TRIPLE MOSAICISM WITH TWO AUTOSOMALLY UNBALANCED CELL LINES IN A PHENOTYPICALLY NORMAL OLIGOSPERMIC MAN

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The chromosomal analysis of a phenotypically normal, moderately oligospermic man is reported. He presented a triple mosaic complement with two autosomally unbalanced cell lines. The cytogenetic results are discussed, and the importance of such investigations in men attending infertility clinics emphasized.

INTRODUCTION

Chromosome studies in male secretory infertility have recently been considered of primary importance for diagnostic and prognostic purpose (Kjessler 1972, Chandley et al. 1975, Koulischer and Schoysman 1975, Millet et al. 1975, Van Zyl et al. 1975). In fact, a significant proportion of chromosomal aberrations has been found in azoospermic as well as oligospermic men, decreasing sperm counts being usually related to an increasing incidence of chromosomal anomalies (Chandley et al. 1975, Koulischer and Schoysman 1975, Millet et al. 1975). Usually, numerical or morphological aberrations of the sex chromosomes are involved, but sex chromosome/autosome and autosome/autosome balanced or Robertsonian translocations as well.

Supernumerary marker microchromosomes frequently satellited or in satellite association have also been found (Smith et al. 1965, Hulten et al. 1966, Chandley et al. 1975, Ventruto et al. 1976). They may be of heterochromatic constitution as satellites and short arms of acrocentric chromosomes, and therefore genetically inactive on the phenotype.

To our knowledge this is the first report of a phenotypically normal, moderately oligospermic healthy individual with two grossly unbalanced cell lines in a triple mosaic complement.

CASE REPORT

A 30-year-old man, the only son of Jewish Polish parents, was referred for cytogenetic analysis by a male-infertility clinic. In three years of marriage his wife never conceived, although a complete gynecological examination revealed no abnormalities.

He was moderately oligospermic (25-30 million/ml), with a motility of 30-40% and some immature and abnormal forms of the spermatozoa. Physically, the patient was a normally developed, healthy male. Distribution and density of facial, body, pubic and axillary hair were normal. The penis was of average size, the testes were of normal size and consistency. Biopsy was not performed.

Urinary 17-ketosteroids and gonadotropins and plasmatic LH and FSH were within the normal limits. Buccal smears were negative for sex chromatin. Drumsticks were not observed in peripheral blood neutrophils.

His I.Q. was normal and he was working as an electronics engineer. Cytogenetic analysis was not thought necessary, but it was performed to complete the clinical picture of the patient.

CHROMOSOME STUDIES

Peripheral blood lymphocytes were cultured in three different flasks by a modification of the technique of Moorhead et al. (1960). G-banding was obtained by the trypsin method of Seabright (1971), slightly modified by using a 0.06% trypsin in Ca^{2+} and Mg^{2+}free Hanks solution. C-banding was according to the method of Arrighi and Hsu (1971). Hundred cells were counted and all had a modal number of 46 chromosomes, except for few cells

CODEN: AGMGAK 26 177 (1977) — ISSN: 0001-5660
Acta Genet. Med. Gemellol. (Roma), 26: 177-180

with random loss. By the standard Giemsa stained karyotypes was already evident that some cells had an abnormal complement: some with a supernumerary chromosome in the Y-G group and a missing C chromosome, some with a supernumerary chromosome in the C group and one missing in the G-Y group. Fifty banded metaphases in different slides from the three cultures were examined and the two abnormal lines identified in a proportion of 6% each (see Table). One line had a deleted chromosome 9: 46,XY,del(9)(q11), resulting in 9q monosomy (Fig. 1). The second presented an unbalanced translocation with the whole long arm of chromosome 9 translocated to the short arm of chromosome 21: 46,XY,—G21,+t(9;21)(q11;p13), and two normal 9 also present (Fig. 2), resulting in 9q trisomy.

DISCUSSION

Normal phenotype with two grossly abnormal cell lines, even if in a relatively small proportion, must be a very rare event, in a healthy person. Cases with trisomy 8 or 7 mosaicism in variable proportion and normal phenotypes have been respectively reported by Caspersson et al. (1972) and De Bault and Holmi (1975). The authors explain the lack of physical abnormalities on the basis of cellular distribution of the trisomy condition, or the possibility that unknown factors may influence the phenotypic expression of a chromosomal abnormality.

