Original article

Characterization of extra euchromatic band in the 9qh region

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Objective The objective of this study was to evaluate the extra G-positive band in the 9qh (9q12) region of two fetuses detected prenatally.

Methods Metaphase chromosomes were prepared from fetal blood samplings of two fetuses. G-banding, micro-FISH, mBAND, and FISH techniques were used to evaluate the chromosomal structure of the extra band.

Results Chromosome analysis using the G-banding technique revealed an extra G-positive band in the 9qh (9q12) region on one of the chromosomes 9 of both fetuses. The micro-FISH technique showed an extra band in the first case with a similar fluorescent signal to that in band 9q12. The extra band from the mBAND technique revealed a fluorescent signal, and the intensity profile resembled band 9q13. In the second case, the extra band showed no fluorescent signal when it was stained with DAPI and hybridized with labeled human Cot-1 DNA. Both children were born at term with a normal phenotype.

Conclusion The extra band in the first case originated from chromosomal material in the 9q12-9q13 region, while that in the second case comprised non-repetitive sequence DNA. The extra band had no clinical effect in either case. **Chiang Mai Medical Journal 2015;54(2):57-63.**

Keywords: extra band in 9q12, micro-FISH, mBAND, human Cot-1 DNA

Introduction

Up to 5% of the human genome is made up from duplicated human chromosomal segments, which are enriched at pericentromeric and pretelomeric sites[1]. More than 90% of the segmental duplications are identical at the sequence level and vary in size from several to hundreds of kilobases in length. Among the non-acrocentric chromosomes, chromosome 9 presents with a very high degree of morphological variations. Variants, such as 9qh+, 9qh- or inv (9) (p11q13) are very common findings in routine cytogenetics[2]. The common pericentromeric inversion of chromosome 9 is believed to be mediated by homology between

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the chromosomal segments that flank the 9qh region. The 9q12 euchromatic variant was first reported by Medan[3]. Extra GTG-positive and CBG-negative bands in the 9q12 region, without clinical consequence, have been reported in several families[4-7]. The variations that were observable at the cytogenetic level have been described as euchromatic variations of 9p12, 9q12/qh, and 9q13, which extend to several megabases. It has been proposed that the 9q12/qh euchromatic variants derived from the 9p12 or 9q13–q21.1 regions[1,8].

This investigation describes an extra band in the 9qh region, which normal fetuses inherited from their father.

Methods

Informed consent for prenatal diagnosis was obtained from the family of each subject. This study was approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University. The mother in the first case was 39 years old and seen for prenatal diagnosis due to her advanced maternal age. She under-went uneventful fetal blood sampling. Metaphase chromosomes were prepared from the fetal blood for prenatal chromosome analysis, and stained using the G-banding technique. Chromosome analysis revealed an abnormal chromosome 9 with an extra band in the 9qh region. The chromosome complements of the parents also were investigated. The maternal karyotype was normal, but the paternal one revealed the same abnormal chromosome 9. Thus, the abnormal chromosome in the fetus was inherited from the father. The extra band was characterized further by using microdissection in combination with fluorescence in situ hybridization (micro- FISH), and the multicolor banding (mBAND) techniques. The fetal metaphase chromosomes were prepared on a cover glass using the micro-FISH technique, and stained with the G-banding technique. The abnormal chromosome 9 was dissected with a glass microneedle equipped with a micromanipulator attached to an inverted microscope. Twenty chromosomes were collected, and the DNA on the dissected ones was amplified by degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR)[9]. The PCR product was labeled with fluorochrome and used as a whole chromosome-9 specific probe. Fluorescence in situ hybridization (FISH), with the abnormal chromosome 9-derived probes was performed on normal and abnormal metaphases (forward and reverse paintings). This study also used the mBAND technique for precise characterization of the extra band[10]. The second case was seen 7 years after the first one. The mother was 35 years old and seen for prenatal diagnosis due to her advanced maternal age. She underwent fetal blood sampling. The chromosome analysis using G-banding revealed an extra band in the 9qh region of a chromosome 9, and it was characterized using DAPI-bands. This study also applied the FISH technique using labeled human Cot-1 DNA as a FISH probe[11]. Human Cot-1 DNA is composed of repetitive sequences that could be used for identifying repetitive sequence DNA in a heterochromatin on a chromosome. The parental chromosomes were investigated and the maternal karyotype was normal, but the paternal one revealed the same extra band in the 9qh region, which was transmitted to the fetus.

