SEX-CHROMOSOME ABNORMALITIES IN A POPULATION OF MENTALLY DEFECTIVE CHILDREN

BY

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In the past few years a number of surveys using the buccal-smear method have been made on various populations both of normal and of mentally defective individuals (Prader *et al.*, 1958; Moore, 1959; Ferguson-Smith, 1959; Fraser *et al.*, 1960; Barr *et al.*, 1960; Israelsohn and Taylor, 1961). In the present paper we report the nuclear sex findings of the entire Fountain Hospital population of low-grade mentally defective children. In three subjects a sex-chromosome abnormality appeared likely and their clinical and cytological findings are described in detail.

Material and Methods

Of the 425 subjects of both sexes of ages ranging from a few months to 20 years examined, 89 (20.8%) were mongols (Down's syndrome) and the remaining 336 (79.2%) were low-grade mental defectives, some of whom had associated somatic anomalies. Of this latter group 169 (39.7%) had "cerebral palsy." These data, together with the sex distribution of the population, are summarized in Table I.

TABLE I.—Data on the Fountain Hospital Population

	Male	Female	Total
Spastic mental defective ..	87	82	169 (39.7%)
Non-spastic „ „ ..	93	74	167 (39.5%)
Down's syndrome ..	49	40	89 (20.8%)
	229	196	425

From each patient three or four smears were taken, fixed in 95% ethanol, and stained with cresyl echt violet (Moore and Barr, 1955). Satisfactory slides were obtained from the entire population. At least 100 flattened, oval-shaped nuclei were scored from each patient. Rigid scoring criteria were adopted and adhered to throughout the survey. Nuclei were regarded as positive only when a distinct chromatin body lying adjacent to the nuclear membrane could be seen. Chromosome studies were carried out using short-term cultures of suspended leucocytes from peripheral blood (Moorhead *et al.*, 1960).

Results

In this series the nuclei of buccal mucosa cells from male patients, with the exception of Case 3, did not show any typical sex-chromatin masses. The mean

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frequency of chromatin-positive nuclei in females was 17.4% and the range 8–53%. One female (Case 1) showed a high percentage (10%) of double chromatin masses (Fig. 1). Another female was found to have several somatic characteristics of "Turner's syndrome" and normal female sex-chromatin pattern (Case 2). The results of sex chromatin, neutrophil counts, and chromosome analyses of these three patients are shown in Table II. One boy with a normal male buccal smear and hypospadias was found to have a normal male karyotype.

FIG. 1.—Case 1. Nucleus of buccal mucosa cell, showing two sex-chromatin masses. (Cresyl echt violet, $\times 5,000$.)

TABLE II.—Chromosome Count Distribution in the Three Abnormal Cases

Case No.	Sex-chromatin Percentage		Neutrophil Drumstick Counts	Summary of Chromosome Counts						Cells Analysed	Chromosome Analysis
	+	++		<45	45	46	47	>48	Total		
1	22	10	2/374	1	4	9	28	—	42	22	Triplo-X female
2	22	Nil	5/637	14	3	32	—	—	49	18	X/iso-chromosome-X
3	20	„	5/420	3	1	—	2	13	19	5	XXY trisomy-21

Figures in italics indicate modal number.

Case 1

A 23-year-old woman (Fig. 2), born after a normal pregnancy and delivery at a maternal age of 21 years and paternal age of 27 years. She weighed 3.4 kg. (7 lb. 7 oz.) at birth, no abnormalities being noted; there was no family history of congenital abnormality or mental deficiency. She has two normal sisters aged 24 and 20 years.

The patient first attempted to sit up at the age of 6 months and allegedly walked and talked at the age of 1 year; however, at the age of 2 years, after an accident when she was burnt by falling into a fire, she had a grand-mal seizure. Seizures have continued intermittently to the present. At 5½ years she was admitted to the Fountain Hospital, where she was thought to be an idiot with spastic quadriplegia and microcephaly. At 16 years two menstrual periods with an

FIG. 2.—Case 1.

interval of eight months were noted. None have occurred since.

She was a very kyphoscoliotic, undersized, low-grade defective—weight 7 st. 6 lb. (47.4 kg.); height 4 ft. 9 in. (147 cm.); cranial circumference 19½ in. (49.1 cm.). She had several contractures, paresis, and spasticity of all four extremities, more marked in the lower limbs, with a measure of head control. Fundi were normal. She could not speak, but could turn her head to a noise and reacted to pinpricks on different body sites. There was minimal breast development with pigmentation of areolae. The pubic and axillary hair was normal in growth and female in distribution. The external genitalia were those of a normal female, and chromosome studies (Table II) suggested that this individual was a triplo-X female.

The patient's mother had 57% chromatin-positive nuclei in the buccal smear and a normal female karyotype. Other members of the family were not available for examination.

Case 2

A 13-year-old illegitimate low-grade mental defective was born at a maternal age of 19 years. The mother had a prison record and had been a prostitute. There was allegedly no consanguinity of the parents. The mother was seen two months before term because of pre-eclampsia, and labour was surgically induced. It lasted nine hours with a second stage of one and a half

hours. The patient weighed 3.1 kg. (6 lb. 14 oz.). A hare-lip and cleft-palate were noted at birth.

