CHROMATIN-POSITIVE PRESUMED KLINEFELTER'S SYNDROME

SURVEY OF BOYS IN LONDON SCHOOLS FOR EDUCATIONALLY SUBNORMAL CHILDREN

BY

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A number of surveys have been made of the incidence of Klinefelter's syndrome in various populations, mostly among mentally defective children or adults. As a result of these and of the study by Pasqualini *et al.* (1957), the belief has arisen that the incidence of the abnormality is higher among mentally defective than among normal males (although tests of nuclear sex were not made in every case in Pasqualini's study). The present survey was made to establish the incidence of chromatin-positive patterns among educationally subnormal boys in London, and to obtain a number of children with this condition for a longitudinal study of their growth and development.

Material

Through the generous co-operation of Dr. J. A. Scott and Dr. D. Pirrie, of the London County Council, and the head teachers and parents of the children concerned, we were able to survey the entire population of 1,928 boys in the L.C.C. day schools for educationally subnormal (E.S.N.) children. Oral smears were taken from 1,556 of the boys, representing 81% of the total population. The remainder had refused examination or were absent from school at the time of examination. The I.Q. and age range of the sample are analysed in Table I.

TABLE I.—Analysis of Sample Studied

	Age Range		I.Q. Range			
	5-9	10-18	0-29	30-49	50-69	70-90 +
No. smears	288	1,268	0	34	851	671
% of sample	18.5	81.5		2.2	54.7	43.1
No. chromatin-positive cases	1	6		0	5	2

Method

Duplicate smears were made from oral mucosal scrapings on each child. Fresh smears were taken from each of the boys whose initial slides were chromatin-positive; the microscopical diagnosis was confirmed in every case. Blood films were taken from each of the chromatin-positive cases and were examined by Professor W. M. Davidson, of King's College Hospital. The chromatin-positive cases were examined clinically and true hemaphroditism was excluded in all cases. Several staining methods were tried; the Feulgen procedure was found to be the most satisfactory, and was used throughout with Davidson's fixative and with light green as counterstain. Seventy normal adults (35 females and 35 males) were studied as a preliminary control sample, and all were identified correctly by both of us. At least 50 suitable nuclei on each smear were examined for the presence of a chromatin body. In view of the occurrence of other heterochromatic material in the nucleus, especially that often associated with the nucleolus, we have accepted as positive only those nuclei with a chromatin body at the nuclear membrane. In the male controls the proportion of positive nuclei ranged from 0 to 6%, with a mean value of 0.8%. The proportion of positive nuclei in the female controls ranged from 32 to 76%, with a mean of 48.7%.

One of us (A. I. T.) examined all 1,556 smears from the E.S.N. boys under the high-power objective. The other (W. J. I.) examined, under oil-immersion, a series of 450 of these smears throughout which all the chromatin-positive slides were dispersed in random order. Although the number of chromatin-positive slides present was unknown to the examiner, all were identified and no additional ones were found. As rigid criteria were observed, and duplicate slides stained in cases of inadequate smears and poor staining, we feel confident that no chromatin-positive cases were missed among the slides examined. A close watch was kept throughout for cells with double chromatin bodies, in view of the cases described by Barr *et al.* (1959) and Ferguson-Smith *et al.* (1960).

Results

Among the 1,556 boys studied, seven were found to have chromatin-positive oral smears. This is an incidence of 0.45% with an S.E. of 0.17% and 95% confidence limits of 0.1 to 0.8%. In every case the blood films were positive. The percentage of nuclei with single chromatin bodies ranged from 44 to 75, with a mean of 62.2. Cells with two chromatin bodies were

TABLE II.—Age, I.Q., and Family History of Chromatin-positive Boys

Cases:	1	2	3	4	5	6	7
Age (years) at examination	9	10	12	12.8	13	13	15
I.Q. (Terman-Merrill)	66	73	59	65	64	72	61
Family history:							
Mother's age at birth of patient	33	14	45	20	33	37	39
Father's age at birth of patient	31	15	41	26	31	33	39
Mental deficiency	—	Mother "slow mentally" Mother's eldest sister certified M.D. Mother's brother in institute for M.D.; uncontrollable temper; abnormal E.E.G.	—	Father's younger brother attended school for backward children	—	—	—
Other defects	Father's 3rd youngest brother born blind. Sight restored by operation	Father: "multiple defects including deformed hands and ears"	—	Father had epileptic fits till age 23. Has attacks of headache, depression. Eye defect	—	Brother age 19 has "small testes and unusually long arms and legs" (parent's description)	—

seen, but in no case represented more than 2% of the chromatin-positive nuclei. Oral smears were taken from all parents and sibs of the chromatin-positive subjects, with the exception of Case 2, Case 7 (whose father had died), and one male sib of Case 6. All were consistent with the phenotype. Ishihara tests of colour-vision on the chromatin-positive subjects were all normal. The ages of these boys with their I.Q.s and family histories are given in Table II.

