

Symptom Domains in Autism and Related Conditions: Evidence for Familiality

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Heterogeneity in autism impairs efforts to localize and identify the genes underlying this disorder. As autism comprises severe but variable deficits and traits in three symptom domains (social interaction, communication, and repetitive behaviors) and shows variability in the presence and emergence of useful phrase speech, different genetic factors may be associated with each. The affected cases ($n = 457$) in multiply affected sibships ($n = 212$), including a proband with autism and one or more siblings with either autism or marked deficits in autism symptom domains, were assessed using the Autism Diagnostic Interview, Revised. Symptom domain scores and language features were examined to determine their similarity within sibships. The variance within sibships was reduced for the repetitive behavior domain and for delays in and the presence of useful phrase speech. These features and the non-verbal communication subdomain provided evidence of familiality when we considered only the diagnosis of autism to define multiply affected sibships (cases: $n = 289$; sibships: $n = 136$). In addition, the same familial features identified also appeared familial for those with autism-related conditions. Finally, the level of severity of almost all of the familial features varied within multiplex sibships indepen-

dently. The features identified as familial replicate the combined set suggested in earlier, smaller studies. Furthermore, the familiality of these features extend to related conditions of milder severity than autism and appear to be independent. Making distinctions among families by the severity of these features may be useful for identifying more genetically homogeneous subgroups in studies targeted at genes for specific autism-related symptom domains.

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INTRODUCTION

Twin study data implicate genetic factors in autism [Folstein and Rutter, 1977; Steffenburg et al., 1989; Bailey et al., 1995]. In addition, studies of autistic proband families have found autism in about 2.7% of their siblings. Accounting for stoppage rules (i.e., the increased tendency for parents of autistic children to have fewer children following their first autistic child) leads to a recurrence risk of around 8.6% [Ritvo et al., 1989]. Assuming the prevalence rate of autism in the general population is between 5 and 17 per 10,000 [Fombonne, 1999; Gillberg and Wing, 1999; Chakrabarti and Fombonne, 2001], the sibling relative risk ranges from 50 to 175. This makes the familiality of autism very many times higher than many other psychiatric disorders (e.g., schizophrenia), which are also believed to have strong, but complex, non-Mendelian genetic determinants.

Despite evidence for genetic transmission in autism, progress in identifying genes involved in autism has

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likely been hampered by both variable expressivity and etiologic heterogeneity. Variable expressivity, i.e., the same causal factors leading to a range of phenotypes, is supported by family and twin studies, suggesting a genetic relationship between autism and other phenomenologically related disorders and traits [Bailey et al., 1998]. While the boundaries of the autism-related phenotype are not fully delineated, at least partially included within them are not only relatively well-defined and still potentially rather severe conditions (e.g., Asperger disorder) [DeLong and Dwyer, 1988], but also milder deficits and traits associated with the three core autism domains: social/interpersonal, language/communication (both verbal and nonverbal components), and repetitive behaviors/stereotyped patterns [Landa et al., 1992; Bolton et al., 1994; Piven et al., 1994, 1997].

Etiologic heterogeneity, i.e., the same disorder arising from several independent causes, is evident in the small proportion of autism cases associated with a variety of other known genetic conditions (e.g., fragile X, tuberous sclerosis, phenylketonuria [PKU]) [Bailey et al., 1993; Rutter et al., 1994] and is likely too among the vast majority of autism cases lacking such conditions. Etiologic heterogeneity in these latter cases suggests a vicious circle: undetected, it reduces the power to identify any given genetic locus in molecular genetic investigations, yet definitive evidence for etiologic heterogeneity requires knowledge of specific genes or other causes involved.

Indirect methods, however, are available, which may help detect and characterize etiologic heterogeneity in autism. Specific but variable features of the illness that are more similar in affected relatives than in unrelated cases may be phenotypic markers for either different causes of the disorder as a whole or specific but variable clinical components of the disorder that partially contribute to the emergence of autism. A focus on the concordance of delayed speech in autism, for example, substantially increased more modestly positive evidence for linkage at loci on chromosomes 2 and 7 [Bradford et al., 2001; Buxbaum et al., 2001]. In the present study, we tried to determine whether intra-familial similarity for key autism clinical features is greater than interfamilial similarity. Such findings would be consistent with etiologic heterogeneity and would suggest that selecting comparable families for the feature identified may strengthen the power of genetic investigations by identifying more homogeneous subgroups. This approach has been used before on clinical symptoms in a small number of studies with relatively modest samples [Spiker et al., 1994; Le Couteur et al., 1996; Szatmari et al., 1996; MacLean et al., 1999], but positive results have been sparse and inconsistent. In the present study, we report on 212 sibships with at least one autistic proband and another sibling with either autism or an autism-related condition, a sample size approximately four times larger than the largest study to date [MacLean et al., 1999] and with far more power to identify familiarity in specific symptom domains.

