Am J Hum Genet 31:634–641, 1979

Brief Communication

Human Male Infertility, Probably Genetically Determined, Due to Defective Meiosis and Spermatogenic Arrest

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SUMMARY

A family is reported in which infertility affected three men related through their mothers. The propositus, from whom testicular tissue was obtained, exhibited desynapsis, lack of chiasmata, and degeneration of spermatocytes during the first meiotic division. These observations lead us to postulate that a gene for meiotic disturbance, spermatogenic arrest, and azoospermia is segregating in this family; its mode of inheritance conforms to either an X-linked recessive or a sex-limited autosomal dominant transmission.

INTRODUCTION

Studies of meiosis and its variation in plant and insect species have established that the meiotic process is under intricate genetic control. Monogenic as well as polygenic control of meiosis has been well established in these organisms. The genetics of meiosis has been reviewed by a number of investigators [1–5].

Evidence for genetic control of human meiosis is just now beginning to emerge. Sporadic cases of meiotic breakdown in men studied for infertility or subfertility suggest the existence of mutations affecting meiosis. It has been shown, for instance, that spermatogenic arrest associated with meiotic abnormalities occurs in a proportion of infertile and azoospermic men with apparently normal karyotypes [5–9]. A genetic

Received February 5, 1979; revised April 19, 1979.

This study was presented at the annual meeting of the American Society of Human Genetics, Portland, Oregon, October 16–19, 1974 and was supported by grants HL-09011 and HD-04134 from the National Institutes of Health and by the American Cancer Society.

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basis for at least some of these cases is suggested by the observations that two of the azoospermic men were from consanguinous marriages [5, 7] and another had a childless sister [5, 7].

We report a family in which infertility affected three men related through their mothers. The propositus, from whom testicular tissue was obtained, exhibited desynapsis, lack of chiasmata, and degeneration of the spermatocytes during first meiosis. All three men had azoospermia or oligospermia. On the basis of these observations, we postulate that a gene for a meiotic disturbance, spermatogenic arrest, and azoospermia is segregating in this family; the distribution of the three affected in the pedigree conforms to either an X-linked recessive or a sex-limited autosomal dominant transmission.

ASCERTAINMENT AND CLINICAL HISTORY

The propositus (III.8 in fig. 1) is a healthy, well-developed, 28-year-old banker of Polish ancestry who was admitted to The New York Hospital in April, 1974, for an investigation of his infertility. He had been married for 6 years, but his wife had never conceived. He had onset of puberty at the usual age, shaved daily, and had normal sexual interests. He described no unusual exposure to radiation or chemicals. His external genitalia were normally developed, the right and left testes measuring 15 ml and 12 ml, respectively; the pubic hair distribution was masculine. This man was shown to be azoospermic on both of the two examinations he has had. His semen was first studied elsewhere in 1972, and sperm were found to be lacking completely. A specimen studied in 1974 at The New York Hospital, two weeks prior to the testicular biopsy, revealed complete absence of mature sperm, although some spermatids and some aberrant but unidentifiable forms were present. His testicular histology is illustrated in figure 2*A*.

The propositus reported that a 44-year-old uncle (II.9 in fig. 1) and a 30-year-old first cousin (III.6 in fig. 1) were also infertile. Clinical histories were then obtained from each of these men.

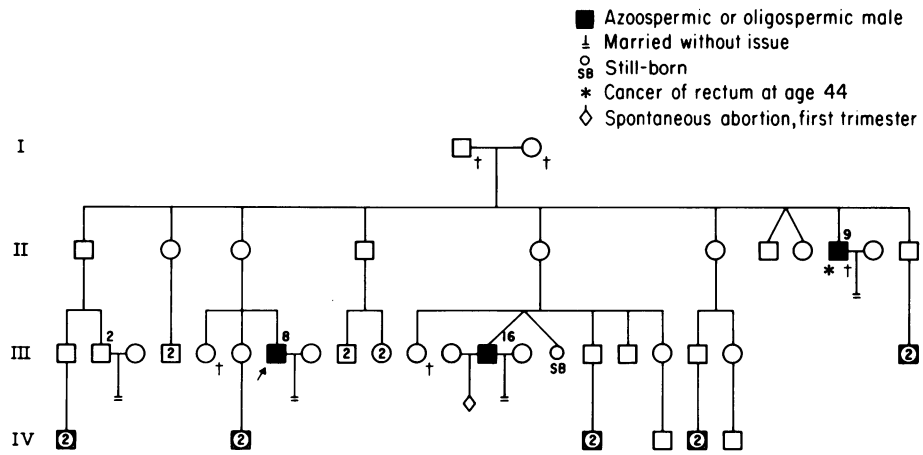


FIG. 1.—Pedigree of family with male infertility due to azoospermia or oligospermia. Propositus indicated by *arrow*.

