

# Reifenstein's syndrome: Investigation of linkage to X-chromosomal loci

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Xg<sup>a</sup> linkage data on a large family with Reifenstein's syndrome is reported. The pedigree was analyzed directly and the data were combined with that from the other linkage study in the literature to allow a numerical estimate of the likelihood of the recombination fraction, based on all the linkage data available. It was concluded that there is not close linkage between Xg<sup>a</sup> and Reifenstein's syndrome. A suggestion was, however, found of tight linkage between the genes for color blindness and Reifenstein's syndrome, but this result was not definite because of the small amount of data available.

*Received 13 March, accepted for publication 25 April 1974*

Reifenstein (1947) originally described a syndrome of hereditary male pseudohermaphroditism with hypogonadism, hypospadias and gynecomastia. Similar families have been reported by others (Peters et al. 1955, Bowen et al. 1965).

Patients with Reifenstein's syndrome are noted at birth to have some degree of pseudohermaphroditism, ranging from simple hypospadias to a small phallus with a perineal urethral opening and a cleft scrotum. Buccal smear and chromosome studies exhibit a normal male pattern. At puberty, these patients develop gynecomastia, are noted to have small testes and do not develop male secondary sex characteristics to a normal degree. Azoospermia is a constant finding and testicular histology after puberty reveals severe impairment of spermatogenesis with tubular hyalinization, but preservation of the Leydig cells. Urinary gonadotropin titers are normal to elevated, urinary testosterone glucuronide titers are normal

to low. The severity of the syndrome varies from family to family, but is relatively constant within an affected family.

The mechanism of inheritance of this syndrome is unclear. The pedigrees of the reported families are consistent with X-linked recessive or male-limited autosomal dominant inheritance. Since the affected members are infertile, this uncertainty cannot be resolved from examination of the pedigree. Linkage studies with genes known to be X-linked, such as colorblindness and blood group antigen Xg<sup>a</sup>, provide one possible way of resolving this question. Bowen et al. (1965) have used this technique on two families with Reifenstein's syndrome. Investigating the genetic linkage of Xg<sup>a</sup> and Reifenstein's syndrome, they found a minimum of three crossovers in six opportunities in the informative family and interpreted this as evidence against close linkage. We report here the second large family with Reifenstein's syndrome in which an

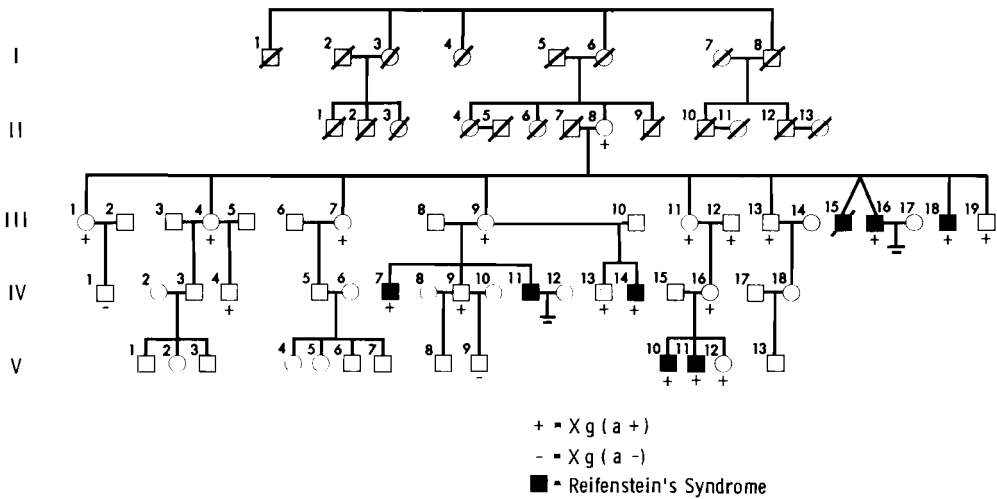


Fig. 1. Family pedigree

Table 1

Clinical data for affected family members

	Hypospadias	Gynecomastia	Buccal smear	Testicular biopsy
III-15	+	pre-pubertal death	0	0
III-16	+	+	negative	done
III-18	+	+	negative	done
IV-7	+	+	negative	done
IV-11	+	+	0	0
IV-14	+	+	0	0
V-10	+	pre-pubertal	0	0
V-11	+	pre-pubertal	0	0

