MOLECULAR AND EXPERIMENTAL BIOLOGY IN MEDICINE

CASE REPORT

INTERSTITIAL 14q31.3-q32.13 DELETION: THE ROLE OF MOLECULAR KARYOTYPING IN CLARIFYING THE ETIOLOGY OF DEVELOPMENTAL DELAY

Kristina Crkvenac Gornik[1], Ivana Tonkovic Durisevic[1], Anita Pokupec Bilic[1], Sanda Huljev Frkovic[2]

Abstract:
Aim: With the exception of ring chromosome 14 or translocations, interstitial deletions of the long arm of chromosome 14 are very rare. All patients with these deletions share common phenotypic characteristics, primarily mild dysmorphia and developmental delay. Molecular karyotyping (array CGH) enabled the precise breakpoint determination and improved the analysis of genotype-phenotype correlations.
Case presentation: In a 7-year-old girl, array CGH was performed due to developmental delay. The array CGH study showed 8.3Mb de novo interstitial deletion of the 14q31.3–q32.13 region.
Conclusions: Comparison of our patient´s phenotype with previously reported chromosome 14q interstitial deletion cases confirmed the presence of common clinical features and highlights the utility of array CGH as a diagnostic tool in clarifying the developmental delay etiology.

[1]*Division of Cytogenetics, Department of laboratory diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia*
[2]*Division of Genetics and Metabolism, Department of Pediatrics, University Hospital Centre Zagreb, Zagreb, Croatia*

Corresponding author:
Kristina Crkvenac Gornik
Division of Cytogenetics, Department of Laboratory Diagnostics
University Hospital Centre Zagreb
Kispaticeva 12, 10000 Zagreb, Croatia
Tel: +385 1 23 67 347
e-mail: kcrkvena@kbc-zagreb.hr

Submitted: December, 2018
Accepted: February, 2019

Key words: chromosome 14q interstitial deletion, array CGH, developmental delay

INTRODUCTION

Cytogenetic diagnostic has been highly improved/transformed through the implementation of new molecular technologies. Routine analysis of metaphase and prometaphase chromosomes using the GTG banding technique with a resolution of 400-500 bands enables the detection of structural aberrations in the size of ≥5-10 Mb. Clearly, chromosome banding techniques continue to be widely used, although the resolution depends mostly on the chromosome length and the resolution limits of light microscopy. However, a great number of clinical phenotypes are caused by cryptical chromosome rearrangements that are less than 3Mb in size, and as such represent microdeletions and microduplications that are impossible to detect by using the GTG banding technique.
The application of high-resolution molecular techniques in research of developmental delay and the etiology of multiple congenital anomalies have led to exponential growth in discovering new microdeletion and microduplication syndromes. The most improvement in detecting genomic aberrations has been accomplished by using the molecular karyotyping technique (aCGH - Array-based comparative genomic hybridization), which enables whole genome screening for duplications and deletions of specific DNA sequences. Literature data confirms the importance of aCGH technique implementation in the evaluation of patients with developmental delay and congenital anomalies. Therefore, this method is recommended as the first genetic test in children and adults with undiagnosed clinical manifestations. The application of this method detects 14-20% of aberrations: 9-10% are interstital aberrations and the rest are subtelomeric aberrations.[1, 2]
This method is limited by the inability to detect balanced chromosomal abnormalities and low-level

mosaicism, but it detects copy number variations (CNV). CNVs are DNA segments, ranging in size from 1 kilobase to a few megabases, causing genome imbalance. It appears that CNVs may be responsible for differences among people and evolution processes, but they can also affect clinical phenotype. The results of the aCGH analysis are classified into five categories: pathogenic, likely pathogenic, variation of uncertain significance (VUS) detected in one of the parents and VUS that has not been detected in parents, likely benign and benign. CNVs classified as likely benign and benign were not reported to the patients.[3]

Discovering VUS makes interpretation of the results a challenge. For a given VUS, it is not clear whether it is associated with clinical features because there is still insufficient knowledge about correlation between detected microduplications or microdeletions and their effect on the phenotype. In such cases it is important to search literature and databases to find patients with the same disorder in the chromosome region and clinical features. Only careful phenotype-genotype comparison can improve the interpretation of such results. Genetic counselling is necessary before and after testing, and patients with VUS need to be monitored and evaluated again for the status of discovered change.