Discussion

Family Background.—The age of the parents is given to augment the scanty data on the relation between age and non-disjunction in man. It will be seen that non-disjunction occurred both in the immature (Case 2) and in the ageing (Cases 3 and 7) gonad. The absence of any significant abnormality in the sibs of the cases (with the possible exception of the only male sib who refused examination and who was said by his parents to have small testes and excessively long arms and legs requiring special tailor-made trousers) is in contrast to the study of Mosier *et al.* (1960) with twinning and mongolism in the sibs of 2 of his 10 chromatin-positive cases, and of Ferguson-Smith's (1958) study with twinning in the families of three of his four cases.

Comparison of Surveys

In Table III we have reviewed and analysed the results of comparable surveys in this field. The surveys are grouped according to the I.Q. range of the sample surveyed. Standard errors of incidence and 95% confidence intervals have been worked out wherever possible to facilitate comparison of results. We are greatly indebted to Professor M. L. Barr, University of Western Ontario, London, Canada; Dr. M. A. Ferguson-Smith, Johns Hopkins Hospital, Baltimore, Maryland, U.S.A.; and Dr. J. G. Cornwell, Yale University School of Medicine, New Haven, Connecticut, U.S.A., for allowing us to report the preliminary results of their unpublished work.

In comparing these different surveys we were interested in the following questions: (1) The frequency of chromatin-positive patterns at different levels of intelligence: (a) Do these chromatin-positive cases concentrate in a particular range of intelligence? (b) Is there a steady gradient of increasing incidence with either increasing or decreasing intelligence? (2) Geographical differences in incidence which might throw light on the causes of chromosomal aberration.

In comparing the survey figures we have used the chi-square and binomial probability difference techniques, together with a simple comparison of confidence intervals where possible. These comparisons have shown:

1. No significant difference in the incidence of approximately 0.3 to 1.5% of chromatin-positive patterns in the three large surveys in the predominantly under 50 I.Q. range (Table III, Panel 4).

2. In the surveys in the predominantly over 50 I.Q. range (Table III, Panel 3) our survey is the only one in which the incidence is significantly lower than that of Ferguson-Smith at the 5% level and of Prader at the 1% level. The frequency of chromatin-positive patterns in this range is approximately 0.5 to 2.5%.

3. With the exception of the survey of Prader *et al.* (1958) there is no significant difference in the incidence of chromatin-positive patterns in the surveys in the over 50 I.Q.

TABLE III.—Incidence of Chromatin-positive Nuclear Patterns in Males at Different Intelligence Levels

General Intelligence Level	Survey	Source	No. Cases Studied	% Total Popln. Sampled	I.Q. Range	I.Q. Level of Majority of Sample	Age Range	No. Chromatin-positive Cases	% Incidence	Standard Error	95% Confidence Limits of % Inc	I.Q. Range Chromatin-positive Cases	Mean I.Q. Chromatin-positive Cases
High intelligence	Prader <i>et al.</i> (1958)	School children, Switzerland	390	Very small		Above 120	Grammar school	0	0.00		0.0-0.1		Unknown
General population: intelligence unknown (includes proportion defectives)	Moore (1959)	Newborns, Winnipeg General Hospital	1,911	All males in 3,715 births over 12-month period		Unknown	Newborn	5	0.26	0.12	0.0-0.5	Unknown	Unknown
Mild mental defective in E.S.N. schools	Prader <i>et al.</i> (1958) Ferguson-Smith (1959) Israelovitch and Tavor (1961) *Cornwell (1960) De la Chapelle and Horring (1960)	Zurich, Glasgow London Connecticut, U.S.A. Helsinki, Finland	336 663 1,556 409 342	50 85 81 22.7	<75-95 45-85 37-101 Above 40 55-85	75-85 Unavailable 50-69: 55% 70-90: 43% Unavailable	7-15 5-12 5-18 Unavailable	8 8 7 3 3	2.4 1.2 0.45 0.73 0.88	0.84 0.42 0.17 0.42 0.51	0.7-4.1 0.4-2.0 0.1-0.8 0.1-6 0.1-9	62-94 50-77 59-73 50-60 63-83	79 63 66 Unavailable 74
Major mental defect	Mosier <i>et al.</i> (1960) *Barr (1960) *Ferguson-Smith (1960)	M.D. Hosp. California, U.S.A. M.T.V. Institution, Ontario, Canada State Training School, Maryland, U.S.A.	1,252 1,506 916	83.5	<20-70+ <20-70+ <20-86	<50: 74% <50: 75% <50: 78.8%	<10-50+ 10-50+ 6-50+	10 11 cases single chromatin body 13, incl 2 cases XXXY syndrome 7 cases single chromatin body 9, incl 2 cases XXXY syndrome	0.80 0.73 0.86 0.76 0.98	0.25 0.22 0.24 0.29 0.33	0.3-1.3 0.3-1.2 0.4-1.3 0.2-1.3 0.3-1.6	22-66 10-64 28-58	49 46 40