When examined at 13 years (Fig. 3) her height was 4 ft. 5 in. (134 cm.), she was microcephalic—head circumference 17 in. (43.3 cm.)—and muscular, and showed many of the signs of "Turner's syndrome," such as webbing of the neck, a low posterior hair-line, deformed pinnae, etc.

FIG. 4.—Karyotype of Case 2 (Denver system). (Arrow indicates isochromosome.)

She had cubitus valgus, valgus knees and ankles, a contracture deformity of the fingers of the right hand, lumbar scoliosis, and bilateral cervical ribs. There was a capillary haemangioma of the left shoulder. External genitalia were those of a normal pre-pubertal female. No pubic or axillary hair and no breast development were present.

The patient could stand, walk, and respond somewhat inaccurately to simple commands. She made guttural sounds but could not speak. Sex-chromatin and chromosome studies are summarized in Table II. A detailed analysis of the karyotypes (Fig. 4) showed 15 chromosomes in the X-6-12 range, with only one presumptive X chromosome, and three chromosomes morphologically resembling chromosome No. 3. One of these was interpreted as an isochromosome of the long arm of the X.

Case 3

A 2-year-old boy with Down's syndrome was born at the parental ages of 43 years. Pregnancy, labour, and delivery were all normal; he weighed 4.2 kg. (9 lb. 5 oz.). There was no history of consanguinity; the mother had a left congenital cataract and the father was deaf. There are three maternal half and three full sibs, all normal, their ages ranging from 6 to 22 years.

The patient (Fig. 5) was a typical case of Down's syndrome. His head circumference was 42.5 cm. (16½ in.). The penis was very small and the testes were not palpable, sex chromatin was positive and chromosome studies (Table II) revealed a chromosome number of 48. Detailed analysis of the karyotypes showed trisomy of chromosome No. 21, a Y chromosome, and 16 chromosomes in the X-6-12 range, giving a presumptive XXY sex chromosome constitution (Fig. 6). Cytologically and clinically this patient was diagnosed as an example of chromatin-positive Klinefelter's syndrome associated with Down's syndrome.

The patient's mother had 38% chromatin-positive cells in the buccal smear and a normal female karyotype. The patient's father was chromatin-negative and had a normal male karyotype. The sibs were not examined, but were stated to be normal.

FIG. 3.—Case 2.

Discussion

The frequency of Klinefelter's syndrome among defective male children in this population is 0.43%. This is lower than but in general agreement with the results of previous surveys of populations of low-grade mental defectives (Prader *et al.*, 1958; Mosier *et al.*, 1960; Barr *et al.*, 1960; Ferguson-Smith *et al.*, 1961; Israelsohn and Taylor, 1961).

Cytological details of three other cases of Klinefelter's syndrome associated with Down's syndrome are known (Ford *et al.*, 1959; Lehmann and Forssman, 1960; Lanman *et al.*, 1960) and are identical with the present findings. Mosier *et al.* (1960) have reported two families out of ten selected for the presence of chromatin-positive Klinefelter's syndrome, in which Down's syndrome was found in members of the same sibship; however, no chromosome studies were published of this material. It is perhaps of interest to consider whether the association of these two syndromes occurs with a greater frequency than might be expected by chance. In the present series 1 out of 49 males with Down's syndrome also has Klinefelter's syndrome, while in the only other comparable series the nuclear sex of 84 male patients with Down's syndrome was determined, and of these one was found to be chromatin-positive, and to have Klinefelter's syndrome, an XXY sex chromosome constitution, and trisomy of chromosome No. 21 (Ford *et al.*, 1959; Shapiro and Ridler, 1960; Shapiro, personal communication).

If the present data are combined with those of Shapiro we obtain a total of 133 males with Down's

syndrome, and of these two also have Klinefelter's syndrome, a frequency of 1.5% (95% confidence limits, 0.17%–5.3%). This estimate cannot therefore be said to differ significantly from estimates of the frequency of Klinefelter's syndrome obtained from other mentally defective male populations (see Israelsohn and Taylor (1961) for summary of data). It is, however, significantly higher than the *best estimate* of 0.26% for the frequency of Klinefelter's syndrome in the normal population (Moore, 1959; Maclean *et al.*, 1961). Harnden *et al.* (1960) reporting further on the case described by Ford *et al.* (1959) estimated that the coincidence of both aneuploid conditions in the same individual might be expected with a frequency of 1 in 560,000 total births. They suggested, however, that this might be reduced if there were genes in man tending to produce non-disjunction or asynapsis. In any calculation of this type the age of the mother at birth of the individual must be taken into account. In the present instance it was 43 years, while in the case of Harnden *et al.* (1960) it was 40. At these late maternal ages the frequency of Down's syndrome rises to about 1 in 100 births (Carter and McCarthy, 1951) and there is some evidence that a similar rise in frequency of Klinefelter's syndrome may also occur (Lenz *et al.*, 1959; Polani, personal communication). If so, the expected coincidence of the two conditions in the same individual might be as high as 1 in 10,000 total births.