MATERIALS AND METHODS

Ascertainment of Families

Opportunistic recruitment of multiple-incidence families for family/genetic studies of autism and related disorders began in 1994 at the Seaver Autism Research Center in the Mount Sinai School of Medicine. Families were ascertained from Ireland (by B.A.L. and M.F.) and the United States. Many of the U.S. families were ascertained and studied in collaboration with the Autism Genetic Research Exchange (AGRE) and then reviewed by our group. Families with a member known to have a medical condition associated with autism (e.g., fragile X, tuberous sclerosis, PKU) were excluded.

Assessment of Family Members

With parents providing written informed consent, we assessed nuclear family members suspected to have autism or autism-related deficits or developmental delays on the basis of parental report. For such children, we administered the Autism Diagnostic Interview, Revised (ADI) [Lord et al., 1994]. The ADI is a standardized, investigator-based semistructured instrument useful in the differentiation of autistic from nonautistic mentally disabled individuals with a mental age of 18 months or greater. It employs a diagnostic algorithm based on ICD-10 criteria, with application to DSM-IV criteria, too. The ADI is conducted with the subject's primary caregiver, usually the mother, and requires approximately 1–3 hr to complete. In addition to a demographic and early development section, the interview is divided into three sections and used to score impairments in three domains: communication, with separate verbal and nonverbal subdomains; qualitative impairments in reciprocal social interaction (hereafter called social); and repetitive behaviors and stereotyped patterns (hereafter called repetitive behavior). In those with functional and spontaneous phrase speech (hereafter called useful phrase speech and defined as ADI item 19 "level of language" score = 0), the communication domain comprises both the nonverbal and verbal subdomain scores. In individuals without useful phrase speech, only the nonverbal subdomain is used. Within each domain there are four categories (the verbal and nonverbal communication subdomains each have two) scored by totaling associated ADI items. An ADI algorithm diagnosis of autism depends on the onset of symptoms and each domain score reaching or exceeding a threshold level. The communication score threshold and the group of items used for scoring varies slightly depending on whether the individual has useful phrase speech.

Most assessments were conducted by ADI raters (including C.J.S., P.G., and K.D.) trained by Dr. Catherine Lord at the University of Chicago. Additional raters were trained by C.J.S., who has had extensive additional training with Dr. Lord and is credentialed as an offsite trainer and a reliable rater against which another's reliability can be assessed. All raters achieved reliability of better than 90% with either Dr. Lord's group or C.J.S.

Diagnosis of Autism and Related Conditions

An ICD-10/DSM-IV diagnosis of autism was established using the ADI diagnostic algorithm. Beyond the diagnosis of autism, we wanted to identify and classify family members who did not meet criteria for autism, but who had autism-related disorders or deficits. While the ADI was designed as a diagnostic tool for autism, the information collected identifies autism-related deficits even when full criteria for autism is not met. The ADI may lack sensitivity for some autism-related phenotypes, but the characteristics identified by the ADI are related to autism at least on a phenomenological level. Thus, to assess deficits among siblings who did not meet full criteria for autism but had autism-related deficits, we established a classification hierarchy. Borderline autism (previously developed and called “not quite autism” by other investigators [International Molecular Genetic Study of Autism Consortium, 1998]) was given to those who failed to meet the ADI algorithm criteria for autism by no more than one point in the social domain and either the communication or repetitive behavior domain but not both, or alternatively those with all three domains above threshold who did not meet the onset criterion. Asperger disorder was given to those who did not meet criteria for either autism or borderline autism but met DSM-IV criteria for Asperger disorder. Two other successively lower categories were also used: autism spectrum and autism-related developmental deficits. For the autism spectrum category, we identified individuals who met ADI autism onset criteria and had just marginally subthreshold deficits or greater in the social domain and either had marked deficits in both of the other two domains, or had just marginally subthreshold deficits (i.e., stronger than marked) or greater in one other domain. Thus, for this category, we required an ADI social domain score ≥ 9 (one below the autism threshold), onset ≥ 1 and either (a) communication score ≥ 4 (if verbal) or ≥ 3 (if nonverbal) and a repetitive behavior score ≥ 1 or (b) a communication score ≥ 7 (if verbal) or ≥ 6 (if nonverbal) or a repetitive behavior score ≥ 2 . Finally, in order to identify individuals with some notable autism-related developmental deficits in at least one domain, we required that ADI onset criteria be met and one or more of the following: social score of ≥ 4 ; communication score ≥ 5 (if verbal) and ≥ 4 (if nonverbal); or repetitive behavior score ≥ 1 .