Table. *Results of the cytogenetic studies*

Culture	Number of cells examined	Number of cells 46,XY,del(9)(q11)	Number of cells 46,XY,—G21,+t(9;21)
A	35	2	2
B	40	1	2
C	25	3	2
Total	100	6	6

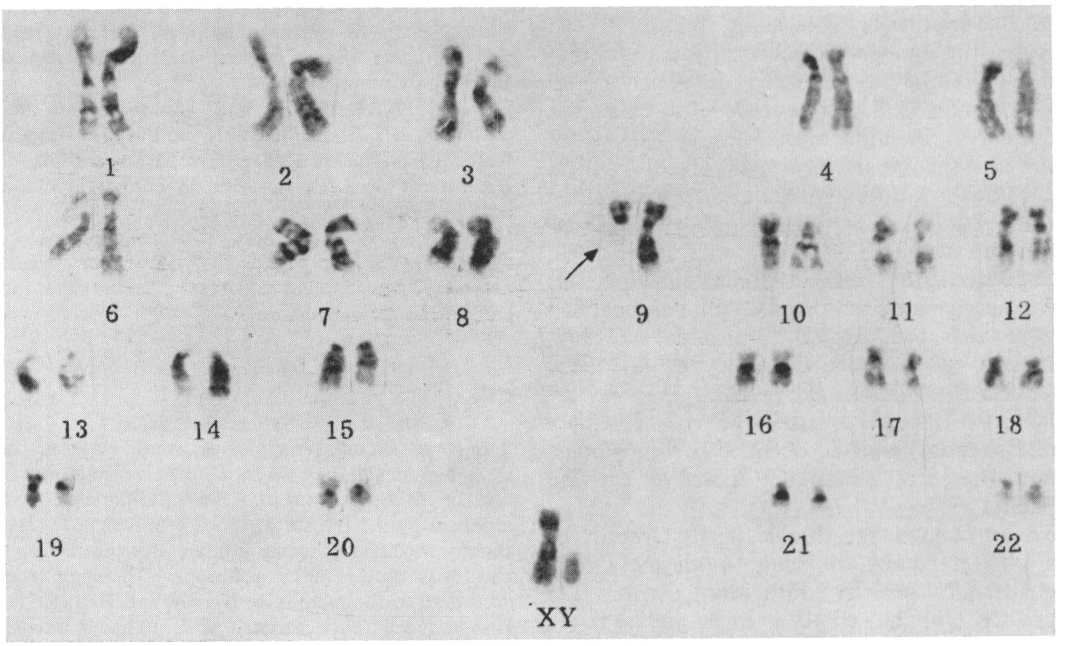

Fig. 1. Karyotype of the 9q monosomic line, with two normal G21 and a normal 9 chromosome.

https://doi.org/10.1017/S0001566000009995 Published online by Cambridge University Press

