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The D Trisomy Syndrome and XO Gonadal Dysgenesis in Two Sisters*

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INTRODUCTION

RECENTLY a first case of trisomy for a chromosome of the D group (in the classification by Patau 1960; identical with group 13–15 of the Denver system: Am. J. Human Genet. *12*: 384–388 (1960)) was described by Patau, Smith, Therman, Inhorn, and Wagner (1960). In an addendum a second case of trisomy for evidently the same chromosome was briefly announced. This second D trisomic is case 20 of the present communication. Remarkably enough, a sister of this patient was found to be affected with XO gonadal dysgenesis (Turner's syndrome); she will henceforth be referred to as case 42.

TISSUE CULTURE AND CYTOLOGICAL METHODS

Except for some earlier results obtained by means of short-term cultures of bone marrow (for the technique followed see Patau *et al.*, 1960), the chromosome studies were based on tissue cultures derived either from skin or from bone marrow. The tissue culture techniques were largely those described by Tjio and Puck (1958a). The following remarks pertain not only to the present investigation but also to other work which will be reported elsewhere.

Fresh biopsy specimens were introduced into stoppered tubes of culture medium in which they sometimes had to remain as long as 12 hours, occasionally even as long as 1½ days, before further manipulation. In the case of bone marrow, the specimen, ordinarily about 0.5 ml in volume, was distributed among four 60 mm Petri dishes and placed in the CO_2 incubator. Usually, after two days clearings became visible in the layers of red cells. Apparently all cells in these clearings were fusiform, much larger than hematopoietic cells, often associated with fatty droplets, and with a relatively high mitotic rate. Because of the similarly fusiform appearance of the cells in the cultures finally used for chromosome analysis, it is questionable whether these cultures had descended from blood-forming elements of the bone marrow. The medium in marrow-derived cultures was replaced every third day after the first week. The first subculture was usually started three to four weeks after biopsy. Cover-slip preparations

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were made one week later at the second subculturing or, if necessary, from later subcultures.

Each skin biopsy specimen was minced but not trypsinized, and placed in a single 60 mm Petri dish in the CO_2 incubator. Within a few days small fragments of tissue attached to the glass. After one or two weeks they were surrounded by areas of outgrowth. After the second week the medium was renewed every third day. When the cultures contained 10^5 or more cells, *i.e.* usually during the fourth week after biopsy, the first subcultures were started. Cover-slip preparations were sometimes made at this time but usually at the second or, occasionally, at a later subculturing. In every case the cultures consisted of fusiform cells.

The over-all success in establishing cultures for chromosome analysis has been close to 90 per cent for skin specimens and 70 per cent for marrow specimens.

Cells grown on cover slips were fixed as recommended by Tjio and Puck (1958a), but they were not permitted to dry. Instead, the slides were preserved until use in 70 per cent alcohol in the cold. After staining with acetic-orcein or Feulgen reinforced by orcein the preparations were made semi-permanent by framing with Kroenig cement. Individual cells were squashed as needed under microscopic control. The chromosomes were counted by a method (*cf.* Patau *et al.* 1960) that precludes errors arising from subconscious bias.

PARENTS AND SIBS

Both parents are Caucasian; their medical history is unremarkable and there are no known cases of congenital anomaly or mental retardation among their relatives. The parents do not seem to be related to the previously described D trisomic. At the time of conception of the first child, case 42, the mother was 22 years and the father 27 years old. The next two children, also girls, are described as normal. One of them died in an accident at two years of age, the other is now six years old. When the fourth and last child, case 20, was conceived, the maternal age was 35 years. There is no record of abortions.

CASE 42 (XO GONADAL DYSGENESIS)

The patient was 14 years, six months of age when examined by one of us (D.W.S.). She appeared as a typical case of XO gonadal dysgenesis, displaying shortness of stature, posterior webbing of the neck, slight puffiness over the dorsum of the fingers and toes, lack of estrogen effects, and other minor anomalies. Buccal smears were chromatin-negative.