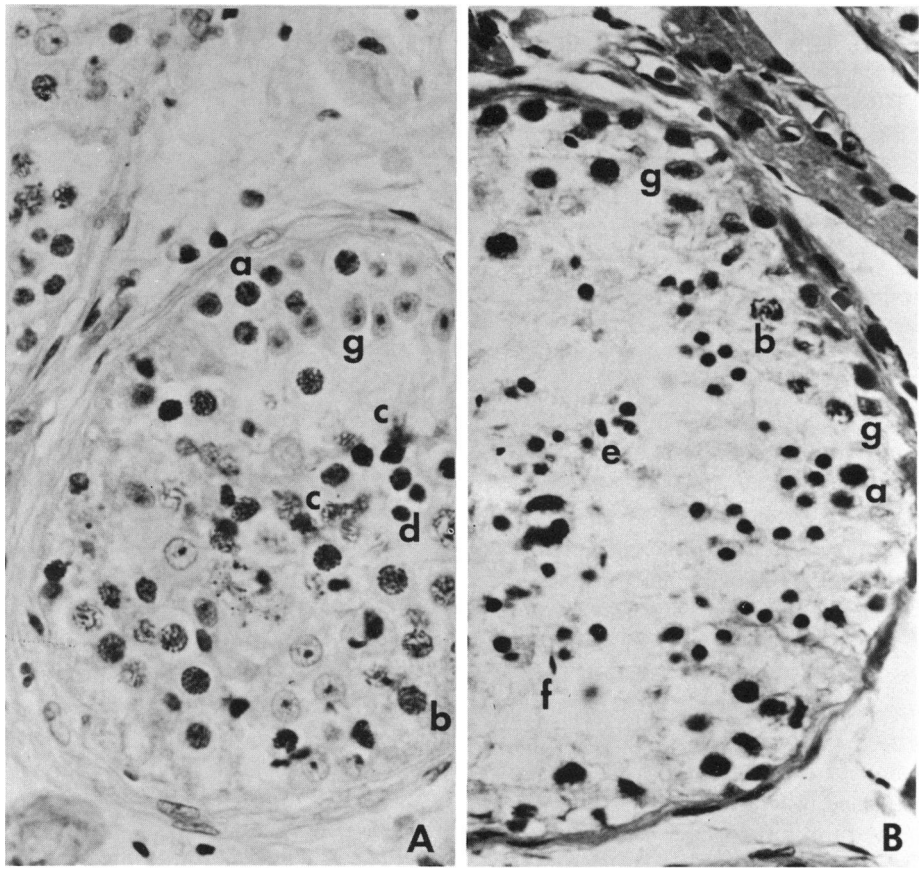


FIG. 2.—Testicular histology. *A*, section of seminiferous tubule from testicular biopsy of propositus; *B*, similarly stained seminiferous tubule from normal testis of individual who underwent orchiectomy during treatment for cancer of the prostate. In *A*, note normal-appearing, plentiful spermatogonia, *a;* primary spermatocytes, predominantly in the early prophase stage of first meiosis, *b;* degenerating spermatocytes, *c;* and darkly-staining spermatid-like elements, *d;* absence of spermatocytes in second meiosis, normal spermatids, and spermatozoa. In *B*, note normal-appearing spermatogonia, *a;* primary spermatocytes, *b;* spermatids in various stages of maturation, *e;* and spermatozoa, *f*. In both *A* and *B*, normal-appearing Sertoli cells, *g*, are present. H&E stain, magnification × 500.

