Brief Report

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Steroid sulfatase activity in cultured fibroblasts of XX males

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More than 100 cases of phenotypic males with a 46, XX chromosome constitution have been reported since the first description in 1964 (de la Chapelle et al., 1964; Court Brown et al., 1964; Therkelsen, 1964). Clinically, these cases resemble Klinefelter's syndrome patients except for their low-normal body size. The etiology of this syndrome has been the subject of much speculation. In a 1972 review article, de la Chapelle discussed three main etiologic mechanisms: that a mutant autosomal gene causes XX zygotes to develop into phenotypic males; that these males are derived from 46, XY or 47, XXY zygotes by means of mitotic nondisjunction in early embryogenesis and subsequent loss in the majority of tissues of cells with karyotypes other than 46, XX; and

that most XX males result from meiotic reciprocal translocations between the Y and the X (X/Y interchange), so that parts of the Yp are translocated onto the Xp (where-Supported in part by DFG grant Wie 458/2 to

T.F. Wienker and H.H.R. C.R.M. is a postdoctoral fellow supported by DFG grant Wo 148/18. as Xp material is lost to the Y). There is now at least circumstantial evidence for any of these three models. For example, as summarized by Miro et al. (1978), 46, XX/ 47, XXY mosaicism has been observed in about 17% of the patients described so far.

The autosomal mutation theory is supported by the existence of animal models, such as the "polled" gene in Saanen goats (Soller et al., 1969) and the Sxr mutation in mice (Cattanach et al., 1971). Evidence in humans is presented by reports of familial occurrence of the 46, XX male syndrome (Kasdan et al., 1973; Armendares et al., 1975; de la Chapelle et al., 1977). Indeed, these data could indicate that in some 46, XX individuals an autosomal masculinizing gene is responsible for the development of phenotypic maleness. The possibility is not, however, excluded that this hypothetical gene predisposes for mitotic errors occurring early in postzygotic development, thereby resulting in mosaicism of the sex chromosomes. Such a gene has been defined recently in BALB/c Wt mice (Eicher et al., 1980).

There are several reports of abnormal X chromosomes in XX males which could have originated by XY translocation (Madan and

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Walker, 1974; Madan, 1976; Wachtel et al., 1976; Bernstein et al., 1978). Recently, Evans et al. (1979) observed that the two X chromosomes were morphologically different in 8 of 12 46, XX males, but de la Chapelle et al. (1979) could not confirm these results in five additional cases, although two of these were closely, and one distantly, related. In summary, these data point to genetic heterogeneity in the XX male syndrome.

As outlined by de la Chapelle (1972), the distribution of Xg phenotypes and the incidence of color-vision defects in XX males are potentially informative with regard to the etiology of this syndrome. Recent data on 76 XX males kindly provided by Dr. Ruth Sanger (personal communication, 1980) show that the observed distribution of Xg phenotypes in XX males is not different from that expected in normal 46, XY males, but significantly so for females and Klinefelter's syndrome patients (the respective χ^2 values are 0.49, 26.9, and 13.2). This result can be interpreted by assuming either (1) that both X chromosomes are genetically identical or (2) that in most XX males there is only one functional Xg locus.

Roughly 8% of the Caucasian male population have X-chromosomally inherited defects of color vision, whereas in females, the proportion of color-blindness is only 0.4%. So far, color vision has been tested in 38 XX males, and in all of them it was normal (de la Chapelle, personal communication to R. Sanger, 1980). This distribution is significantly different from that expected for males ($P < 0.05$) and suggests that XX males have two nonidentical copies of the color-vision gene cluster.

Taken together, the data derived from Xg and color-vision testing suggest that in most XX males, a portion of one X chromosome including the Xg locus is missing (a formal alternative would be selective silencing of the Xg locus on one X chromosome). These findings can be easily reconciled with (and strongly argue for) the XY translocation hypothesis. Thus in the majority of cases, the Xg gene may be included in the portion of Xp that is translocated to the Y.

Recently, in collaboration with our group, Tiepolo et al. (1980) have assigned a gene controlling the expression of steroid sulfatase (STS) activity to the same chromosomal region on the tip of the short arm (Xp223). Because this locus is not inactivated (Mohandas et al., 1979; Müller et al., 1980a), it is theoretically possible to infer the number of functional STS gene copies from intracellular STS levels. Such gene-dosage differences have indeed been detected in cultured fibroblasts of males and females (Müller et al., 1980b). Thus, STS determinations in XX males offer a potential opportunity to substantiate the XY translocation hypothesis. In this context we have determined STS activities in cultured fibroblasts of nine XX males. None of these had cytogenetically detectable XY or XXY mosaicism in blood and/or fibroblasts. Three further cases are included in this series in which gonad histology revealed the presence of testicular and ovarian tissue. All patients were referred to our institute for determination of H-Y antigen and were consistently H-Y positive. The results of the STS determinations are illustrated in fig. 1. In the XX males and normal females, mean STS activities are almost identical and clearly higher than in male controls, although there is some individual overlap. While the standard deviation is nearly identical for females and XX males, the range is consid-