For the cytological analysis a skin tissue culture was available. The chromosome number was found to be 45 (table 1). A detailed analysis was possible in most of the scored cells. There were always four G chromosomes of the kind found in normal females (Fig. 1). Evidently there was no Y chromosome. The presence of only 15 C chromosomes, in conjunction with the buccal smear findings, demonstrated the presence of only one X chromosome. The chromosomes of the autosome groups A, B, D, E, and F appeared normal in number and shape. The cytological finding, a complement as first described by Ford, Jones, Polani, DeAlmeida, and Briggs (1959), thus confirmed the clinical diagnosis of XO gonadal dysgenesis.

TABLE 1. CHROMOSOME COUNTS, NEAR-DIPLOID CELLS ONLY

Patient	Material		≤43	44	45	46	47	48	≥49	Total
	Source	Method								
Case 42	skin	tissue culture	—	1*	19	—	—	—	—	20
Case 20	bone marrow	short-term culture	—	—	—	—	6	—	—	6
	" " (diff. loc.)	tissue culture	—	—	—	—	4	—	—	4
	skin	" "	—	—	—	—	14	1†	—	15
	Total		—	—	—	—	24	1	—	25

* Cell appears unbroken, count reliable.
† Counting error possible.

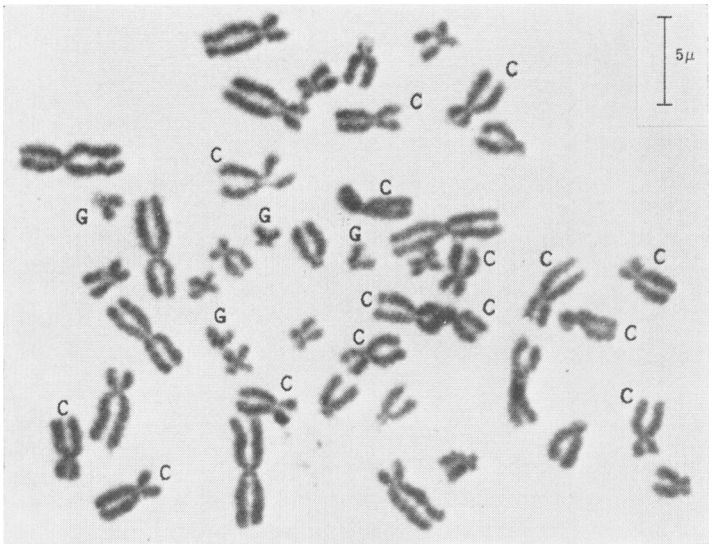

FIG. 1. The 45 chromosomes of case 42. Note 15 C chromosomes and four G chromosomes: XO gonadal dysgenesis. Tissue culture. Feulgen, orcein.

CASE 20 (D TRISOMY SYNDROME)

The patient, a female infant, was born in November, 1958, and is, after two years, still alive. Her mother had a "bad case of flu" with fever and chills during the first trimester of the full-term pregnancy. At birth the patient weighed 7 lb. 7 oz. During her first two days she showed respiratory distress and cyanosis but, as far as is known, not thereafter. Over the posterior fontanelle area of the skull, the skin was absent with the effect that this area appeared "raw and meat-like". This healed spontaneously within three weeks. During her first month the patient was inactive and she seldom cried. When she was nine months old she could, in the prone position, hold up her head, but was not able to sit alone. The parents

