Microarray Analysis of 8p23.1 Deletion in New Patients with Atypical Phenotypical Traits

Hela Ben Khelifa^{1,2} Molka Kammoun^{1,2} Hanene Hannachi^{1,2} Najla Soyah³ Saber Hammami⁴
 Hatem Elghezal^{1,2} Damien Sanlaville⁵ Ali Saad^{1,2} Soumaya Mougou-Zerelli^{1,2}

¹ Cytogenetic and Reproductive Biology Department, Farhat Hached University Teaching Hospital, Sousse, Tunisia

² Common Service Units for Research in Genetics, Faculty of Medicine of Sousse, University of Sousse, Tunisia

³ Pediatric Department, Farhat Hached University Teaching Hospital, Sousse, Tunisia

⁴ Pediatric Department, Fattouma Bourguiba University Teaching Hospital, Monastir, Tunisia

⁵ Cytogenetic Department, Biological and Pathological Center EST, Bron, Lyon- France

Address for correspondence: Hela Ben Khelifa, PhD, Cytogenetics and Reproductive Biology Department, Farhat Hached University Teaching Hospital, Street Ibn El Jazzar, 4000, Sousse, Tunisia (e-mail: bkhelifahela@yahoo.com).

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Abstract

Keywords

- 8p deletion
- *TNKS* gene
- CGH array
- intellectual disability

We describe two patients carrying deletions of chromosome 8p23.1 with a commonly critical region identified by means of oligonucleotide array comparative genomic hybridization (array CGH). They didn't present congenital heart defects or behavioral problems. Only one patient presented with intellectual disability and carrying deletion of *TNKS* gene. We presumed the inclusion of *TNKS* gene in the mental impairment.

Introduction

Several regions within 8p are proposed to be hotspots leading to the formation of recurrent genomic rearrangements, mainly because of the existence of two olfactory receptor gene clusters (REPD [repeat-distal] and REPP [repeat-proximal]) flanking a 5-Mb region of 8p23.1.^{1–3} Various interstitial or terminal deletions have been reported in this region associated with a spectrum of anomalies that includes intellectual disability (ID) and congenital heart malformations.⁴ The variable clinical spectrum could be explained by the extent of the deletion or variations in the breakpoints.^{5–7} Most 8p23.1 deletions are de novo, and only terminal deletions have been reported due to paternal transmission.^{5–7} Recently, prenatally case of 8p23.1 interstitial deletion with maternal origin has been reported.⁸ In this article, we describe two patients carrying deletions at 8p23.1 with a commonly critical region. A role of suggested candidate genes is estimated. The *TNKS* gene could be involved in ID.

Clinical Description

Patient 1

Patient 1 is a 5-year-old boy from unrelated parents. The results of prenatal ultrasonography were normal in term of fetal growth. After a full-term pregnancy and uncomplicated delivery, the child had been hypotonic since the first months of life. The clinical examination at age of 5 years showed a weight of 12 kg (−2.85 SD [standard deviation]), a length of 90 cm (−4.2 SD), and a head circumference of 50 cm (−1.25 SD). Until this age, the boy didn't sit or walk independently. Also, he presented eye movement disorders with intermittent ptosis due to oculomotor paralysis. He had dysmorphic features, including long face with sloping forehead, prominent nasal bridge, hypoplastic alae nasi, long and flat philtrum, hypertelorism, and branch with curled ears of the helix that crosses the conch (► Fig. 1). His heart as well as his behavior is normal. The patient presented with moderate ID without seizures. Magnetic resonance imaging (MRI) indicated normal profile. His

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Fig. 1 Photographs of our patients: (A) Facial picture of patient 1. (B) Facial picture of patient 2.

mother presented with moderate ID without cardiac anomalies or behavioral problems.

