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Case Report

Molecular cytogenetic and phenotypic characterization of ring chromosome 13 in three unrelated patients

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Abstract. We report on the cytogenetic and molecular investigations of constitutional de-novo ring chromosome 13s in three unrelated patients for better understanding and delineation of the phenotypic variability characterizing this genomic rearrangement. The patient's karyotypes were as follows: 46,XY,r(13)(p11q34) dn for patients 1 and 2 and 46,XY,r(13)(p11q14) dn for patient 3, as a result of the deletion in the telomeric regions of chromosome 13. The patients were, therefore, monosomic for the segment 13q34 → 13qter; in addition, for patient 3, the deletion was larger, encompassing the segment 13q14 → 13qter. Fluorescence in situ hybridization confirmed these rearrangement and array CGH technique showed the loss of at least 2.9 Mb on the short arm and 4.7 Mb on the long arm of the chromosome 13 in patient 2. Ring chromosome 13 (r(13)) is associated with several phenotypic features like intellectual disability, marked short stature, brain and heart defects, microcephaly and genital malformations in males, including undescended testes and hypospadias. However, the hearing loss and speech delay that were found in our three patients have rarely been reported with ring chromosome 13. Although little is known about its etiology, there is interesting evidence for a genetic cause for the ring chromosome 13. We thus performed a genotype-phenotype correlation analysis to ascertain the contribution of ring chromosome 13 to the clinical features of our three cases.

Keywords: Array-CGH, FISH, intellectual disability, ring chromosome 13

1. Introduction

Ring chromosome 13 is a rare cytogenetic disorder that was first described by Lejeune et al. [1] and is relatively uncommon, with an estimated incidence of 1/58,000 in live births [2]. In common with other ring chromosomes, r(13) are formed by breakage in both-arms of the chromosome with fusion of the points of fracture and variable degree of loss of the distal fragments. While loss of p-arm and satellite material has minimal clinical consequences, loss of critical genes on 13q34 may contribute to the phenotype [3,4]. The most consistent finding of individuals with r(13) are, intellectual disability ranging from moderate to severe,

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marked short stature and small for gestational age, frequently associated with neural tube, brain and heart defects, microcephaly, genital malformations in males including undescended testes and hypospadias and dysmorphic features with high hairline, hypertelorism, prominent and broad nasal bridge, upslanting palpebral fissures, small mouth, thin lips and shortened survival.

The hearing and speech delay is a relatively common birth defect. Although little is known about its etiology, there is interesting evidence that it has a genetic cause. Both chromosomal abnormalities have been described in patients with hearing and speech delay as the 13q-syndrome. We present here the molecular and phenotypic characterization of three r(13) patients, differing both in severity of phenotype and size of genomic material loss on 13q. We thus performed a genotype-phenotype correlation analysis to ascertain the contribution of ring (13) to the clinical features of our patients.

2. Materials and methods

The patients were referred to our laboratory for hearing and speech delay. We obtained a detailed clinical history and a physical examination was carried out by the same medical geneticist. Informed consent was obtained to publish clinical and molecular investigations for all patients. The parents of patients 1 and 3 refused to share the photos of their son.

2.1. Patient 1

The patient was a 3-year-old boy. The pregnancy history was negative for significant complications or tetatogenic exposures. He was born at full term by vaginal delivery with birth weight of 2100 g, (-3SD); length 46 cm, (-2SD); head circumference 30 cm (-4 SD). The neonatal period was complicated by discovery of congenital heart defect at 30 d after birth, with an intraventricular communication that was repaired at the age of 3 yr. He was found to be unresponsive to sound. A hearing test revealed bilateral severe sensorineural hearing loss. Clinical exam showed hypotonia, growth retardation and microcephaly. His ears were low-set with over-folded helices. He had a sloping forehead, wide-spaced eyes with upslanting palpebral fissures, depressed nasal bridge and a long philtrum. The palate was noted to be high-arched and his teeth were irregular and dysplastic. Brain magnetic resonance imaging discovered agenesis of the corpus callosum andcerebral white matter abnormalities. Additional imaging studies showed normal kidneys.

