Clinical and cytogenomic characterization of de novo trisomy 9 mosaicism in an Egyptian family: phenotype/karyotype correlation

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Abstract

Trisomy 9 mosaicism is a rare chromosome abnormality with developmental disabilities, delayed speech, facial dysmorphism and organ pathologies, the phenotype usually differs among individuals. This study presents a proband and his sib with mosaic trisomy 9 but the clinical manifestations of the boy were more severe than his sister as the number of abnormal cells were more in the boy, this could highlight the existence of phenotypic/karyotype correlation and the association of the level of mosaicism with the severity of the phenotype. This report expands the clinical manifestations of this chromosomal abnormality as multiple areas of hyperpigmentation were noticed in these cases that to the best of our knowledge have not been reported before.

Introduction

Trisomy 9 (T9) syndrome and trisomy 9 mosaicism are rare chromosomal disorders. Trisomy 9p may result from a parental reciprocal translocation between chromosome 9 and another autosome or may be due to a de novo non familial aberration. In the majority of cases, phenotypic variability occurs due to the variable size of the trisomic fragment and the frequent concomitant monosomy or trisomy [1]. Children born with T9 mosaicism usually survive into adulthood [2,3]. T9 mosaicism usually present with a low birth weight and a wide range of developmental disabilities, dysmorphism and organ abnormalities, though the phenotype may differ widely between the patients [4,5]. T9 mosaicism is characterized by growth retardation, developmental delay, delayed communication and speech and learning difficulties. The typical physical features include microcephaly, craniofacial abnormalities such as cleft palate, low set ears, loose joints, skeletal abnormalities of the skull, hands and feet [6].

Abnormalities involve different organ systems such as the brain and CNS, heart, kidneys, digestive tract, muscles (hypertonia), genital and urinary tract [5,7]. However, growth retardation and developmental delay were mainly less severe in patients with a low level of T9 mosaicism. NTRK2, DMRT1 and NTRKR2 recessive genes related to growth and development on chromosome 9, may also contribute to the overall clinical disease phenotype [3].

Case report

In this study, we report on two de novo sibs with variable phenotypic and clinical features of trisomy 9p. A 3-years old male patient, the first born of a healthy consanguineous couple was referred to our clinic for chromosomal analysis due to delayed milestones and dysmorphic features. The karyotype of the parents was normal. There was prenatal onset of growth deficiency. The dysmorphic features included triangular face, recession of anterior hair line, downwards slanting of eyes, bulbous nose, low set ears, micrognathia, short neck and clinodactyly in the 5th finger of the right hand. There were areas of hyperpigmentation on the face and abdomen. Anthropometric measurements revealed growth retardation as the head showed microcephaly 41.5 cm (-4.4 SD), the weight was 6 kg (-5 SD) and the height was short 73.5 cm (-3.9 SD). Cardiovascular abnormalities were patent foramen ovale, patent ductus arteriosus (PDA), and mild pulmonary hypertension. X-Rays of the left hand revealed hypoplastic terminal phalanges. Growth hormone levels before and after stimulation were normal. Abdominal and pelvic sonar and CT brain were normal. No fundus, kidney or genital, abnormalities were detected. Intellectual disability and delayed speech were detected. The karyotype of our patient revealed: 47,XY,+ 9, 45,XY, - 9, 47,XY,+mar and 46,XY. After one year of admission, the parents got a daughter, but they did not seek prenatal diagnosis, then they brought her at the age of 3 years for consultation. She had delayed milestones, growth retardation and dysmorphic features but to a less extent than her brother, her echocardiogram was normal, the speech development was milder and her karyotype was 47,XX,+ mar and 46,XX.

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Patients and Methods

Patients were assessed by full medical history, meticulous clinical examination, pedigree analysis, language evaluation and karyotype analysis.

Language evaluation was performed using the Modified Preschool Language Scale - Fourth (modified PLS-4) Arabic edition [8]; a language test, standardized on normal Arabic speaking children from Egypt. It is composed of two subscales; Auditory Comprehension and Expressive Communication subscale used mainly to identify children who have a language disorder or delay; with age range between 2 months till 7 years and 5 months, as well as elder children who function developmentally in this age range.

G-banding: Chromosomal analysis was performed on phyto-haemagglutinin (PHA) - stimulated peripheral blood lymphocytes, then GTG-banding technique according to Verma and Babu [9], to record the presence of chromosome aberrations. Metaphases were analyzed and karyotyped following the International System for Human Cytogenetic Nomenclature [10]. Conventional cytogenetic results were further confirmed by Fluorescence in situ hybridization (FISH) according to Pinkle et al. [11].

Fluorescence in-situ hybridization (FISH) technique was applied on interphase and metaphase cells from peripheral blood according to modification of Pinkel et al. [11] by using Whole Chromosome Painting Prope (WCP) supplied by Cytocell Aquarius Diagnostics. The origin of the extra marker chromosome was identified and confirmed by FISH using Whole Chromosome Paint 9 probe (WCP9) spectrum green.