+ = present 0 = not done

erations and includes eight affected members, seven of whom are alive. The manifestations of the syndrome (Table 1) are typical. Hypospadias was a consistent finding. Buccal smears in three affected males were chromatin negative. Testicular histology in three members was similar to that of previously reported cases. Gynecomastia occurring at puberty and infertility characterized all affected members. In the immediate post-pubertal period, affected members reported normal libido and potentia, and two are married. However, by approximately 10 years post-pubertally, androgen therapy seemed to be required to maintain this adjustment.

Xg<sup>a</sup> linkage study has been carried out. In addition, we have analyzed our data in conjunction with those of Bowen et al. (1965) to obtain numerical estimates of the linkage of Xg<sup>a</sup> and of colorblindness with Reifenstein's syndrome.

Case Report

This family (Fig. 1) covers four living gen-

Methods and Results

Linkage Data

Xg<sup>a</sup> blood grouping results are shown in Fig. 1. This data was analyzed in two ways. First, the traditional analysis of the pedigree was done, and segregation of the two markers was used to deduce crossovers that must have occurred if the locus for Reifenstein's syndrome were on the X chromo-

some. Second, a mathematical analysis of the present data and of those of Bowen et al. (1965) was done to give overall confidence limits to the recombination fractions based on all the families in which linkage studies have been reported.

#### *Pedigree Analysis*

Since IV-1 is  $Xg(a-)$  (Fig. 1), III-1 must carry  $Xg$ , an allele of  $Xg^a$  which has no associated antigenic activity. This, in turn, means that either II-7, or II-8 carried a similar allele. Since II-8 is known to be  $Xg(a+)$ , two possibilities for her  $Xg$  genotype exist; first, she could be homozygous  $Xg^a/Xg^a$  (making II-7 hemizygous for the "silent"  $Xg$  allele) or she could be heterozygous  $Xg^a/Xg$ .

Considering the first possibility (which is more likely since she had four  $Xg(a+)$  sons), and assuming that the locus for Reifenstein's syndrome is on the X-chromosome, III-9 is heterozygous  $Xg^a/Xg$  with the syndrome locus on the chromosome carrying the  $Xg^a$  allele. However, of her four sons who are known to be  $Xg(a+)$  ( $Xg^a$  data on IV-11 is not available), two inherited the syndrome and two did not, so there must have been two crossovers in this generation. There are no necessary crossovers between III-13 and II-19.

The second possibility is that II-8 is heterozygous  $Xg^a/Xg$ . Assuming that the locus of Reifenstein's syndrome is on the X chromosome, it could be either in repulsion or in coupling with  $Xg^a$ . If it were in repulsion, crossovers occurred at II-13, III-16, III-18, III-19, IV-7, IV-9, IV-13 and IV-14. If it were in coupling, crossovers occurred at III-13, III-19, IV-9 and IV-13. Thus, whichever possibility is assumed for the genotype of II-8, there were at least two crossovers in eight opportunities in each case.

Family members were not considered opportunities for crossover if  $Xg^a$  data were not available for them. V-10 and V-11 were

not considered opportunities for crossover since they could have inherited an X chromosome from III-12.

#### **Mathematical Analysis**

As shown in the previous section, it is clear from an inspection of the pedigrees that there cannot be close linkage between the locus for Reifenstein's syndrome and  $Xg^a$ . However, since results of a linkage investigation are best reported in terms of lod-scores (Edwards 1972), the program LIPED (Ott 1974) was used to compute the lod-scores under the assumption that Reifenstein's syndrome is an X-linked recessive trait with  $p_G=0.675$ ,  $p_R=0.01$  (gene frequencies for  $Xg^a$  and Reifenstein's syndrome, respectively). The results are listed in Table 2. Analyses with different values for  $p_R$  showed that the values of the lod-scores do not change within five decimal places when  $p_R$  is between 0.01 and zero. Since the test for homogeneity (Morton 1956) of the pedigrees with respect to the recombination fraction  $r$  is far from being significant (which may be due to its low power with these small families), the scores are summed over the single pedigrees.