2.2. Patient 2

A 4-year-old girl was the second child born to healthy and consanguineous parents. She was born at term at 38 wk of gestation by caesarean section after an uncomplicated pregnancy. At birth, her weight was 2000 g, (-2SD); length 42 cm (-4SD); head circumference 32 cm (-2 SD). The neonatal period was normal without hypotonia, and she sat at 6 mo and walked at 18 mo. She presented congenital dislocation of the hip. At age of 4 yr, she had significant, severe intellectual disability with speech delay and abnormal hearing. Her weight was 16 kg, length 90 cm (-2SD) and head circumference 46 cm (-3SD).

She had dysmorphic features with microcephaly, postnatal short stature, flat profile, broad forehead, high hairline, hypertelorism, rounded nose lip, long and flat philtrum, thin lips, high arched eyebrows, prominent ear lobes, widely spaced teeth, short neck, blepharophimosis, small hands and short feet. Genital examination and brain magnetic resonance imaging were normal. Electroencephalography, electrocardiography, gastroesophageal reflux and ophthalmology exams were normal.

2.3. Patient 3

The proband, a 3-year-old boy, was the second child of healthy and unrelated parents. The child's birth weight was 2350 g (-2 SD9; length 48 cm (-1SD); head circumference was 34 cm (-1SD). The milestones were delayed, with sitting independently by 10 mo and walking with support by 3 yr. On examination at the age of 3 yr, the child's weight was 13 kg (-1SD); length 80 cm (-4SD); head circumference 48 cm (-2SD). The face showed dysmorphic features including a high forehead, a broad and flat nasal root, arching of the eyebrows, a long and flat philtrum, thin lips, low-set ears and widely spaced teeth. He also had clinodactyly of the fifth fingers and abnormal external genitalia with hypospadias. At the examination, he could only speak single words and he had hypotonia. Cranial magnetic resonance imaging showed agenesis of the corpus callosum. During the cytogenetic testing, we were informed that the child died after rapid regression of his health caused by acute leukemia.

2.4. Karyotype

Chromosomal analysis was performed according to standard procedures for the patients and their parents. Peripheral blood lymphocytes were cultured in RPMI 1640 medium (Gibco®, Grand Island, NY, USA) enriched with 20% fetal calf serum, L-glutamine, antibiotics (penicillin and streptomycin) and phytohemagglutinin. The cells were cultured for 72 h in a 37 ºC incubator with 5% CO2. Culture was stopped using colcemid solution (0.05 μg/mL) for 45 min. After harvesting, the cells were exposed to hypotonic solution (0.075 mol/L KCl) and fixed with methanol/acetic acid (3:1). The slides were prepared and stained using R-banding (reverse banding). A minimum of 50 metaphases were analyzed from each sample using the Applied imaging CytoVision Karyotyping System®. Karyotypes were assigned according to the recommendation of the International System of Human Cytogenetic Nomenclature (ISCN) 2009 [5].

2.5. Fluorescence in situ hybridization (FISH)

FISH was, performed on blood lymphocytes of all the patients. The ring chromosome 13s were further characterized by a whole chromosome 13 painting probe, WCP 13 (Vysis®, Downers Grove, IIL, USA) for patient 1 and 2 and metaphases of all the patients were analyzed by means of probes using the specific 13q subtelomeric probe, TelVysion D13S327 in 13q34 (75 Kb) and the Vysis LSI13 in 13q14 (440 Kb). Patient 3 was also investigated by means of a specific proximal probe, Vysis LSI D13S319, in 13q14.3 (130 Kb) between the flanking regions 13q34 and 13q14 with the aim of explaining the origin of the acute leukemia. Hybridizations were performed according to the manufacturer's instructions. Depletion of chromosomal materials has prevented further molecular investigations.