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2000γ

X

• 1500-

46XX♀ 46XY♂ 46XXc/
normal controls patients

Fig. 1. Steroid sulfatase activity in cultured fibroblasts of XX males and normal male and female controls. Mean $\pm$ sd for XX males: 1105 ± 77 pmoles/mg xh ($n = 12$; true hermaphrodites are marked by an arrow); normal females: 110 ± 74 ($n = 9$); normal males: 775 ± 35 ($n = 9$). Details of STS measurements and cell culture have been described previously (Hameister et al., 1979). erably wider in the latter group. Although this may, in part, reflect true genetic heterogeneity, at least some of the variation found in patients is probably due to differential expression of the *STS* gene in cells from different biopsy sites. This assumption is based on the observation that in fibroblasts of inguinal origin, STS levels are lower than in cells from nongenital sites (Migl, Müller, and Ropers, unpublished). In controls, all cell cultures were established from forearm biopsy material, whereas for most patients, the biopsy site is not known to us (and presumably varies).

Thus, with a few possible exceptions, XX males appear to have a double dose of the *STS* gene. This result is puzzling since the *STS* gene is localized very distally on the short arm of the X, in the close vicinity of the $\frac{7}{8}$ locus. Unlike *Xg*, however, the *STS* locus does not seem to be affected by the postulated meiotic X-Y interchange. Provided that most XX males originate by reciprocal XY translocations, as suggested by the male distribution of *Xg* phenotypes, one has to conclude from our results that the *Xg* gene is located more distally on Xp than the *STS* gene. In fact, linkage data indicate that the genetic distance of these loci is about 11 cM (Adam et al., 1966), whereas cytogenetic evidence argues for localization at almost identical chromosome sites on the telomere of Xp. These data are not really conflicting, because it is known that compared to the cytogenetic map, the linkage map of telomeric regions appears extended. For the long arm of the X, this point is well documented by two other loci, *G6PD* and *HPRT*, both are located near the end of Xq (for review, see Miller et al., 1978), although linkage studies suggest that they are about 35 cM apart (Francke et al., 1974).

Recently, it has been postulated that a gene involved in the control of the male-specific antigen H-Y is located near the end of Xp (Wolf et al., 1980), while another H-Y controlling gene is on the short arm of the Y chromosome (Wolf, 1978). It may well be that these two regulating genes are homologous. Further circumstantial evidence for homology of parts of Xp and Yp is provided by recent data on the expression of a new polymorphic red blood cell antigen

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which can be detected with a specific monoclonal antibody (Goodfellow and Tippett, 1980). It appears that the expression of this antigen is controlled by the *Xg* locus and, possibly, by a corresponding gene on the Y chromosome, "*Yg*". Hence, a small portion of Xp and Yp, i.e., presumably the one that pairs during meiosis, may be genetically homologous.

It is clear that, in general, chromosomal exchange in this region must be prohibited, but the assumption seems reasonable that on the rare occasions when it does take place, meiotic interchange tends to involve homologous segments rather than generating unequal rearrangements. If this is the case, then the fact that irregular inheritance of *Xg* has been observed in otherwise inconspicuous probands (Sanger et al., 1968) suggests that *Xg* (and the hypothetical *Yg*) are located nearer to the respective telomers than the H-Y controlling genes on the X and the Y. In these cases, the interchange must have included *Xg*, but not the H-Y controlling loci. Abnormal inheritance of *Xg* seems to be a regular feature in XX males. In 9 of 12 informative

matings compiled by Evans et al. (1979), the paternal *Xg(a)* gene was not expressed in (and probably not transmitted to) the XX male offspring. Since XX males are genetically heterogeneous, as discussed above, expression of the paternal *Xg* allele in three other cases may indicate that here, phenotypic maleness resulted from factors other than X-Y interchange.

A final point to be considered is the known close linkage between *Xg* and the chronic granulomatous disease (*CGD*) locus. Until now, only two recombinations between both loci are known (Marsh, personal communication, 1980; Sanger and Tippett, personal communication, 1980) among more than 20 informative cases. These data suggest a genetic distance of less than 10 cM (with, however, considerably wide confidence limits). Thus, there is the possibility that *Xg* is more closely linked to *CGD* than to *STS*. To further analyze this question, white blood cells of XX males could be examined with the NBT test (Ochs and Igo, 1973), because deletions of Xp including the *CGD* locus would be expected to result in detectable granulocyte mosaicism. On the other hand, recent evidence strongly argues for the *CGD* locus being proximal to the *STS* locus (Tiepolo et al., 1980). These authors described a boy in whom *STS* deficiency, but not *CGD*, resulted from a deletion of the distal part of Xp (Xp223→Xpter). Therefore, in the telomere-to-centromere orientation, the gene order on Xp may be *Xg*, the H-Y antigen controlling gene, *STS*, and *CGD*.

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