Sex ratio and heterozygote advantage in cystic fibrosis families

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SUMMARY The families of aunts and uncles of cystic fibrosis (CF) patients were compared with one another and with controls in order to test claims of increased fertility and sex ratio in families containing the CF allele and to identify the more fertile sex. Uncles' families were found to contain a significant excess of sons, particularly at primiparous births, as well as a significantly increased sex ratio. Reanalysis of published records of CF sibships confirmed the effect of parity on sex ratio. The families of uncles were also exceptional in exceeding the size intended, although their mean size was not raised significantly. It is suggested that these effects could be related to an interaction between CF-related, paternally-derived antigens and the maternal immune system.

Cystic fibrosis (CF) is the most common severe disease in white people to follow a recessive mode of inheritance. At least one in 2500 white babies is affected, about five times as many as with any other severe genetic disease, and about one in every 25 is a carrier.

According to the Hardy-Weinberg law, the frequency of the CF allele should be about 0.02 and it has been suggested that such a high frequency could be accounted for only if the heterozygote has some selective advantage, relative to normal homozygotes. In other words, it is considered that the CF allele and its normal homologue could constitute, or be approaching, a balanced polymorphism.^{1,2} The precise nature of the presumed heterozygote advantage is unknown, but grandparents of CF patients, ignorant of their carrier status and married mainly to normal homozygotes, have been shown to have produced significantly larger families than the grandparents of control children. In one study by Knudson *et al.*³ matings of the type $AA \times Aa$, where a is the allele for CF, had an average of 4.34 children, compared with 3.43 produced by control, $AA \times AA$ matings, a highly significant difference, corresponding to a relative selection against normal homozygotes of 0.21. The general finding of an increased family size associated with CF is supported by Danks *et al.*,⁴ who also noted a striking excess of males in the sibships of index cases.

The data suggest the basis of the heterozygote advantage may lie in the superior fertility of one sex, with respect to male offspring. In the present study,

an attempt is made to identify the more fertile sex, by comparing the composition and size of the families of aunts and uncles of CF patients, half of whom would be expected to be CF heterozygotes, with homozygous normal marriage partners. In considering Knudson's data, Stern⁵ comments: 'It is not certain . . . whether the existence of some subtle bias leading to preferential ascertainment of the more fertile grandparents does not exist, which, if excluded, would remove the difference'. It was considered that a comparison between the families of aunts and uncles should eliminate any such bias in the selection of controls, but equivalent data for unrelated families were also collected in order to identify effects on family composition which might apply to CF heterozygotes of both genders.

Methods

Parents of CF patients attending hospital clinics in Newcastle upon Tyne and West Sussex were interviewed in order to determine the structures of their families. Two copies of a questionnaire were then posted to each aunt and uncle of the patients, together with instructions to pass the second copy to a friend or neighbour. It was hoped that selection of normal controls in this way would eliminate any major biases due to social class, geography, or parental age. The questionnaire requested information about dates of birth and occupations of the parents, the numbers of their children, alive or dead, with details of their gender, and dates of birth and death. Details were also collected concerning the

numbers and dates of abortions and stillbirths, the incidence of CF in the family, what was considered to be the ideal family size before and after starting a family, and whether they had considered limiting their families because of CF, or any other disease. No indication was given of the precise purpose of the enquiry. Responses from control families were divided into two sets, depending on whether the father or mother had completed the questionnaire. This distinction was relevant to the concept of ideal family size.

Results

Completed questionnaires were received from 33 aunts and 24 uncles of CF patients and 40 control families, 10 of the last group being completed by fathers and 30 by mothers. The replies from aunts and uncles were completed in all cases by the blood relatives of the patients. Replies were received from Britain and overseas, each set of families covering a similar variety of business occupations. One exceptional reply, from an uncle, described 5 abortions and no livebirths. Since it was felt there could be additional problems, this reply was excluded.

The mean number of livebirths in the uncles' families was 2.78, that of aunts 2.19, and that of (combined) controls 2.35 (Table 1). The apparent increase in the size of families born to uncles of CF patients is not statistically significant ($P > 0.1$).

The mean number of girls, was similar in all the families. In the controls and aunts' families the mean number of boys was similar to that of girls, but in the

uncles' families there were many more boys. A χ^2 test shows this to be a highly significant departure from the 1:1 ratio ($P = 0.024$).