II.9 was healthy until the onset of adenocarcinoma of the rectum in 1974 at the unusually young age of 44. (Only 3% of patients with cancer of the colon and rectum reported in the *Third National Cancer Survey* [10] were below this age.) He died of metastasis in 1976. When we examined him in 1974, he was found to be well developed in general; his genitalia were normally developed except that his testes were abnormally small (estimated 5 ml) and of softer than normal consistency. He had been infertile throughout the 15 years of his marriage. Prior to the onset of his cancer, his semen had been analyzed on five different occasions: four times by his private urologist (June and November, 1962; May and June, 1966) and once in 1967 at the New York Fertility Institute. These studies revealed a semen pH of 7.0 to 7.5 with normal viscosity

but an abnormally low sperm count of $7-10 \times 10^6$ (normal range for the urologist's laboratory being $65-200 \times 10^6$); the sperm motility was drastically depressed after one hour. In the 1967 analysis, only half of the sperm appeared normal morphologically; in addition, poor motility and low count were noted. This man gave no history of radiation exposure; he had been exposed intermittently to pesticides, weed killers, and fungicides while working on a farm between the ages of 19 and 21.

III.16 is a healthy, normally developed policeman. His semen was analyzed on three different occasions in 1971 by a private urologist. The semen consistency was found to be "unusually thin"; the sperm counts were "very low," and the sperm were nonmotile. His external genitalia, including testicular size, are reported by his urologist to be normal. He married at age 21 in 1965, but after about a year the marriage was annulled. During that marriage and reportedly while he was home on Christmas leave from the armed services, his wife conceived. The pregnancy ended in a spontaneous abortion. He remarried at age 25. His second (and present) wife menstruates and ovulates regularly (evidence from basal body temperature) but during the 9 years of marriage has never become pregnant. Contraception (the pill) was used for one year, after which no contraceptive measures have been taken. All three of these men and their wives desired children, and II.9 and III.8 have adopted.

METHODS

Cytogenetics

The chromosomes of somatic cells were studied using phytohemagglutinin-stimulated blood lymphocytes from III.8 and II.9 and dermal fibroblasts from a cell line developed from a skin biopsy from III.8. The karyotypes were analyzed by G-banding techniques, and the incidence of chromatid gaps and breaks was determined in orcein-stained preparations. The incidence of sister chromatid exchanges (SCE) was determined in the cultured dermal fibroblasts of III.8 after growth in BrdU-containing medium.

Meiosis was studied in right and left testicular biopsy specimens from III.8. Within a few minutes after their surgical removal, the specimens, which consisted mainly of tubules, were washed in isotonic sodium citrate. Small portions were fixed, as tubules, and Feulgen squash preparations were made following the method described by Darlington and La Cour [11]. The remaining material was used for air-dried preparations following the method described by Evans et al. [12]. These preparations were stained with orcein or Giemsa.

DNA Repair

Fibroblasts from III.8 were studied by Dr. R. S. Day (National Institues of Health) for their ability to repair damage to DNA caused by UV radiation [13] and by Dr. C. F. Arlett (University of Sussex) for their sensitivity to $\gamma(Co^{60})$ radiation, UV radiation, and mitomycin C [14].

RESULTS

Cytogenetic Studies

Mitotic chromosomes. The G-banded karyotypes of lymphocytes from III.8 and II.9 were normal male—46,XY. No evidence for increased chromosome breakage was found during the study of 100 orcein-stained metaphases from each individual. Dermal fibroblasts of III.8 had a mean of 5.7 SCE per cell which is within the limits of normal studied in our laboratory. Spermatogonial mitoses were found in the squash as well as

the air-dried preparations of the testicular specimens; those analyzed had 46 chromosomes.

Meiotic chromosomes. The Feulgen squash preparations of testis from III.8 revealed an abundance of early prophase cells (leptotene, zygotene, and pachytene). Cells in diakinesis and metaphase were very infrequent, while second meiotic configurations were absent. In addition, many apparently degenerating cells of varying sizes were present; these cells had tightly condensed chromatin which was stained dark purple by the Feulgen reaction (fig. 3). A sex vesicle was present in most pachytene cells; in many of these cells pairing appeared normal. In a proportion of pachytene cells, the bivalents exhibited unpaired or desynaptic regions (fig. 4). Only two cells in diplotene were observed, and both were abnormal; they showed extensive failure of pairing (fig. 5). The few cells in diakinesis seen on the squash preparations could not be interpreted because their bivalents appeared to be clumped. Stages of meiosis beyond diakinesis were not observed; however, a few cells were seen in which as many as 60 small and fragmented-appearing chromosomes were present (fig. 6). The absence of post-diakinetic cells on the preparations suggests that the cells with the fragmented-appearing chromosomes represent spermatocytes at diakinesis undergoing fragmentation and degeneration. In the air-dried preparation, 26 cells were classified as being in diakinesis. Of these, 19 showed univalents only (fig. 7), and the chromosomes of these cells were tightly coiled. Both bivalents and univalents were present in the remaining cells, possibly bivalents only in one (fig. 8). None of these seven cells was suitable for analysis of chiasmata.