2.6. CGH-array

An 180,000 Agilent Technologies oligonucleotides array was used according to the manufacturer's protocols (Human Genome OligoMicroarray Kit) in order to analyze the patient's deoxyribonucleic acid (DNA) [6]. DNA from patient 2 and the reference DNA were digested with Rsal I and Alu I, respectively. Each digested DNA produced was labeled by random priming with Al5-dUTP or Al3-dUTP. After purification, each probe was denatured and pre-annealed with 50 g of human Cot-1 DNA (Invitrogen, Calif., USA). The hybridization was performed at 65 ºC for 24 h. After washing, the array was analyzed by feature extraction 9.1 software. Interpretations of the results was carried out with the CGH analytics 4.5 software with the following parameters: z-score threshold: 2.0, window: 0.5 Mb. A copy number variation was noted if at least three contiguous oligonucleotides presented an abnormal $\log^2$ ratio ($> +0.5$ or < -0.5).

3. Results

Analysis of 20 metaphase chromosome spreads revealed a simple ring chromosome 13 in all cells of the patients. The patient karyotypes were: 46,XY,r(13)(p11q34)dn for patient 1, 46,XX,r(13)(p11q34)dn for patient 2 and 46,XY,r(13)(p11q14)dn for patient 3 as a result of the deletion in the telomeric regions of chromosome 13 (Figs 1A-1C). They were, therefore, monosomic for the segment 13pter and for the segment 13q34 → 13qter. In addition, for patient 3, the deletion was larger and encompassed the segment 13q14 → 13qter. All the parents had normal karyotypes.

The results of the FISH analysis of our three patients suggest a break at both ends of chromosome 13 with a loss of subtelomeric region as a mechanism of ring formation and not a simple telomere-to-telomere fusion. FISH performed on metaphasic lymphocytes of peripheral venous blood (Patients 1, 2 and 3), using subtelomeric probe (D13S327, Vysis) of chromosome 13q, showed hybridization only to the normal chromosome 13 in three patients, confirming the deletion of the terminal regions of ring chromosome 13 in all the patients (Figs 1: A2, B2 and C1). The second probe mapping to the LSI 13 in 13q14 (Vysis) showed hybridization to the normal and the ring chromosome 13 in all the patients. The FISH study of LSI D13S319 in 13q14.3 showed hybridization of only one signal confirming the deletion of this probe. The 13q breakpoint 3 (Fig. 1: C2) in patient 3 was localized to 13q14.3, resulting in a deletion of 66 Mb from 13q14.3 → 13qter.

CGH-array exploration of DNA from patient 2 revealed a loss of at least 3.86 Mb on chromosome 13p with the base position of the deleted probes: A_14_P131321 (18601703- 18601762) and A_14_P103583 (22464962- 22465021,Hg18), while the first normal probe on 13q11 wasA_14_P136382 (22647431-22647490) and a deletion of 4.7 Mb

