Case-control study of whether subfertility in men is familial

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Abstract

***Objective*—To test the hypothesis that subfertility in men is familial and to examine the distribution of subfertility within families for consistency with a genetic cause.**

***Design*—Case-control study and segregation analysis.**

***Setting*—Two teaching hospitals in Leeds.**

***Subjects*—Cases (probands) were men with an abnormal sperm count who attended a subfertility clinic and whose partners had no major factor contravening fertility. Controls were fathers of two or more childen recruited through vasectomy clinics or a maternity department.**

***Main outcome measures*—The incidence of involuntary childlessness among brothers with partners and among sisters and second and third degree male relatives. When possible clinical and laboratory details were obtained from involuntarily childless brothers.**

***Results*—Seventeen of the 148 (11·5%) brothers of probands but none of the 169 brothers of controls had sought medical advice for childlessness ($P<0·0005$). Four probands had more than one involuntarily childless brother. There were six further brothers whose childlessness was thought to be involuntary bringing the total prevalence of subfertility among brothers of probands to 16%. Segregation analysis was consistent with an autosomal recessive mode of inheritance accounting for 60% of subfertility in men. Seventeen of the 346 (4·9%) uncles of probands and 10 of 420 (2·8%) uncles of controls were reported to be involuntarily childless ($P=0·09$), but there was no difference in childlessness among sisters. In three families sperm counts from "affected" brothers confirmed the diagnosis and showed considerable similarities within but not between families.**

***Conclusion*—Subfertility in men has a familial component, and the observations are consistent with an autosomal recessive mode of inheritance in over half the cases. Several different genes are probably involved.**

Introduction

Subfertility due to oligoasthenoteratozoospermia is common in humans,[1,2] and environmental and genetic factors have been implicated. The former include viral infections—for example, mumps and chickenpox—radiation, chemotherapy, drugs—for example, sulphasalazine and cimetidine—and possibly environmental oestrogens.[3]

Chromosome abnormalities are an infrequent cause of subfertility in men.[4-7] Known single gene defects include Kartagener's syndrome,[8] partial deficiency of androgen receptors,[9] and possibly oligochiasmate maturation arrest.[10] Subfertility in men is also associated with medical conditions such as pituitary deficiency or cryptorchidism, which may themselves have a genetic component.

A common genetic mechanism for subfertility in men might seem unlikely because of reproductive selection pressures. Human survival, however, is not critically dependent on maximising the number of offspring,[11] and subfertility may be a recessive phenotype in which the effect on reproductive fitness is restricted to homozygotes. Several specific genetic causes of subfertility in men have been discovered in mice,[12] and brothers of subfertile men have poor sperm characteristics when compared with fertile controls.[13] We conducted a case-control study to investigate genetic factors in subfertility in men.

Patients and methods

The study was approved by the Leeds ethics committee.

Subfertile men with abnormal results on semen analysis were identified through the infertility clinics at St James's University Hospital and Leeds General Infirmary. Our definition of abnormal results was adapted from the World Health Organisation's manual —sperm count under 20×10^6 per ml with less than 50% motility on two separate occasions.[14]

All probands had normal growth of body hair, and their partners had been investigated to exclude tubal disease or anovulation. Subjects with a history of radiotherapy or drugs known to interfere with fertility were excluded. Relevant medical factors such as an undescended testis were recorded, although we did not exclude such cases as we were interested in any genetic cause underlying subfertility without prejudging the mechanism of action.

The number of male relatives, their position in the family tree, and whether they had a regular female partner was recorded and inquiry made as to whether such relatives had children. If they did not have children further inquiry was made as to why this was so. In certain cases the subject had discussed this directly with his brother and had been told that the childlessness was voluntary or involuntary. The remaining cases were classified as completely unknown or "probable" involuntary childlessness if this impression had been gained because of an indirect remark or through another family member. We also made a note of men who had children after years of subfertility associated with abnormal results on semen analysis. This provided us with an estimate of subfertility as opposed to childlessness.