Statistics

Evidence for familiarity was defined as significantly reduced variability by analysis of variance and covariance (F-statistic) in autistic-related domains within sibblingships. Family membership was treated as a random-effect independent variable. For features found to be familial, intraclass correlation coefficients were calculated to measure the extent of the similarity within families. Sex and age were entered as covariates when they were significantly associated with the dependent variable. Conventional *t*- and chi-square statistics were also employed.

To limit spurious results arising from multiple testing, we used a “protected drill down” rule: only in domains or communication subdomains providing statistically significant ($P < 0.05$, two-tailed) evidence for familiarity were the specific categories that comprise the higher-level score examined. Similarly, familiarity with respect to individual ADI items was examined only if the higher-level category score suggested familiarity at a significant level. In this way, we tried to identify the specific underlying factors that might be driving the higher level result without casting an overly broad net. Two key ADI items concern if and when useful phrase speech developed. Item 19, level of language, classifies the subject for useful phrase speech and determines whether verbal communication items are used in the diagnostic algorithm. Item 13, age at phrase speech, assesses the age (in months) when phrase speech first arose. The presence of language difficulties has been previously suggested for increasing homogeneous subgroups in autism [Folstein et al., 1999] and the familiarity of each of these two items was considered without preconditions. Siblings without phrase speech by interview time were coded by their current age (months) if < 6 years, or 72 months if > 6 years.

RESULTS

Families Ascertained, Cases Identified

We assessed 238 multiple-incidence families (25 from Ireland, 213 from the United States) and completed 523 ADIs. Excluding families where the affected pairs were not first-degree siblings (e.g., MZ twin pairs, cousin pairs, half-sibling pairs) left 212 families, called broadly defined multiply affected sibblingships, with two or more affected siblings ($n = 457$; males = 355 (78%); mean age = 9 ± 6) in which at least one sibling met criteria for autism and another met criteria for either autism or a related condition. Table I shows for each classification, descriptive statistics for the domains, communication subdomains, and the phrase speech items. Twenty-one families (10%) had more than two affected siblings (three: $n = 19$; four: $n = 1$; six: $n = 1$). For brevity, the term *multiplex sibblingship* here refers to only the affected cases in a sibblingship.

Within the broadly defined multiplex sibblingships, there were 136 autism multiplex sibblingships, i.e., sibblingships with two ($n = 129$) or more (three: $n = 6$; four: $n = 1$) cases of ADI-diagnosed autism. Although some of these sibblingships ($n = 10$) in the subgroup also included other members with other autism-related conditions ($n = 12$), unless otherwise indicated, analyses with autism multiplex sibblingships included only siblings with autism ($n = 289$; males = 223; 77%; mean age = 8 ± 5).

Age and Sex Associations With Clinical Features

With the unsurprising exception of age with age at phrase speech (broadly defined multiplex sibblingships: $r = 0.28$; autism multiplex sibblingships: $r = 0.20$),

TABLE I. ADI Domain, Subdomain, and Phrase Speech Item Scores in Autism and Autism-Related Disorders