The significance of this difference was also tested by Student's t test. When compared with either the aunts' or control families, the mean excess of boys born to uncles came just outside the significance level ($P = 0.06$), but compared with the combined values from aunts and control families, it was significant at the 5% level of probability.

Our data therefore support those of Danks *et al.*⁴ in showing a swing in the sex ratio, due to an increase in the numbers of sons born to uncles of CF patients. Although we have not demonstrated a significant increase in total family size, our values show a similar trend.

The mean ideal family size, as considered by control women before starting their families, was similar to that of the CF aunts, (Table 2). The men in both groups gave appreciably lower figures. In the light of experience, all groups revised their estimate of the ideal family size, aunts of CF patients and control mothers and fathers, all reducing their concept to a value almost midway between their original estimate and the actual present family size. In contrast, the uncles of CF patients now consider the ideal family size to be similar to its actual size and larger than their original concept. It is uncertain to what extent the actual family size of CF uncles is a result of this revision of outlook, but CF uncles clearly do not wish to increase their families any further, whereas the other three sets of parents wish to do so.

Table 1 Comparison of families of aunts and uncles of CF patients with control families, in relation to parental age

	No of families	Livebirths										Abortions and stillbirths		Mean age at each livebirth (years)		Mean parental age differential (years)
		Both genders			Boys			Girls			Sex ratio					
		Total	Mean	SE	Total	Mean	SE	Total	Mean	SE	(Boys/girls) × 100					
											Total Mean	Of mother	Of father			
Controls	40	94	2.35	(0.132)	47	1.18	(0.130)	47	1.18	(0.132)	100	11	0.275	25.3	28.0	2.7
Aunts	32	70	2.19	(0.122)	38	1.19	(0.145)	32	1.00	(0.135)	119	8	0.242	25.5	27.7	2.2
Uncles	23	64	2.78	(0.320)	41	1.78*	(0.280)	23	1.00	(0.166)	178†	2	0.087	26.7	28.3	1.6

*Significantly raised, as assessed by Student's t test. †Significantly raised, as assessed by χ^2 analysis, $P = 0.024$.

Table 2 Parents' concepts of the ideal size for their own family in relation to the actual size

	No of families	Ideal family size (mean)			Actual family size (mean)
		Original concept	Present concept	Difference	
Controls					
Response from mothers	30	2.90	2.67	-0.23	2.47
Response from fathers	10	2.40	2.30	-0.10	2.00
Aunts of CF patients	33	2.92	2.35	-0.57	2.19
Uncles of CF patients	24	2.38	2.75	+0.37	2.78

Table 3 Livebirth sex ratio and abortions in relation to parity and parental age at first livebirth

	Sex ratio at each livebirth									Mean ages at first livebirth (years)	
	First birth			Second birth			Subsequent births			Of mother	Of father
	Boys	Girls	A	Boys	Girls	A	Boys	Girls	A		
Controls											
Numbers	18	17	4	17	17	1	11	10	3	23.8	26.4
Sex ratio		106			100			110			
Aunts											
Numbers	16	16	0	14	9	6	7	8	2	24.4	26.6
Sex ratio		100			156			88			
		P>0.05			P>0.05			P>0.05			
Uncles											
Numbers	15	5	2	12	8	0	12	9	0	24.5	26.1
Sex ratio		300*			150			133			
		P=0.025			P>0.05			P>0.05			

A=abortion. *Significantly raised, as assessed by χ^2 analysis.

The mean maternal and paternal ages and the age differentials of the parents are shown in Table 1 and are not appreciably different. The frequency of stillbirths and abortions was similar in the aunts and controls but considerably lower among the uncles' families. This difference would be negated if the exceptional family described above were included and does not account for the increased proportion of boys born to uncles.

Control families and those of the aunts showed no significant variation in sex ratio with respect to parity, but in contrast, the uncles' families had a highly significant excess of boys at the first birth, producing a sex ratio of 300 ($P=0.025$, Table 3). Later births in uncles' families also produced an excess of boys, as did the aunts' second births, but these deviations were not significant. The mean parental ages at the first births are shown in Table 3 and are similar in the three groups.