A control group of men attending vasectomy clinics (154) or visiting their partners in the maternity department (42) was also studied. Only men who had fathered at least two children were included to obtain an unambiguously normal group. For both groups we excluded adopted, step, and half brothers.

Permission was sought to obtain a detailed history and sperm count from any identified subfertile brothers, although this was often withheld.

STATISTICS

The prevalence of subfertility among relatives of probands and controls was compared with a χ^2 test, and the consistency of the data with a genetic cause was tested by segregation analysis—a statistical technique applicable to families in which relatives have been assessed for the same trait.[15] The proportion of "affected" members among different types of relative is compared with that predicted by the laws of genetics. For instance, if all subfertility in men was due to an autosomal recessive gene then only rarely would fathers be subfertile, whereas on average one quarter of the brothers would be subfertile. In such a case, if

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every proband had two "exposed" brothers, then in one out of 16 of the families both brothers would be subfertile, in six one would be subfertile, and in the remaining nine neither brother would be subfertile. A statistical comparison between the observed numbers of such families with no, one, or two subfertile brothers with those expected indicates the consistency of the hypothesis with the data. In practice, the analysis is slightly more complicated because not all families have the same number of exposed brothers, and we would not expect all cases to be due to genetic factors. We assumed that x% of all cases are due to a recessive gene while the remainder are "random"—that is, not inherited. In the analysis we examined the fit of this model to the observed family distribution of subfertility among brothers of cases (other than those who were voluntarily childless) and in the process estimated the value of x, which makes the expected distribution of subfertile brothers closest to that observed.

Results

PROBANDS (CASES) AND CONTROLS

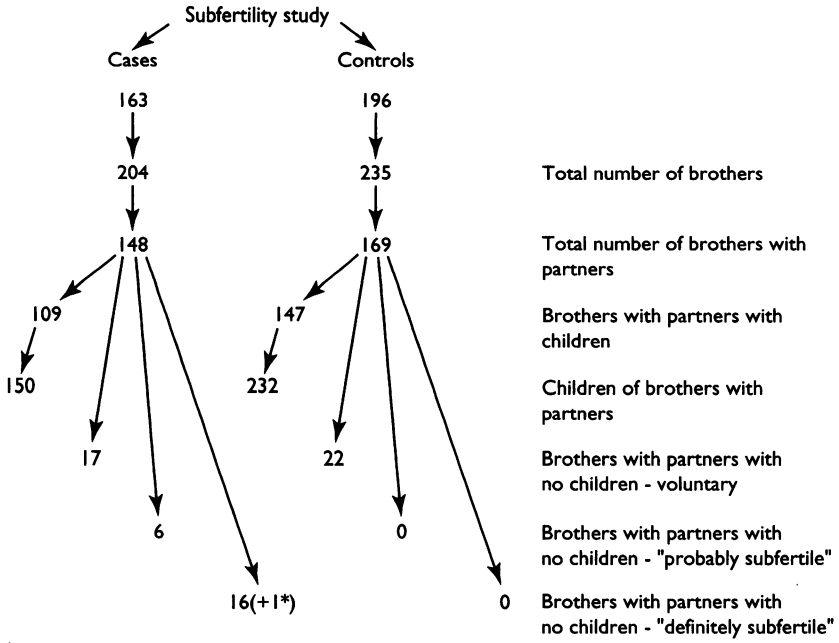
One hundred and sixty three probands and 196 controls were recruited. The mean ages of probands and controls were 33·2 and 34·3 years, respectively (P=0·689, t test) and the number of brothers was 204 and 235, respectively; an average of 1·25 and 1·19 (P=0·69, t test). There was a history of undescended testes in six probands and two controls and of an orchidectomy in three probands and one control.

Some subjects had brothers who were either too young, or who had never established a regular relationship with a woman. We therefore carried out our analysis among probands and controls who had at least one brother who was married or had been cohabiting for at least one year. We refer to these brothers as brothers with partners. Eighty nine probands versus 108 controls had at least one brother with partner, and the total number of such brothers was 148 and 169, respectively (χ^2=0·68).

