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Issues in analysis of data on paternal age and 47,+21: implications for genetic counseling for Down syndrome

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Summary. Data and analyses on paternal age and 47,+21 are reviewed. It is concluded that there are few, if any, grounds to justify the inference of a paternal age effect independent of maternal age for those paternal age–maternal age combinations on which there are prenatal diagnostic data. It is suggested that genetic counseling as to increased (or decreased) risk of Down syndrome associated with various paternal ages is not justified at present.

Main implication of the reanlaysis of previous New York State data by Stene et al. (1987)

The reanalysis by Stene et al. (1987) of previously published New York data (Hook and Cross 1982) with regard to paternal age effects raises several new questions but lays to rest an important old one.

The most significant point about the note by Stene et al. (1987) is that they have in essence retracted the strong statements and implications in their earlier papers that elevated paternal age is an ubiquitous and strong independent risk factor for Down syndrome or 47,+21 (e.g., Stene et al. 1981; Stene and Mikkelsen 1983). In the past Stene et al. (1981) stated that data that differed from this view were biased or erroneous. They now, however, explicitly endorse one possible explanation that Hook and Cross (1982) had suggested for differences among studies: temporal and geographic variation in putative trends. The difference made by the addition of "environmental" to these alternatives by Stene et al. (1987) is not clear since environmental variation results in general in geographic variation and often in temporal variation.

Of course this is now and was then consistent with all the observations. Whether it is the correct explanation must still be regarded as moot. Other alternatives are still possible, including the suggestion that there is a relatively weak effect with increasing paternal age, and statistical fluctuation accounts for the variation in such effects at older ages (Hook and Cross 1982). Certainly the likelihood that this explanation is correct was diminished but not nullified by a subsequent paper by Roecker and Huether (1983) that found a statistically significant negative effect of paternal age in a case-control analysis of Ohio data. The issue of the correct explanation for the variation has some implications for genetic counseling. This issue is discussed further below.

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Methodological issues

The most serious problem with the analysis of Stene et al. (1987), which claims to have found paternal age effects in the earlier New York State data, is their post hoc selection from the data of hypotheses to test. This is a well-known type of statistical misunderstanding. A hypothesis should be structured *before* formally testing a data set. One may *explore* a data set for trends, but *proof* that observed trends are not the result of chance should be sought in a new data set, once a hypothesis has been established. As the well-known statistician Diaconis has noted: "There is a difference between data analysis and actually testing a well-formulated hypothesis. These shouldn't be confused" (De Groot 1986). These comments were made with regard to some statistical analyses in the field of parapsychology, but they are also applicable to other fields of endeavor.

In previous work on data from amniocentesis, Stene et al. (1981) proposed a boundary of age 41 and above in a claim of paternal age effect. What happens if our earlier data are examined with this hypothesis in mind? Andrew Carothers has calculated ratios of observed and expected numbers derived from our original data, and the results of his analysis appear in Table 1. (I thank Dr. Carothers for permission to cite his calculations.) He followed Stene et al. (1987) in considering all those ages 35 and over. The expected numbers at different paternal ages were calculated from the data by assuming that the ratios of Down syndrome cases to normals at any specific maternal age is the same at all paternal subdivisions. On this assumption, there are 39 cases at paternal age 41 and over in the data set reanalyzed by Stene et al. (1987) compared to 40.3 expected, a ratio of less than 1, contrary to their claim. Shifting the boundary by 1 year eliminated the "effect"! Ubiquitous effects at ages 41 and over may not be inferred from trends in the ad hoc analysis in those "40 and over" in this data set. If a shift upward in a single year makes such a difference, how plausible is it that there is a real biological effect here? With regard to the younger ages, there was to our knowledge no prior hypothesis by Stene et al. (1987) on effects at "younger" ages. Age 33 (i.e., 33 and below) was presumably picked by them in the reanalysis as that boundary that maximized the "effect." If age 35 (35 and below) is picked instead, then the ratio is 23 observed to 20.0 expected, 1.15, and is not significantly different from 1.0.