Patient 2

Patient 2 is a 14-year-old girl from unrelated parents. Her birth weight was 2,100 g. Psychomotor development was slightly delayed (she sat at 12 months, walked at 17 months). On clinical evaluation, her weight was 36 kg (−1.58 SD), her length was 141 cm (−2.5 SD), and her head circumference was 47.5 cm (−5.75 SD). According to these clinical parameters, the patient suffered from growth retardation. Regarding overall health, she did not experience medical complications such as cardiac anomalies. At school, she presented poor academic performance with limited intelligence. The dysmorphic features were evident and included almost the same features of patient 1 (►Fig. 1). Her behavior as well as her heart was normal. Electroencephalography did not reveal any epileptic anomalies. The MRI showed a normal morphology.

Methods

Karyotype

Conventional cytogenetic analysis was performed on the peripheral blood lymphocytes at 450 to 550 Giemsa Reverse Banding (RHG). Chromosomal analysis of the patients and their families was performed according to standard procedures. Peripheral blood lymphocytes were cultured in RPMI-1640 medium (Gibco, Grand Island, NY) enriched with 20% fetal calf serum, L-glutamine, antibiotics (penicillin and streptomycin), and phytohemagglutinin. Cells were cultured for 72 hours at 37°C in an incubator with 5% CO₂. The culture was stopped using Colcemid solution (Gibco BRL-KaryoMAX, Carlsbad, CA) (0.05 mg/mL) for 45 minutes. After harvesting, cells were exposed to hypotonic solution (0.075 mol/L KCl) and fixed with methanol/acetic acid (3:1).

Slides were prepared and stained using R-banding. A minimum of 20 metaphases were analyzed from each sample, using the Applied Imaging CytoVision Karyotyping System (Santa Clara, California, United States). Karyotypes were assigned according to the recommendations of the International System of Human Cytogenetic Nomenclature. We obtained a consent form from the patient's families.

Array Comparative Genomic Hybridization

Oligonucleotide comparative genomic hybridization (CGH) array was performed with Agilent Human Genome CGH array Kit 44K (Feature Extraction 9.1, CGH Analytics 4.5, Santa Clara, California, United States). An in-silico analysis of the unbalanced region indicated by the analysis was made using the March 2006 release of the UCSC Genome Browser (<http://genome.ucsc.edu/>).

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) used commercial probes, Bac clone RP11-77B14 (Blue FISH) on metaphase chromosomes of patient 1, his mother, and on interphase chromosomes of patient 2. Sub-Telomere 8pter (Kreatech Diagnostics, Amsterdam, Netherlands) was used on metaphase chromosomes of patient 2. And 10 µL of the probe mixture was applied to metaphase slides and co-denatured for 5 minutes at 72°C. After overnight hybridization at 37°C, slides were washed for 5 minutes in 2XSSC/NP40 (Vysis, Illinois, United States) at 72°C. Finally, the slides were mounted with 15 mL of 4,6-diamidino-2-phenylindole (Vysis) and analyzed using a FISH station (CytoVision, Applied Imaging, Illinois, United States).

Results

The chromosomal analysis of patient 1 indicated normal karyotype in all metaphases. Array CGH analysis revealed interstitial deletion on the short arm of chromosome 8 involving the 8p23.1 region. The deletion encompasses 1,413,535 pb: arr 8p23.1 (8,229,404–9,642,938) X1(hg18) (►Fig. 2).

In the second case, the conventional karyotype revealed a terminal deletion of the short arm of chromosome 8 (►Fig. 2). CGH array characterized this deletion. It encompasses 8,883,636 bp: arr 8p23.3–8p23.1 (191,530–9,075,165) X1(hg18) (►Fig. 2). The parental karyotype was normal.

FISH analysis confirmed the *TNKS* gene deletion in patient 1 and showed the same result as his mother (►Fig. 3). In patient 2, FISH analysis confirmed the 8pter deletion, but the clone RP11-77B14 targeting *TNKS* gene appeared to be not deleted.

