, and short up-slanting palpebral fissures (Figure 1).

A brain MRI was recently performed and showed severe cortical and cerebellar atrophy, corpus callosum hypotrophy, periventricular leukoencephalopathy and lateral ventricles hypertrophy. Cerebral perfusion scintigraphy revealed severe hypoperfusion in frontal, parietal and temporal regions, hippocampus and basal nuclei.

A normal conventional karyotype evaluation was followed by molecular assessment through aCGH. It identified a 1.65Mb duplication at 3q29 (chr3:195,979,518-197,638,922, GRCh37 coordinates) (Figure 2).

According to the aCGH result, the molecular karyotype of the patient is arr 3q29 (197,190,786-198,850,190) x 3 dn. The duplicated region encompasses 29 genes including several protein coding genes such as *RNF168, WDR53, TFRC, PIGX, PAK2, NCBP2, PIGZ, MFI2, BDH1, FBXO45* or *DLG1* and a non-coding mRNA: *mir4797*. Genetic testing was refused at the time by both parents. The informed consent was obtained from patient`s parents as legal representatives, to collect blood, take pictures and access medical data.

Figure 1. Clinical presentation: Note: Dystonic position of the head. Facial dysmorphism with downturned corners of the mouth; depressed nasal bridge; small bulbous nose; short up-slanting palpebral fissures. Consent for publication was obtained.

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Figure 2. Array CGH result on chromosome 3. Image generated with Nexus 6.1 software (Nexus BioDiscovery, El Segundo, CA). Data extraction (signal intensities) was performed with DEVA software (Roche Nimblegen).Methods

Genomic DNA was extracted from peripheral venous blood using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA), following the manufacturers protocol. DNA concentration was measured using a spectrophotometer (Eppendorf Biophotometer) and DNA quality was further assessed using the Agilent 2010 Bioanalyzer (Agilent Technologies Inc., US). 0.5µg of genomic DNA was used for aCGH analysis following the manufacturer's protocol for the whole-genome 135K oligonucleotide microarray platform, 3×720K slide (Roche NimbleGen, Madison, WI, USA).

Copy number data was analysed with Nexus 6.1 software (Nexus BioDiscovery, El Segundo, CA).

Discussion

The 3q29 microduplication syndrome involves a 1.6Mb region on the short arm of chromosome 3. The syndrome is characterized by heterogenous phenotype including cognitive disability, developmental and speech delay, recurrent chronic *Candida* esophagitis, mildly dysmorphic features along with ocular, palate, renal and cardiac anomalies [6,8,12].

Delineating a recognizable phenotype is a challenge for 3q29dup and overlap with the 3q29del has to be carefully explored [15].

Mild-to-moderate intellectual disability, slightly dysmorphic facial features, autism and musculoskeletal defects are the most frequent clinical features described in patients with 3q29 duplication [4-9]. The present case shows severe intellectual disability, as seen in previously reports, with particularities in structural brain changes and possibly immunodeficiency signs, as suggested by the multiple infections and the chronic mucosal candidiasis.

Usually, the onset of 3q29dup is in the first year of life, with feeding problems, failure to gain weight, hypotonia, and respiratory distress, and higher importance for seizures [15].

In the presented case, the parents reported an uneventful infancy and early childhood.

In the presented case, the duplication region overlaps the typical 1.6Mb 3q29dup region previously reported [6-9,12].

The commonly involved region has its breakpoints flanked by high homology low copy repeats, which could explain sub-telomeric rearrangement of the region occurring during meiosis. Non-allelic homologous recombination is the mechanism producing the anomaly. In a fourth of the cases, it appears to be a reciprocal duplication product of the 3q29 microdeletions [6,8].

However, the exchange events have widely varying defect sizes that flank, span, or partially overlap the common deletion region (chr3:195,726,835-197,344,663 GRCh37, https://decipher.sanger.ac.uk/).

The condition is autosomal dominant and seems to be mostly inherited from either parent. Germinal mosaicism cannot be excluded in cases of apparent *de novo* inheritance. Unfortunately, no such assessments were performed for the presented case.

This region encompasses 29 genes, including *PAK2, DLG1, BDH1, FBXO45, TFRC, ZDHHC19, PIGX* and *RNF168*. The heterogeneity in size of duplicated region can only in part explain why the phenotype seen is highly variable in the several described cases of 3q29 syndrome. Dosage sensitivity of the genes included in the region may play an important role, but alterations outside the 3q29 region could also be involved [15,18].

It was shown that *PAK2, DLG1, FBXO45, BDH1*, and *ZDHHC19* genes have an important role in central and peripheral nervous system

development and neuro-synaptic maturation [19,20].

Besides these roles, *PAK2* and *DLG1* are autosomal homologues of *PAK3* and *DLG3*, two genes involved in X-linked intellectual disability etiopathogeny [4,21,22].

PAK2 and *DLG1* genes might be candidate genes responsible for the development of intellectual disability found in patients with 3q29 microduplication syndrome. Based on the presentation of two cases with smaller affected regions, it has been suggested that the smallest region of overlap in this syndrome is limited to *DLG1* and *BDH1* [17], whose gain-of-dosage may explain main features of the phenotype.

Congenital heart anomalies like auricular/ventricular septal defects or bicomissural aortic valve, described in five out of ten previously published cases, have not been found in our patient, despite the fact that she shared the duplicated region that contained the *WDR53, TFRC, PIGX, MFI2* and *ZDHHC19* protein-coding genes and a non-coding RNA: *mir4797*. *ZDHHC19* gene is homologous to *ZDHHC9* that encodes for an enzyme called palmitoyltransferase and it was described to be involved in X-linked mental retardation with Marfanoid habitus [23].

RNF168 gene encodes the E3 ligase RING finger 168 and is involved in ubiquitylation and control of 53BP1 response to DNA double-strand breaks [24,25].

Defects of this gene have been identified as the cause of a novel immunodeficiency by the use of whole exome sequencing [26].

Patients with mutations in this gene have been reported to suffer from radiosensitivity, primary immunodeficiency, dysmorphic features, and learning difficulties [25,26].

We found that our patient suffered from chronic oral and oesophageal candidiasis that might be related to a immunodeficiency arising from defects in *RNF168* gene, but other causes cannot be excluded. Unfortunately, immunological functional tests at this time, but future studies are warranted.

In summary, we describe a case with 3q29 microduplication syndrome with apparently late onset and more severe phenotype. Consideration should be given to the above-mentioned locus (3q29) in patients with intellectual disability and/or abnormal brain MRI findings.

In-depth studies are needed to understand the pathophysiological mechanisms leading to the traits seen in this very rare syndrome.

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Conflict of interests

None to declare.

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