Domain/Subdomain/ phrase speech items	Autism (n = 364)	Asperger disorder (n = 18)	Borderline autism (n = 22)	Autism spectrum (n = 23)	Autism related developmental deficits (n = 80)	Total (n = 457)
Qualitative impairments in reciprocal social interaction	22.1 ± 5.1	10.3 ± 8.9	18.4 ± 5.6	16.5 ± 5.9	5.0 ± 2.7	20.1 ± 7.0
Repetitive behaviors and stereotyped patterns	6.3 ± 2.2	4.7 ± 2.2	2.6 ± 1.8	2.2 ± 3.0	3.1 ± 2.7	5.6 ± 20.1
Communication	15.5 ± 3.5	8.9 ± 4.0	11.8 ± 3.0	10.4 ± 4.8	6.5 ± 4.2	14.3 ± 4.5
Nonverbal communication	11.0 ± 2.8	4.4 ± 3.7	9.1 ± 3.4	7.9 ± 4.5	3.5 ± 3.2	10.0 ± 3.4
Verbal communication ^a	6.4 ± 2.2	4.5 ± 1.4	4.3 ± 2.4	3.3 ± 2.0	3.1 ± 2.1	5.7 ± 2.4
Useful phrase speech	0.7 ± 0.9	0 ± 0	1.0 ± 1.0	0.7 ± 0.8	0.1 ± 0.4	0.7 ± 0.9
Age (months) at phrase speech ^b	52 ± 20	23 ± 8	49 ± 14	51 ± 14	40 ± 17	50 ± 20

^aThe samples used for verbal subdomain included only those cases (autism: n = 207; Asperger: n = 18; borderline autism: n = 10; autism spectrum disorder: n = 11; and autism related developmental deficits: n = 28) with useful phrase speech (level of language = 0).

^bAge at phrase speech for those who never achieved it was counted as current age (in those younger than 6 years) and 72 months in those age 6 or more.

correlations for clinical features with age or sex were all < 0.20. Nevertheless, these factors were entered as covariates when the correlation was statistically significant.

Familiarity of Domains

Familiarity among broadly defined multiplex siblingships was observed for repetitive behavior and level of language and age at phrase speech, but not for the social and communication domains or for verbal or nonverbal communication subdomains (Table II). Using the “protected drill down” rule, the repetitive behavior category scores were examined and significant differences were found for encompassing preoccupation/circumscribed patterns of interest and apparently compulsive adherence to nonfunctional routines/rituals. All the ADI items contributing to these categories were significant. Intraclass correlations for these significant variables ranged from 0.12 to 0.26 (Table II).

Among the autism multiplex siblingships, significant differences between families were observed for repetitive behavior, nonverbal communication, level of language, and age at phrase speech (Table II). For repetitive behavior, the same two categories and ADI item scores were identified as significant as in the previous analysis. For nonverbal communication, the category “lack of/delay in spoken language and failure to compensate through gesture” was significant, as were three of the four ADI items associated with this category. Intraclass correlations in the autism multiplex siblingships for the significant variables ranged from 0.14 to 0.34 (Table II). The evidence for familiarity was unchanged when we controlled for different raters or the source of the family.

To show the patterns of score in siblingships for familial and nonfamilial features, the repetitive beha-

viour score in autism siblings and the social domain score are plotted in Figure 1. What is key in these plots is the proximity of the points in vertical alignment with each other as they represent scores within multiplex siblingships. To better discern these patterns, for each domain the siblingships are ordered along the X-axis first by the lowest-scoring sibling and then by the within-siblingship mean. For repetitive behavior, extended troughs, indicating similar within-siblingship scores, are evident across the X-axis, while no pattern of within-siblingship similarity is evident for the social domain.

Domain Levels in Autism and Autism-Related Conditions in Siblingships With Both

As discussed later, because the autism-related disorders had by definition subthreshold scores, the intraclass correlations were likely to be reduced for the broadly defined multiplex siblingships. To investigate more directly whether the autism-related disorders show familiarity for the autism clinical features, we next examined the relative consistency of the domain score between cases of autism and those with related conditions in the same family. As displayed in Figure 2, siblingships (n = 84) with at least one sibling with autism (n = 94) and at least one sibling with other related conditions (n = 93 siblings) were ranked for each domain and subdomain score independently, solely by the autism cases in these families. For each ranking, the families were classified as falling in the high, middle, or low tertiles (third). Siblingships whose cases of autism fell within only the middle tertile on the given domain or subdomain of interest as well as siblingships with multiple cases of autism at different tertiles were then excluded. Finally, domain scores (and specific category and item scores using the “protected drill down” rule) were compared in siblings