Written informed consent was obtained from the parents of the children for the publication of this report and any accompanying images.

Results

Table 1 summarizes the clinical findings of the studied patients. Testing of both parents revealed normal peripheral blood karyotypes denoting de novo non familial trisomy 9p.

The pedigree of the family is presented in Figure 1. The dysmorphism and hyperpigmentation in the proband and the female sib (Figure 2).

The language skills of the two sibs were evaluated using the Modified Preschool Language Scale - Fourth (modified PLS-4) Arabic edition [8]. The male sib, aged 6 years and 1 month, revealed receptive language age equaling 5 years and 2 months (standard receptive language score = 55), expressive language age equaling 4 years and 8 months (standard expressive language score = 53), and total language age equaling 5 years and 1 month (standard total language age = 51) Table 2. The female sib, aged 2 years and 4 months, revealed receptive language age equaling 2 years and 5 months (standard receptive language score = 54), expressive language score = 53), and total language age equaling 52 (standard total language age = 53).

Table 1. Summarizes the clinical findings of the studied patients

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Prenatal onset of growth deficiency</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Broad forehead</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Upplunting of the eyes, blepharophimosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Deeply set eyes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Micrognathia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Low set ears</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Facial asymmetry</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Short neck</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Single palmar crease</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Joint anomalies</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Other medical conditions</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Congenital heart defect</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hydrocephalus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hearing loss</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>13 ribs and thoracic vertebrae</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Genitourinary anomalies</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Areas of hyperpigmentation</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Karyotype</td>
<td>47,XY,+9 10%</td>
<td>47,XX,+mar 60%</td>
<td>47,XX,+mar 60%</td>
<td>46,XY 40%</td>
</tr>
<tr>
<td>46,XY</td>
<td>27%</td>
<td>47,XX,+9 3%</td>
<td>47,XY,+9 10%</td>
<td>47,XY,+9 10%</td>
</tr>
</tbody>
</table>

Table 2. The modified PLS-4 Arabic edition in the male sib, with the chronological age of 6 years and 1 month

<table>
<thead>
<tr>
<th>Test domain</th>
<th>Language age</th>
<th>Standard score (mean=100 ± 22.5)</th>
<th>Degree of language delay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptive language</td>
<td>5 years, 2 months</td>
<td>52</td>
<td>moderate</td>
</tr>
<tr>
<td>Expressive language</td>
<td>4 years, 8 months</td>
<td>53</td>
<td>moderate</td>
</tr>
<tr>
<td>Total language</td>
<td>5 years, 1 month</td>
<td>51</td>
<td>moderate</td>
</tr>
</tbody>
</table>

Figure 2. a and b: The patient at the age of 1 year and 5 months. c and d: the patient at the age of 7 years showing the dysmorphic features: microcephaly, broad forehead, downwards slanting of eyes, hemangioma under the right eye, full lips, large low set ears, bulbous nose, micrognathia, and short neck. e: areas of hyperpigmentation on the abdomen. f: clinodactyly of 5th finger in the right hand. g and h: The patient and his sister with the same dysmorphism.

Figure 1. The pedigree of the family
The karyotype analysis of the male sib was: 47,XY, +9 in 10%, 45,XY, -9 in 10%, 47,XY, +mar in 40% and 46,XY in 40% of the studied cells (Figures 3a, 3b and 4).

The karyotype analysis of the female sib was: 46,XX in 60% of the studied cells and 47,XX, +mar in 40% of the cells (Figure 5).

Table 3. The modified PLS-4 Arabic edition in the female sib, with the chronological age of 2 years and 4 months

<table>
<thead>
<tr>
<th>Test domain</th>
<th>Language age</th>
<th>Standard score (mean=100 ± 22.5)</th>
<th>Degree of language delay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptive language</td>
<td>2 years, 5 months</td>
<td>105</td>
<td>Average</td>
</tr>
<tr>
<td>Expressive language</td>
<td>2 years, 2 months</td>
<td>98</td>
<td>Average</td>
</tr>
<tr>
<td>Total language</td>
<td>2 years, 3 months</td>
<td>103</td>
<td>Average</td>
</tr>
</tbody>
</table>

Conventional cytogenetic results were further confirmed by Fluorescence in situ hybridization (FISH).

The origin of the extra marker chromosome was identified in the male sib (Figure 6a) and the female sib (Figure 6b) and confirmed by molecular cytogenetic analysis FISH according to Pinkle et al. [11] using Whole Chromosome Paint 9 probe (WCP9) spectrum green.