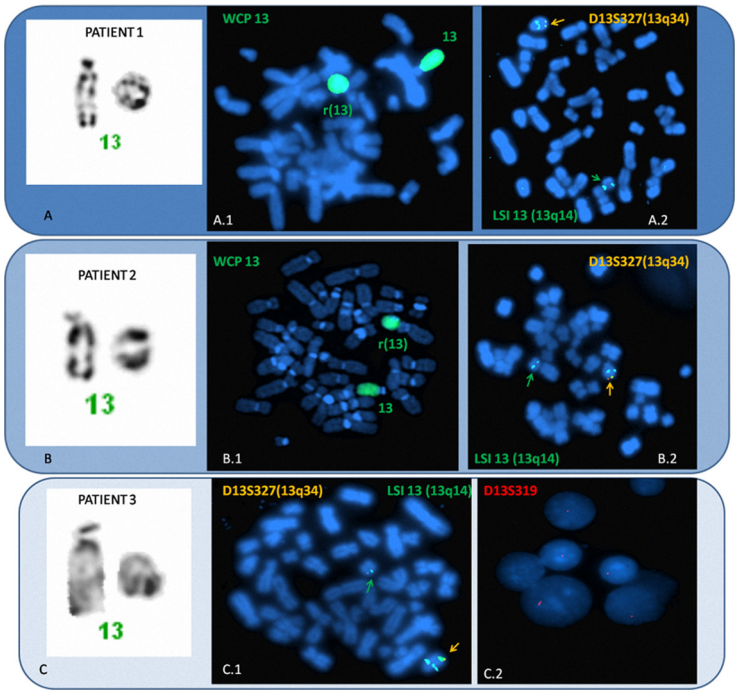

Fig. 1. Illustration of standard and fluorescence in situ hybridization findings in our three patients. (A, B, C): Partial karyotype showing the ring 13. (A1, B1): WCP13 painting probe hybridized to blood lymphocytes of patients 1 and 2 confirming that the r(13) were entirely derived from chromosome 13. (A2, B2, C1): Locus-specific fluorescence in situ hybridization commercial LSI13(13q14) and D13S327 (13q34) probes hybridized to blood lymphocytes showing only a signal (green) of LSI 13 (13q14) on the ring chromosome 13 confirming the terminal 13q34 loss. (C2): LSI D13S319 probe showing only a signal (red) of D13S319 confirming the distal deletion 13q14.3.

on the long arm of the same chromosome 13: the first deleted probe was A_14_P139655 (109599699-109599758), and the last deleted was A_14_P128085 (113964307-113964366), while the last normal probe on 13q34 was A_14_P132413 (109664184-109664239) (Fig. 2). Initially FISH defined, the chromosome breakpoints were precisely localized by CGH-array on 13p13-q12.11 - [arr 13p13q12.11 (122,132-21,593,561)×1]dn deletion at least 2.9 Mb instead of 13p11 and 13q34 [arr 13q34 (109,599,699- 113,964,366)×1]dn deletion at least 4.7 Mb (Fig. 2). No other imbalances were detected. The patient's karyotype was therefore refined to 46,XX,r(13).arr13p13q12.11(122,132-21,593,561)×1,13q34(109,599,699-113,964,366)×1 dn through use of the CGH-array.

4. Discussion

Partial deletions resulting from ring chromosome 13 lead to variable phenotypes dependent on the size and position of the deleted region. The phenotypic spectrum of r(13) syndrome is broad and can range from mild to severe [3,4]. The phenotypes of reported cases are currently known to share several common semiologic features, including overall intellectual disability ranging from moderate to severe, short stature with marked intrauterine growth retardation, microcephaly and dysmorphic traits. Behavioral anomalies, congenital heart defects, cerebral malformations, hearing and speech disorders and abnormal external genitalia in males have also been reported [3,4,7–9]. The most clinical features of patients with ring chromosome 13 overlap with those of 13q33-34 deletion syndrome, except for large deletions, which are often associated with more severe phenotype [3–10]. A comparison between the overall results of our patient's clinical features and a series of r(13) and another of 13q terminal deletion revealed that they were roughly compatible and showed common clinical features, particularly the microcephaly, developmental delay, hypotonia, short stature, congenital heart defects, abnormal external genitalia, cerebral malformations, hand and foot anomalies and dysmorphic features (Table 1) [3,4,7–11]. In addition, hearing and speech disorders were reported in our patients. Heterogeneity

