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The Y-chromosomal and autosomal testis-determining genes

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Summary

It has been conclusively shown that a majority of XX males has acquired Y-chromosome-specific DNA (Y-DNA) sequences from their father's Y chromosome. Testicular differentiation in these XX males is very likely induced by the testis-determining factor, *TDF*, normally located on Yp. The phenotypic expression of *TDF* in the presence of two X chromosomes is a male habitus, dysgenetic scrotal testes and no ambiguity of the genitalia.

Among those XX males who do not have Y-DNA detectable by presently used methods, some, but not all, have ambiguity of the genitalia with or without hypospadias. XX true hermaphrodites are characterized by the presence of both testicular and ovarian tissue and have ambiguous genitalia. They do not have Y-DNA.

Several instances of familial XX maleness are critically analysed. In these pedigrees, most of the affected individuals are true hermaphrodites or XX males with ambiguous genitalia; in at least one family no Y-DNA occurred in these individuals. Typical autosomal dominant inheritance of XX testicular differentiation occurs in informative pedigrees. The suggested conclusion is that an autosomal dominant testis-determining factor, *TDFA*, exists. *TDFA* shows somewhat variable expression in XX individuals often causing genital ambiguity or true hermaphrodism. *TDFA* has no phenotypic effect on XY individuals. It is argued that XX males without presently detectable Y-DNA are caused either by *TDF* or *TDFA*.

Key words: Y chromosome, X chromosome, autosome, sex chromosome, sex determination, testis-determining factor, DNA probe, gene map, genital ambiguity, hypospadias, XX male, true hermaphrodite.

Introduction

While the existence of a testis-determining factor (*TDF*) on the Y chromosome is now accepted, virtually nothing is known about its nature or mode of functioning. Several papers in this volume deal with the mapping of *TDF* on the Y chromosome. In the present paper, some facts and hypotheses related to *TDF* will be presented and reviewed. It will be shown that an autosomal dominant gene mutation with somewhat different phenotypic effects also can cause testicular differentiation in XX individuals.

The testis-determining factor on the Y chromosome, *TDF*

Much of our knowledge regarding the gene map of Yp and *TDF* has come from the study of XX males. There is now sufficient evidence to show conclusively that most XX males carry a portion of Y-specific DNA in their genomes, Y(+) XX males. As shown by Guellaen *et al.* (1984), Vergnaud *et al.* (1986) and others referred to in this volume, it is highly likely that *TDF* is among the Y-chromosome-specific sequences acquired by such males. On the other hand, in some XX males and several XX true hermaphrodites, no Y-chromosome-specific sequences have been detected, Y(−) XX testis determination. In the following, we shall examine the proportions between Y(+) and Y(−) individuals.

XX males and hermaphrodites with and without Y DNA

Guellaen *et al.* (1984) reported on three Y(+) XX males and one Y(−) XX male. When studied with a more comprehensive battery of Y-chromosome-specific DNA probes (Vergnaud *et al.* 1986), the latter patient still remained Y(−). Including the four patients reported by Guellaen *et al.* (1984), we published extensive studies on one XX hermaphrodite and 20 XX males (Vergnaud *et al.* 1986). It should be noted that patient LGL208 was erroneously described

Table 1. *Presence and absence of Y-chromosome-specific DNA in five series of XX males and XX true hermaphrodites tested with different sets of cloned probes*

Source	No of XX males		No of true hermaphrodites	
	Y(+)	Y(−)	Y(+)	Y(−)
Vergnaud *et al.* (1986)*	13	7		1
D Page & A. de la Chapelle (unpublished)	1			1
Affara *et al* (1986)	12	2		
Muller *et al.* (1986)	9	2		2†
Seboun *et al.* (1986)	24	16		
Total	59	27	0	4

* See text for explanations

† One of these patients was reported as weakly positive with a probe recognizing repetitive sequences; chimaerism was suggested by the authors.

as a true hermaphrodite; he is in fact an XX male with hypospadias described in detail earlier (de la Chapelle *et al.* 1971, case 2). Moreover, in patient LGL203 in whom no Y-DNA was demonstrated, later studies have disclosed the presence of a newly detected Y-chromosome-specific DNA sequence localized in interval 1 on the short arm of Y (D. Page & A. de la Chapelle, unpublished data). With these changes, the series presented by Vergnaud *et al.* (1986) comprises one XX true hermaphrodite and seven XX males who were Y(−), while thirteen XX males were Y(+). Similar data emerge from the series studied by Affara *et al.* (1986), Müller *et al.* (1986) and Seboun *et al.* (1986) who used other probes to study the same question in different patients. These and other recent data summarized in Table 1 indicate that all of the XX true hermaphrodites reported are Y(−), while in XX males 27 out of 86 (31%) are Y(−). The higher proportion of Y(−) patients in the study of Seboun *et al.* (1986) is probably exceptional due to several factors, such as differences between the probes and the modes of ascertainment of the patients.