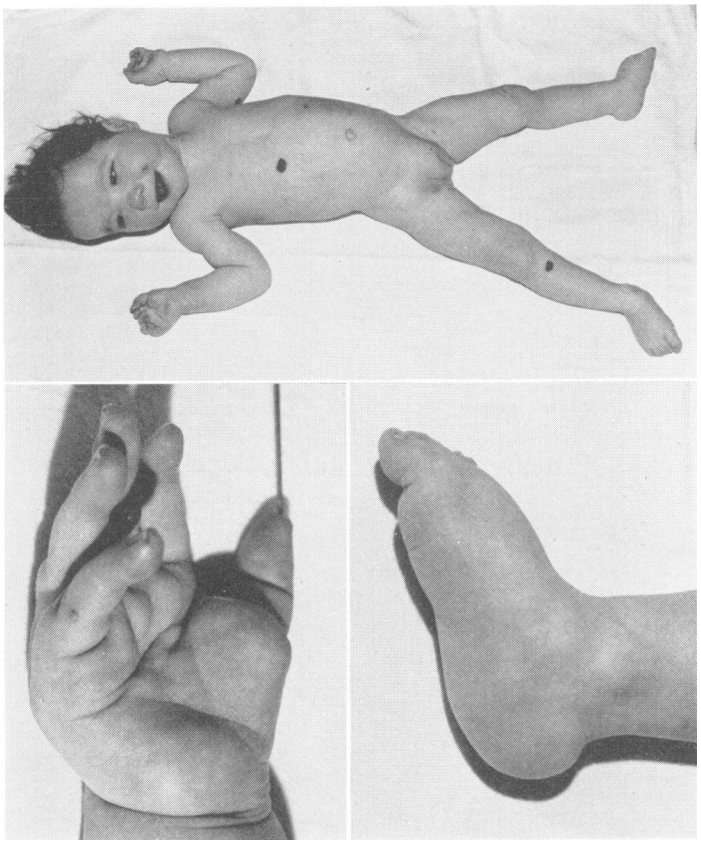

FIG. 2. D trisomic (case 20).

had the impression that she could distinguish light from dark, but did not follow a light with her eyes; they also noted that she did not respond to sound.

When the infant was first seen by one of us (D.W.S.) she was 13 months of age (Fig. 2). Her weight, 18 lb., was 4 lb. under the average for her length of 31.0 in. The head circumference was 17.2 in.

The patient reacted to a strong light by blinking but did not follow moving objects with her relatively small eyes. Each iris had an inferior-medial coloboma, the back of each lens was partially covered by a vascularized white membrane, there were abnormally few retinal vessels, and the optic discs were large, pale, and vertically ovaloid with undercut medial margins.

The patient showed no response to a loud sound, she did not respond even to a slapping of the bed that visibly shook her. The auricles were slightly low-set. The palate had a 2.0 cm long posterior cleft. The frenulum extended to the tip of the tongue, which was slightly cleft. Eight teeth were present and appeared normal.

Both hands had horizontal palmar creases and relatively slim terminal phal-

anges with unusually convex fingernails. The thumbs could be bent backward to an abnormal degree at their rather narrow base, two small "clicks" being sometimes noticeable. This condition of the thumbs resembled that described for the first case of D trisomy (Patau *et al.*, 1960) as "trigger thumb", except that the thumbs of the present patient occasionally assumed the backward bend spontaneously. We have since learned that the term "trigger thumb" has been used by others for a different condition. For this reason, we shall henceforth call the anomaly of the thumb as observed in both D trisomics "retroflexibility" of the thumb.

At the ulnar side of the left fifth finger a 2 mm wide nodule of skin was present. The feet were maintained in a position of moderate downward flexion and had a dorsal cover of non-pitting soft tissue. Their soles were flat and they had posterior projections (1.0 cm) of the heels, an anomaly known as rocker bottom feet.

Raised cherry-red hemangiomata, ranging in diameter from 1 to 3 cm, were present on the trunk, left upper arm, and on the right lower leg.

Judged by her performance, the mental development was below the two-months level. She smiled, but otherwise showed little spontaneous activity and tended to lie on her back with legs extended and arms partially flexed. An occasionally occurring sudden extension of the extremities resembled a myoclonic jerk. The infant was unable to support herself in the sitting position. On passive movement of the extremities, particularly the lower, the muscles were found to be moderately hypotonic. The deep tendon reflexes appeared to be normal.

Roentgenograms disclosed a spina bifida posterior of the first cervical vertebra. The heart was normal in size and contour (blood pressure: 105/70). An electroencephalogram, which showed frequencies of 4 to 8 cycles per second, was interpreted as normal. Further studies, including a pyelogram and laboratory tests of blood and urine, revealed no abnormality.

The patient was subsequently admitted to the Northern Wisconsin Colony and Training School for Retarded Children where she was last seen by us when she was 19 months old. She then followed a moving hand with her eyes but seemed still unable to roll over or sit without support.

For the cytological analysis three different samples were available (table 1). The basic chromosome number of the patient is 47, the extra chromosome belonging to the D group (Figs. 3 and 4). A detailed analysis of all suitable cells revealed that the chromosomes of all groups other than the D group are those of a normal female complement, not only in regard to number but also to chromosome morphology as far as this can be ascertained in favorable mitoses. Buccal smears were chromatin-positive.