Results

In the first case, the extra band in the 9qh region was G-band positive [Figure 1]. By using micro-FISH, the abnormal chromosome 9-derived probes were painted on only the two normal homologue chromosomes 9 on the normal metaphases. The probes on the abnormal metaphases were painted on the normal and abnormal chromosomes 9. Band 9q12 and the additional band on the abnormal chromosome revealed the same intensity as the fluorescent signals [Figure 2]. By using the mBAND technique, the extra band revealed the color and intensity profiles of the band 9q13 [Figure 3]. With the assistance of micro–FISH and the mBAND techniques, this study concluded that the extra band in the 9qh region originated from the amplification and inversion of genetic material in the 9q12-9q13 region.

In the second case, the extra band was G-band positive (Figure 4) and DAPI- band negative (Figure 5). The FISH technique using labeled human Cot1-DNA found signals on the heterochromatic regions of all chromosomes, but no signal on the extra band (Figure 6).

Ultrasonography of both fetuses was unremarkable, the pregnancies were continued to term, and the infants were born at term with normal phenotypes.

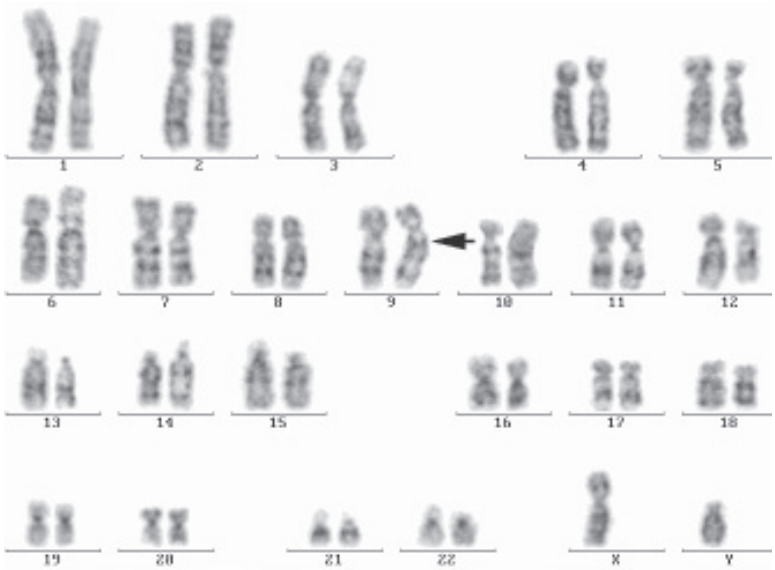

Figure 1. GTG-banding of the fetal karyotype in the first case revealing an extra G-positive band in the 9q12 region (arrow).

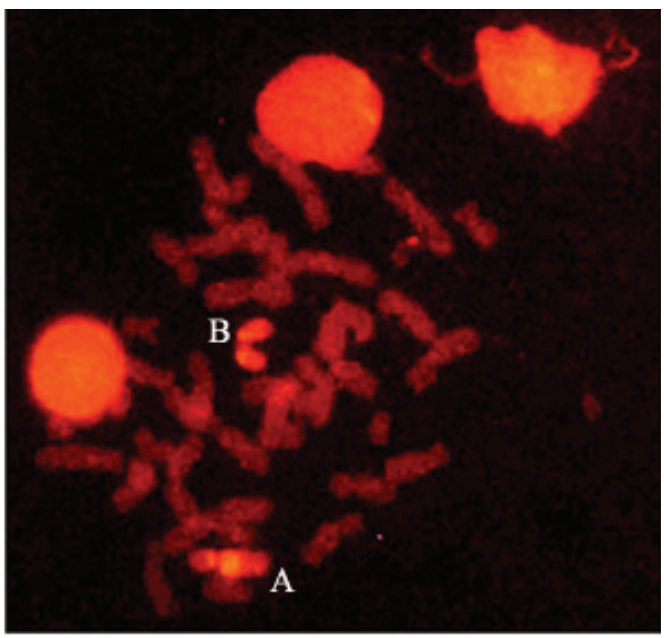

Figure 2. Abnormal metaphase chromosomes in the first case, hybridized with an abnormal chromosome 9-derived probe; on the abnormal chromosome 9 (A), band q12 and extra band, revealing the same intensity.