* Preliminary unpublished results.

range as compared with those in the under 50 I.Q. range. The incidence of single-body chromatin-positive patterns in Prader's sample is significantly higher (5% level) than in all three surveys in the under 50 I.Q. range. However, the incidence in Prader's group does not differ significantly from that in Ferguson-Smith's sample when the two cases of XXXY syndrome are included in the comparison, or from Barr's sample when the nuclear-positive case, not Klinefelter's syndrome (Barr, 1959, Case 3), is included.

4. There is no significant difference between the number of cases in the 20-29, 30-49, and 50-69 intelligence levels in Mosier's sample, and in the 50-60 and 70-90 levels in the L.C.C. sample. The incidence in the under 20 I.Q. level in Mosier's sample is significantly lower than that in the over 50 I.Q. level.

5. The incidence of 0.26% in Moore's (1959) sample differs significantly only from that of Ferguson-Smith (1959) and of Prader *et al.* (1958) in all the surveys on groups of subnormal intelligence.

From these results we can conclude that the incidence of single-bodied chromatin-positive patterns in males does not increase as the intelligence level of the group surveyed falls. The only evidence that the incidence of these cases is highest in the upper levels of subnormal intelligence is the significantly higher incidence in Prader's sample (I.Q. 75-95) as compared with those in the under 50 I.Q. range. This may, however, be due to a greater absolute incidence of the condition among males in Zurich. Further surveys are needed among boys in the upper levels of subnormal and low normal intelligence before this fact is established.

The present survey is conspicuous for its difference from that of Ferguson-Smith (1959) and that of Prader *et al.* (1958) in the over 50 I.Q. range. Both the London and the Glasgow surveys may be regarded as representative of an entire population of comparable I.Q. range. There are several possible explanations for this difference. (a) Only 43% of the London sample belong to the over 70 I.Q. range. It may be that cases of Klinefelter's syndrome in London tend to concentrate in the lowest streams of the normal primary and secondary modern schools. (b) A result which might be expected statistically on the basis of the fact that if 20 comparisons are made there is a 5% chance of one of them being outside the 2-S.D.-from-mean range. (c) An overall reduction in the incidence of chromatin-positive pattern in males in London reflecting a diminished tendency to chromosomal aberration in this area.

Moore's (1959) survey provides interesting information on the incidence of chromatin-positive males in the general population in Canada, and Prader *et al.* have found no cases in their small sample of highly intelligent Swiss boys. • Ferguson-Smith has had experience of a number of adult chromatin-positive subjects who are distinctly above average in intelligence. Further surveys on groups from known normal intelligence range, and intelligence studies on chromatin-positive cases diagnosed in male-fertility clinics and their families, would be valuable not only in the prognosis of a condition that can now be diagnosed early and with relative ease, but also to provide evidence of the extent to which the brain can develop and function normally in the presence of an abnormal sex-chromosome complement.

Summary

A survey of 1,556 of a total of 1,928 boys (I.Q. of the majority, 50-90) attending schools for educationally subnormal children in the London County Council area

revealed seven with single-bodied chromatin-positive nuclear patterns and presumed Klinefelter's syndrome (true hermaphroditism excluded by clinical examination). This is an incidence of 0.45%, S.E. 0.17, with 95% confidence limits 0.11 to 0.79%.

This figure is significantly lower than those found in Ferguson-Smith's survey (1.2%) and in Prader's survey (2.4%) on educationally subnormal boys with I.Q.s over 50. A comparison of surveys on boys with minor and major mental defect revealed no statistically significant difference between the incidence of these cases in surveys of the over 50 I.Q. range as compared with surveys of the under 50 I.Q. range, with the exception of Prader's survey. Prader's incidence of cases in the 75-95 I.Q. range is significantly higher than in the three chief surveys of the under 50 I.Q. range. The only study of newborns (which would sample all I.Q.s in their proportionate incidence in the population) is Moore's, with an incidence of chromatin-positive patterns of 0.26%. This is significantly lower than only those of Ferguson-Smith (1959) and of Prader *et al.* (1958) in males of subnormal intelligence.

Further surveys are required if the impression that cases of chromatin-positive Klinefelter's syndrome have their highest incidence in the borderline or low-normal-intelligence range is to be sustained. Intelligence studies on a larger number of chromatin-positive cases and their families are needed to provide evidence on the extent to which the brain can develop and function normally in the presence of an abnormal sex-chromosome complement.

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