SUBFERTILITY AMONG BROTHERS OF PROBANDS AND CONTROLS (FIGURE)

Among the families of probands, 16 brothers with partners had told their brother that their childlessness was involuntary, and one more brother had fathered a child after nine years of investigation for oligoasthenozoospermia. (We also encountered a subfertile (maternal) half brother with oligoasthenozoospermia, who is not represented in the above analysis as only full genetic brothers have been included.) Thus there were 17 cases of subfertility among 148 brothers of probands, a prevalence of 12%. There were no involuntarily childless brothers among the controls (P=0·0005).

The incidence of voluntary childlessness among the brothers of probands and controls was 17/148 (11·5%) and 22/169 (13%), respectively. There were also six brothers with partners (all among probands) who were childless but who had not specifically told the interviewee whether this was voluntary or involuntary. In all of these the interviewee had reason to believe that the childlessness was involuntary. If these "probable" subfertile brothers with partners are included then 23/148 (15·5%) brothers among probands (versus none among controls) were subfertile.



* Subfertile rather than childless - see text

Numbers of subfertile childless brothers among cases (men attending fertility clinic) and controls (men with two or more children)

PATTERN OF SUBFERTILITY WITHIN FAMILIES

Four probands had two subfertile brothers with partners, but none had more than two. Thus the 23 cases of subfertile brothers were distributed across 18 families. None of the brothers with probable subfertility was in the same family.

MEDICAL FACTORS IN PROBANDS WITH FERTILE AND SUBFERTILE BROTHERS WITH PARTNERS

Two of the probands with a subfertile brother had a medical factor. This was an undescended testis in both instances and both had only one subfertile brother (one of whom also had an undescended testis). Thus, medical conditions relevant to subfertility were present in two out of 19 (11%) cases with a subfertile brother compared with 19 out of 163 (12%) overall.

GENETIC ANALYSIS OF THE PATTERN OF FAMILIAL SUBFERTILITY AMONG BROTHERS

Segregation analysis was carried out among brothers with partners who were "exposed" in that they wished to have children—that is, excluding brothers who were intentionally childless (table I). The model best fits the data when 60% of cases are assumed to be due to a recessive gene and 40% due to random non-genetic factors. The goodness of fit χ^2 is 7·9 with 8 df (P=0·24), showing that we can accept the hypothesis that the above autosomal recessive explanation is compatible with the data.

SUBFERTILITY IN OTHER FAMILY MEMBERS

Nineteen of the 163 (11·7%) probands and 10 of the 196 (5·1%) controls reported a history of subfertility in an uncle or cousin. The proportion of uncles who were subfertile among cases and controls was 4·9% and 2·8%, respectively (χ^2 P=0·09) and was similar among maternal and paternal uncles (data available on request). Three of the probands with affected second or third degree relatives but none of the controls reported two affected family members apart from themselves. Two of the controls but none of the cases reported a subfertile sister.

DETAILED ANALYSIS OF SPERM COUNTS IN AFFECTED FAMILIES

Three families with subfertile brothers allowed us to obtain sperm counts for comparison. Table II gives the results; there was considerable similarity within but not between the families. Family 1 seems to have a problem with motility rather than sperm density, whereas family 2 has low sperm counts, and family 3 has reasonable counts and motilities but abnormal sperm function (wash and swim) tests.

Discussion

As in all case-control studies we need to be concerned with factors that might have biased our results. Factors against bias and in support of a common familial aetiology include that the overall reported incidence of voluntary childlessness (12%) in this study is similar to that reported elsewhere[16 17] and similar between the brothers with partners of cases (11·5%) and controls (13%), suggesting that any bias in ascribing childlessness to voluntary or involuntary mechanisms was small. Assuming that all of the difference in recorded voluntary childlessness was due to underrecording of subfertility among controls, the results would still be highly significant ($P<0·001$). Even if we made the implausible assumption that all the cases of recorded voluntary childlessness were subfertile, the results would still be significant ($P=0·03$) while the segregation analysis would be essentially unchanged (60% of subfertility due to a recessive gene, $P=0·27$). The existence of four probands with more than one subfertile brother adds weight to a genetic hypothesis, and this is supported formally by the segregation analysis. The abnormality in the sperm count was confirmed in each case in which we were able to get the results from brothers who were reported to be subfertile. An intermediate incidence of subfertility among second degree relatives (uncles) of probands and controls adds further support to the genetic hypothesis as does observation of more than one affected second degree relative in three proband but no control families. The similar incidence in paternal and maternal uncles argues against a high incidence of sex linked inheritance. It is unlikely that bias is selective to male siblings. Thus our failure to find an increased number of infertile sisters in cases argues against strong reporting bias.