Discussion

Our patients had prenatal onset of growth deficiency similar to that reported by Jones et al.; Pruksanussak et al. and Pejcic et al. [2,5,12]. They presented with delayed milestones and dysmorphic features comprising triangular face, recession of anterior hair line, downwards slanting of eyes, bulbous nose, low set ears, micrognathia, short neck...
and clinodactyly of 5th finger in right hand. Schinzel [13] suggested that the pattern of radiographic findings in trisomy 9p appears to be unique among chromosome aberrations. Roentgenograms of the skeleton demonstrated hypoplastic pubic bones, and bilateral hypoplastic distal phalanges of the feet, pes valgus and bilateral clinodactyly of both 5th fingers [14]. Both sibs presented with hypertelorism with downwards slanting of the eyes, similarly, Dhangar et al. [15] affirmed hypertelorism in their patient. Although our patients did not have hearing loss, Zhou et al. [16] and Dhangar et al. [15] described a trisomy 9 patient with sensorineural hearing loss and proposed that 9q13→9q21 region may be responsible for hearing. This may be because the critical region of trisomy 9 in our cases was different. Also, they found clinodactyly of both of the 5th fingers, a bilateral simian crease, joint hyperlaxity and hypoplasia of the toenails, however the male sib had hypoplastic terminal phalanges and clinodactyly of the fifth finger on the right hand while, his sister did not have any bone abnormality. Wilson et al. [17] reported that the clinical severity in trisomy 9p generally associates with the extent of trisomic chromosome material. However, De Ravel et al. [18] narrowed the critical region of trisomy 9p phenotype between 9p22.1 and 9p22.2. Our studied cases revealed trisomy of the distal part of the short arm of chromosome 9.

Our patient and his sister presented with areas of hyperpigmentation, which to the best of our knowledge was not reported before. They also had short neck comparable to that reported by Brambila et al. [7] and Zhou et al. [13]. The echocardiogram displayed patent foramen ovale, patent ductus arteriosus (PDA), and mild pulmonary hypertension in the male sib but not in his sister, this may be due to the increased number of affected cells in the proband compared to his sib. While, Tonni et al. [4] noted that the cardiovascular abnormalities were characterized by a right-sided, U-shaped aortic arch associated with a ventricular...
septal defect (VSD). On the contrary, Zhang et al. [14] did not find any cardiac abnormality. Furthermore, our male sib had growth retardation and intellectual disability more than that in the female sib, analogous to previous investigators findings [14,15,19]. The incidence and severity of the malformation and the intellectual disability correlates with the percentage of trisomic cell [12]. This may emphasize that patients with a high percentage of affected cells (high mosaicism) might have more severe symptoms than those with a low percentage of affected cells (low mosaicism). Although, Ma et al. [3] claimed the lack of phenotypic/genotypic correlation and absence of correlation of the level of mosaicism with the severity of the disease phenotype. Haddad et al. [20] stated that, Trisomy 9 patients are more severe and variable in their phenotype than partial trisomy 9 patients. The genes associated with growth and development on Chromosome 9 as DMRT1, NTRK2 and NTRKR2 may have led to the growth retardation [3]. The growth hormone levels before and after stimulation were normal in our patients. Though, Tiong et al. [21] stated that his patient had growth hormone deficiency. Abdominal and pelvic sonar was normal in both patients although, Tonni et al. [4] reported multicystic dysplastic kidney disease in their study.

CT brain of both patients were normal, this corroborates with the findings of Zhang et al. [14]. Whereas, Tonni et al. [4] found hypoplastic corpus callosum, and a Dandy-Walker malformation in their study.

The range and severity of the phenotype relies on the percentage of cells with extra chromosome 9 [15]. Speech development was severely retarded in the proband but was milder in his sister, Gulten et al. [21] and Bruns and Campbell [19] noted delay in language, communication and social-emotional development.

Speech development in the male sib showed a moderate delay in his total language skills, especially in the expressive domain, while the female showed average language skills. This diversity in language skills was recorded in several studies describing trisomy 9 mosaicism [6,16,22]. However, the young female sib should be followed up to delineate any delay in her language skills in the future.

Mosaic trisomy 9 appears to result from errors of chromosomal separation during meiosis or occur during mitosis, these abnormalities are known as a "pericentric inversion" in one of the parents. Cytogenetic studies allow the differentiation of several mechanisms causing trisomy 9p [23]. The karyotype of the proband was confirmed as 47,XY,+9; 45,XY,-9 and 47,XY,+mar and 46,XY while his sister karyotype was 47,XX,+mar and 46,XX similar to Luo et al., Tong et al. [24,25]. FISH is a focused and targeted approach for diagnosing mosaic trisomy facilitating genetic counseling and prevention of the birth of further affected offspring. In our study, the mother did not come for prenatal diagnosis during the pregnancy of her daughter. The accompanying features in trisomy 9 usually depend on the percentage of trisomic cells. Therefore, the clinical presentation of the proband was more severe than his sib.

Conclusion: Trisomy 9 mosaicism comprises a wide spectrum of congenital abnormalities, the present study broadens the phenotype of this rare aneuploidy, multiple areas of hyperpigmentation were noticed in these cases that to the best of our knowledge have not been reported before. Furthermore, a phenotype/karyotype correlation was revealed. Chromosome studies and genetic counselling are typically recommended for parents with an affected child.

Competing interests

The authors declare that they have no competing interests.

References


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