In our laboratory, the aCGH method is used to evaluate patients with apparently balanced chromosomal rearrangements and normal karyotype individuals with mental retardation, dysmorphic features and/or multiple congenital anomalies.

CASE REPORT

A 7-year-old Caucasian girl was referred to our Department of Genetics by a neuropediatrician due to developmental delay of uncertain origin. She was born at term after an uncomplicated pregnancy to healthy non-related parents. Their first child is a healthy girl and the family history is unremarkable. At birth, the patient's weight was 3100 g, length 50 cm and Apgar score 10/10. Evaluation due to developmental delay started at the age of 3. The clinical examination at 7 years of age showed that her body weight was at the 75th percentile, height at the 25th and head circumference at the 50th percentile. Dysmorphic features of the face were observed, bulbous nose, micrognathia, thin upper lip, low-seat ears and high-arched palate without clefts. Developmental milestones were mildly delayed; she started walking at 20 months. No malformations of internal organs were discovered. Psychological evaluation showed mild cognitive delay with restricted language expression. During childhood she had frequent ear infections. Left-sided conductive loss of hearing was found on audiologic examination. She did not have any epileptic or non-epileptic events despite focally changed EEG, regularly monitored by a neuropediatrician. Classical cytogenetic analysis revealed a female karyotype 46,XX with a suspicion of 14q terminal deletion (Figure 1). Cytogenetics of peripheral blood

Figure 1. Classical karyotyping – suspicion of 14q terminal deletion (arrow)

lymphocytes from both parents revealed normal karyotypes. Available FISH (Fluorescent In Situ Hybridization) - probe set (centromeric probe, whole chromosome 14 paint probe, and locus-specific probes 14q11.2, 14q32, t(11;14) and sub-telomeric 14qter) was not informative. Thus, no conclusion could be drawn considering the presence of 14q terminal deletion. DNA was extracted from peripheral blood and analyzed using array-CGH (aCGH) on the platform SurePrint CGH 8x60K G3 ISCA (Agilent Technologies) and the CytoGenomics software. Molecular karyotype analysis revealed de novo 8.3 Mb interstitial deletion encompassing chromosomal region 14q31.3-q32.13 and designated as arr[hg19]14q31.3q32.13(86,933,738-95,234,796)x1 (Figure 2).

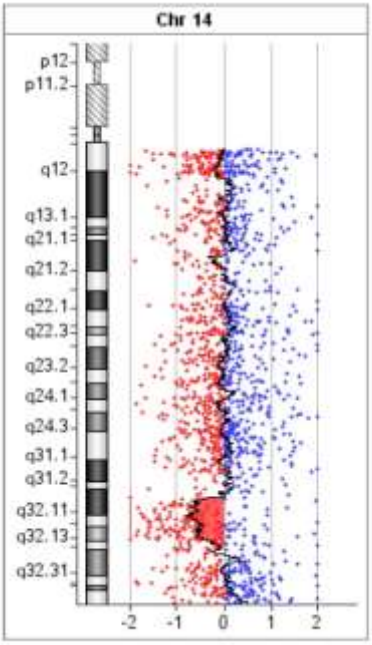

Figure 2. Array detected interstitial deletions of the long arm of chromosome 14

DISCUSSION

Interstitial deletions at the long arm of chromosome 14 are considered very rare. Only a limited number of cases with such a defect can be found in literature, with few of them partially including the region affected by deletion verified in our patient. The type and the severity of symptoms depend on the size and location of deletion. Table 1 summarizes the clinical presentation of our patient in relation to cases from literature. Phenotypic comparison reveals common clinical features. The neurodevelopmental features were quite consistent with some frequent and non-specific dysmorphic signs including hypertelorism, bulbous nose with a depressed bridge, thin upper lip and low-set ears, while malformations of internal organs were not found. The mild phenotypic presentation may indicate that the deleted segment does not contain genes important for early organ development. Therefore, we could conclude that it would be difficult to recognize similar cases on the basis of clinical findings alone.