It remains impossible to state to which of the three pairs of the D group the extra chromosome belongs (*cf.* Patau *et al.* 1960, and Patau 1960). There would be no need to say more were it not for a recent publication (Hayward and Bower 1960) in which our first case of D trisomy is referred to with the additional, and to the present authors surprising, information that the "trisomic chromosome" is No. 15. No reason for this identification is given, but as the authors use the Denver "standard system" in which No. 15 is defined only by the sentence "no

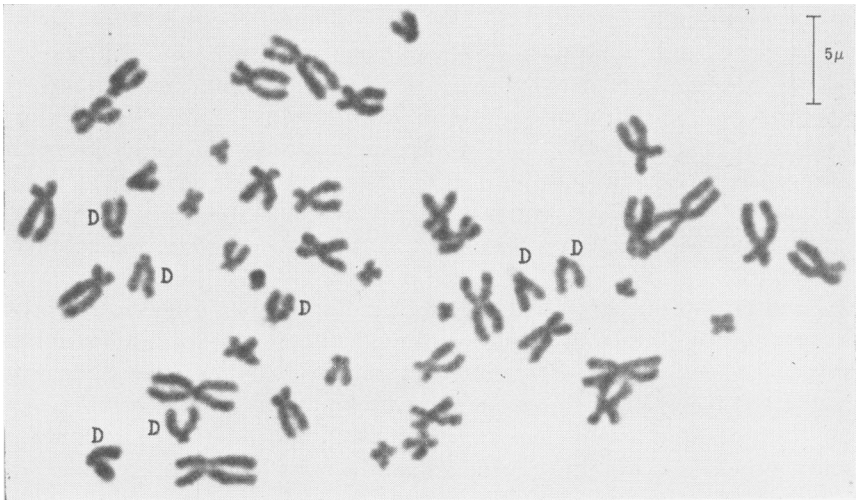

FIG. 3. The 47 chromosomes of case 20. Note seven D chromosomes. Tissue culture. Feulgen, orcein.

FIG. 4. The seven D chromosomes from four cells of case 20. Tissue culture. Feulgen, orcein. ×3040.

satellite has been detected on chromosome 15", it would seem that Hayward and Bower assume the extra chromosome to have no satellite. To this we offer the following comment.

The satellite of any human satellite chromosome is more often than not unrecognizable, either because its position relative to the short arm that carries it precludes it from being seen as a distinct body (and nothing less justifies speaking of a satellite) or, possibly, because it was torn off during the preparation. To realize the difficulty one needs only to remember that all authors who first described satellites in man assumed that they occur only at one pair of D chromosomes (Tjio and Puck 1958, b; Chu and Giles 1959; Böök, Fraccaro, and Lindsten 1959; Levan and Hsu 1959) although now they seem to agree that two pairs of

TABLE 2. EXPECTED FREQUENCIES (BINOMIAL) OF NUCLEI WITH DIFFERENT NUMBERS OF DISTINGUISHABLE SATELLITES, ASSUMING THAT THE MEAN NUMBER OF VISIBLE SATELLITES PER NUCLEUS IS ONE

D Chromosomes with Visible Satellites, Number per Nucleus	Number of Actually Satellited D Chromosomes:	
	4	6
0	31.64%	33.49%
1	42.19	40.19
2	21.09	20.09
3	4.69	5.36
4	0.39	0.80
5	—	0.06
6	—	0.00
Total	100.00%	99.99%

D chromosomes, No. 13 and No. 14, have satellites (Denver system). We can confirm this, for we have in a few instances seen three D chromosomes with satellites in metaphases from persons with an evidently normal D group. We also have noted that satellites occur at both the smallest and larger D chromosomes. The observations published by other authors do not indicate that they have had much more success than we in demonstrating satellites with any regularity. Neither does it seem that the situation could be greatly improved by any special staining method, as the visibility of orcein stained satellites that are sufficiently set off from the chromosome is satisfactory.

