DELETION OF Y CHROMOSOME IN A FAMILY WITH MUSCULAR DYSTROPHY AND HYPOSPADIAS

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Although there have been many attempts at differentiating the various types of muscular dystrophy the classification is singularly confusing, and the contribution from genetics has not clarified the situation much. The condition of the affected members of the family here studied will be described simply as "limb-girdle muscular dystrophy," which seems to be the condition in all the affected members. This term has been used by Natrass (1957), and the essential characteristics are as follows: (a) expression in either sex; (b) onset usually in the first or in the second or third decade, but sometimes in middle age; (c) transmitted usually as an autosomal recessive character but occasionally as a dominant; (d) primary involvement of either the shoulder-girdle or the pelvic-girdle muscles with frequent asymmetry of wasting when the upper limbs are first involved; (e) spread from the upper to the lower limbs or vice versa, in most cases within 20 years; (f) rarely abortive cases; (g) rarely muscular pseudohypertrophy; (h) variable rate of progression with sometimes periods of clinical arrest; (j) muscular contractures uncommon and skeletal changes slight; (k) death frequent in middle-age but later with cases of late onset.

Affected Family

The inheritance of the muscular dystrophy in the present family could be caused by an autosomal or X-linked dominant. Five members are affected (Fig. 1). In the first generation a mother of four children died from cancer at an early age and was too young to have symptoms, which tend to appear late in this family. Her eldest son died from anaemia after having been bedridden for many years owing to the paralysis. The eldest daughter was also affected and carried the gene to one of her two children, a daughter, while her son

FIG. 1.—Pedigree showing the inheritance of muscular dystrophy and hypospadias.

and his family appear to be unaffected. The daughter started her first symptoms about middle-age, but there has been some clinical arrest for a long time and she is still not badly affected. She has two sons, both of whom exhibit typical symptoms of moderate severity. One of the sons has two small boys.

The chromosome investigation started with one of the affected members, the father of the two small boys, wanting his chromosomes analysed. Cultures were prepared from venous blood, and all the examined cells showed the presence of an abnormally small Y chromo-

FIG. 2.—Early metaphase in leucocyte from original patient showing the deleted Y chromosome. (Note the intact short arm and the parallel longer arms.)

some (Fig. 2). The missing part represented from one-third to one-half of the long arm of the normal Y chromosome, presumably depending upon the degree of coiling of the chromosome (Fig. 3).

A point about the structure of the Y chromosome which was revealed from a detailed study of an XXYY-male (Muldal and Ockey, 1960), but not previously reported, is the existence of a short secondary constriction in the Y chromosome at about the middle of the long arm. This band or constriction is not too often seen, but can be more regularly deduced from coiling abnormalities, which gives the appearance of a quartet of knobs marking off the constriction in the two chromatids.

FIG. 3.—Diagrammatic representation of chromosomes in group VII. Top row, the normal chromosomes in this group. Below, deleted 21 (the Philadelphia chromosome), and, on the right, the deleted Y.

Since the constriction is either absent or terminal in the deleted Y it would appear that the deletion comprises the terminal part of the long arm stretching from the constriction to the telomere. It is possible that the constriction itself actually is present terminally on the deleted Y chromosome, since it appears to be rather sticky at this end, indicated by a tendency of

association with the telomeres and centromeres of other chromosomes.

The reasons for thinking that we have identified the deleted chromosome correctly are the following standard criteria. (1) The lack of satellites, whereas these can be seen clearly on the other small chromosomes. (2) The aberrant chromosome is not associated with nucleoli; cells can be found where the other four chromosomes as well as members of group IV are associated with one or more nucleoli, while the aberrant chromosome is lying separately. (3) The aberrant chromosome also shows the general tendency of the arms of the Y chromosome to lie parallel instead of opening out as chromosome 21 and 22 often do after colchicine treatment. This behaviour may be due to delayed D.N.A.-synthesis in heterochromatin (Taylor, 1960), so that coiling in the Y as compared with the autosomes is retarded. As contraction proceeds the difference will gradually disappear. (4) The four chromosomes form two pairs, one pair (No. 21) with long satellites and a smaller pair (No. 22) with shorter satellites. Even in cells where the Y chromosome of a normal male is not bigger than the 21 pair, there are clearly three larger and two smaller chromosomes in group VII. In the present cases there are always two larger and three smaller, one of which is very small.

