

Variants of Chromosome 9 in Phenotypically Normal Individuals

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Abstract

The human chromosome 9 displays the highest degree of structural variability. Four different types of variants are described including pericentric inversion, extra G-positive band in the q arm, additional G-positive band in the p arm and duplication of band 9q21-q22. It is important to demonstrate inheritance from a phenotypically normal individual in order to differentiate between a variant chromosome and an abnormal chromosome.

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Introduction

For some unknown reasons, the human chromosome 9 displays the highest degree of structural variability in the secondary constriction region, an area whose function remains obscure. Numerous investigations have reported unusual findings involving this region.¹⁻⁴ It is well known that chromosome 9qh shows polymorphism in length and position. There have been reports of variant bands within the long arm heterochromatin^{3,5} as well as the proximal short arm of chromosome 9.

Consequently, the confusion between a normal heteromorphic variant and a chromosome abnormality has aroused much anxiety.

In our laboratory, we had observed four different types of variants of chromosome 9 at the secondary constriction region.

The aims of this study were to review the different variants of chromosome 9 in phenotypically normal individuals and to show their importance for prenatal diagnosis.

Materials and Methods

All prenatal diagnosis specimens sent to our cytogenetic laboratory between January 1990 and December 1995 were reviewed. The specimens received were for analysis of any abnormal morphology present in the chromosomes. Cytogenetic analysis of amniotic fluid, chorionic villi and fetal blood specimens is mainly recommended to women with advanced maternal age.

Other indications are abnormal ultrasound findings, and previous family history of abnormalities. Standard cytogenetic procedures of culturing the amniotic fluid cells were carried out on all the specimens. The cultured cells were then harvested and the chromosomes from the cells spread on a glass slide. The slides were stained with the GTG banding technique (G bands by using Trypsin and Giemsa) and a minimum of 20 metaphases were analysed.

CBG banding technique (C-bands by using Barium-Hydroxide and Giemsa) was applied on cases where chromosome 9 deviated from the usual findings. This technique produces selective staining of constitutive heterochromatin. The method involves treating the chromosome with an alkali, barium-hydroxide [Ba(OH)₂], to denature the chromosomal DNA and subsequent incubation in a salt solution, followed by staining with Giemsa.

In the case, where an extra G-positive band was observed on the long arm of chromosome 9, fluorescent-in-situ-hybridisation was performed using Oncor (Gaithersburg, USA) Coatsome for chromosome 9. The technique applied was according to the protocol recommended by the supplier.

Results

A total of 4969 chromosome studies for prenatal diagnosis were done between 1990 and 1995. Sixty-six cases of variant chromosome 9 were detected. Out of

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these, a total of 62 cases showed a pericentric inversion at breakpoint p11-q13 of the secondary constriction region of chromosome 9 (Fig. 1). This has been observed with such frequency, that some cytogeneticists would consider it as a normal variant. It has been described in various populations and the incidence rates had been reported to be between 1% and 1.6% of the general population. Our experience in the Singapore General Hospital showed an incidence rate of 1.2%. This compares well with reports quoted elsewhere. Our study also showed that there was no untoward effect on the person carrying the inversion.⁶ This structural alteration is regarded as a normal familial variant, and is inherited in a Mendelian fashion without any apparent phenotypic consequences.⁷

The next pericentric inversion observed had breakpoints located at region p22-q21.2 (Fig. 2). This pericentric inversion differs from the usual familial variant at breakpoint p11-q13 and involves a much larger area. The chance of a viable abnormal offspring resulting from this inversion is increased as compared to the smaller p11-q13 pericentric inversion. At meiosis, there will be more crossing over within the inverting loop of the chromosome which might lead to an abnormal recombinant chromosome with duplication and deletion. Since the anomalous chromosome 9 was also found in the proband's father, who appeared phenotypically normal, the couple were advised that the predicted risk of an abnormal phenotype in the

foetus was low. Upon delivery, the baby was found to be a phenotypically normal male.