Discussion

The heterochromatin in the pericentromeric region and the proximal long and short arms of human chromosome 9 revealed a homology of between 9p12 and 9q13-21.1. Human chromosome 9 exhibits a high degree of morphological variation owing to the heterochromatin component in the area of 9p12–9q13. The variants of heterochromatin are 9qh+, 9qh- and inv (9) (p11q13). Molecular cytogenetic techniques have demonstrated that the centromere and pericentromeric heterochromatin are not homogeneous structures. The heterogeneity of these regions is presumably due to the accumulation of different satellite DNA sequences. The centromeric region contains alphoid satellite DNA; while the pericentromeric regions contain β satellite and satellite III DNA[4,12].

There are two euchromatic variants on chromosome 9: the short- and long-arm. The short arm variant has additional chromosom-

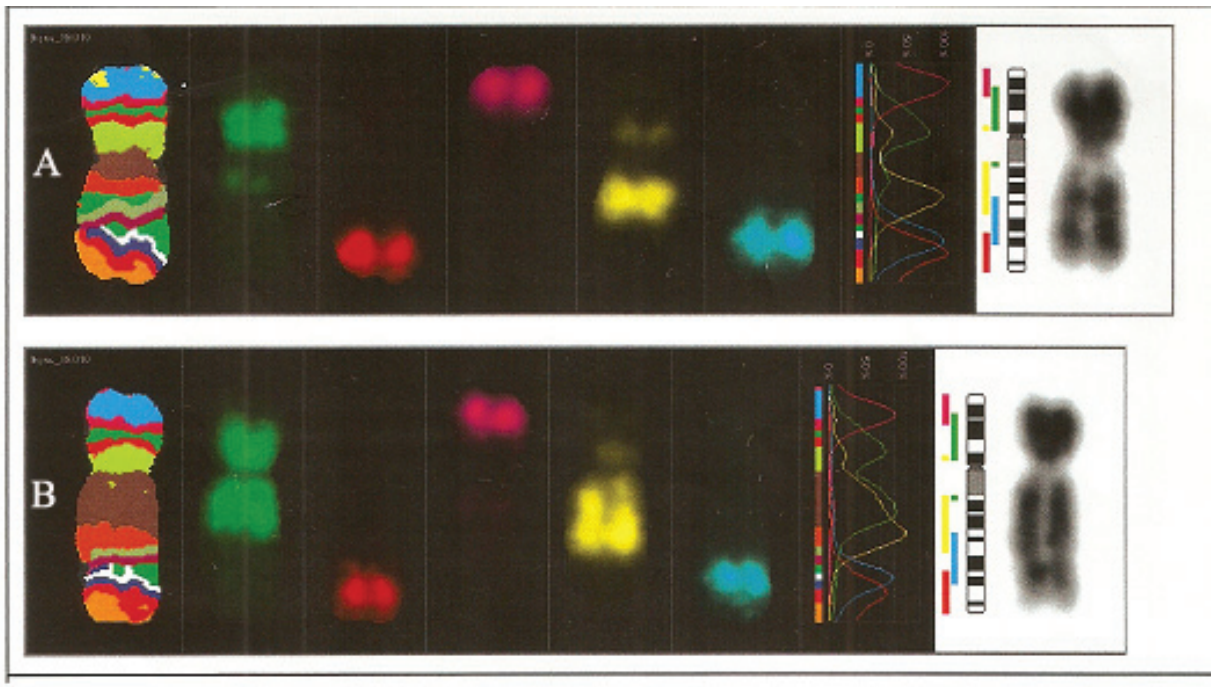

Figure 3. The mBAND technique on the fetal chromosome in the first case, showing multicolor patterns and an intensity profile on the normal (A) and abnormal (B) chromosomes 9, with the extra band revealing the color and intensity profile of the band, q13.