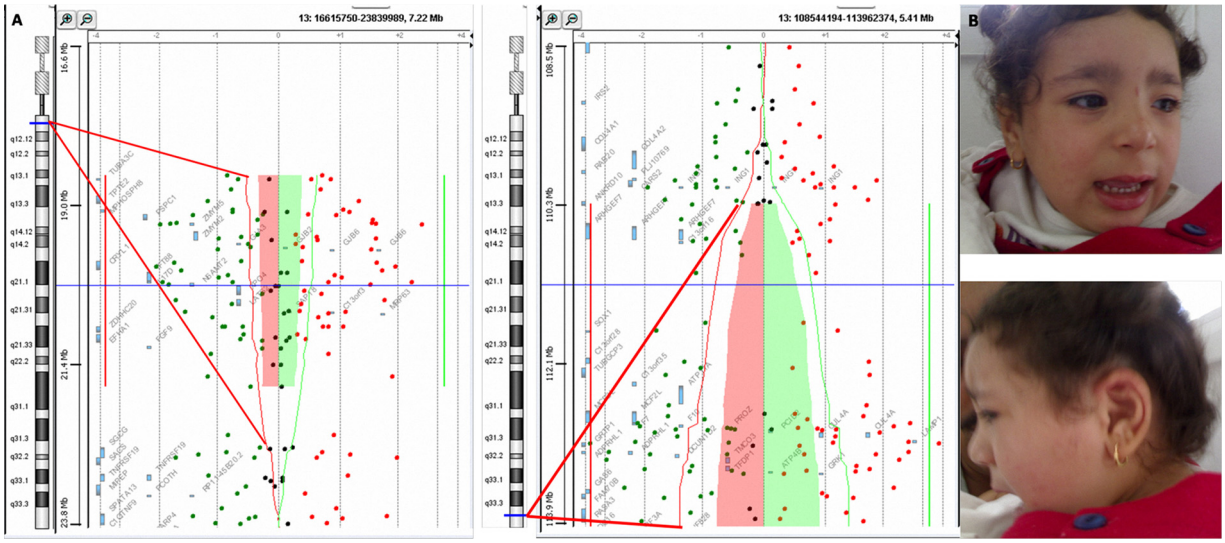

Fig. 2. (A) aCGH profiles of the patient 2 showing a 13p13 → 13q12.11 deletion of at least 3.86 Mb in size (left) and a 13q34 → 13qter deletion of 4.7 Mb in size (right). Dotted lines represent log2 ratios. (B) Photo of the patient 2 showing dysmorphic features with flat profile, broad forehead, high hairline, hypertelorism, rounded nose tip, long and flat philtrum, thin lips, high arched eyebrows, prominent ear lobe, widely spaced teeth, short neck and blepharophimosis.

of the phenotype and the difference in penetrance and expressivity of ring 13 phenotypic features could be explained by many possible hypotheses.

a. The mitotic instability of the ring chromosome 13 and level of mosaicism.
b. The correlation with the amount of subtelomeric genetic material lost from the 13q when the chromosome 13 breaks.
c. The unmasking of recessive alleles on the normal chromosome 13 by the 13qter deletion.
d. The occurrence of an intermediate inv dup del chromosome 13 that circularizes to stabilize itself.
e. The loss or retention of the minimal critical region involving 13q34 and implication of other genes proximal to this region.

Mitotic instability of ring chromosome 13 due to dynamic mosaicism occurs very frequently, but this was not evident in our patients in whom a simple ring 13 was seen in the all examined metaphases (Fig. 1) [12,13]. It has long been suggested that this phenomenon contributes to the phenotype and variable clinical presentations have been attributed to dynamic mosaicism caused by the ring's mitotic instability and resulting in subsequent aneuploidy within the chromosome [10,14–16]. Aneuploidy generated by continuously evolving mosaicism was suggested to explain the occurrence of pre- and postnatal growth retardation and microcephaly as consistent features in r(13) syndrome [13–17].

4.1. Correlation of deletion size and phenotype

A large series of reported studies suggested an overall positive correlation between phenotype severity and deletion size, although this was not evident for some clinical features such as retinoblastoma. In contrast with most patients with germ line RB1 mutations, a relevant proportion of those carrying a cytogenetically visible deletion involving the 13q14 band show unilateral disease and some develop no tumors at all [18,19]. This is the case in our patient 3.

Our three patients 1, 2 and 3 have a common deleted region in 13p13q11 (R1) and 13q34qter (R2) (Fig. 3). In addition, patient 2 has a proximal deleted region R3 in 13q21.11 and patient 3 has a distal deletion that is not overlapping with the patients 1 and 2 in 13q14.3q33.3 (R4) (Fig. 3).

The small copy variants in the terminal end of 13p13q11 (R1) have been detected in healthy individuals Database of genomic variants revealed seven reported cases of de novo deletions in the region 13p13q11 (122,132-18194570) in association with normal phenotypes [20,21]. This analysis suggested

Table 1
Comparison of our patients' phenotypic features with those of partial deletion 13q and ring 13 syndromes