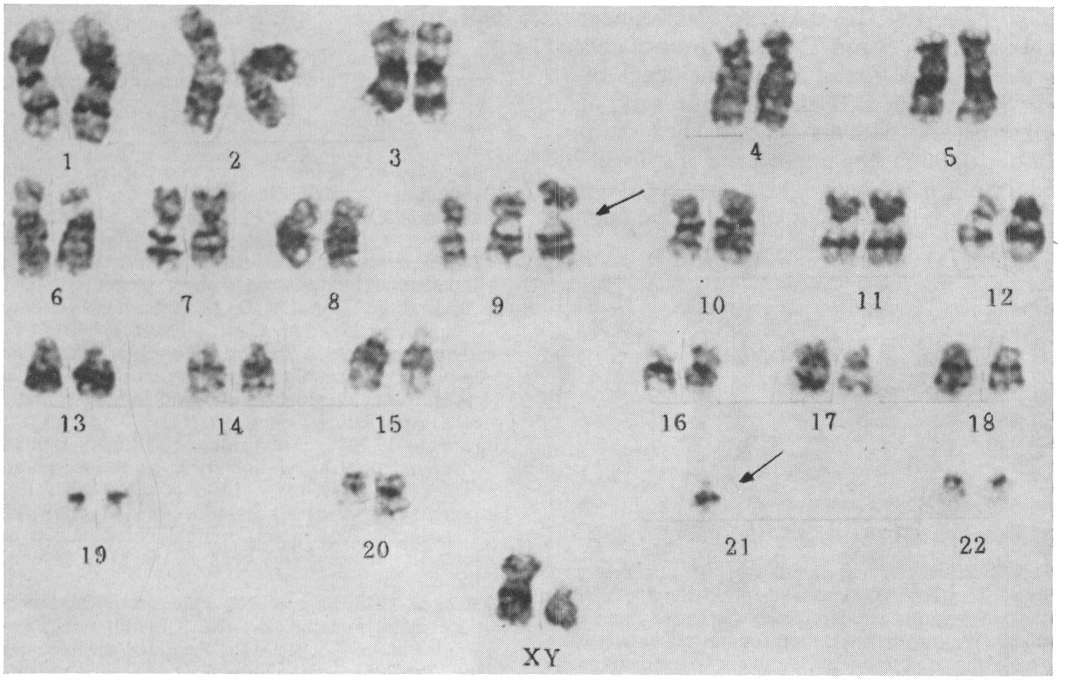

Fig. 2. Karyotype of the 9q trisomic line. Only one G21 is present. The other is in translocation with 9q: t(9;21)(q11;p13).

We should also suggest that in our case, the two abnormal lines, in equal proportion, the one with 9q monosomy, the other with 9q trisomy, may have in some way compensated each other.

Unfortunately, no biopsy of the testes, nor cultures from the skin or bone marrow or some other tissues of the patient, or of the other members of his family, could be examined, because we were not able to contact him again. At the time of examination, no hematologic disease was detected, but a follow-up of the patient might have been recommended.

A multiple-step event in a late stage of development must have been the origin of both lines. Probably, a break happened in C9 (q11) and the whole long arm of chromosome 9 translocated to the short arm of a chromosome 21, which was next to chromosome 9. Attraction of acrocentric satellited chromosome to the heterochromatic portion of 9q has been observed (Ferguson-Smith and Handmaker 1963).

All the more so, as our patient had a very prominent secondary constriction in chromosome 9, which has also been noted to be frequently subject to breakages (Hansmann 1976). On the other hand, at the time of examination, no increased incidence of breakages was noted in the patient's cultures, even 9q11, and no particular incidence of attraction between 9q and acrocentric chromosomes.

After the translocation was formed, inequal division of genetic material must have happened at anaphase (Figs. 3, 4).

It is quite surprising how such unbalanced lines survived and replicated. The cytogenetic findings in this young healthy man seem to us most unusual. Although the possibility exists of "de novo" clone formation in vitro, even in short-term cultures, in our case it seems an in vivo event as aberrant cells of the same type were found in different cultures.

The question remains whether the cytogenetic findings are causally related to the

https://doi.org/10.1017/S0001566000009995 Published online by Cambridge University Press

oligospermia. However, the involvement of chromosomes 9 and 21 seems to us of special interest, as we found a high incidence of 9h variants and of G21 sat+ in our azoo- and oligospermic patients (unpublished data).
In our opinion, the importance of cytogenetic analysis (in its many-sided aspects) in men attending infertility clinics has to be emphasized.

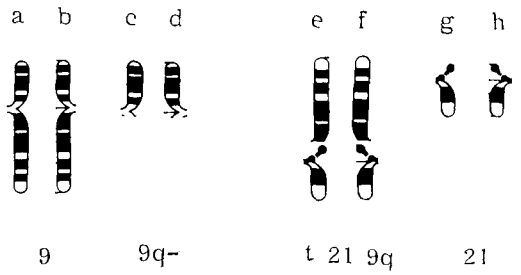

Fig. 3. Schematic representation of chromosomes 9 and 21 after the translocation, at anaphase, when their chromatids are completely separated from each other. We may assume that, for inexplicable reason, *a b e g* migrated to one pole and *c d f h* to the opposite one.