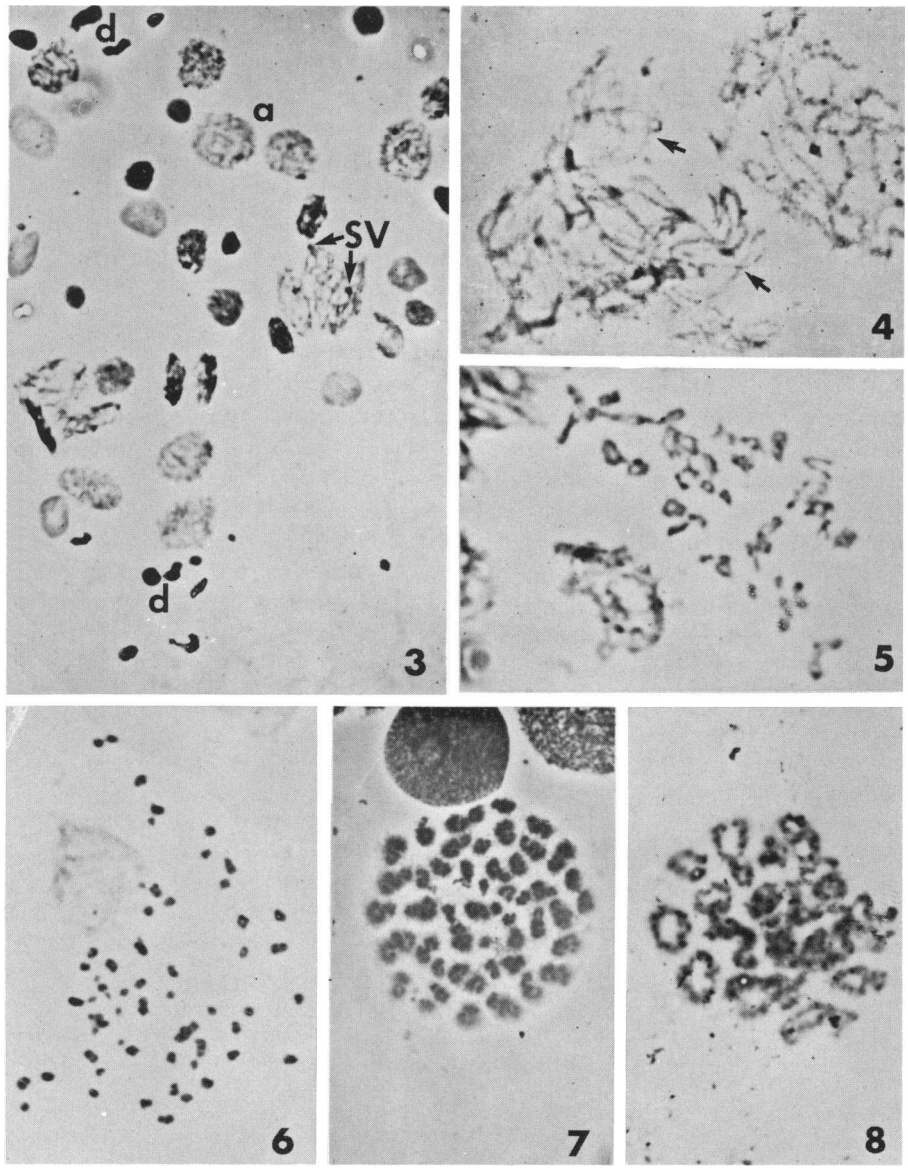
DNA Repair Studies

UV-irradiated adenovirus 2, when introduced into fibroblast cells of III.8, underwent host cell reactivation to a level comparable to that achieved in normal cells [13]. Thus, fibroblasts of III.8 are normally proficient in repairing UV-induced damage to DNA. Their cloning efficiency following treatment with $\gamma(Co^{60})$ radiation and mitomycin C was similar to that of normal control cells.