Clinical features	Bed0yon et al. [7] 2004	Ballarati et al. [4] 2007	Ballarati et al. [4] 2007	Walczak-Sztulpa et al. [8] 2008	Quélin et al. [9] 2009	Quélin et al. [9] 2009	Kirchoff et al. [3] 2009	Kirchoff et al. [3] 2009	Witters et al. [11] 2009	Our study		
Case reference	Case IA	Patient 3	Patient 14	Patient 4	Patient 11	Patient 12	Patient 2	Patient 3	No data	Patient 1	Patient 2	Patient 3
Deleted segment	13q34qter	13q14.3q32.1	13q34qter	13q33qter	13q33.1qter	13q33.3qter	13q34qter	13q34qter	13q34qter	13q34qter	13q34qter	13q14qter
Sex	Female	Male	Female	Male	Female	Female	Male	Female	Male	Male	Female	Male
Inherited/de novo	Inherited	No data	No data	De novo	No data	No data	De novo	De novo	De novo	De novo	De novo	De novo
Small for gestational age	+	+	+	+	+	+	+	+	+	+	+	+
Microcephaly	+	No data	+	+	+	−	+	+	−	+	+	+
Low set ears	−	+	−	−	−	−	+	+	−	+	−	+
Clinodactyly of fifth finger	−	+	−	−	−	−	+	−	−	−	−	+
Intellectual disability	+	+	+	+	+	+	+	+	+	+	+	+
Hypotonia	−	+	+	+	−	−	+	+	−	+	−	+
High hairline	−	−	−	−	−	−	+	+	−	+	+	−
High forehead	−	−	−	−	−	−	+	+	−	+	+	+
Hypertelorism	−	+	+	−	+	−	−	+	−	+	+	−
Upslanting palpebral fissures	+	−	−	−	−	−	−	−	−	+	−	−
Prominent and broad nasal bridge	−	+	−	−	+	−	+	+	−	+	−	+
Long philtrum	−	−	−	−	−	−	−	−	−	+	+	+
High arched palate	−	−	−	−	−	−	−	−	−	+	−	−
Hearing abnormal congenital/acquired	−	−	−	−	−	−	−	−	−	+	+	−
Speech delay/defect	+	+	−	−	−	−	−	−	−	+	+	+
Short stature	+	+	+	−	+	+	+	+	−	+	+	+
Congenital heart defect	−	+	−	−	Atrial septal defect	−	−	−	−	Interventricular communication	−	−
Hand anomalies	−	+	−	−	−	−	−	−	−	−	+	−
Foot anomalies	−	+	+	−	−	−	+	+	−	−	+	−
Abnormal external genitalia	−	−	−	+Hypospadias and penoscrotal transposition	−	−	+Hypospadias	−	−	−	−	+Hypospadias
Kidney malformations	−	−	−	+	−	−	−	−	−	−	−	−
Irregular/dysplastic teeth	−	−	−	−	−	−	+	+	−	−	+	+
Cerebral malformations	−	−	−	−	−	−	−	−	Agenesis of corpus callosum	Agenesis of corpus callosum and cerebral white matter abnormalities	−	Agenesis of corpus callosum
Epilepsy	−	−	−	−	−	−	−	+	−	+	+	−