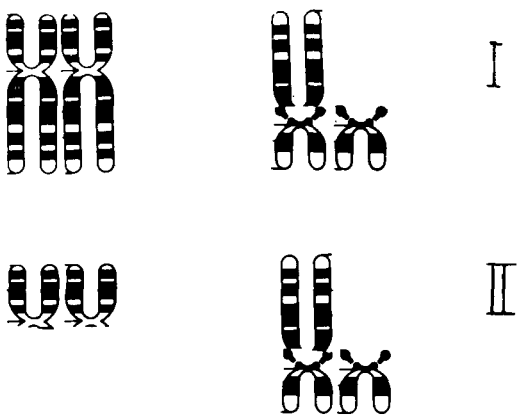

Fig. 4. Schematic representation of the two abnormal lines that had to be formed, after cleavage at telophase.
Line I trisomic for 9q was found, indeed. Line II was not found, because apparently the 9q of the 9/21 translocation joined back the 9p. So, instead of it, a line was found with two normal 21, a normal 9 and a deleted 9 chromosome: del (9)(q11) (see Fig. 1).

REFERENCES

Arrighi F.E., Hsu T.C. 1971. Localization of heterochromatin in human chromosomes. Cytogenetics, 10: 85.
Caspersson T., Lindsten J., Zech L., Buckton K., Price W. 1972. Four patients with trisomy 8 identified by the fluorescence and Giemsa banding techniques. J. Med. Genet., 9: 1.
Chandley A.C., Edmond P., Christie S., Gowans L., Fletcher J., Frackiewicz A., Newton M. 1975. Cytogenetics and infertility in man. I. Karyotype and seminal analysis. Ann. Hum. Genet., 39: 231.
De Bault L.E., Holmi K.A. 1975. Familial trisomy 7 mosaicism. J. Med. Genet., 12: 200.
Ferguson-Smith M.A., Handmaker S.D. 1963. The association of satellited chromosomes with specific chromosomal regions in cultured human somatic cells. Ann. Hum. Genet., 27: 143.
Hansmann I. 1976. Structural variability of human chromosome 9 in relation to its evolution. Hum. Genet., 31: 247.
Hulten M., Lindsten J., Fraccaro M., Mannini A., Tiepolo L. 1966. Extra minute chromosome in somatic and germ-line cells of the same person. Lancet, 2: 22.
Kjessler B. 1972. Facteurs génétiques dans la subfertilité mâle humaine. In: Ch. Thibault (ed.): Fecondité et Stérilité du Mâle. Acquisitions Récentes (pp. 205-225). Paris: Masson et Cie.
Koulischer L., Schoysman R. 1974. Chromosomes and human infertility. I. Mitotic and meiotic chromosome studies in 202 consecutive male patients. Clin. Genet., 5: 116.
Millet D., Plachot M., Lety M.A., De Grouchy J., Netter A. 1975. Les remaniements chromosomiques dans la stérilité masculine. J. Gynecol. Obstet. Biol. Reprod., 4: 689.
Moorhead P.S., Nowell P.C., Wellman W.J., Battips D.M., Hungerford D.A. 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell Res., 20: 613.
Seabright M. 1971. A rapid banding technique for human chromosomes. Lancet, 2: 971.
Smith K.D., Steimberger E., Steimberger A., Perloff W. 1965. A familial centric chromosomic fragment. Cytogenetics, 4: 219.
van Zyl J.A., Menkveld R., van W. Kotze T.J., Retief A.E., van Niekerk W.A. 1975. Oligozoospermia. A seven-year survey of the incidence, chromosomal aberrations, treatment and pregnancy rate. Int. J. Fertil., 20: 129.
Ventruto V., Coli A., Farina L., Festa B., Ricciardi I., Sebastio L. 1976. A case of hypogonadotrophic hypogonadism with anosmia (Kallmann's syndrome) in a male, with familial incidence of a small metacentric chromosome (47,XY, mat?+). J. Med. Genet., 13: 71.

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