An assumption in our study is that paternity is as stated. Arguably, any bias would work against the genetic-familial hypothesis on the grounds that the opportunities for conception through another man are greatest among partners of subfertile men. Another bias working against the familial hypothesis is the concept that the factors responsible may reduce rather than eliminate fertility. Thus some men may have poor sperm results on sperm analysis but have fathered children. We have evidence of this in one of our families (see table II).

Segregation analysis is unable to distinguish between the involvement of one or multiple genes. The observation of familial specific patterns of sperm motility suggests there may be a number of different genes involved, while the lack of subfertility in sisters argues against common occurrence of a common gene causing subfertility in both sexes—for example, by disrupting meiosis.

Further research should aim at comparing family histories and sperm characteristics within and between a larger number of families. Studies of gene expression by using candidate genes or regions of the human genome with homology to genes known to cause subfertility in male mice[12] would be worth while in families with similar results on semen analysis. Microdeletions in the Y chromosome have already been found in some subfertile men[18]—although this is presumably a somatic and not a genetic mutation—and further candidate genes are likely to be found through cloning and identification of unique messenger RNA species found in spermatozoa.[19]

TABLE I—*Numbers of families in which cases (men being treated for abnormal sperm count) have no, one, or two subfertile brothers*

No of brothers with partners wanting to have children*	No of families by No of involuntarily childless brothers with partners		
	0	1	2
0	83	—	—
1	40	7	—
2	14	3	3
3	5	3	0
4	2	2	1

*There were 131 brothers with partners who wanted to have children—that is, 148 minus 17 who were voluntarily childless. Table shows how many families had no, one, and two subfertile brothers as function of number of "exposed" brothers with partners. For example, among families with two brothers with partners who wanted to have children, both had children in 14 families, one was childless in three families, and both were childless in three families.

TABLE II—*Details of seminal analyses among index cases with one or more subfertile brothers*

Family	Index case		Brother 1		Brother 2	
	Density ($\times10^6$/ml)	Motility (%)	Density ($\times10^6$/ml)	Motility (%)	Density ($\times10^6$/ml)	Motility (%)
1	57	1	55	2	75*	5
2	0·3	33	5	80	NA†	
3	15	33	24‡	91	30‡	57

*This man has fathered three children and experienced no difficulty in enabling his wife to conceive. He organised sperm count out of curiosity when he discovered two of his four brothers were childless and had had sperm counts indicating low motility.
†NA=not available. Known to have sought hospital treatment for subfertility but it was thought unwise by both his brothers to approach him for details of sperm counts.
‡Wash and swim test: final motile preparation $<0·1\times10^6$/ml.

Clinical implications

- Subfertility in men is not often caused by chromosome abnormalities
- A common genetic mechanism for subfertility in men is not implausible as human survival is not dependent on maximising the number of offspring and many autosomal recessive conditions are lethal before the individual reaches maturity
- A higher proportion of brothers (with partners) of men treated for subfertility were involuntarily childless compared with brothers of men with at least two children
- Segregation analysis was consistent with an autosomal recessive mode of inheritance accounting for 60% of subfertility in men

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Agism as explanation for sexism in provision of thrombolysis

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See papers on pp 563 and 566 and editorial by Jackson

Evidence exists that physicians manage coronary heart disease less aggressively in women than in men,[1-3] even though heart disease in women may be more severe.[1] We assessed whether thrombolysis is provided on a different basis in men and in women.