The total lod-scores for Reifenstein's versus  $Xg^a$  are highest at  $r=0.5$ . The Bayesian interval estimate (using a uniform prior for  $r$  in the whole range  $0 \leq r \leq 0.5$  and cutting off 5% or 1% from the left of the area under the posterior density) turned out to be  $0.23 < r < 0.50$  (95%) and  $0.17 < r < 0.50$  (99%), respectively.

In one pedigree (Bowen's family 1), colorblindness (deuteranopia) can be shown to be segregating. The corresponding lod-scores are listed in Table 2. The gene frequency for deuteranopia was assumed to be 0.08 (Cavalli-Sforza & Bodmer 1971). The highest lod-score of 1.26 occurs at  $r=0$ , which allows for a suspicion of tight linkage. However, a score of 1.26 cannot be consid-

**Table 2**  
Lod-scores for various recombination fractions

	0	0.05	0.10	0.15	0.20	0.30	0.40
Reifenstein vs Xg <sup>a</sup>							
Present family	-∞	-1.30	-0.78	-0.51	-0.34	-0.14	-0.04
Bowen, family 1	-∞	-2.38	-1.55	-1.09	-0.79	-0.41	-0.16
Bowen, family 2	0.19	0.16	0.13	0.11	0.08	0.04	0.01
Total	-∞	-3.52	-2.20	-1.49	-1.05	-0.51	-0.19
Reifenstein vs Deutanopia							
Bowen, family 1	1.26	1.16	1.05	0.93	0.80	0.52	0.23

ered significant and more data are needed to clarify the situation.

### Discussion

The results of the present study strongly suggest that there is not close linkage between Xg<sup>a</sup> and Reifenstein's syndrome. Direct analysis of the pedigree revealed a minimum of two crossovers in eight opportunities. This is comparable to the results of Bowen et al. (1965) who found three crossovers in six opportunities. Mathematical analysis of the present data, combined with those of Bowen et al. (1965), revealed the most likely estimate for the recombination fraction for Xg<sup>a</sup> and Reifenstein's syndrome to be 0.5 with a 95 % Bayes' confidence limit of 0.23 and a 99 % Bayes' confidence limit of 0.17.

The fact that there is no demonstrable close linkage between Xg<sup>a</sup> and Reifenstein's syndrome does not, however, rule out X-linkage for Reifenstein's syndrome (Race & Sanger 1968, Giblett 1969). The locus for Reifenstein's syndrome may be located on the X-chromosome but far enough from the Xg locus that linkage was not demonstrated. Our analysis of Bowen's colorblindness data in subjects with Reifenstein's syndrome raises the possibility of tight linkage between these two loci, which

would imply X-linkage of Reifenstein's syndrome. However, there are too few affected subjects to have confidence in this association. Unfortunately, the family reported here refused testing for colorblindness.

The syndrome of testicular feminization (Naftolin & Judd 1973) is analogous to Reifenstein's syndrome in that the pedigree does not differentiate X-linked recessive from male-limited autosomal dominant inheritance, since the affected males are infertile. Testicular feminization in the mouse has been shown to be X-linked by linkage studies using coat color (Lyon & Hawkes 1970). This can be interpreted as evidence for X-linkage of testicular feminization in the human because of the strong tendency for genes that are carried on the X chromosome in one species to be carried there in other species (Ohno 1969). Studies of testicular feminization in the human have not, however, shown linkage to Xg<sup>a</sup> (Sanger et al. 1969).

### Acknowledgements

+Xg<sup>a</sup> blood grouping was done courtesy of Dr. Eloise R. Giblett at the King County Central Blood Bank.

We appreciate the assistance of Dr. George Stamatoyannopoulos of the Division of Medical Genetics, University of

Washington School of Medicine in interpretation of data, of Dr. J. Felsenstein, Department of Genetics, University of Washington, in providing helpful discussions about the mathematical aspects, and of Dr. Eloise R. Giblett of the King County Central Blood Bank for a critical review of the manuscript.

This work was supported in part by the National Institutes of Health grants HD 05105, AM 05161 and GM 15253 (Dr. Ott). Parts of the data have been presented in abstract form (*Clin. Res.* **21**, 261. 1973).

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