Cytogenetic evaluation of a patient with ring chromosome 9 presenting failure to thrive and developmental delay

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= Abstract =

We report clinical, cytogenetic, and fluorescence in situ hybridization (FISH) studies of a patient with ring chromosome 9. She presented with failure to thrive, facial dysmorphysm and mild psychomotor development delay in the absence of major malformations. Peripheral blood karyotype of the patient was 46,XX,r(9)(p24q34). G-band analysis suggested no loss of material in the ring chromosomes. FISH analysis using the subtelomere-specific sequences on chromosome 9p and 9q, revealed 46,XX,r(9)(p24q34),ish r(9)(D9S913-,D9S325+). Failure to detect any hybridization of a probe for the subtelomeric sequences in the ring 9p terminal suggested that this ring arose from breakage in the distal short arm. The cytogenetic and FISH data in our case provided further evidence for the existence of a "complete ring" phenotype with incomplete subtelomeric sequences. (Korean J Pediatr 2008;51:426-430)

Key Words : Ring chromosome 9, Clinical implication, Cytogenetic, FISH studies

Introduction

A change in chromosome topology from linear to circular may totally disrupt the chromosome sequence. In individuals with Chromosome 9 Ring, the variability of associated symptoms and findings may depend upon the amount and location of genetic material lost from the 9th chromosome, the stability of the ring chromosome during subsequent cellular divisions, or other factors. One of possible mechanisms is ring chromosome deletion of terminal segments of the chromosome followed by fusion of arms, with loss of genetic material distal to the breakpoints. The second mechanism of ring formation is end-to-end fusion of palindromic sequences at telomeres without genetic loss[1, 2]. The formation of the ring chromosome in these cases may be regarded as a "structural mutation" or alteration in the structure of the genetic material, rather than a loss or gain of genetic dosages[3]. The phenotypes may be attributes to loss of variable portions of 9p and 9q lost in the formation of the ring (Table 1). The term, "ring syndrome," was proposed to describe a phenotype of growth failure without major malformations due to a ring autosome[2]. This can be characterized by extreme somatic retardation together with an otherwise almost-normal appearance, viz. no major anomalies, and mild or moderate mental retardation. The severe somatic retardation, i.e. the sole major sign of this phenotype, can not be explained by organic, biochemical, or endocrine causes. The growth failure is thought to be caused by instability of the ring chromosome leading to aneusomy and cell death[3]. Using FISH, complete rings were demonstrated by intact subtelomeric sequences[4].

We report clinical and molecular cytogenetic studies in a patient with ring chromosome 9.

Case report

The patient was a 4 year-old girl and was ascertained by failure to thrive and short stature. She was born to a 30-year-old mother at 39 weeks after uncomplicated pregnancy. Birth weight was 2.8 kg, length 45 cm, and head circumference 30.5 cm. Family history was unremarkable. The parents were healthy without any dysmorphic feature. Father's height was 170 cm and mother's height 158 cm. Her two elder sisters also were normal. At age of 4 years and 5 months, her growth showed less than the third percentile in weight (13 kg), height (91 cm) and head cir-

Table 1. Clinical Features in 9p- and 9q- Syndromes

Clinical features	9p-	9q-
Cranium	Trigonocephaly, prominent forehead, flat occiput, midface hypoplasia	Microcephaly, dolichocephaly, prominent occiput, midface hypoplasia
Eyes	Upslanting or downslanting palpebral fissures, epicanthal folds, arched thick eyebrows	Downslanting or oblique palpebral fissures, epicanthal folds
Mouth	Micrognathia, long philtrum high arched palate	
Ears	Low set pinnas with abnormal ear lobules short nose with flat bridge, anteverted nostrils	Low set malformed pinnas
Nose	Short neck with low hairline	Small nose with flat nasal bridge and anteverted nares
Neck	Widely spaced nipples	
Chest	Long fingers and toes secondary to elongation of second phalanx, flat feet, dermatoglyphic abnormalities (excess whorls on fingers)	Pectus excavatum
Extremities	Cardiovascular anomalies	Talipes equinovarus, short third metarsals, single tranverse palmar crease
CVS	Scoliosis/kyphosis	Supraventricular tachcardia
Skeletal	Epilepsy	
Neurologic	Primary sex reversal with ambiguous genitalia with varying grades of mixed gonadal dysgenesis	Hypotonia, arrhinencephaly, absent deep tendon reflexes
Genital		Hypospadias
Respiratory		Tracheomalacia
Hematologic		Capillary hemangiomas of head and neck
Gastrointestinal		Intestinal malrotation, gastroesophageal reflux, feeding difficulties
Growth and mental development	Mental and growth retardation	Mental and growth retardation

Reference: http//www.nlm.nih.gov/mesh/

cumference (44.7 cm) in spite of good appetite. Her psychomotor development was mildly delayed. The face showed some dysmorphic facial features including prominent eyebrows, short nose and fifth finger clinodactyly. The extremities revealed prominent great toes and hyperextensible joints. Laboratory evaluation included CBC, biochemical parameters, thyroid function, IGF-1, amino acids and organic acids. The bone age corresponded to 3 years and 6 months old according to Greulich and Pyle standards. Spine x-ray, brain MRI and echocardiogram were normal.