Elsewhere in this issue of *Human Genetics* appear results of an analysis of *new* New York State data on paternal age (Cross and Hook 1987) in accord with the recommendations of Diaconis (1985) on the formulation and testing of statistical

Table 1. Analysis by A. Carothers of those data of Hook and Cross (1982) that were reanalyzed by Stene et al. (1987). The expected numbers are calculated assuming ratios of 47,+21 to those with normal karyotype at any maternal age are the same at all paternal age subdivisions (Obs, Observed numbers of 47,+21; Exp, expected numbers of 47,+21 on the above assumption)

Mother's age (years)	Father's age (years)							
	<35		36–40		≥41		Total	
	Normals	47,+21 Obs/Exp	Normals	47,+21 Obs/Exp	Normals	47,+21 Obs/Exp	Normals	47,+21
35	798	4/2.9	776	3/2.8	359	0/1.3	1933	7
36	520	6/3.6	798	3/5.5	415	3/2.9	1733	12
37	422	3/3.0	660	5/4.7	471	3/3.3	1553	11
38	269	3/2.6	456	5/4.3	436	3/4.1	1161	11
39	178	4/2.9	274	2/3.7	371	5/5.0	823	11
40	93	1/2.9	157	5/4.9	322	12/10.1	572	18
41	52	2/0.9	78	2/1.4	210	2/3.7	340	6
42	28	0/0.4	40	2/0.6	151	1/2.1	219	3
43	13	0/1.1	23	2/1.9	61	6/5.0	97	8
44	6	0/0.4	15	0/0.9	49	4/2.8	70	4
≥45	3	0/0	5	0/0	23	0/0	31	0
Total	2382	23/20.0	3282	29/30.6	2868	39/40.3	8532	91

hypotheses. These data do not reveal evidence for paternal age effects in men under 30, under 34, 40 and over, 40 to 49, or 41 and over, either in comparison with expected numbers derived from rates on those in the middle age range, or with expected numbers derived from a different data source. This does not prove that such effects did not occur in the population from which data of the first study were gathered, but they make it more likely that any such "effects" claimed in a post hoc analysis are simply chance results, despite the formal calculations of Stene et al. (1987).

Another analogous statistical issue, which is less important overall, is the calculation by Stene et al. (1987) of the probability of observed events in the data of Hook and Cross (1982) at age 40. This calculation is erroneous because it assumes that age 40 had already been chosen as an age of concern prior to analysis. This is similar to the post hoc specification of an age boundary discussed above. Such a calculation has to be adjusted for all other possible ages at which "outliers" might be observed. A second difficulty is the choice of reference rates used in their comparison; if the data on those aged 35–39 alone had been used and not 39–41 only, the chi square value calculated would have been lower. Stene et al. (1987) chose for reference the two ages that maximized the calculated values of chi square. Nevertheless, despite these objections to their analysis, it is certainly possible that there was distortion of some sort in the reporting of ages of the 47,+21 cases in the series analyzed, leading to the apparent excess exactly at age 40. But why it occurred for 47,+21 and not other abnormalities, or in the normals in the same series, or for 47,+21 in the rest of the collected date is difficult to explain by this hypotheses. Indeed because of the inconsistent nature of this peak, I presumed it was most likely the result of statistical fluctuation.

In this regard I emphasize that coincidences do occur in any data set, indeed throughout nature. Unusual events happen for many reasons, the critical question is: what is their implication for inference? I did not see a reason to reject the data source for inference on trends with increasing paternal age because of a peak at one specific age.

The same considerations apply to an additional claim of Stene et al. (1987) that the observed numbers of cases are higher in the series of 10,000 than the number expected if calculated from maternal-age-specific rates in the series of 50,000 reported from Edinburgh. Statistical fluctuation may well account for the difference despite ad hoc probability calculations of Stene et al. (1987). But whatever the explanation, these considerations are irrelevant to the main issue of putative paternal age effect in the data.