Discussion

Deletion of 8p23.1 is known as pathogenetic syndrome. It was not abundantly present in the normal population according to the publicly available database (Database of Genomic Variants, www.projects.tcag.ca). Many studies suggest a common underlying mechanism predisposing

Fig. 2 44 0000 Agilent Technologies oligonucleotides array profiles of our patients showing: (A) 8p23.1 deletion of 1,413,535 pb, (B) partial karyotype showing chromosome 8 of patient 2, and (C) 8p23.3→8p23.1 deletion of 8,883,636 bp. Dotted lines represent log2 ratios.

to the deletion, in which two previously described LCRs (low-copy number repeats), REPD and REPP, are located. This led to an abnormal recombination, during meiosis, of mispaired copies of the repeated sequences.^{1–3} The classic clinical features associated with this anomaly included developmental impairment, mild to moderate ID, microcephaly, congenital heart disease, diaphragmatic hernia, hypospadias, and a particular behavior, including extreme

hyperactivity, impulsiveness, and aggressiveness, with destructive episodes.^{6,7}

Using the oligo 44K CGH array, we succeeded in revealing a 1,413,535pb deletion at 8p23.1 region in patient 1 and characterized a larger terminal deletion of 8,883,636 bp in patient 2. Familial investigations were performed. FISH analysis showed that patient 1 as well as his mother had the deletion of *TNKS* gene (Tankyrase, TRF1-interacting ankyrin-related gene). In

Fig. 3 Fluorescence in-situ hybridization (FISH) analysis: (A, B) FISH analysis using the probe RP11-77B14. (A) FISH analysis of patient 1 no orange spot was detected, suggesting deletion of the *TNKS* gene. (B) FISH analysis of the mother of patient 1, only one orange spot was detected, suggesting deletion of the *TNKS* gene. (C) FISH of patient 2, the *TNKS* gene is intact. (D) FISH analysis of patient 2 using the probe Sub-Telomere 8pter and showing deletion of 8pter. SE 8(D8Z1) and 8qter were used as control probes.

fact, the mother, who presented with moderate ID, had transmitted the deleted copy to her son. The breakpoints of the microdeletion at 8p23 were flanked by LCRs suggesting nonallelic homologous recombination (► **Fig. 4**). In patient 2 who presented with limited intelligence, the *TNKS* gene was not deleted. The breakpoints of the microdeletion at 8p23 were proximal to the telomere. Therefore, we presumed that the ID genes within the LCRs were deleted or disrupted. In fact, most of the reviewed patients carried deletions within the LCRs. Their mental statuses were characterized by mild ID (► **Table 1** and ► **Fig. 4**). It has been suggested that a region corresponding to the overlapping deletions may be critical in this regard.^{9,10} In this region, *TNKS* gene is suspected to be related to the mental impairment,^{9,10} especially that *TNKS* gene is highly expressed in the brain.¹¹ Therefore, our comparative analysis detains the hypothesis supporting *TNKS* as a proposed gene responsible for ID.^{12–14}

Also, our data are in conformance with the findings of Ballarati et al suggesting that *TNKS* is not a plausible candidate gene responsible for Cornelia de Lange syndrome

(CdLS).¹³ In fact, neither of these two patients has facial anomalies that meet the dysmorphic features of CdLS. Moreover, some of the patients reviewed in this article (► **Table 1** and ► **Fig. 4**), lacking the *TNKS* gene, could not be clinically assessed because of the absence of pictures, or the dysmorphic features mentioned did not meet the facial features of CdLS.

The hallmark clinical sign in 8p23.1 deletions is congenital heart disease, typically in the form of atrioventricular septal defect (AVSD), atrial septal defect (ASD), and commonly pulmonary stenosis (PS).^{14–16} The GATA-binding protein 4 gene (*GATA4*, OMIM: 600576), which encodes a zinc finger transcription factor, has been considered a likely candidate for heart defects, as it is deleted in most patients with heart anomalies.^{14,15} Thus, the absence of cardiac malformations, in our patients, could be explained by the retention of *GATA4* gene. On the other hand, we revealed a commonly deleted region. In this region of 845,762 pb (position: 8,229,404–9,075,165), at least 10 genes are located. Interestingly, *PRAGMIN* (pragmin) and *CLDN23*

Fig. 4 Schematic illustration of the 8p23.1 region. Some relevant genes according to UCSC: *CLDN23*, Homo sapiens claudin 23, mRNA; *GATA4*, GATA binding protein 4; *MCPH1*, microcephalin; *TNKS*, tankyrase, TRF1-interacting ankyrin-related. Black bars describe deleted genes at 8p23.1 in our patients and in some published cases.