Assume there is in technically favorable mitoses (which implies among other things a low degree of chromosome contraction) a mean number of one recognizably satellited D chromosome per cell, probably a too optimistic estimate. On this basis and under the further assumption of statistical independence of chromosomes in regard to the visibility of satellites table 2 was computed. It will be seen that a very large number of mitoses would have to be scored before any case could be made for the existence of only two pairs of satellited D chromosomes. As long as such a demonstration is lacking, the Denver definition of the individual chromosomes 13–15 is meaningless.

We suspect that this definition will remain meaningless, for there are indications that all D chromosomes have satellites. We have found in a tissue culture of skin from a patient with an apparently normal group of six D chromosomes a prophase nucleus with five clearly satellited D chromosomes. One cell cannot decide the issue but there is some supporting evidence. In blood culture mitoses we have very often found two or more acrocentric chromosomes that were associated by their short arms, *i.e.* the arms that may carry satellites. The association, which has independently also been discovered by Dr. Ferguson-Smith (personal communication), is non-homologous, D and G chromosomes being combined in a random fashion. It seems highly likely that the association is a remnant of an association brought about by nucleolar fusion, the stems of satellites being nucleolar organizers. Polani, Briggs, Ford, Clarke, and Berg (1960) have ably discussed the role that nucleolar fusion in man may play in promoting special types of chromosomal rearrangements and in non-disjunction. Rele-

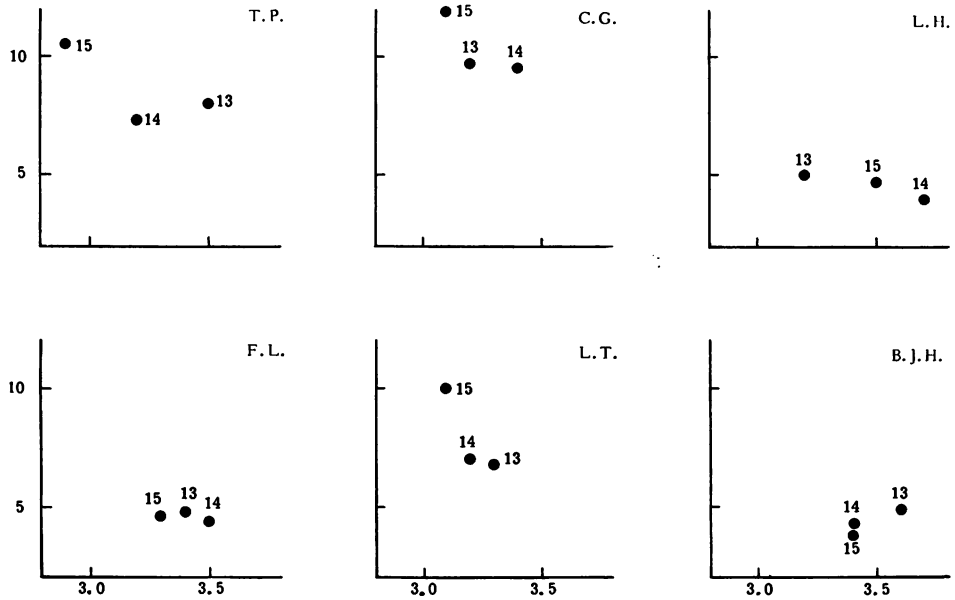

FIG. 5. Karyograms of the D group based on the means of measurements by different authors (identified by initials) as given in Table 2 of the Denver Report. Abscissae: chromosome lengths in percent of haploid total. Ordinates: arm ratios.

vant in the present context is the occurrence, in blood cultures from euploid persons, of mitoses in which five (the highest number found so far) D chromosomes are associated with each other or with G chromosomes. This, again, suggests that all three pairs of D chromosomes have satellites.

If there should be any notion that the individual D chromosomes might be identified, if not by satellites, by their arm lengths, a study of the karyograms (Patau 1960) of Fig. 5 should dispel it. Differences in preparation and measuring technique can cause considerable discrepancies in relative chromosome length and arm ratio between measurements by different authors. This reflects systematic errors that should affect all of the very similar D chromosomes in about the same manner, resulting in shifts of the pattern of points in the karyogram but not in major alterations of the pattern itself. Instead, we find a variety of patterns that clearly contradict each other. Evidently the authors of the Denver Report themselves felt that the relative chromosome length and the arm ratio do not yield a meaningful definition of the chromosomes 13 through 15. As pointed out above, the attempted alternative, definition by satellites, is not practical either. We repeat the previous suggestion (Patau 1960) to discontinue the practice of assigning numbers to chromosomes unless there are well-defined criteria by which they can be identified with assurance. Such criteria do not exist for D chromosomes.