Biological evidence on paternal age effect for 47,+21

Recent data by Martin and Rademaker (1987) and Martin (personal communication) on chromosomes in human sperm are also pertinent. In five men in each of six different age intervals the proportion of +21 sperm were as follows: 2/275 at ages 20–24, 0/282 at ages 25–29, 1/376 at ages 30–34, 0/250 at ages 35–39, 0/213 at ages 40–44, and 0/186 at ages 45 and over. (There was also a +G at age 22 and at age 35, and a +22 at age 29.) The percentages for all hyperploidy were 3.2%, 1.1%, 1.3%, 1.3%, 0%, and 0% at these ages, respectively. These data provide no evidence for any increase with paternal nondisjunction for +21 with father's age. And at the younger ages the apparent increase was in those aged 20–24, a group on which the reanalysis by Stene et al. (1987) had essentially no data. (There were no 47,+21 cases in 143 men reported on at these ages in the series of Hook and Cross 1982.) While the trends in the data of Martin and Rademaker (1987) are of great interest and must of course be investigated in other sources, they provide no confirmation for the trends claimed by Stene et al. (1987).

Other issues in the epidemiology of Down syndrome

Stene et al. (1987) introduce a number of matters in consideration of other aspects of the epidemiology of Down syndrome.

They state unequivocally that (putative) ethnic variation in rates of Down syndrome is solely because of environmental

influences. But what grounds are there for this statement? There is a great deal of evidence from lower organisms that there are genes that influence nondisjunction (see, e.g., Baker et al. 1976). Why cannot there be similar genes in humans and ethnic variation in such genes? Moreover, there may be genetic variation in factors affecting survival of Down syndrome fetuses to a gestational stage at which they are recognized. It is of interest in this regard that at least one study, that of Alfi et al. (1980) on Kuwaitis reported higher rates of consanguinity in Down syndrome livebirths. Studies of consanguinity and Down syndrome in European populations have not revealed such effects (see review in Hook 1982). Such data are at least consistent with some genetic ethnic variation, i.e., genes predisposing to 47,+21 livebirths specifically in the Kuwait population studied by Alfi et al. (1980). It is also of interest that the only direct evidence for ethnic differences in maternal-age-specific rates of Down syndrome are also from the Middle East. The rates are higher in Jews from Africa or Asia than those from Europe (Hook and Harlap 1979). Both of these studies need confirmation before unequivocal conclusions may be drawn. And the Israel results certainly could result from environmental differences between the two Jewish groups. But genes associated with Down syndrome in at least some Middle Eastern populations are also consistent with both studies.

Stene et al. (1981) also introduced an additional issue in endorsing the "main conclusions" of the paper of Ayme and Lippman-Hand (1982) on selection. The implication of that paper was that selective differences in survival of 47,+21 fetuses *after usual recognition of pregnancy* contribute to the *maternal* age effect. The whole point of that paper was an attempt to prove this hypothesis by considering trends in the available spontaneous abortion data. But, there are major statistical difficulties with such claims (see Carothers 1983; Warburton et al. 1983; Hook 1983). The contention of Stene et al. (1987) that their analysis of subsequent data proves what may be called a "relaxed selection" effect (Hook 1983) is subject to these same objections. Briefly, pooling of data on the many nonviable and the few viable trisomies will confound inferences pertinent to the latter. It is certainly possible that there are "relaxed selection" effects for +21 conceptuses (or analogously some type of preferential fertilization of +21 gametes) with increasing maternal age, but such effects if they contribute to a maternal age effect would appear to be operative *before* the usual recognition of pregnancy (Hook 1983). In addition, some further ad hoc hypotheses must be invoked to explain why there is no notable maternal age effect upon 47,+21 fetuses born to 47,+21 mothers (Hook 1983) or for cases with Down syndrome caused by translocations (Hook 1984).

Implications for genetic counseling

At least one practical implication of any paternal age effect if it exists is for accurate genetic counseling. Concern about older paternal age usually arises in advising "younger" women, e.g., those under 35 married to men at the upper extremes of age. In many jurisdictions, younger women are counseled against or denied prenatal diagnosis. But if some other factor such as the father's age should raise the risk for these younger women then amniocentesis might well be recommended or allowed.