An extra G-positive band on the long arm of chromosome 9 (Fig. 3), detected during a prenatal chromosome study, was present in all cells examined while all other chromosomes appeared normal. This extra band was located within a longer than average heterochromatin region. A small extra G-positive band on some individuals with 9qh+ has been reported.⁸⁻¹¹ It is of interest that the extra G-positive band within the 9qh has been associated with an increase amount of heterochromatin. Therefore, it seems unlikely that the extra band represents a simple insertion. It is more likely that the whole region has become amplified.^{12,13} Fluorescent-in-situ-hybridisation with paint probe for chromosome 9 demonstrated that the extra G band is composed entirely of chromosome 9. This excludes the possibility of a translocation from another chromosome. The extra G-positive band on the long arm of chromosome 9 was also observed in C-banding. This further implied that it is mostly made up of heterochromatin material which is similar to that of the centromeric region. Whatever the origin of the extra G-positive material, it has no apparent phenotypic effect, and does not appear to contain any active genes. This is possibly because it is between blocks of heterochromatin as reported by Docherty and Hulten.¹⁴

We observed an additional dark G-positive band at the 9p13 region (Fig. 4) of a phenotypically normal woman as well as in her amniotic fluid specimen during

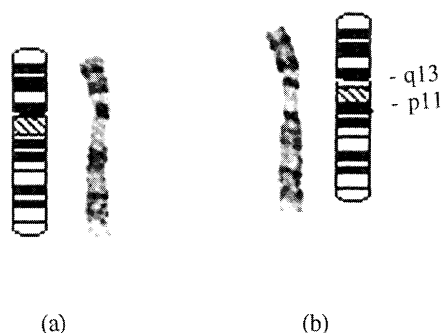


Fig. 1a. Normal chromosome 9 with corresponding ideogram.
Fig. 1b. Pericentric inversion at breakpoint p11-q13.

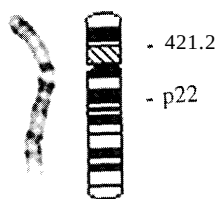


Fig. 2. Pericentric inversion at breakpoint p22

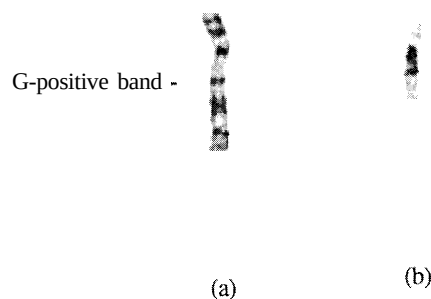


Fig. 3a. Extra G-positive band on q arm of chromosome 9.
Fig. 3b. C-banding shows that it is made up of heterochromatin material.

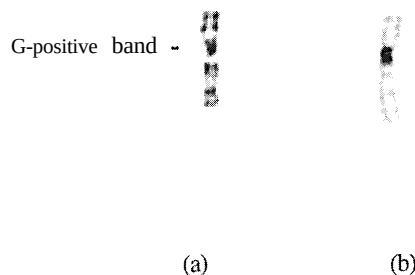


Fig. 4a. Additional dark G-positive band in region 9p13.
Fig. 4b. C-banding shows that it is made up of heterochromatin material.

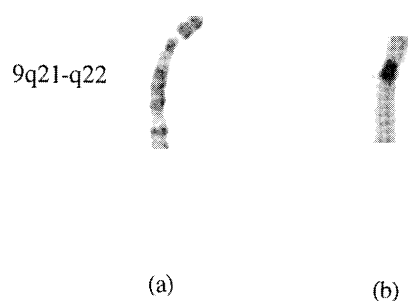


Fig. 5a. Excess material involving region 9q21-q22.