DISCUSSION

The meiotic process exhibits a remarkable morphological similarity throughout the eukaryotic phyla. Recent evidence points to a biochemical similarity as well in certain key events that take place during the early stages of meiosis [15–17]. Plant and insect studies have demonstrated that the various phases of meiosis, such as chromosome synapsis, disjunction, and chiasma formation and crossing over, are under genetic control. Little is known of the biochemical basis of this genetic control.

In mammals, also, information is accumulating regarding the genetic control of cell division. In the mouse, three recent reports describe genetically controlled disturbances in chromosome pairing or disjunction: Purnell [18] observed a mouse, the product of brother-sister mating in a highly inbred strain, with severely depressed numbers of chiasmata in spermatocytes; Philips et al. [19] discovered a semidominant sex-linked gene, *Bare patches* (*Bpa*), which is associated with the production of a high frequency of 39,X females; Beamer et al. [20] reported that in early embryonic cells of *BALB/cWt*



FIGS. 3–8.—Meiosis in the propositus. Figures 3–6 from Feulgen-squash preparations of seminiferous tubules; figures 7 and 8 from air-dried preparations. FIG. 3.—Low-power view of squash preparation showing many primary spermatocytes, *a*, in early prophase of meiosis [a sex vesicle (*SV*) is present in many of them] and darkly-staining and degenerating cells, *d*. Note absence of spermatozoa. FIG. 4.—Primary spermatocytes with abnormal-appearing pachytene exhibiting unpaired (desynaptic) chromosomal regions (*arrows*). FIG. 5.—Diplotene-like cell showing mostly univalent chromosomes. FIG. 6.—Cell with about 55 small chromosomes, many of which appear to be undergoing fragmentation. FIG. 7.—Spermatocyte at diakinesis with only univalents. FIG. 8.—Spermatocyte at diakinesis with bivalents.

mice, there is a high probability of Y-chromosome nondisjunction leading to mosaicism for 39,X and 41,XYY cell lines and hermaphroditism.

Reference was made earlier to the possible meiotic mutants already recognized in humans. They have been of either the asynaptic type (complete or partial absence of pachytene pairing) or the desynaptic (apparently normal pairing succeeded by the falling apart of homologues prematurely during diplotene) [6, 7]. Either disturbance results in reduced numbers of visible chiasmata. Meiosis in our propositus is desynaptic, being characterized by normal zygotene and pachytene pairing but premature desynapsis, with a depressed chiasma frequency [5].

In yeast, *Neurospora,* and *Drosophila,* a number of mutations that affect meiotic recombination also affect DNA repair processes in somatic cells, pointing to the existence of common genetic controls for these two phenomena [5, 21]. In addition, in *Drosophila,* mutants at six of seven recombination-defective loci studied affect mitotic chromosome stability as well [21]. Therefore, it is to be expected that in humans also, some of the mutations affecting chiasma formation will affect DNA repair ability, chromosome stability in somatic cells, or both. So far, such studies have been performed on only five individuals with defective synapsis and chiasma formation (including the present study). Pearson et al. [8] reported a reduced ability to repair damage to DNA caused by X-ray and UV radiation by the lymphocytes of an azoospermic man with desynaptic meiosis. Sasaki and Tonomura [9] observed that somatic cells derived from three infertile men with desynaptic meiosis had a normal ability to excise UV-induced damage, a normal response to treatment with γ-rays, UV, and a variety of chemical mutagens, and a normal amount of SCE in untreated cells. Dermal fibroblasts from our propositus were fully competent to repair damage caused by $\gamma(Co^{60})$ radiation, UV radiation, and mitomycin C. They also repaired UV-damaged DNA in viruses introduced into them. They exhibited no spontaneous chromosome instability. Relevant to this question is the study of Hultén et al. [22] which demonstrated a normal level of chiasma formation in a patient with xeroderma pigmentosum, an autosomal recessive disorder in which somatic cells display an inability to repair damage to DNA caused by UV radiation.

ACKNOWLEDGMENTS

We are grateful to Dr. William H. Wier for providing us with the semen analysis data on II.9 and III.16 and to Dr. Rufus S. Day III of the National Institutes of Health and Dr. Colin F. Arlett of the MRC Cell Mutation Unit, University of Sussex, Brighton, England, for the DNA repair and cell sensitivity studies described in the text.

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