We may conclude, therefore, that there is little evidence at present of either a lowered viability in this type of double aneuploidy or of a gene, genes, or other factors present in man which would tend to promote chromosome non-disjunction.

The frequency (0.5%) of triplo-X females found in this population is of the same order as that reported by Fraser *et al.* (1960), who found 0.7% of the females in a mental hospital in Glasgow to have double sex-chromatin masses and three presumptive X chromosomes.

The third case reported here is one of the cytological variants of chromatin-positive ovarian dysgenesis with webbing of the neck. Fraccaro *et al.* (1960) reported briefly three cases of chromatin-positive ovarian dysgenesis with identical karyotypes to that of our patient. They interpreted these findings to represent one X chromosome and one isochromosome for the long arm of the second X. Six other cases are known (Jacobs *et al.*, 1961; Lindsten, 1961; Blank *et al.*, 1961; Hamerton, Northfield, and Polani, unpublished); two of which may be examples of XO/XX — isochromosome mosaicism. If we accept the interpretation of the chromosome findings as correct, then these females are formally monosomic for the short arm of the X chromosome and trisomic for the long arm, and genes

FIG. 5.—Case 3.

FIG. 6.—Karyotype of Case 3 (Denver system).

on the short arm of the X chromosome are thus present in a hemizygous state. It would be tempting to postulate that the genes associated with the somatic stigmata of these forms of ovarian dysgenesis are located on the short arm of the X chromosome, as the clinical appearance of these females resembles closely that shown by females with 45 chromosomes and an XO sex chromosome constitution. However, before such a statement can be made much more knowledge is required about gene expression and dose effects of different genes in man. Studies on colour vision and other sex-linked conditions in these and similar cases of morphological sex chromosome abnormality may enable us to take some further steps in mapping the X chromosome.

Finally, in reporting Case 2 we have deliberately omitted any consideration of the size of either the sex-chromatin body or the drumstick, as such observations can only be subjective, and therefore of little value, unless absolute measurements of size, D.N.A. content, or both are made on normal control subjects and abnormal individuals.

To conclude, it is becoming clear that abnormalities of the sex chromosomes have a higher frequency in mentally defective populations than in the normal population. It does, however, seem likely that the association of intellectual subnormality with sex-chromosome aberrations is not due specifically to genes or gene complexes on the sex chromosomes, but to a generalized imbalance of the chromosome set as a result of aneuploidy. Furthermore, it seems probable that the relatively mild malformations resulting from abnormalities of the sex chromosomes when compared with malformations caused by autosome anomalies (Edwards *et al.*, 1960; Patau *et al.*, 1960) are due to the relative genetic inertness of the sex chromosomes when compared with the autosomes.

Summary

Clinical and cytogenetic findings are reported from three patients revealed during the course of a nuclear sex survey of a population of low-grade mental defectives at the Fountain Hospital, Tooting. The three are, respectively, a triplo-X female, a mentally defective case of chromatin-positive ovarian dysgenesis (Turner's syndrome), and a case of Klinefelter's syndrome associated with Down's syndrome. It is concluded that the frequency of Klinefelter's syndrome in a population of males with Down's syndrome is no greater than might be expected in any mentally defective male population. The relationship of sex-chromosome anomalies to mental deficiency and the possibility of mapping some genes on the X chromosome is discussed.

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USE OF AMIPHENAZOLE IN RESPIRATORY FAILURE

BY

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A large number of cases of chronic bronchitis show some degree of carbon dioxide retention in the blood. Many of these cases may show few clinical signs or symptoms of this and live without untoward incident for many years, but if an acute infection arises the respiratory insufficiency is increased, leading to a drop in blood-oxygen tension and a rise in that of carbon dioxide. The latter condition is further aggravated by the injudicious use of oxygen, thus removing the anoxic stimulus to respiration.

Several methods have been tried to decrease the carbon dioxide tension in such patients, with varying degrees of success: acetazolamide (Galdston, 1955), salicylates (Wégria *et al.*, 1955; Westlake and Campbell, 1959), dichlorphenamide (Naimark *et al.*, 1960), trihydroxymethylaminomethane (THAM) (Berman *et al.*, 1960; Manfredi *et al.*, 1960), nikethamide (Westlake and Campbell, 1959), and assisted respiration with and without tracheostomy. These methods have had only partial success and may even be harmful and uncomfortable to the patient.

Amiphenazole was first introduced by Shaw and Bentley (1952) as a morphine antagonist and has largely been used for that purpose. It was, however, known to be a respiratory stimulant, and Simpson and Hawkins (1959) reported briefly on its use in the treatment of respiratory failure.

The purpose of this paper is to report 16 cases in severe carbon dioxide retention treated with amiphenazole. Although not successful in all cases, this drug has, at any rate in my experience, had no severe toxic effects.

Methods.—Samples of blood were taken from the brachial artery, using a Riley needle, before and after giving amiphenazole. Arterial carbon-dioxide and