Hypotheses to explain Y(−) XX males

Two main hypotheses will be considered:

(1) Y(−) XX males have *TDF* on a smaller piece of Y-DNA than Y(+) XX males so that it has so far escaped detection.

(2) Testicular determination can also occur by mechanisms other than through the action of *TDF* and so Y(−) XX males have no Y-specific DNA at all.

It is possible to find evidence pertaining to both hypotheses.

(1) This hypothesis predicts that as more and more Y-chromosome-specific DNA probes are detected and applied to Y(−) XX males, some should turn Y(+). This has already happened as indicated above for XX male LGL203. As probes closer to *TDF* become available, more Y(−) XX males will probably turn positive.

(2) Of the principal mechanisms that might account for testis determination in the apparent absence of *TDF*, two should be mentioned.

First, mosaicism involving a cell line with a Y chromosome or Y-chromosomal DNA may occur and trigger testis determination, but may not be detectable if it is extremely tissue-limited, circumscribed or even eliminated altogether during the development of the individual. This hypothesis is difficult to test, but sporadic cytogenetic observations (reviewed by de la Chapelle, 1981) have not provided solid evidence in its favour, and neither has molecular evidence in its favour been produced.

Second, an autosomal or X-chromosomal gene mutation might turn a gene with other functions into a testis-determining one. This hypothesis predicts that familial cases should occur. Another prediction is that the clinical features resulting from a gene mutation might be different from those arising from the acquisition of *TDF*. In order to examine the evidence regarding what effects *TDF* has on the sex phenotype, it is necessary to make some clinical distinctions.

Definitions of XX maleness

For the purpose of this paper, maleness or the presence of testicular tissue in the presence of an XX karyotype is divided into three types:

(1) *XX males* are individuals with a male habitus and gender, scrotal gonads histologically verified as testes, and male external and internal genitalia without ambiguity. If this definition is literally followed, an individual can only be classified as an XX male if both gonads are surgically exposed, biopsied and found to be of testicular histology. However, in practice such biopsies are neither feasible nor advisable, so the macroscopic and microscopic evidence is usually not available.

(2) *XX males with ambiguous genitalia and/or hypospadias* are individuals with a male habitus and gender, gonads in which testicular but not ovarian tissue is found, but whose external or internal genitalia show some degree of ambiguity, often just hypospadias.

(3) *XX true hermaphrodites* are individuals with male or female habitus and gender, gonads containing both testicular and ovarian tissue, and usually ambiguity of the genitalia (van Niekerk & Retief,

1981). The diagnosis of true hermaphrodism requires gonadal biopsies.

Familial XX maleness caused by TDF

Most XX males are sporadic, but familial cases occur. In a previous paper, the families presented by Nicolis *et al.* (1972), Kasdan *et al.* (1973), Minowada *et al.* (1979), and de la Chapelle *et al.* (1977, 1978) were reviewed. Molecular studies have been reported only in the latter family (Page, de la Chapelle & Weissenbach, 1985). The two XX males who are second cousins are Y(+) and possess identical portions of Y-DNA. Since the patients are related through their fathers who are cousins, and the fathers' fathers, who were brothers, the two XX males do not share a common ancestral X chromosome so X-linked inheritance of the Y-specific DNA is excluded. It follows that, provided that the Y-DNA they possess is localized on the paternal X as predicted by the Y–X interchange seen in many XX males including the more distantly related third affected XX male in the pedigree (Andersson, Page & de la Chapelle, 1986) then a *de novo* identical interchange must have occurred in both fathers. This has been tentatively explained to be the result of a structural peculiarity on the molecular level affecting the Y chromosome in this family (de la Chapelle, 1986). Alternatively, it could be due to a rare gene predisposing to Y–X exchange. In any event, the maleness in these two second cousins is very likely due to the presence of *TDF* in their genomes so it should not be explained by a gene mutation. However, as argued below, this family may well be unique because, in all other families described, the XX males had ambiguous genitalia, or hypospadias, or occurred together with one or several true hermaphrodites.

