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Increased Rate of Twins among Affected Sib Pairs

To the Editor:

Recently, Greenberg et al. (2001) and Betancur et al. (2002) reported an excess of twin pairs among affected sib pairs with autism (MIM 209850). Greenberg et al. (2001) reported an excess of both MZ and DZ pairs, whereas Betancur et al. (2002) found an excess of MZ pairs only. Both studies tested the rates of twin pairs among a sample of affected sib pairs against the population rates. The hypothesis put forward was that being a twin is in itself a risk factor for autism. The purpose of this letter is to show that an excess of twin pairs among affected siblings—in particular, an excess of MZ pairs—is what would be expected if genetic factors are implicated in the etiology of a disorder and does not in itself suggest that being a twin confers a risk. Hence, the reported results could be a logical consequence of the affected sibling ascertainment scheme.

The proportion of twin pairs among a random sample of affected siblings from the population depends on the population incidence of twinning and on the concordance rate for the disorder. Let p be the incidence of the disorder in the population; f_{MZ} and f_{DZ} the population rates of MZ twins and DZ twins, respectively; and r_S, r_{DZ}, and r_{MZ} be the (casewise) concordance rates (i.e., the probability that one sibling is affected, given that the other sibling is affected) for nontwin siblings, DZ, and MZ twins, respectively. For each of the three kinds of sib pairs, the probability of 0, 1, and 2 affected individuals is, for $r = r_S, r_{DZ}, r_{MZ}$,

$$P(0 \text{ affected}) = (1 - p) - p(1 - r)$$

$$P(1 \text{ affected}) = 2p(1 - r)$$

$$P(2 \text{ affected}) = rp \ .$$

It follows that the proportion of MZ pairs among all pairs of affecteds is

$$f^*_{MZ} = \frac{f_{MZ} r_{MZ}}{f_{MZ} r_{MZ} + f_{DZ} r_{DZ} + (1 - f_{DZ} - f_{MZ}) r_S} \ .$$

Note that this proportion is independent of the population incidence. For small DZ and MZ population rates, $f^*_{MZ} \approx f_{MZ} r_{MZ} / r_S$; that is, we would expect an increase in the rate of MZ twins that is proportional to the increase in the concordance rate relative to nontwin siblings. From epidemiological studies, the estimates for the concordance rates for autism in MZ pairs, DZ pairs, and nontwin siblings are approximately 0.4–0.7, 0.0–0.03, and 0.03, respectively (see Lauritsen and Ewald [2001] and Folstein and Rosen-Sheidley [2001] for reviews), consistent with a very high heritability on a liability scale and the existence of nonadditive genetic variation for liability (see, e.g., Smith 1970). These estimates suggest that the proportion of MZ twin pairs in a random sample of affected sib pairs is approximately 13–23 times larger than the population MZ twinning rate. The observed increases in the MZ rate in the Greenberg et al. (2001) and Betancur et al. (2002) reports are 13 and 16, respectively; they are in accordance with the published concordance rates.

Greenberg et al. (2001) also report a significant increase (a nearly fivefold increase) in the proportion of DZ twins among the affected sib pairs. Estimates of DZ concordance rates have been similar to or lower than the rates among nontwin siblings but have been based on small numbers of observations (Folstein and Rosen-Sheidley 2001; Lauritsen and Ewald 2001). An increase in the rate of DZ twins relative to nontwin siblings could be due to common environmental factors or due to the "stoppage" phenomenon, in which parents with one affected child choose not to have more children. Lastly, Greenberg et al. (2001) compare their observed increased rates of autism in affected twin pairs with the rates for insulin-dependent diabetes mellitus (IDDM). They found a deficit of DZ twin pairs but an excess of MZ twin pairs. These results are also consistent with the genetic epidemiology of IDDM, with reported concordance rates of 0.06, 0.11, and 0.30–0.50 for nontwin siblings, DZ twins, and MZ twins, respectively (see, e.g., Kyvik et al. 1995; Field 2002).

In this letter, I have suggested another explanation for the observed excess of twin pairs among affected sibling pairs; that it is simply the effect of ascertaining pairs of affected siblings. Is multiple birth an important risk factor for autism? The data presented by Greenberg et al. and Betancur et al. do not allow the testing of this hypothesis. A population-based study, in which the incidence of autism among MZ twins, DZ twins, and nontwin siblings is estimated should clarify this important issue.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for autism [MIM 209850])

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Response to Visscher

To the Editor:

We must admit that Dr. Visscher (2002 [in this issue]) is quite correct and that in our two reports of twins in autism (Greenberg et al. 2001; Betancur et al. 2002) we overlooked the elementary application of Bayes's rule in this situation, namely: If MZ twin pairs are more likely to be concordant than nontwin pairs, then sampling concordant pairs will produce an excess of MZ twin pairs relative to nontwin pairs. This excess says nothing about the relative strengths of genetic or nongenetic effects in autism, contrary to what we concluded in our papers.