Fig. 5b. C-banding shows that it is made up of euchromatin material

chromosome studies. C-banding test showed that the dark G-positive band on the short arm was made up of heterochromatin material. Since the mother was phenotypically normal, the presence of the additional dark G-positive band would suggest it to be a harmless variant. A phenotypically normal girl was born. Subsequently, the same variant appeared in her second pregnancy prenatal testing also resulting in a normal girl. Although it is extremely rare, this type of variant has been previously reported.^{4,15}

The last variant observed involved excess material at region 9q21-q22 (Fig. 5). The anomalous chromosome 9 was also found in the phenotypically normal mother. She gave birth to a normal baby boy. In this family study, the extra G-positive band is totally euchromatic and does not include an extra C-band.¹⁶ The mechanism involved is not clear and some authors have suggested gene amplification.^{13,17} Thus, it is assumed that there is a duplication in region 9q21-q22.

Discussion

The parents' blood chromosomes were studied on all the cases mentioned and it was found that one parent who appears phenotypically normal had a similar variant. The interpretation, evaluation and awareness of variants is crucial in the practice of prenatal diagnosis, as it does not always imply a clinical abnormality.

Parental chromosome studies should be done to demonstrate the inheritance of the variant from a phenotypically normal individual. This will enable differentiation between an abnormal chromosome and a normal variant. Parental chromosome analysis is thus

of utmost importance in evaluating the significance of an anomalous chromosome and for an accurate genetic counselling of the couple.

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REFERENCES

1. Hansmann I. Structural variability of human chromosome 9 in relation to its evolution. *Hum Genet* 1976; 31:247-62.
2. Madan K, Bobrow M. Structural variation in chromosome 9. *Hum Genet* 1974; 17:81-6.
3. Madan K. An extra band in human 9qh+ chromosome. *Am Genet* 1978; 43:259-64.
4. Sutherland GR, Eyre H. Two unusual G-band variants of the short arm of chromosome 9. *Clin Genet* 1981; 19:331-4.
5. Silengo MC, Davi GF, Franceschini P. Extra band in the 9qh chromosome in a normal father and his child with multiple congenital anomalies. *Hum Genet* 1982; 60:294.
6. Teo SH, Tan M, Knight LA, Yeo SH, Ng I. Pericentric inversion 9—Incidence and clinical significance. *Ann Acad Med Singapore* 1995; 2:302-4.
7. Luke S, Verma RS, Conte RA, Matthews T. Molecular characterization of the secondary constriction region (qh) of human chromosome 9 with pericentric inversion. *J Cell Sci* 1992; 103:919-23.
8. Jalal SM, Kukulich MK, Garcia M, Day DW. Euchromatic 9qh+ heteromorphism in a family. *Am J Med Genet* 1990; 37:155-6.
9. Joe JJ. A new chromosome 9 variant an extra band within the 9qh+ region. *Clin Genet* 1992; 41:157-8.
10. Roland B, Chernos JE, Cox DM. 9qh+ variant band in two families. *Am J Med Genet* 1992; 42:137-8.
11. Verma RS, Luke S, Brennan JP, Matthews T, Conte RA, Macera MJ. Molecular topography of the secondary constriction region "qh" of human chromosome 9 with an unusual euchromatic band. *Am J Hum Genet* 1993; 52:981-6.
12. Donlon TA, Magenis RE. Structural organisation of the heterochromatic region of human chromosome 9. *Chromosoma* 1981; 84:353-63.
13. Docherty Z, Hulten MA. Extra euchromatic band in the qh region of chromosome 9. *J Med Genet* 1985; 22:156-7.
14. Docherty Z, Hulten MA. Rare variant of chromosome 9. *Am J Med Genet* 1993; 45:105-6.
15. Spedicato FS, Comite AD, Akvad T. An unusual variant chromosome 9 with an extra C-negative G-dark segment in the short arm. *Clin Genet* 1985; 28:162-5.
16. Knight LA, Goon MS, Tan M. Extra G-positive band on the long arm of chromosome 9. *J Med Genet* 1993; 30:613.
17. Luke S, Verma RS, Pebenito R, Macera P. Inversion-duplication of bands q13-q21 of human chromosome 9. *Am J Genet* 1991; 40:57-60.