DISCUSSION

The patient described above is the second example known to us of trisomy for a D chromosome, the first having been published by Patau *et al.* (1960) (this

patient, henceforth referred to as case 5, died recently; the autopsy findings will be reported elsewhere). The two cases have in common mental retardation, apparent deafness, myoclonic seizures, an eye defect (case 5: apparent anophthalmia, case 20: microphthalmia, colobomata of the iris, etc.), retroflexibility of the thumbs (called "trigger thumbs" by Patau *et al.* 1960), horizontal palmar creases, cleft palate, and hemangiomata (capillary-case 5, or raised ones-case 20). In addition, both cases have abnormal feet (case 5: polydactyly, case 20: rocker bottom feet). A hare lip and a heart anomaly were found only in case 5. Clearly, the sets of anomalies displayed by both patients are similar enough to be regarded as representatives of the same syndrome.

Hayward and Bower (1960), referring not only to the one trisomy case each that was published by themselves and by Edwards *et al.* (1960) but also to D trisomy as reported by Patau *et al.* (1960), express the opinion that "it has still to be unambiguously demonstrated that autosomal trisomy is the cause rather than an accompanying phenotypic effect expressed at the nuclear level, or a chance association of events". A strange remark. When Patau *et al.* (1960) mentioned in an addendum their second case (20) of D trisomy with a "similar set of congenital anomalies" the association of D trisomy and the syndrome, which obviously are both very rare events, became statistically significant to a degree that amounted to an unambiguous demonstration of this not being a "chance association". There remains the possibility of trisomy being an "accompanying phenotypic effect". How are we to envisage that? Are we, for instance, to assume that the agent responsible for the syndrome also causes non-disjunction not just of any chromosome but of a D chromosome during the first cleavage division? If so, what happens to the cell which is deficient of a D chromosome? It does not seem that the present patient is a mosaic. We must confess that we are unable to think of any remotely plausible alternative to the conclusion that the syndrome is caused by the presence of a specific D chromosome in triplicate, which in turn we take to have resulted from non-disjunction, most likely during the first meiotic division, in one of the parents.

With the methods available to date it is impossible to decide which of the three different D chromosomes is present in triplicate in the two trisomics. We do infer from the similarity of the two patterns of anomalies that in both cases of D trisomy the responsible chromosome is the same. We propose to call it D_1, suggesting that in general chromosomes that are identifiable by the phenotype of trisomics but not with the microscope be denoted by a subscript attached to their group symbol. It is unknown whether trisomics for a D chromosome other than D_1 are viable. So long as none has been observed it will suffice to refer to the "D_1 trisomy syndrome" as the "D syndrome".

More cases will have to be discovered before a satisfactory characterization of the D syndrome can be given. No doubt it will be found to contain anomalies as yet unrecognized or that happened not to manifest themselves in the two cases discussed here. In view of the situation in mongolism, it is also to be expected that some, or even all of the anomalies displayed by these two patients will not always be present. The D syndrome is obviously very rare. We have screened clinically some thousand patients, mostly young and/or low grade, in three state

colonies for the mentally retarded and found only one with the D syndrome, the present case 20, whom we had seen before. In the same sample, there may have been some 250 to 300 mongoloids. Assuming an incidence of mongolism of 1 in 700, this would correspond to a frequency of the D syndrome of 1 in 200,000. Allowing for bias and a very large sampling error, we may safely estimate that the D syndrome is rarer than 1 in 10,000. It should be added that among these thousand patients there were a number who displayed some of the anomalies of the D syndrome without giving the impression of belonging to the same clinical entity as the present two patients. Almost all of these proved to have 46 chromosomes, but evidence has turned up to support the working hypothesis that at least certain "partial D syndromes" are due to the presence of a translocated extra piece of D_1, in other words, that they are duplication effects. The investigation of such cases is being continued.