However, the points made by Dr. Visscher explain only part of our observations, and they also highlight the sensitivity of the conclusions to the accuracy of the population data. Because twin concordance rates vary from study to study, the issue of increased autism risk to twins is not yet settled. In particular, interpreting the findings from the DZ twins remains problematic.

We begin with some calculation issues. First, Visscher's formulas contain an error, although the error does not affect his conclusions and may even strengthen them. The probability of both members of a sib pair being affected, his $P(2 \text{ affected})$, does not equal rp, as he states. (We use his notation of r for the "pairwise concordance rate," but note that p should represent disease population prevalence, not incidence.) Rather, $P(2 \text{ affected})$ is given by Kp, where K is the "recurrence risk" for that particular kind of sib pair (James 1971; Risch 1990).

To see why, let us use π_i for Visscher's $P(i \text{ affected})$—that is, the probability that a sib pair has i affected sibs. The standard definition (also used by Visscher) says that the pairwise concordance rate r gives the probability that both sibs are affected, given that *at least one* is affected—that is, $r \equiv \pi_2/(\pi_2 + \pi_1)$. In contrast, the recurrence risk K is defined as the recurrence risk to the sib of an affected individual—that is, $P(\text{sib \#2 is affected} \mid \text{sib \#1 is affected})$, which can be written as $\pi_2/P(\text{random individual is affected}) = \pi_2/p$. Since $K = \pi_2/p$,

$$\pi_2 = P(2 \text{ sibs affected}) = Kp \; . \qquad (1)$$

(The recurrence risk K is the same as the "probandwise concordance rate" for twins [Smith 1974]; also see Wickramaratne and Hodge 2001). K is always $\geq r$, approaching twice r when both are small:

$$K = 2r/(1 + r) . \quad (2)$$

We now recalculate Visscher's f^*_{MZ}, defined as $P(\text{MZ}|2 \text{ affected})$. We incorporate the above correction from equation (1) (i.e., replace r with K), and we use the values for f (the population probability of each type of sib pair, among all sib pairs) from Greenberg et al. (2001): $f_{MZ} = .008$, $f_{DZ} = .016$, $f_S = 1 - .008 - .016 = .976$. The formula becomes

$$f^*_{MZ} \equiv P(\text{MZ}|2 \text{ affected}) = \frac{(.008)K_{MZ}}{(.008)K_{MZ} + (.016)K_{DZ} + (.976)K_S} . \quad (3)$$

Visscher approximates this quantity by $(.008)K_{MZ}/[(.976)K_S]$ and examines the effect on the ratio f_{MZ}/f_S, but we prefer to work directly with f^*_{MZ}. Combining data from the three epidemiologically based twin studies of autism cited by Folstein and Rosen-Sheidley (2001) (Folstein and Rutter 1977; Steffenburg et al. 1989; Bailey et al. 1995), we note that 25 of 36 MZ twin pairs are concordant. (This figure comes from $r_{MZ} = 15/25 = 0.60$ in the work by Bailey et al., which includes the Folstein and Rutter data, as well as 10 of 11 concordant MZ pairs in the work by Steffenburg et al.) This yields $r_{MZ} = 25/36 = 0.69$, which we convert to $K_{MZ} = 0.82$, using equation (2). As for nontwin sib pairs, several studies (August et al. 1981; Piven et al. 1990; Bolton et al. 1994, cited in Lauritsen and Ewald 2001) agree on a sib recurrence risk of ~3%. Note that this is a *recurrence* value, so we use it *un*changed. Ritvo et al. (1989), in the largest published study of sibs, reported a slightly higher figure of 4.5% among all sibs of the firstborn subject. We will also assume K_{DZ} equals K_S for the purposes of these calculations. Using equation (3), we calculate f^*_{MZ} as 18% or 13%, for $K_S = 3\%$ or 4.5%, respectively. These predictions agree reasonably closely with the rates of MZ twin pairs observed by us: 10%–14% (Greenberg et al. 2001) and 10%–13% (Betancur et al. 2002). Thus, we agree with Visscher that our observed rates fit right within what would be predicted by the respective MZ twin and sibling recurrence rates. (We have used somewhat different input figures than Visscher, because we went back to the original studies, but the point remains the same.)