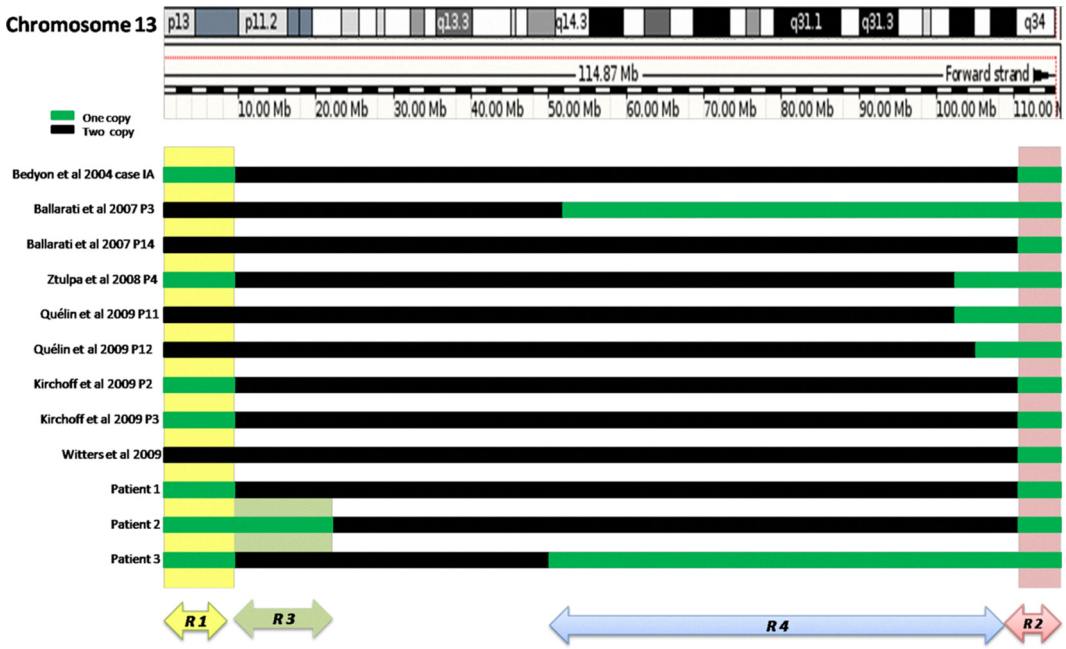

Fig. 3. Schematic illustration of the long arm of chromosome 13, comparing the size and location of previous 13q deletions reported in table 1. The genomic distances from the p telomere expressed in base pairs and shown in the figure were according to "Ensembl genome browser 59". For each patient, a normal copy number is illustrated as a black line, and deleted segment as a green line. The region marked R1 seems to be a benign deletion. Note that all the patients have loss of the region R2 which is the critical region presumed to be involved in the occurrence of intellectual disability ranging from mild to severe, microcephaly, genital malformations in males including undescended testes, hypospadias and penoscrotal inversion and agenesis of corpus callosum. The hearing and speech delay could be explained by the deletion of the third segment marked R3 including the *GJB2* and *GJB6* genes. Note that the candidate region 13q14.2, involved in the occurrence of retinoblastoma, was retained in all patients of this series.

that in our patients, terminal 13p13q11 micro deletion could only be a benign deletion, and our patients' phenotypes should result from the distal deletion rearrangement. The genomic rearrangements affecting chromosome 13q (47,357,604-114,077,122) are often caused by structural features that cause non-recurrent rearrangements with different sizes and distinct breakpoints. The non-recurrent rearrangements in partial monosomy 13q share a common genomic region-of-overlap, the smallest region of overlap is located in the 13q34qter region (109,599,699-114,077,122), that encompasses many genes associated with the common features of partial monosomy 13q syndrome, including intellectual disability ranging from mild to severe, microcephaly, genital malformations in males including undescended testes, hypospadias and penoscrotal inversion and agenesis of corpus callosum (Fig. 3: R2).

Cerebral malformations and microcephaly have been described in many patients with partial deletion of 13q34qter, so we propose that it is more likely that one or more subtelomeric genes in the deleted region on 13qter is a progenitor to the development of cerebral malformations (Fig. 2: R2) [22–24]. The deleted region, 13q34qter, encompasses several genes that could play a role in the development of cerebral malformation based on their known functions. The *SOX1* gene, located at 13q34, is a protein sharing a DNA-binding domain known as the HMG box. Bylund et al. [23] found that SOX1 is expressed in neural progenitor cells. Gene knockout and lower expression experiments showed that *SOX1* was essential for initiated neural differentiation prematurely. Another deleted gene is *ARHGEF7*, known for its role in the functioning of the human cerebral cortex [24–28]. Therefore, haploinsufficiency of *SOX1* and *ARHGEF7* genes may contribute to microcephaly and congenital cerebral malformations in patients with partial monosomy 13q34qter, as is the case of our two patients (Patient 1 and 3).

The *EFNB2* gene is considered as an excellent candidate for anal atresia with penoscrotal transposition and hypospadias in male with deletion 13q (OMIM: 602553). It was proposed that the *EFNB2* gene plays

an important role in urorectal development [25]. Therefore, the haploinsufficiency of *EFNB2* gene may contribute to hypospadias in patients with terminal deletion 13q, as is the case of our proband.