(claudin 23, OMIM: 609203) may contribute to the neurodevelopmental issues: *PRAGMIN* encodes an enzyme that belongs to the tyrosine protein kinase family. A similar protein in rat binds to Rho family GTPase 2 (Rnd2) and regulates neurite outgrowth via activation of the Ras homolog gene family, member A (RhoA),¹⁷ and *CLDN23* encodes a member of the claudin family. Katoh and Katoh¹⁸ defined claudins as integral membrane proteins and components of tight junction strands, and they demonstrated the critical roles of these components in maintaining cell polarity and signal transduction. Consequently, we suggest that similar clinical features in both patients, mainly the dysmorphic features and growth retardation, could be associated with these deleted genes.

Only patient 2 presented with microcephaly. This could be explained by the deletion of *MCPH1* gene. Indeed, the frequently observed microcephaly in patients with microscopically visible deletions of 8pter suggests that the gene(s) causing the microcephaly were centromeric to the deleted region.¹⁹ Trimborn et al.²⁰ showed that the hap-

loinsufficiency of *MCPH1* (microcephalin, OMIM: 607117) gene could be associated with the microcephaly. Also, mutations in this gene have been reported in association with primary autosomal recessive microcephaly 1 and premature chromosome condensation syndrome.^{20,21}

Here, we report on two new patients with 8p23.1 deletion. The apparent involvement of *TNKS* gene as an ID gene suggests that attention should be focused on it. Besides, more sophisticated technologies such as next-generation sequencing technology are highly recommended to better elucidate the role of the discussed genes.

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Table 1 Detailed phenotypes of previously reported cases with 8p23 deletions and our patients

References	Intellectual disability	Facial dysmorphisms	Growth restriction	Microcephaly	Behavioral problems (type)	Cardiac anomalies	Karyotype
Devriendt et al. (1999) Pt 2	Mild	N/D	N/D	N/D	+++	AVSD, PVS, HRV, AVS	del(8)(p23.pter)
Devriendt et al (1999) Pt 3	Mild	N/D	N/D	N/D	+++	ASD, PVS	del(8)(p23.pter)
Devriendt et al (1999) Pt 5	Mild	N/D	N/D	N/D	+++	ASDH, PVS	del(8)(p23.pter)
Devriendt et al (1999) Pt 6	Mild	N/D	N/D	N/D	+++	ASDH, PVS	del(8)(p23.pter)
Devriendt et al (1999) Pt 7	Mild	N/D	N/D	N/D	+++	Normal	del(8)(p23.pter)
Paez et al (2008) Pt 1	+	–	+	+	N/D	Complex cardiac anomaly (HRV, pulmonary artery stenosis, ASD, MS)	del(8)(p22-p23.3)
Ballarati et al (2011)	+	High and narrow forehead, broad nasal bridge, low set or malformed ears, puffy hands	–	+	ADHD	ASD, PS	Normal
Current pt 1	Moderate	Long face, sloping forehead, prominent nasal bridge, thin lips, malformed ears	–	+	Normal	–	Normal
Current pt 2	Limited intelligence	Long face, Sloping forehead, prominent nasal bridge, thin lips, spaced teeth	+	+	Normal	–	del(8)(p23.pter)

Abbreviations: (–), absent; (+), present; ASD, atrial septal defect; ASDH, arteriosclerotic heart disease; AVS, aortic valve stenosis; AVSD, atrioventricular septal defect; HRV, heart rate variability; MS, mitral stenosis; N/D, not determined; PS, pulmonary stenosis; Pt, patient; PVS, programmed ventricular stimulation.

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