However, additional questions remain:

(1) Although the sib recurrence rates seem to be fairly consistent among studies, that is not true of the published MZ twin concordance rates, which vary widely and depend highly on issues of ascertainment, diagnosis, etc. (Smith 1974). It appears to be more difficult to collect unbiased, clearly diagnosed samples of twin pairs than of nontwin sib pairs. Thus, the K_{MZ} rates are "soft"; if they turned out to be lower than those used here, then the conclusions could be quite different.

(2) If the reasoning outlined by Visscher and discussed above completely "explains away" the striking increase in twin pairs among affected sib pairs observed by both our groups, then why has that increase not been observed in other autism data sets as well? Is this phenomenon wholly due to most investigators rejecting MZ twin pairs for their studies, which our two groups did not do? And/or is it due to investigators simply not examining their data sets for excess twins? We do not know the answer, but this situation illustrates the usefulness of tracking how and what kind of families enter a study.

(3) Similarly, if the above reasoning explains the increase in twin pairs, why is a similar increase not observed in data from other diseases? In one of our original articles (Greenberg et al. 2001), for example, we had looked at affected sib pairs (ASPs) with insulin-dependent diabetes mellitus (IDDM) for just this reason, to provide a control. A relatively recent study (Kyvik et al. 1995) of IDDM concordance in Danish twins found recurrence risks (probandwise concordances) of $K_{MZ} = 0.70$ and $K_{DZ} = 0.13$ (cumulative age-adjusted). This study was based on 20,888 twin pairs from a population-based nationwide register and was probably more free of ascertainment problems than earlier studies. Sib recurrence risks for IDDM are usually estimated at ~0.06 (Thomson et al. 1988; Dorman et al. 1995). Inserting these values into equation (3) yields $f^*_{MZ} = .085$, yet, in our control sample of ASPs with IDDM, collected in the same manner as the ASPs with autism, we observed only 13/649 = 0.02 MZ twin pairs—nothing like the kind of excess predicted by equation (3). In fact, our observed proportion would represent a highly significant *deficit* relative to what was expected. Moreover, one can also calculate f^*_{DZ}—that is, $P(\text{DZ}|2 \text{ affected})$—by replacing $(.008)K_{MZ}$ with $(.016)K_{DZ}$ in the numerator of equation (3), yielding $f^*_{DZ} = 0.03$. So we should have observed a proportion of 0.03 DZ twins among the IDDM sib pairs, higher than the population rate of .016; yet, we observed again a statistically significant *deficit* of DZ twin pairs (1/649 = 0.002). This remains puzzling.

On the other hand, a 1992 Finnish study of IDDM twin concordance (Kaprio et al. 1992) found much lower recurrence rates: $K_{MZ} = 0.23$ and $K_{DZ} = 0.05$. Using those values, one would predict observing $f^*_{MZ} \approx 0.04$ and $f^*_{DZ} \approx 0.016$ (we set both K_{DZ} and K_S to 0.05 for that calculation), which is much closer to what we did observe in the IDDM data. This discrepancy between the two studies, only 3 years apart, highlights again the

"softness" of twin concordance rates and the difficulty of drawing firm conclusions from them. An older study by Cahill (1979) had also reported a low K_{MZ} of only 5/28 = 18%.

(4) The DZ twin pairs in the autism data sets are puzzling as well. We did observe increased numbers of these pairs—a striking and significant increase under both narrow and broad diagnostic criteria by Greenberg et al. (2001) and a slight (not significant) increase by Betancur et al. (2002). Yet the literature reports K_{DZ} no higher than K_S for autism, so there should have been *no* increase over population proportions. Visscher mentions the "stoppage" phenomenon as possibly explaining the excess in DZ twins, but we did account for stoppage in the original Greenberg et al. (2001) article.

We agree with Dr. Visscher that to provide a definitive answer to these questions will require a population-based twin study examining the prevalence of autism among MZ and DZ twins. One of us (C.G.) is currently undertaking such a study in Sweden, and we hope this will help answer these questions. Moreover, a just-published epidemiological study examined >3.5 million live births in California between 1989 and 1994, of whom 4,381 were diagnosed with what those authors call "full-syndrome" autism (Croen et al. 2002). This study found an increased autism risk associated with multiple births: relative risk was 1.7 (95% CI 1.4–2.0), when adjusted for all other factors considered by the authors. Thus, we feel that whether twinness represents a risk factor for autism is still an open question. However, for the time being, we must agree with Dr. Visscher that the observed proportions of twins in both our autism data sets argue neither for nor against the hypothesis that being a twin is itself a risk factor for autism.

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