The deleted segment R3 in our patient 2 harbors the *GJB2* and *GJB6* genes. Among these, *GJB2* is considered as an excellent candidate for hearing and speech delay. Screening of the *GJB2* gene in 576 unrelated patients with recessive or sporadic deafness showed a total of 23 different mutations in *GJB2* and confirmed the major role of this gene in non-syndromic sensorineural hearing impairment, leading to the suggestion that deletion of *GJB2* gene could represent a risk factor for hearing disorders [26]. In addition, patients having rearrangements proximal to *GJB2* were recently reported to have cognitive deficits, with affected hearing and speech. This suggests a potential implication of other genes proximal to *GJB2* in hearing and speech differences associated with 13q11q21.11 deletions, like the *GJB6* gene, acting either in the-expression of hearing and speech impairment [27]. Such a hypothesis could explain the hearing loss of our patient 2.

FISH analyses showed that the locus specific D13S319 probe was deleted in the blood lymphocytes of our patient 3, so, he has an additional deleted region R4 with 67 Mb of chromosome loss, involving the deletion of at least 200 genes, suggesting their potential involvement in the phenotypic severity and neurodevelopmental features of this proband. This result is corroborated by the previous report in DECIPHER Database of a 1-year-old girl with an interstitial 10 Mb deletion ranging from 105,086,123 bp to 115,107,733 bp. The deleted region in this girl coincides with the non-overlapping deleted region of our patient (Fig. 2: R4) and her clinical features, including facial dysmorphism with low-set ears, round face, microcephaly and single transverse palmar crease, intellectual disability, congenital heart defect and aphasia, are partially compatible with those of r(13) syndrome. Like our patient, this girl does not carry a deletion including the RB1 locus (13q14.2) and no signs of retinoblastoma were evident at postnatal examination in patient 3. Contrary to the available literature with deletion 13q14 and retinoblastoma, our patient does not have this malformation. It was reported by Caselli et al. [28] that the retinoblastoma in patients was due to deletion of 13q14.2 region with loss of the *RB1* gene; this deduction was based on the proximal location of the breakpoints in patients with retinoblastoma that started at 13q14.2. In comparison to this, our patient 3 did not develop retinoblastoma, and the breakpoint was located more distally at 13q13.4 (Fig. 2).

Moreover, acute leukemia has been described with deletion 13q14.3 with loss of specific locus D13S319 [29]. We proposed that increased expression of this marker in bone marrow may be implicated in the acute leukemia observed in our proband. Nevertheless, given the death of the patient, no investigations were processed for the diagnosis of the type of leukemia and a genotype-phenotype correlation is still impossible in order to establish a definitive conclusion.

It was recently discovered that some ring chromosomes can occurr with unexpected inverted duplication contiguous to the 13q terminal deletion and this mechanism has been highlighted in some ring chromosome genotype-phenotype correlation studies. It was reported in seven patients from a series of 33 ring chromosomes, three of them had a ring 13 with duplication and contiguous deletion [30]. Knijnenburg et al. [31] proposed different mechanisms for this complex rearrangements by an interchromosomal U-type recombination during meiosis I, or a loop formation combined with one or two recombination events between homologous alleles, so an intermediate inv dup del chromosome is formed and then circularizes to stabilize itself. This mechanism leads to further phenotypic anomalies might confuse the genotype-phenotype correlation, given the additional material implicated [32].

Obviously, array-CGH studies, which can exclude any concurrent duplication as in the case of our patient 2, should also solve any discrepancy for the origin of the ring of the patients 1 and 3. Array-CGH can show additional aneuploid segments, either deletions or duplications or both; unfortunately, DNA from patients 1 and 3 was not available.

In conclusion, our study provides three additional r(13) patients, thus contributing to the clinical and genetic delineation of this rare disorder, which is known to have a widely variable phenotype. Our study supports ring chromosome 13 as major causative factor of intellectual disability, microcephaly, short stature, dysmorphism and hearing and speech delay. The advent of new technology such as array-CGH and SNP arrays and their increasing implementation in diagnostic centers, will help with the characterization of haploinsufficiency in additional carriers of r(13). The plausibility of such hypotheses should be easier to check in the few next years, together with family studies for understanding the transmission of ring chromosomes.

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