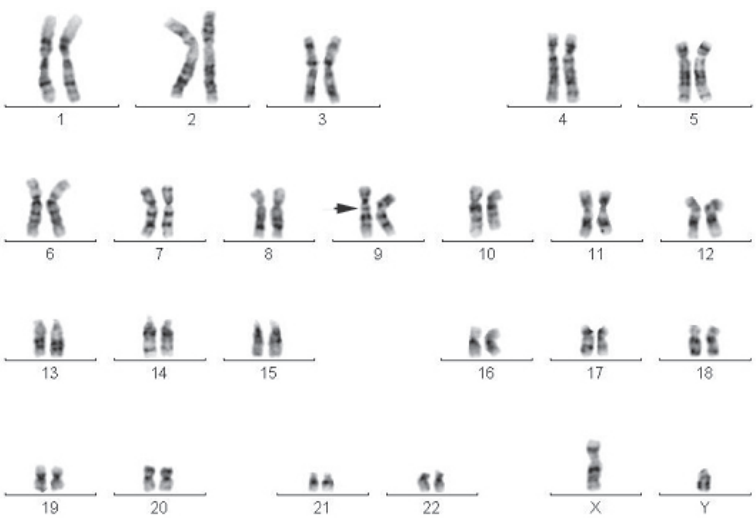

Figure 4. GTG-banding of the fetal karyotype in the second case revealing an extra G-positive band in the 9q12 region (arrow).

al material inserted into the 9p12 region[13]. The long-arm variant has an extra G-positive band located within the 9q12 region[6]. These variants are considered to have no clinical effects[14]. It has been proposed that the 9q12/qh euchromatic variants derived from 9p12 or 9q13-q21.1[15]. Di Giacomo *et al*[16] found a duplication of 9p11.2-p13.1 in two unrelated fetuses and in their phenotypically normal mothers. In the trisomy of 9p12-p21.3, the size of the trisomic segment in three generations of normal individuals is about 21 Mb, as reported by Stumm *et al*[17]. Verma *et al*[18] characterized the extra G-positive band in the 9qh region in a normal individual by using the FISH technique. They postulated that the G-positive band was

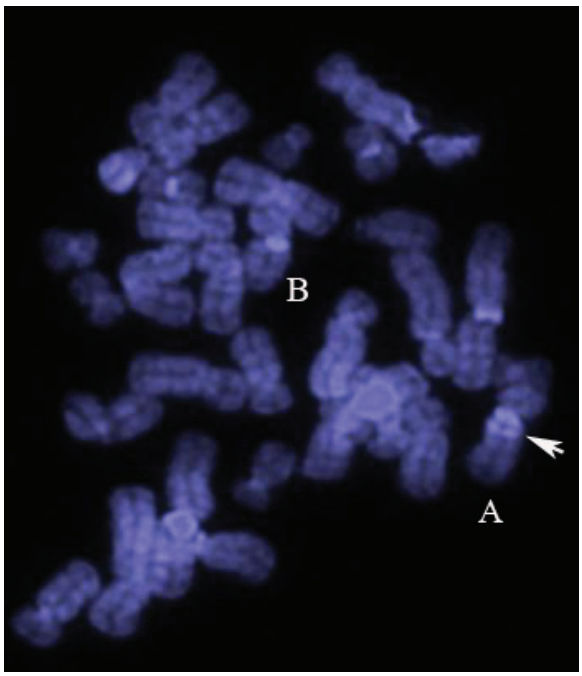

Figure 5. Partial metaphase stained with DAPI in the second case. The normal chromosome 9 (B) and abnormal chromosome 9 (A), with the extra band showing negative staining (arrow).

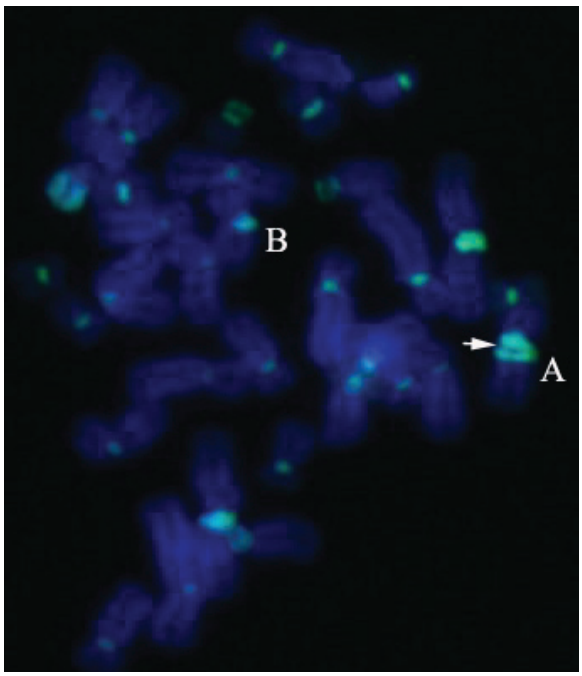

Figure 6. Partial metaphase in Figure 5, hybridized with labeled human Cot-1 DNA, with the extra band on the abnormal chromosome 9 (A) showing no fluorescent signal (arrow).

euchromatic, and originated from band p12. Macera *et al*[19] reported a case with the G-positive band in the 9qh region, which originated from duplicated β-satellite and satellite III DNA sequences and the band, 9q13q21.1.

