Increased rate of twins among affected sibling pairs with autism

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To the Editor:

There is consistent evidence from twin and family studies implicating genetic factors in the etiology of autism (MIM 209850), but no specific genes associated with autism have yet been identified. In a recent article in the *Journal*, Greenberg et al. (2001) reported a striking excess of twin pairs, both MZ and DZ, in the cohort of families with at least two siblings with autism or autism-related conditions recruited by the Autism Resource Exchange (AGRE) of the Cure Autism Now (CAN) Foundation. The proportion of twins among autistic sib pairs (18%, 30/166) was significantly higher than the expected twinning rate per sib pair (2.4%). Greenberg et al. (2001) demonstrated that in order to ascribe this excess of twins with autism to a sampling bias would require very large ascertainment factors, which seem unlikely. These findings suggest that being a twin represents a risk factor for autism, and have important implications for the etiology of autism. However, as Greenberg et al. (2001) pointed out, these results need to be replicated in other data sets. We report a similar excess of twins in a sample of affected sib pairs recruited by the Paris Autism Research International Sibpair (PARIS) study.

Families with two or more children with autism or autism-related disorders (Asperger syndrome or pervasive developmental disorder not otherwise specified [PDD NOS]) were

recruited by the PARIS study at specialized clinical centers in eight countries (Austria, Belgium, France, Israel, Italy, Norway, Sweden, and the United States). Patients were included after a complete clinical and neuropsychological assessment described previously (Philippe et al., 1999); subjects demonstrated to suffer from organic conditions associated with autism, such as tuberous sclerosis, fragile X syndrome, or other established chromosomal disorders were excluded from the study. We divided the affected sib pairs into two diagnostic categories: "narrow" when both affected sibs had autism, and "broad", when one or both of the affected sibs had either Asperger syndrome or PDD NOS. Patients in the narrow diagnostic category fulfilled the DSM-IV and ICD-10 criteria for autistic disorder/childhood autism and met the Autism Diagnostic Interview-Revised (ADI-R) algorithm (Lord *et al.,* 1994). All the families were Caucasian except one of mixed ethnicity (Caucasian/Asian).

To make our results directly comparable to those obtained by Greenberg et al. (2001), in the current analysis we included only families having exactly two affected offspring, i.e., families with triplets, or with an affected twin pair and one or more nontwin affected siblings were excluded. We also excluded families with mixed twin pairs (one affected twin, one unaffected cotwin, and a nontwin affected sib) and families with half-siblings.

Table 1 shows the distribution of affected sib pairs divided according to diagnostic category, twin status, and sex. Table 2 shows the observed proportion of twins in our data set compared both to the population rates reported by Greenberg et al. (2001) and to the rate observed in the AGRE families. In agreement with the results of Greenberg et al. (2001), we observed a remarkably high proportion of MZ twin pairs among affected sib pairs. Of 79 affected sib pairs (narrow + broad diagnoses), 11 were twin pairs (2 DZ and 9 MZ). This represents a 14-fold increase for MZ twins, compared to the population frequency, and is statistically significant. In contrast, we did not observe a significant increase in the proportion of DZ twins.

We did not include in the calculations: 1) one family with affected triplets (two MZ and one DZ); 2) one family with two affected MZ twins plus an affected nontwin; 3) one family with two affected twins (zygosity unknown) plus two affected nontwins; 4) one family with one affected MZ twin, one cotwin deceased during the first year of life, and a nontwin affected sib; and 5) three sets of discordant DZ twins (one affected and one unaffected). Together with the other twin pairs, these families further contribute to reinforce the hypothesis that twinning per se is a significant risk factor for autism.

The increase in the proportion of twins among affected sib pairs with autism observed by Greenberg et al. (2001) and confirmed in our data set strongly suggests the involvement of

biological factors rather than an ascertainment bias. Moreover, different ascertainment methods were used in our study and that of Greenberg et al. (2001): the AGRE families were recruited exclusively via mailings and presentations to autism support groups, whereas the majority of our families were collected by clinicians members of the PARIS study among their clinic cases.

The PARIS study collects affected sib pairs for linkage studies but also parent-offspring trios for association studies. We do not preferentially collect MZ twins, but neither do we turn them away, because these families can be used for association studies. In our data set, only MZ twins were overrepresented among affected sib pairs. Although Greenberg et al. observed an excess of both DZ and MZ twins among their families, the deviation from population rates was more important among MZ twins (12-fold) than among DZ twins (4-fold). As mentioned by Greenberg et al. (2001), the excess of MZ twins compared to DZ twins in autism suggests that the estimates of heritability based on concordance for autism in MZ pairs versus DZ pairs may be overestimated. These intriguing findings also emphasize the need to explore the participation of nongenetic factors, as well as genetic, in the etiology of autism.

Electronic-Database Information

The accession number and URL for data in this article are as follows:

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

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Table 1
Distribution of affected sib pairs

	Number in diagnostic category[a]		
Group	Narrow	Broad	Total
Singletons	59 (34, 23, 2)	9 (5, 4, 0)	68 (39, 27, 2)
DZ twins	2 (2, 0, 0)	0	2 (2, 0, 0)
MZ twins	9 (7, —, 2)	0	9 (7, —, 2)
Total	70 (43, 23, 4)	9 (5, 4, 0)	79 (48, 27, 4)

[a] Numbers in parentheses indicate breakdown into male-male, mixed sex, and female-female, respectively.

Table 2
Observed proportion of twins compared to population rates and the study by Greenberg et al. (2001)

		Greenberg et al. (2001)[a]		Narrow diagnosis		Narrow + broad diagnoses	
Twin Group	Population Rate[b]	Rate observed	*P*[c]	Rate observed	*P*	Rate observed	*P*
DZ	.016	.072 (12/166)	<.00005	.029 (2/70)	N.S.[d]	.025 (2/79)	N.S.
MZ	.008	.102 (17/166)	<.000001	.129 (9/70)	<.000001	.114 (9/79)	<.000001
All	.024	.181 (30/166)	<.000001	.157 (11/70)	<.000001	.139 (11/79)	<.000005

[a] Includes narrow + broad diagnoses.
[b] From Greenberg et al. (2001).
[c] Two-sided, exact binomial calculations.
[d] N.S. = not significant.