The autosomal testis determining factor, *TDFA*

Familial XX maleness with genital ambiguity; familial true hermaphrodism

A number of families is on record in which XX males or XX true hermaphrodites occur (Fig. 1). In some families, all affected individuals have the same phenotype, such as in A, D and E (only true hermaphrodites) or F (two XX males with hypospadias). In other families, XX true hermaphrodites and XX males coexist. It is notable that of the 21 affected individuals that occur in these pedigrees, only two are XX males, while six are XX males with genital

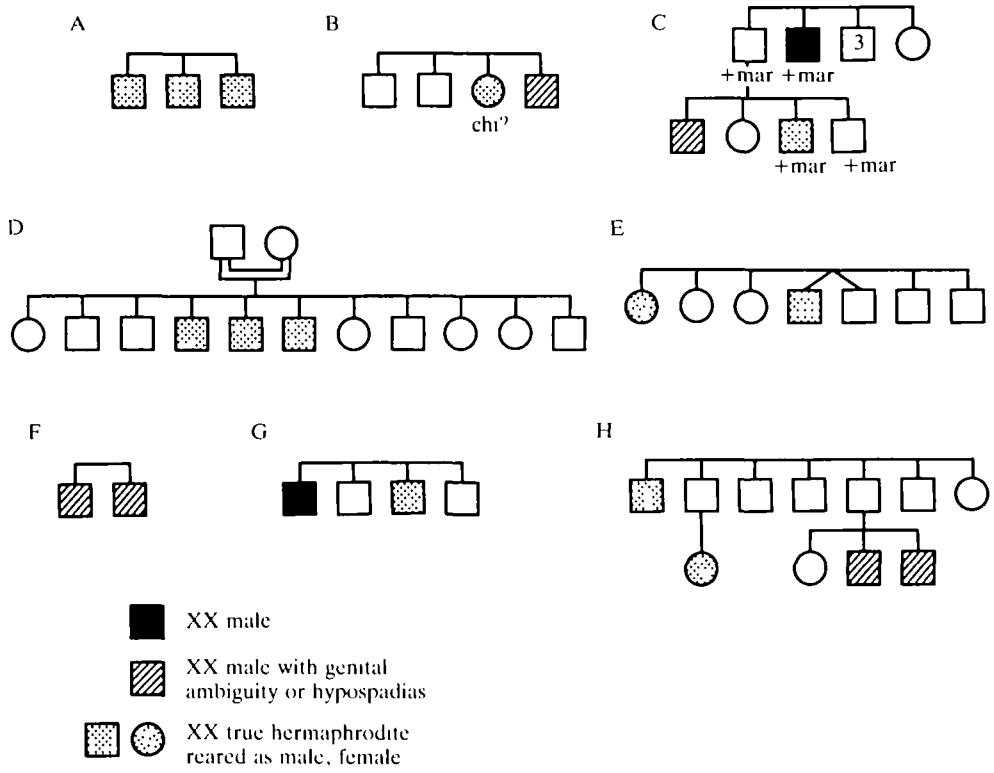

Fig. 1. Simplified pedigrees of families with more than one XX male or XX true hermaphrodite. (A) Clayton, Smith & Rosenberg (1958); Rosenberg, Clayton & Hsu (1963); (B) Berger *et al.* (1970); (C) Kasdan *et al.* (1973); (D) Armendares *et al.* (1975); (E) Fraccaro *et al.* (1979); (F) Minowada *et al.* (1979); (G) Vergnaud *et al.* (1986); Petit *et al.* (1987); (H) Skordis *et al.* (1987). Explanations: +mar indicates the presence of a supernumerary small marker chromosome of unidentified origin. Chi? indicates that the patient had 134 cells with the karyotype 46,XX and 2 cells with the karyotype 46,XY; the authors suggested possible chimerism.

Table 2. *Phenotypes of 21 affected individuals belonging to 8 pedigrees shown in Fig. 1*

	No
XX males	2*
XX males with genital ambiguity or hypospadias	6
XX true hermaphrodites	13

* If the pedigree described by de la Chapelle *et al.* (1977, 1978) with 3 familial XX individuals is added, the number of XX males is 5. See text for explanations.

ambiguity or hypospadias and 13 are XX true hermaphrodites (Table 2).