Material and Method

Peripheral blood lymphocytes were prepared by standard methods and G-banded. Metaphase cells were analyzed at a resolution of 550 bands per haploid set and imaged using the CytoVision karyotyping system (Applied imaging).

FISH analysis was performed using subtelomeric 9p (D9S913, Telvision 9p) and 9q (D9S325, Telvision 9q) probes. All probes were used according to the manufacturer's instruction and imaged using CytoVision system (Applied imaging).

Results

Chromosomal analysis showed 46, XX,r(9)(p24q34). G-banding analysis suggested no loss of material in the ring chromosome (Fig. 1). The finding was confirmed by FISH using subtelomeric probes. Sequences specific for subtelomeric region of 9p was deleted (Fig. 2), but subtelomeric region of 9q was intact (Fig. 3). Cytogenetics study in our case revealed the ring (9) chromosome with subtelomeric deletion 9p by FISH but showed the "ring syndrome" phenotype.

Discussion

Patients with ring chromosome 9 often share typical features of the 9p- syndrome, as well as some features seen in the less frequent deletion of 9q[5]. Atypical manifestations are frequently seen in patients with r (9), indicating heterogeneity of the genotype. An apparent Prader-Willi phenotype has been described[6]; the patient had mental retardation, socially inappropriate behavior, and a karyotype of
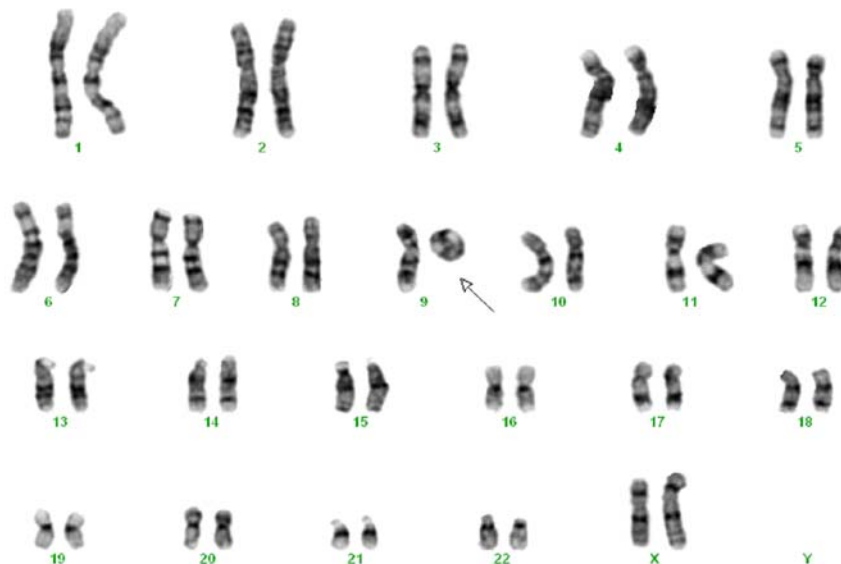
Fig. 1. GTG-banded karyotype of the patient. XX,r(9)(p24q34).
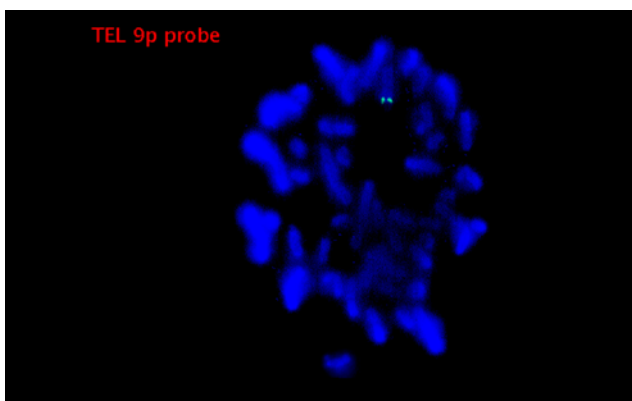
Fig. 2. FISH with subtelomeric 9p probes showing absence of 9p subelomeric region on ring chromosome 9.
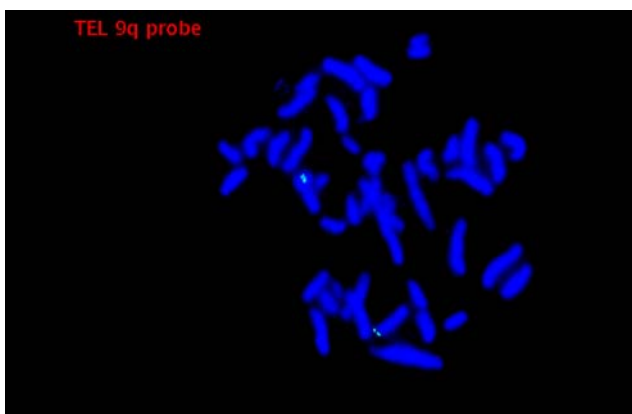
Fig. 3. FISH with subtelomeric 9q probes showing presence of 9q subtelomeric regions on both normal chromosome 9 and ring chromosome 9.