Confusion with the PH chromosome is improbable owing to the following features: the PH-chromosome, although by gross appearance not unlike our aberrant Y, is *satellited* and may be found *associated with nucleoli*; it *lacks the knobs* and shows a tendency to appear as a *cross* in colchicine-treated preparations. As to the actual size of the missing parts of the two chromosomes, these are of the same order of magnitude.

The unmarried brother of the propositus was subsequently examined, and at the same time their mother. The deleted Y chromosome was found to be present in the brother as well, and no differences between their Y chromosomes could be detected. The mother had a normal female complement. The two small boys of the last generation both carry the deleted Y chromosome, as expected—and that concluded the chromosome analysis (Fig. 4).

The deleted Y chromosome has come from the father of the propositus, who presumably carried the chromosome in all his cells and not only as a freak error in spermatogenesis. He came from a family of 10 children, and all the males are now deceased. Eight marriages in this family resulted in only two fertile ones—with

the following generation consisting of only three children—a girl in addition to the two brothers included in the chromosome analysis. Unfortunately, nothing can therefore be done about carrying the analysis of the deleted Y chromosome further. The low rate of reproduction in this branch of the family may possibly be regarded as a sign of infertility.

The youngest of the two boys was operated on for hypospadias a few years ago, and hypospadias is present in the older boy as well, although in a slighter form. Their father also carries this mild form of the defect, but the unmarried brother is said to be quite normal in this respect. We have no information about this condition in any other members of this family, and there appear to be no prospects of getting such information either.

In order to find other possible effects of the deleted Y chromosome we have obtained hormone assays of the two adult males:

24-Hour Urine Specimens			
	17-Ketosteroids	Ketogenic Steroids	Gonadotrophins
The propositus ...	7.5 mg.	8.5 mg.	4 M.U.
Unmarried brother	10.6 „	13.4 „	Below 3 M.U.

The normal range for 17-ketogenic steroids in males of about our patients' age is 5–18 mg., with an average of 10 mg.; the range in females is 3–9 mg., average 6 mg. The gonadotrophin level in a male should range from 5 to 50 M.U. a day, so the values for both brothers are below normal.

Apart from the symptoms of muscular dystrophy and hypospadias no other abnormalities could be found.

Discussion

Although the pattern of inheritance of muscular dystrophy in this family suggests that the deleted Y chromosome has nothing to do with this disease, it seems likely that it is the cause of the hypospadias and the low gonadotrophin level in the adult brothers. The hypospadias could be inherited as an autosomal gene or as a Y-deletion.

Hypospadias is generally regarded as a slight form of intersexuality and occurs in 3 out of 1,000 males. According to Sorsby (1953) it is inherited as an irregular recessive defect; but there are on record, although more rarely, cases of dominant transmission. Rahbek Sørensen (1953) suggests that 75% occurs spontaneously. Hypospadias shows considerable interfamilial and intra-familial variation, often associated with cryptorchidism. Peniscrotal hypospadias and especially the pseudo-vaginal hypospadias are often the conditions in sex mosaics.

The genesis of hypospadias is generally held to be as follows.

At about the 45-mm. stage (3 months) the male genitalia take on approximately their final form as a result of the fusion of the urethral folds in the midline, from the posterior edge of the urogenital orifice towards the tip of the phallus, forming a median raphe over the resulting tubular urethra. As the fusion proceeds from behind forwards, the primitive urogenital orifice is gradually reduced in size and carried forward on to the under surface of the phallus, where it lies below the urethral plate. The glans penis is now defined by the development of a circular coronary sulcus around the distal part of the phallus. Since the urethral groove and folds do not extend beyond the sulcus, a closed penile urethra is formed upon the completed union of the folds.

FIG. 4.—Karyotypes of group VII (21, 22, and Y) chromosomes from A, propositus (43 years); B, his unmarried brother (46 years); C, youngest son (5 years); N₁, normal male (4 years); N₂, normal male (30 years); Ph₁, male (16 years) with chronic myeloid leukaemia showing the Ph chromosome.

A cord of ectodermal epithelium invades through the glans to reach the distal end of the closed urethral tube, and this becomes canalized later to form the glandular portion of the urethra.