Molecular studies have so far been reported in only one of these families (family G). Notably, both affected individuals were Y(−) (Vergnaud *et al.* 1986). This result, especially if confirmed in other families (cf. Seboun *et al.* 1986), suggests that maleness or the presence of testicular tissue is the result of a mutation rather than the presence of *TDF* in these familial cases. The fact that all sporadic true hermaphrodites listed in Table 1 and so far tested with molecular probes are Y(−) strengthens the same argument in that true hermaphrodites are predominant in familial cases (Table 2). It is also notable that of the eight familial XX males reviewed here, only two did not have genital ambiguity or hypospadias.

Mode of inheritance

Most of the pedigrees are compatible with both dominant and recessive inheritance. Indeed, the parents of three XX true hermaphrodites shown in pedigree D are first cousins (Armendares *et al.* 1975) which is compatible with, or even mildly suggestive of, recessive inheritance. However, two of the families show informative segregation as to mode of inheritance. In family C, the putative gene was transmitted to two affected XX individuals by their normal father, because the father's brother was also affected. This suggests dominant inheritance. Unfortunately, the presence of a minute marker chromosome in (both affected and unaffected) members of the family somewhat clouds the interpretation. Family H is most informative. Here the putative gene occurs in three members of the older generation; one is an XX true hermaphrodite, while two are normal XY males who have transmitted it to one and two, of their offspring, respectively. There is little doubt about the dominant mode of inheritance in this family. Moreover, X chromosomal inheritance is excluded, because the sister of the two XX males is healthy even though she must have received the same X from her father as her two affected sibs. If the penetrance of the trait is complete, then the three members of the older generation must have received the putative gene from their father, who also has a normal daughter providing additional evidence against X-linkage. Notably, the gene might still be pseudoautosomal (Burgoyne, 1982).

Thus, available evidence suggests that an autosomal dominant mutation (referred to here as *TDFA*) may be responsible for testicular determination in at least some cases. Its penetrance cannot be determined at present; its expression is variable, in that both XX males, XX males with genital ambiguity, and true XX hermaphrodites may result. The variable expression in pedigree H might well be explained on the basis of modifying genes. *TDFA* appears to have no phenotypic effect on XY individuals. Finally, recessive or X-linked inheritance cannot be excluded in several families.

As an alternative explanation, Y;autosome translocation has often been suggested. It has recently become known that 45,X males can show translocation of Y-specific DNA to an autosome (Maserati *et al.* 1986; Disteche *et al.* 1986). Such a translocation can be excluded in familial and sporadic XX maleness only if there is no Y-DNA. So far the observations in this regard are confined to family G (Vergnaud *et al.* 1986) in whose affected individuals no Y-DNA was found.

Testis determination in Y(−) XX males

As shown above, the existence of an autosomal dominant mutation causing testicular differentiation (*TDFA*) is strongly suggested by the family data. This predicts that a proportion of sporadic XX males should also arise through the same mechanism. The data in Table 1 show that with presently available Y-DNA probes some 31 % of XX males are Y(−). As shown above, some of these Y(−) XX males may turn Y(+) when studied by probes very close to *TDF*. If other Y(−) XX males have *TDFA*, then in analogy with the phenotypic features of familial XX maleness, (Table 2) they should comprise a higher proportion of individuals with genital ambiguity or hypospadias. Unfortunately, the clinical features of many of the XX males presented in the papers quoted in Table 1 have been so poorly described that an evaluation is difficult. Restricting the analysis only to patients studied by the present author, the count is as follows: Of a total of fifteen XX males, thirteen are Y(+); none of these have genital ambiguity. Of two Y(−) XX males studied (Vergnaud *et al.* 1986), one (LGL200) has no genital ambiguity while the other (LGL208) had hypospadias and a bifid scrotum. Strong support for a high proportion of genital ambiguity among Y(−) XX males is briefly provided by Seboun *et al.* (1986) who stated that eight of sixteen Y(−) XX males had ambiguous genitalia. The

data published by Guellaen *et al.* (1984) show the same tendency, in that three Y(+) patients were regular XX males while the fourth, who was Y(−), displayed 'the most severe hypogonadism' even though the presence or absence of genital ambiguity was not stated.

These data are too fragmentary to allow firm conclusions to be drawn; however, further studies may well disclose a consistent phenotypic difference between Y(+) XX males and at least some Y(−) XX males. This would argue in favour of *TDF* as a causative factor in the former and *TDFA* in some of the latter.

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