Table 1. Presented patient phenotype correlated with patients described in literature (adjusted by ref. 4 and 5)

CLINICAL FEATURE	GORSKI ET AL[4]	PICCIONE ET AL[5]	THIS REPORT
Breakpoint location	q31q32.3	q31.3q32.2	q31.3q32.13
Mental retardation	+	+	+
Microcephaly	+	+	-
Strabismus	NR	+	-
Rounded nose tip	+	+	+
Long philtrum	+	+	+
Thin upper lip	+	+	+
Low set ears	+	+	+
Rounded palate	Uvula bifida	Cleft palate	High-arched palate
Growth delay	+	-	-
Parent of origin	not specified	Pat	*De novo*

(+) present, (-) absent, Pat - paternal originate, de novo - a new deletion; NR – nor reported

The deleted region contains 52 OMIM genes, including the following 15 morbid genes: *ATXN3*, *CALM1*, *CCDC88C*, *FBLN5*, *GALC*, *GPR68*, *GSC*, *AAT* (*SERPINA1*), *SERPINA6*, *SLC24A4*, *SPATA7*, *TDP1*, *TRIP11*, *TTC8*, *ZC3H14*. From these, pathogenic variants in some genes have been described in AD diseases or syndromes such as: spinocerebellar ataxia with axonal neuropathy (OMIM 607250), cutis laxa type I (OMIM 219100) and *FBLN5* gene, Machado–Joseph disease (OMIM 109150) and *ATXN3* gene, Long QT syndrome 14 (OMIM 616247), Ventricular tachycardia, catecholaminergic polymorphic, 4 (OMIM 614916) and *CALM1* gene, Spinocerebellar ataxia 40 (OMIM 616053) and *CCDC88C*, Corticosteroid-binding globulin deficiency (OMIM 611489) and *SERPINA6*. The phenotype of the proband does not match conditions listed above. The *AAT* gene, also designated as *PI* (protease inhibitor), is located in the 14q32.1 region. When deleted, it is associated with emphysema and liver insufficiency, thus pointing to the need for α1-AT blood level monitoring among deletion carriers.[6-9] The size of the deleted region, the location of multiple genes in it, as well as the limited number of published clinical-genetic case series, makes genotype-phenotype correlation difficult. Therefore, newly discovered and thoroughly genetically described disease carriers will contribute to the elucidation of the functional relevance of the aforementioned region.

The implementation of aCGH is a practice-changing methodology improvement in the field of cytogenetic workup of patients with developmental delay with or without dysmorphic changes. Classical cytogenetics, with the GTG-banding technique, may lead to diagnosis in about 3% of such patients, excluding well-defined syndromes (Down's syndrome etc.). Molecular karyotyping may significantly increase efficacy in diagnosing the etiology of developmental delay. The presented case illustrates the greater ability of aCGH in defining etiopathogenetic background that could only be suspected, based on GTG-banding cytogenetic finding. Such approach profoundly improves genetic counseling as well as clinical follow-up.

REFERENCES

1. Shaffer LG, Bejjani BA. Medical applications of array CGH and the transformation of clinical cytogenetics. Cytogenet Genome Res. 2006;115(3-4):303-309.
2. Stankiewicz P, Beaudet AL. Use of array CGH in the evaluation of dysmorphology, malformations, developmental delay, and idiopathic mental retardation. Curr Opin Genet Dev. 2007;17(3):182-192.
3. Lovrečić L, Vrečar I, Peterlin B. Molekularna kariotipizacija - nov pristup u kliničkoj i laboratorijskoj genetici. Medicina fluminensis. 2014;50:181-187.
4. Gorski JL, Uhlmann WR, Glower TW. A child with multiple congenital anomalies and karyotype 46,XY,del(14)(q31q32.3): further delineation of chromosoma 14 interstitial deletion syndrome. Am J Med Genet. 1990;37:471-474.
5. Piccione M, Antona V, Scavone V, Malacarne M, Pierluigi M, Grasso M et al. Array CGH defined interstitial deletion on chromosome 14: a new case. Eur J Pediatr. 2010;169:845-851.
6. Cox DW, Markovic VD, Teshima IE. Genes for immunoglobulin heavy chains and for alpha-1-antitrypsin are localized to specific regions of chromosome 14q. Nature. 1982;297:428-430.

7. Denayer E, Legius E. What's new in the neuro-cardio-facial-cutaneous syndromes? Eur J Pediatr. 2007;166:1091-1098.
8. Rabin M, Watson M, Kidd V, Woo SLC, Breg RW, Ruddle F. Regional location of -antitrypsin genes on human chromosome 14. Somat Cell Mol Genet. 1996;12:209-214.
9. Nagayama Y, Kaufman KD, Seto P, Rapoport B. Molecular cloning, sequence and functional expression of the cDNA for the human thyrotropin receptor. Biochem Biophys Res Commun. 1989;165:1184-1190.