If the process of fusion of the urethral folds does not extend the whole length of the phallus hypospadias results—in which the urethral orifice is on the ventral side of the phallus.

At first the glans penis is naked, but at about the 70-mm. stage (fourth month) a proliferation of the ectoderm, the prepuce, comes to overlie it and will eventually cover it completely. This proliferation is adherent by epithelial fusion of the opposed layers until late in foetal life. In the milder forms of hypospadias—the juxtaglandular type—the prepuce will represent a cast of the underlying glans penis, so that the ventral position of the urethra will give the prepuce a characteristic “hooded” appearance. In typical cases no prepuce is present on the ventral side.

In the mildest form of hypospadias one is faced with two possible mechanisms for the abnormality: (1) initial failure of completion of the terminal end of the urethra, (2) a failure of the ectodermal ingrowth through the glans penis directly or an upset in the timing of this ingrowth.

Rahbek Sørensen (1953) found that a classification or a grouping of the various forms of hypospadias had little or no significance. "In the external male genital, there seems to be a completely even morphological transition between normal genitals and the most severe forms of hypospadias. There is no reason, therefore, to interpret the various forms as different lesions." Both severe and light forms may occur in the same family and in brothers. Sørensen also considers slight forms such as "short urethra" and "low-seated orifice" to belong to the condition of hypospadias, although information about the variations in this respect in the general population seems to be lacking. He concludes that the abnormality is inherited as a recessive with irregular manifestation.

Sørensen's study contains, apart from isolated cases, 31 pedigrees where more than one member is affected; and, of these, 10 could support the view of Y-linked inheritance with complete expression, nine pedigrees showed partial expression, but 12 pedigrees directly contradict Y-linkage—that is, show passage through a mother.

Kemp (1940), in his summary of recorded cases up to 1909, finds that hypospadias occurred 11 times in two brothers, seven times in three brothers, twice in four brothers, and once in five brothers; nine times in father and son, and three times in father, son, and grandson. It was recorded twice in five generations, and once in six.

It may also be mentioned that hypospadias has been observed in connexion with the Klinefelter syndrome (Gray, 1961).

Vaharu *et al.* (1961) have described a case of enlarged phallus in a 4½-year-old girl. She had gonadal dysgenesis and partly fused labia ; 45 chromosomes plus a fragment were found in cells from blood cultures. Conen *et al.* (1961) reported a mosaic XO/XY with a deleted Y chromosome in the XY cells. The latter case does not serve to illustrate the genetic effect of a deletion because of the coexisting mosaic condition.

It would appear that the deletion of the Y chromosome in our cases is the cause of the hypospadias—thought to be a slight form of intersexuality, leading to a 75% manifestation of the hypospadias, but not sufficient to impair the fertility of the propositus. It

is also possible, of course, that the deletion carried by the propositus is slightly greater than that of the brother's Y chromosome, the difference not being big enough to be detected under the microscope. Thus the hypospadias may in that case show complete manifestation. The case reported by Vaharu *et al.* suggests that the fragment present is a severely deleted Y chromosome. This deletion seems to involve both arms of the Y chromosome, leaving only the proximal part of the large arm plus the centromere. The effect of this piece is to masculinize a basically Turner condition.

The various deletions of the Y chromosome give phenotypes which are intermediate between the fertile male and the Turner female (Fig. 5). Addition or loss of whole sex chromosomes shows the same relationship of phenotypes (Fig. 6). An increase in Y chromatin might be expected to cause excessive masculinization, although in the balanced condition of the sex chromo-

FIG. 5.—Spatial relationship between the phenotypes produced by progressive deletions of the X and Y chromosomes. Top right shows the two possible isochromosomes derived from the X chromosome—i.e., the double short arm and the double long arm. The former is not yet demonstrated. 1, Muldal and Ockey (see text). 2, Vaharu *et al.* (1961). 3, Fraccaro *et al.* (1960). 4, Jacobs *et al.* (1960).

FIG. 6.—Spatial relationship between the phenotypes produced by numerical variation of whole intact sex chromosomes. Bar indicates mosaicism.

somes the XXYY (Muldal and Ockey, 1960) seems to have no such effect. The XXY and the XXYY Klinefelters are clinically indistinguishable from each other at present.