Methods and results

As part of the Royal College of General Practitioners' myocardial infarction study, 776 general practitioners in Britain supplied information about the management (including the use of thrombolysis) of 2495 patients suspected of having a myocardial infarction. The patients were recruited from March 1991 to September 1992. We examined the use of thrombolysis in hospital among the 1094 patients who had a myocardial infarction that had been confirmed by a hospital and who had no contraindication to thrombolysis.

In all, 214 patients (20%) were excluded from the analysis because of missing data on age (seven), smoking (118), duration of symptoms (41), or use of thrombolysis (54); some patients were excluded for more than one reason. For the remaining 880 subjects crude and age adjusted odds ratios were calculated, with logistic regression, to determine whether the hospitals' use of thrombolysis was affected by the sex, age, and smoking habits of patients, and by the interval between the onset of a patient's symptoms and admission. Information about the time taken to transport patients to hospital and the time that thrombolysis was given was not collected.

In all, 545 patients received thrombolysis in hospital (62% (95% confidence interval 59% to 65%)). Initially, the women seemed less likely than the men to be given thrombolysis (unadjusted odds ratio 0·74 (table)). The difference, however, was explained by the confounding effect of age: the women tended to be older than the men, and age was an important determinant of the provision of thrombolysis. Adjustment for age removed the effect of sex (age adjusted odds ratio 0·96 (0·71 to 1·31)). The confounding effect of age also explained the apparent lower use of thrombolysis in smokers but it did not affect the trend of decreasing use of thrombolysis the longer the duration of symptoms.

Odds ratios (95% confidence intervals) for treatment with thrombolysis in hospital in 880 patients with confirmed myocardial infarction and no recognised contraindication to thrombolysis

Characteristics of patients	No of patients given thrombolysis: Yes (n=545)	No (n=335)	Odds ratio: Unadjusted	Age adjusted
Men*	379	210	1·0	1·0
Women	166	125	0·74	0·96 (0·71 to 1·31)
Age (years):				
<65*	262	112	1·0	1·0
65-	194	93	0·89	0·89 (0·64 to 1·25)
75-	79	109	0·31	0·31 (0·21 to 0·45)
≥85	10	21	0·20	0·21 (0·09 to 0·46)
Significance	Test for trend $\chi^2=43{-}0$, df=1, P<0·01			
Non-smoker*	181	90	1·0	1·0
Smokers	364	245	0·74	0·98 (0·71 to 1·35)
Time between onset of symptoms to admission (hours)†				
<2*	297	144	1·0	1·0
2-	134	85	0·76	0·72 (0·50 to 1·01)
4-	53	38	0·68	0·66 (0·41 to 1·06)
≥6	61	68	0·43	0·38 (0·25 to 0·58)
Significance	Test for trend $\chi^2=20{\cdot}7$, df=1, P<0·01			

*Reference group.
†Additional adjustments for sex and smoking had no effect on odds ratios.

Comment

All of the patients in this analysis had a confirmed myocardial infarction and no recognised contraindication to thrombolysis. It is noteworthy, therefore, that nearly 40% of patients were not given thrombolysis. The study's protocol asked the general practitioners, in the absence of information about the use of thrombolysis in hospital, to contact their hospital colleagues to confirm that this treatment had been withheld. No explanation, however, was sought for this decision on treatment. No reason exists to suspect that any underreporting of treatment that may have occurred was related to the variables examined in this study.

Some patients may have been denied treatment either because they did not meet the criteria on electrocardiography currently recommended for thrombolysis or because they experienced long delays in transportation, which excluded them from the "therapeutic window." A number of patients, however, were probably denied thrombolysis simply because of their age: two fifths of consultants in charge of coronary care units in Britain who responded to a questionnaire in December 1990 operated age related policies on thrombolysis.[4] Inadequate provision of thrombolysis, however, was not restricted to elderly people. In our study 30% (95% confidence interval 25% to 35%) of patients aged < 65 years and 33% (28% to 37%) of those admitted within two hours of onset of symptoms were not given thrombolysis.

These results remind all staff participating in the care of patients with myocardial infarction of the need to review regularly whether all eligible patients are being offered this important treatment.[5] They also illustrate the need to consider confounding factors when exploring epidemiological data.

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