Unfortunately, there are relatively few data specifically on women at this age in prenatal diagnostic studies. All of the prenatal cytogenetic data of Stene et al. (1981) or Ferguson-Smith and Yates (1984) for instance, are on women 35 and over in whom paternal age effects are of lesser importance. Moreover, the reanalysis of Stene et al. (1987) of the data of Hook and Cross (1982) did not consider women under age 35. The results in the available sparse data on such women do not reveal any paternal age effect (Cross and Hook 1987). One *may* be able to extrapolate from those at older ages to women under 35, as implied by Stene et al. (1981) but until this is proven, the most directly pertinent clinical data will be those on younger women.

If there were some type of maternal age–paternal age interaction (Hook 1980), then positive paternal age effects in younger mothers might be obscured by results in men married to older women. Indeed some data of Erickson (1979) are consistent with this possibility although the trend is nonsignificant.

Another issue of practical concern is the possibility of "lower" risks associated with some paternal ages (independent of maternal age). If there is a marked increase in rate with the father's age sufficient to be of clinical significance at the upper extreme of paternal age, then this would imply that at some other paternal ages the rates would be lower, perhaps markedly lower. But great caution is necessary before inferences are made on this point because the consequences might be denial of prenatal diagnosis to some women ages 35, 36, or even older because of their husbands' ages. A great deal more supporting evidence should be sought before counseling a negative ("protective") than a positive paternal age effect.

While awaiting "more investigations" on these matters, what should a genetic counseler do? A woman who postpones a pregnancy to await a firm answer to these questions will only increase her risk as she ages!

Earlier, rates were published (Hook and Cross 1982) on the assumption that the (log) rate of 47,+21 increases about 1% per year with paternal age, an increase that appeared consistent with most studies. These rates did not predict much of an increased risk with elevated father's age even at age 55 (although the regression model used was not optimal, see Hook 1987). Moreover, subsequent publication by Roecker and Huether (1983) indicating overall a *negative* paternal age effect in a case-control study raised the possibility that even these estimates appear too extreme (Hook 1985).

The alternatives now endorsed unequivocally by Stene et al. (1987) – temporal and geographic (including environmental) variation – appear, initially, very difficult to interpret for genetic counseling purposes. If there is temporal and geographic variation as Stene et al. (1987) now claim, then until one can define the reason for that variation and obtain pertinent data on each woman subject to such influence, no risk figures for counseling may be established. The putative underlying factor may vary. For example, a tentative inference I drew from the paper by Stene et al. (1987) is that in the jurisdiction in which their earlier analysis had revealed alleged strong positive effects (Stene et al. 1981), such strong effects no longer can be detected. *If* this is correct, then genetic counseling in that jurisdiction based on the earlier published data would have been inappropriate for the subsequent time interval. Certainly, on the hypothesis of Stene et al. (1987) by the time data are analyzed they may no longer be applicable.

This implication of the hypothesis endorsed by Stene et al. (1987) does have one immediate consequence for genetic

counseling: ignore paternal age as a risk factor for 47,+21! If the risk was low yesterday in one place at paternal age 33 and is high today in another at age 40, perhaps tomorrow rates at paternal age 41 will be low somewhere else. And moreover, should the alleged cause of the underlying variation be defined eventually, then this factor alone should be used in counseling. Consider a hypothetical illustration. There is some evidence consistent with but not proving the hypothesis that maternal cigarette smoking results in selective loss of Down syndrome fetuses during pregnancy, at least at some ages (Kline et al. 1983; Hook and Cross 1985). Suppose this hypothesis is correct and moreover, that maternal cigarette smoking is associated in some complex way with paternal age, but the association varies temporally and geographically. Then variation in maternal cigarette smoking might explain differences among studies in the paternal age effects. But the implications for the genetic counselor would be to cite risk figures adjusted for the mother's smoking status, not the father's age. I emphasize this is only a hypothesis subject to investigation.

My own preference in genetic counseling for paternal age at the present stage of knowledge is to cite the possibility of increased risk of 47,+21 only for men at the upper extreme of paternal age, say 55 and over. This is *not* to claim unequivocally that there is strong evidence even at this extreme, or that there are not significant effects at other ages, but only that this appears a cautious course, at least consistent with the available data.

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