Reddy[20] reported a prenatal case of maternally inherited subtle duplication of the band, 9q21.1-q21.2. The G-positive band became inert when it was sandwiched between two blocks of heterochromatin and became inactive through the position effect, resulting in a phenotypically normal child[21]. Goumy *et al*[22] reported a 6-year-old boy with growth and language delay, an unusual chromosome 9 variant and duplication of the 9p12- q21 region. An identical variant was found also in the boy's mildly language-retarded brother and his phenotypically normal father and grandfather. Variation in the heterochromatic regions of 1phqh, 9qh+, and 16qh- was found more frequently in children with autism[23]. The euchromatic variant of 9q13 was reported originally by Jalal *et al*[24]. They described an extra euchromatic band in the 9qh region in a normal individual. In this study both children had normal phenotypes. The extra band in the first case likely originated from duplication and inversion of genetic material in the q12-q13 region. The extra band in the second case comprised non-repetitive sequences, since it was negative with DAPI staining and had no signal when hybridized with human Cot-1 DNA. This study proposed the method of using labeled human Cot-1 DNA as a FISH-probe for identifying repetitive sequences in an extra band or a supernumerary marker chromosome.

Since heteromorphisms in the 9qh region, including an extra G-positive band, can originate from different mechanisms, and may be associated with normal, abnormal, autistic and leukemic phenotypes, precise investigation and characterization of the extra band is necessary, especially when it occurs de novo in prenatal diagnosis. Long-term follow-up of different cases with precise characterization of the heteromorphism is very useful in genetic counseling, and for understanding the functions of these heteromorphic regions.

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การตรวจวิเคราะห์แถบสียูโครมาทินที่เกินมาในบริเวณ 9qh

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วัตถุประสงค์ เพื่อประเมินโครงสร้างของแถบสีจีบวกที่เกินมาในโครโมโซม 9 บริเวณ qh(9q12) ของทารกในครรภ์ 2 ราย ที่ตรวจพบก่อนคลอด

วิธีการ เตรียมโครโมโซมจากการเลี้ยงเลือดทารก ทำการย้อมโครโมโซมด้วย เทคนิค G-banding, mBAND, micro-FISH และ FISH เพื่อศึกษาโครงสร้างของ แถบสีที่เกิน

ผลการทดลอง ด้วยเทคนิค G-banding พบแถบสีเกินมาในบริเวณ 9qh (9q12) บนโครโมโซมคู่ที่ 9 ของทารกทั้ง สองราย ในทารกรายแรกด้วยเทคนิค micro-FISH พบว่าแถบสีที่เกินแสดงสัญญาณฟลูออเรสเซนต์เหมือนกับบริเวณ 9q12 ด้วยเทคนิค mBAND แถบสีที่เกินแสดงสัญญาณฟลูออเรสเซนต์และความเข้มของแสงเหมือนกับแถบสีตรง 9q13 ในรายที่สองแถบที่เกินไม่ให้สัญญาณฟลูออเรสเซนต์ เมื่อย้อมด้วย DAPI และไม่ให้สัญญาณฟลูออเรสเซนต์เมื่อไฮบริไดซ์ด้วย Cot-1 DNA ที่ติดฉลาก ทารกทั้งสองรายคลอดตาม กำหนดและปกติ

สรุป แถบสีที่เกินในรายแรกมาจากส่วนของโครโมโซมในบริเวณ 9q12-9q13 ส่วนในรายที่สองแถบสีที่เกินประกอบด้วย non-repetitive sequences DNA แถบสีที่เกินในทารกทั้งสองรายไม่ส่งผลให้มีความผิดปกติของร่างกาย **เชียงใหม่เวชสาร 2558;54(2):57-63.**

คำสำคัญ: แถบสีที่เกินใน 9qh ไมโครฟิช เอ็มแบน Cot-1 DNA