46,XX,r(9)(p24q34). Case reports also suggest other features like malformed upper extremities with congenital heart disease in a patient with a karyotype of 46, XY,r(9)(p24q34)[7]; skeletal abnormalities with hypospadias[8]; ambiguous genitalia in a patient with a karyotype of 46, XY,r(9)(p2q3)[9]; corpus callosum hypoplasia and infantile periodic spasm[10]; gatroesophageal reflux[11]. Stumm et al. reported deletion of the DM-domain gene cluster in a fetus with ring chromosome 9 with breakpoints at 9p24 and 9q34 and sex reversal [12]. Severe symmetric growth failure, generalized hyperpigmented rash, cafe au lait spots, and cherry red spots in both eyes were reported in a case of 45,XY,-9/46,XY,r(9) (p24q34.3) [13]; this patient also showed features of the "ring syndrome". The ring syndrome consists of a phenotype of primordial growth failure without malformations and with or without minor anomalies due to a ring autosome[2, 3].

The mechanism for formation of a ring chromosome is generally thought to be breakage in each arm of the chromosome and subsequent fusion of the broken ends. This presumes the loss of some genetic material, particularly the terminal segments. Zuffardi et al.[1] and Cote et al.[2] first proposed that in patients with a ring chromosome associated with minor phenotypic alterations, palindromic DNA base sequences at the chromosome ends might be responsible for ring formation by end-to-end fusion. This would result in alteration of the structure of the genetic material rather than a pure deletion. It is thought that the ring structure itself made the chromosomes unstable during cell

division, which leads to aneusomic daughter cells with increased mortality[2, 3]. Cell death can lead to decreased number of viable cells at any given interval of development. This could play a fundamental role in the determination of growth failure in patients with "complete rings."

According to Kosztolanyi, the size of a ring chromosome may determine the patient's growth: the greater the chromosome involved, the higher is the probability of severe growth failure. This could be explained by the assumption that a greater ring chromosome, being exposed to more sister chromatid exchanges than a smaller one, is more unstable and, consequently, produces an increased in vivo cellular death rate resulting in significant growth deficiency. Also, he suggested that a ring chromosome in humans evolves in many cases by an event which does not involve deletion in the genetic sense. Telomeric fusion provides a plausible explanation for this chromosomal mutation. Ring chromosome without a preceding deletion may manifest itself its behavioral and structural instability resulting in the continuous generation of secondary aneuploid cells with increased mortality.

The phenotypic anomalies in these cases consist of severe growth failure, only a few or no minor anomalies (no major malformation and specific deletion syndrome), and mild to moderate mental retardation.

A ring chromosome may be regarded as a unique genetic abnormality since the primary mechanism whereby this chromosome anomaly results in its phenotypic effect is a "non-DNA or structural mutation," i.e. an alteration in the structure of the genetic materials, rather than a loss or gain of gene dosages[3].

The variability between the cases with r (9) despite similar breakpoints identified by GTG-banding may be explained by submicroscopic differences between deletion breakpoints, ring instability, interaction of other genes on the phenotype, and variation in fetal environmental conditions[5].

We report a patient with ring chromosome 9 with deletion breakpoints at 9p24 and 9q34 determined by GTG-banding. Based on FISH analysis, terminal 9p were deleted. She has severe symmetrical growth failure and nearly normal cognitive abilities in the absence of malformations. Our patient has terminal 9p deletion, but her clinical features are similar to ring syndrome. Ring syndrome confirmed by FISH using chromosome-specific subtelomeric probes might be a useful way to predict developmental prognosis in a case with an apparently complete ring chromosome[14].

We propose that cases of isolated severe short stature should be considered for a cytogenetic evaluation to rule out the presence of a ring chromosome.

한글 요약

성장부진과 발달지연을 보인 환아에서 확인된 환상 9번 염색체 1례의 세포유전학적인 연구

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환상 염색체의 발생 기전은 염색체 말단 부분이 결손된 후 양 끝이 융합되거나 끝분절 염기서열이 앞뒤역순상동서열로 융합될 경우로 생각되고 있다. 환상 염색체는 세포 분열을 하는 동안 불안정하기 때문에 세포핵이 없는 딸세포의 사망률이 증가하게 되어 생존하는 세포수가 감소하고 성장 장애가 발생하게 된다. 표현형은 염색체 손실의 정도에 따라 다양하다. 저자들은 최초로 심각한 저신장을 주소로 내원한 환아의 염색체 검사상 환상 9번 염색체를 확인하였기에 이를 보고하는 바이다.

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