Discussion

The data reported here support the conclusion of Danks *et al.*⁴ that families born to CF heterozygous carriers contain an excess of male offspring. We also extend their deduction by demonstrating that this feature is a property of male, rather than female heterozygotes, that applies particularly to the first birth. Three tests of the data support this conclusion. (1) The increased number of boys born to uncles, especially at the first birth, compared with aunts and controls. (2) The increased sex ratio in uncles' families. (3) The discrepancy between desired and actual family size in uncles' families. Our sample size is too small to be confident of a real increase in family size, as claimed for grandparents of CF patients by Knudson *et al.*³ and previously suggested by Danks *et al.*⁴ but our values certainly

support this concept. A real increase in family size would perhaps be unexpected these days, in view of the widespread use of contraception, but it should be stressed that the uncles' families were the only ones to exceed the size originally intended.

Our information suggests that the sex ratio among the children of uncles is particularly high at the first birth. Reanalysis of Danks's records shows a positive ratio in all except the fourth birth, although it reaches statistical significance only at the first and third (Table 4). The ratio for the first three combined is 133, a very highly significant departure from equality ($P=0.0005$). For the remaining births it is still positive (112), but this deviation from equality is not significant. The overall sex ratio in the CF sibships reported by Danks' group is 128 ($P=0.0006$). Table 3 shows that in our uncles' families, in which about half the uncles should be CF heterozygotes, married to normal homozygous women, the sex ratio is 300 at the first birth. The primiparous sex ratio when all the parents, of both sexes, were CF heterozygotes (Danks's data) was considerably lower, at 130 (Table 4).

A negative relationship between birth order and sex ratio is normal, but the normal excess of males is an order of magnitude lower than that in CF families.^{6,7} Neither our data nor those of Danks suggest any explanation in parental age,⁶ sex of the preceding child,⁸⁻¹⁰ or any social or demographic factor.⁶

In considering the biological basis of the increased number of sons born to CF uncles, we need to distinguish between (a) the ratio of Y chromosome—to X chromosome-carrying sperms, (b) the 'primary sex ratio'—that is the ratio of XY to XX zygotes, and (c) the 'secondary sex ratio', or ratio of male to female livebirths.

Table 4 Summary of data on sex ratio in relation to parity, both parents CF heterozygotes

Parity	No of children	Sex ratio	Probability
1	225	130	0.05*
2	208	121	0.16
3	161	155	0.006*
4	92	88	0.52
5	52	136	0.40
6	25	150	0.30
7-10	20	150	0.36
1-10	738	128	0.0006*
1-3	594	133	0.0005*
4-10	189	112	0.4

Calculated from figures of Danks *et al.*⁴ *Significant by χ^2 analysis.

Shettles¹¹ presents evidence that in normal men the ratio of Y to X sperm may be as high as 2:1 Sex ratio at conception may also be high, values up to 160 having been reported¹¹ and estimates based on abortuses during the first 16 weeks of pregnancy came in the range 112-140.¹² Such figures are challenged by Stern,⁵ who disputes the classification of early fetuses and presents evidence for similar proportions of the sexes at all stages. One line of argument suggests an initially high proportion of Y sperms, or male fetuses, which drops steeply to equal the number of females, at some time after birth; while according to the other the ratio wavers around equality. Explanations for the increased numbers of boys in CF families can be based on overproduction, or hyperfertility of Y sperms, or on selection, or retention by the mother, of male fetuses which would normally be lost.

A significantly increased sex ratio (122) was observed in cousin marriages by Darlington¹³ which was even higher (156) in families with further consanguinity. Kirby *et al.*¹² cite this in support of their theory that Y-linked cell surface antigens are particularly significant at implantation, if mother and fetus are antigenically similar. A related observation is the increased sex ratio among blood groups O and AB, compared with A and B.¹⁴ There seems to be a subtle and complex relationship between the immune status of the mother, with respect to fetal antigens and the selection of XX, XY embryos, for implantation or early abortion.

The observation that excess boys are born to uncles, but not to aunts, of CF patients suggests it is the male carriers which exert the major effect on sex ratio in CF families, but the effect of parity also indicates the influence of maternal physiology. It is noteworthy that the sex ratio at the first birth is

markedly higher (300) if half the fathers are CF carriers and the mothers are normal (Table 3), than if both parents are carriers (130; Table 4). We suggest that the increased fertility of CF families, with respect to male offspring, could be an aspect of interaction between maternal immune system and CF-related antigens originating in the father and carried by sperm. But there remains the possibility of our unconscious selection of atypical families.

We thank the patients, their relatives and the Cystic Fibrosis Research Trust; Dr M Parkin, Dr A Robinson, Dr J M Semmens, Professor D F Roberts, Miss D Johnson and Miss S Mitchinson.

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Received 1 November 1982