The genetic strength of the Y chromosome can hardly be questioned, since in the extreme Klinefelters with more than two X chromosomes, either as a single modal number or in chimeric conditions, the Y chromosome is always capable of manifesting itself to the extent of making these individuals phenotypically males, however sterile they may be. If this point is considered in terms of a polygenic system it might even be predicted that a greater material loss of Y chromatin would still swing the scales to masculinity.

The reduced size of the Y chromosome may be expected to have the effect of lowering the androgen level in the carriers—which ought to cause a reduced anabolism and in the long run bring about a condition somewhat similar to muscular dystrophy. It seems strange that two such characters should flow together in the same family, and one cannot help thinking that one must aggravate the other.

On the basis of the evidence it would appear that the male-determining genes are situated close to the centromere of the Y chromosome. Alternatively, one might envisage a system of pleiotropic masculinizing polygenes located more or less randomly along the whole length of the Y chromosome. At the moment we do not have any certain evidence of Mendelian genes on the Y chromosome (due to the lack of crossing-over between X and Y), but there are possibly genes controlling dimorphism in growth rate between the sexes, genes controlling the degree of infiltration into the embryonic gonads of primordial germ cells, genes controlling the activity of embryonic testes, and possibly genes controlling "hairy ears"—a trait which appears to be Y-linked (Gates, 1946).

The study of the deletions in the Y chromosome may enable us to localize some of these genes or, alternatively, provide evidence of a polygenic system.

Summary

A deletion of the Y chromosome has been found in a family where it occurs associated with muscular dystrophy and hypospadias. Although the evidence with regard to the muscular dystrophy does not suggest a connexion with the deleted Y, it seems most likely that an association exists between the aberrant chromosome and hypospadias.

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RED CELLS OF ALL FOUR ABO GROUPS IN A CASE OF LEUKAEMIA

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A number of cases have now been described showing that certain leukaemic patients develop changes in the A antigen during the course of their disease (van Loghem *et al.*, 1957; Stratton *et al.*, 1958; Gold *et al.*, 1959; Salmon *et al.*, 1959; Salmon and Bernard, 1960; Hoogstraten *et al.*, 1961). The change observed is that the red cells become relatively inagglutinable by anti-A blood-grouping sera. When the patients' cells have been tested initially their blood group has often been difficult to determine because little or no agglutination may occur with anti-A sera. Commonly there has been only a partial (or mixed field) agglutination—that is, both agglutinates and free cells were present though the proportion of each differed from case to case. Examination of the bone-marrow cells has yielded similar results (van Loghem *et al.*, 1957; Stratton *et al.*, 1958).

In some cases records were available of blood-grouping tests carried out prior to the onset of the disease, and the patients were found to be normal group A in these tests (van Loghem *et al.*, 1957; Stratton *et al.*, 1958; Hoogstraten *et al.*, 1961). The suggestion that their A antigens were originally normal was supported by the family study in our previous case (Stratton *et al.*, 1958), which showed that the patient was genetically A₁, and by two other cases in which the family study was suggestive of the presence of a normal A gene (Gold *et al.*, 1959; Salmon *et al.*, 1959). In many of the cases described the change was confined to the A antigen, and the antigens of the other blood group systems appeared to be normal, though changes have been found in the H and Le^a antigens (Tovey, 1959), in the B, H, M, N, and D antigens (Májský and Brabec, 1961), and in the D and E antigens (Tovey *et al.*, 1961).

Although serologically the changed red cells which these leukaemic patients possess when they present themselves for examination are difficult to classify, nevertheless it is clear from these cases that there has been a change in the A antigen on the patients' erythrocytes during the time they were suffering from leukaemia.

Gold *et al.* (1959) showed that there was a partial reversion towards normal in the patient's red cells during a temporary clinical remission. In another case also the patient's cells reverted to normal A₁, but this was in the terminal stage of the disease (Hoogstraten *et al.*, 1961).

The significance and cause of these changes in the A antigen has not been clear, and we thought when we studied our first case that an enzyme produced by the leukaemic white cells might be affecting the A antigen on the red cells, although attempts to reproduce the phenomenon *in vitro* were unsuccessful. In the present case, therefore, the object of the study was to try to