The word "trisomy" implies the presence of three homologous chromosomes and should not be used when an extra chromosome is suspected to be a translocation chromosome that merely happens to have about the same size and shape as a certain pair of homologues. By the same token, the diagnosis of trisomy of a new type remains necessarily somewhat uncertain until another case is found that combines the same chromosome complement with a similar clinical picture. According to this criterion, only three types of autosomal trisomy can be considered as established to date[1]: mongolism, the D syndrome, and the "E syndrome" described by Smith, Patau, Therman, and Inhorn (1960), to which on clinical grounds the case reported by Edwards, Harnden, Cameron, Crosse, and Wolff (1960) may be added, even though their identification of the extra chromosome as No. 17 conflicts with our own as No. 18, which we consider as definitive (the evidence is being published elsewhere: Patau, Therman, Smith, and DeMars 1961). This trisomy syndrome seems to be somewhat more frequent than the D syndrome, but it is still very rare compared with mongolism. There are two equally plausible mechanisms either of which might explain the low incidence of E and D trisomics. Conceivably, the probability of non-disjunction decreases steeply with increasing chromosome length. Alternatively, trisomy for D_1 and trisomy for chromosome No. 18 might ordinarily be lethal conditions, rendered viable only by specific gene mutations.

It is virtually certain that the co-occurrence in a sibship of two conditions as rare as XO gonadal dysgenesis and the D syndrome is no mere coincidence. An environmental causation of an increased frequency of non-disjunction is conceivable but does not fit too well the circumstance that the two patients were born 13 years apart. Alternatively, and perhaps more likely, one of the parents may

[1] Hayward and Bower (1960) found apparent trisomy in a patient with Sturge-Weber's syndrome. In all cases of this disease that have since been investigated cytologically, by others or ourselves, the chromosome number was 46. However, in one of our cases a translocated extra piece was present. Hayward and Bower's extra chromosome may also have resulted from a translocation. It appears likely that Sturge-Weber's syndrome is generally caused by the presence in triplicate of a translocated segment of an unknown donor chromosome even though this segment may usually be undectable in the microscope (Patau, Therman, Smith, Inhorn, and Picken 1961: Am. J. Human Genet., in press).

have a genotype that fails to sustain fully all of the processes required for a successful conclusion of meiosis. That there is a genetic control of meiosis could be taken for granted even if it had not been demonstrated in maize, *Drosophila,* and other organisms. Any explanation, except by pure coincidence, of the present case of familial incidence of aneuploidy involving different chromosomes leads one to suspect that zygotes with other abnormal chromosome complements might also have been formed. If these were abnormal enough, abortion might have taken place before the pregnancy was recognized. The birth of two apparently normal children during an interval of thirteen years does not rule out this possibility.

The present case of different types of aneuploidy in two sibs reminds one of the patient with combined mongolism and Klinefelter's syndrome (Ford, Jones, Miller, Mittwoch, Penrose, Ridler, and Shapiro 1959; Harnden, Miller, and Penrose 1960). Here, too, coincidence is extremely unlikely and a genetic causation appears possible, but there is less reason to resort to a genetic explanation than in our case. Individual cells in an otherwise healthy population of gametocytes may show disturbances, of the spindle for instance, that could result in multiple non-disjunction. An increase of the incidence of non-disjunction with maternal age need not reflect an increased probability of non-disjunction in all oocytes. If it does not, if merely the frequency of "disturbed" oocytes were increased, an above-random incidence of multiple non-disjunction in aging mothers (in the British case her age was 40) would result.

SUMMARY

A patient who represents the second case of trisomy for a D chromosome (for the first see Patau *et al.* 1960) is described. Both cases have in common mental retardation, apparent deafness, myoclonic seizures, an eye defect, retroflexibility of the thumbs, horizontal palmar creases, cleft palate, hemangiomata and abnormal feet.

The two cases establish the existence of a "D syndrome" that is caused by the presence in triplicate of a specific D chromosome, even though it is at present not possible to distinguish the latter microscopically from the other two pairs of D chromosomes.

A sister of the present patient is a typical case of XO gonadal dysgenesis. This familial incidence of aneuploidy may be due to a genotype of one of the parents that does not sustain full regularity of meiosis.

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