TABLE II. Familiarity in Levels of ADI Domain, Subdomain, Category, Language, and Individual Item Scores in Broadly Defined and Autism Multiplex Siblingships

	Broadly defined multiplex siblingships			Autism multiplex siblingships		
	F (214,239) ^a	<i>P</i>	ICC ^b	F (139,145)	<i>P</i>	ICC
Qualitative impairments in reciprocal social interaction	0.82	NS		1.00	NS	
Repetitive behaviors and stereotyped patterns	1.45	< 0.005	0.18	2.06	< 0.001	0.34
D1, encompassing preoccupation/circumscribed pattern of interest	1.76	< 0.001	0.26	1.87	< 0.001	0.30
70, circumscribed interest	1.62	< 0.001	0.23	1.79	< 0.001	0.28
71, unusual preoccupation	1.38	< 0.01	0.15	1.59	< 0.005	0.22
D2, apparently compulsive adherence to nonfunctional routines/rituals	1.47	< 0.005	0.18	1.92	< 0.001	0.31
25, verbal rituals	1.35	0.01	0.14	1.47	< 0.01	0.19
75, compulsion/rituals	1.36	0.01	0.15	1.68	< 0.001	0.25
D3, stereotyped and repetitive motor mannerisms	0.96	NS		1.23	NS	
D4, preoccupation with parts of objects or nonfunctional elements of materials	0.61	NS		0.83	NS	
Communication	1.05	NS		1.13	NS	
Nonverbal communication	1.05	NS		1.48	< 0.01	0.19
C1, lack of/delay in spoken language and failure to compensate through gesture	NA			1.69	< 0.001	0.25
30, pointing to express interest	NA			1.75	< 0.001	0.28
31, conventional instrumental gestures	NA			1.40	< 0.05	0.16
32, nodding	NA			1.06	NS	
33, headshaking	NA			1.59	< 0.005	0.22
C4, lack of varied spontaneous make-believe or social imitative play	NA			1.25	NS	
Verbal communication ^c	1.06	NS		0.96	NS	
Useful phrase speech	1.38	< 0.01	0.15	1.33	< 0.05	0.14
Age at phrase speech	1.45	< 0.005	0.18	1.67	< 0.001	0.25

^aDegrees of freedom may vary by one to two degrees depending on whether age and sex was used as a covariate.

^bICC = intraclass correlations: derived from the F-statistic when the *P* value for this statistic was at least a trend level (*P* < 1.0) or significant.

^cSamples are reduced for this subdomain because only those families with multiple cases having useful phrase speech were included.

with autism-related conditions grouped by their relationship with high versus low tertile autism cases with respect to the same domain score.

Repetitive behavior and nonverbal communication were both significantly elevated in the autism-related disorder cases from families with autism cases who themselves were elevated in these respective areas (Table III). In addition, the same three of six categories providing earlier evidence of familiarity in the autism multiplex siblingships were identified as increased (one at a trend level) in autism-related condition subjects with autism siblings elevated in the associated domain or subdomain. Among the six specific ADI items associated with those categories reaching full statistical significance, four of them were increased (at least at a trend level) in the autism-related condition siblings associated with the high-tertile autism siblingships. Three of these four (not nodding) had previously provided evidence of familiarity in the autism multiplex siblingships. For useful phrase speech, we identified siblingships in which all autistics had a level of langu-

age score of either 0 or 2 (in a scale of 0, 1, and 2 points) and found useful phrase speech to be significantly less likely in the autism-related condition siblings of autistic cases lacking useful phrase speech. Finally, the group with autistic siblings whose age at phrase speech was in the high tertile had themselves significantly more delayed phrase speech than the group with autistic siblings in the low tertile for this feature.

Given these results, we wondered if low (or high) tertile siblingships in one domain were similarly in the low (or high) tertiles in the others. In other words, do siblingships appear to have a general severity level that is similar across the familial features identified or do the severity levels for these features vary within a siblingship? Table IV provides the number of times siblingships were ranked in the low or high tertile for the four identified familial features and shows that siblingship rankings mostly did not overlap. About two-thirds of the siblingships were ranked at least once in the low tertile and two-thirds were ranked at least once in the high tertile. At the same time, more than one-half

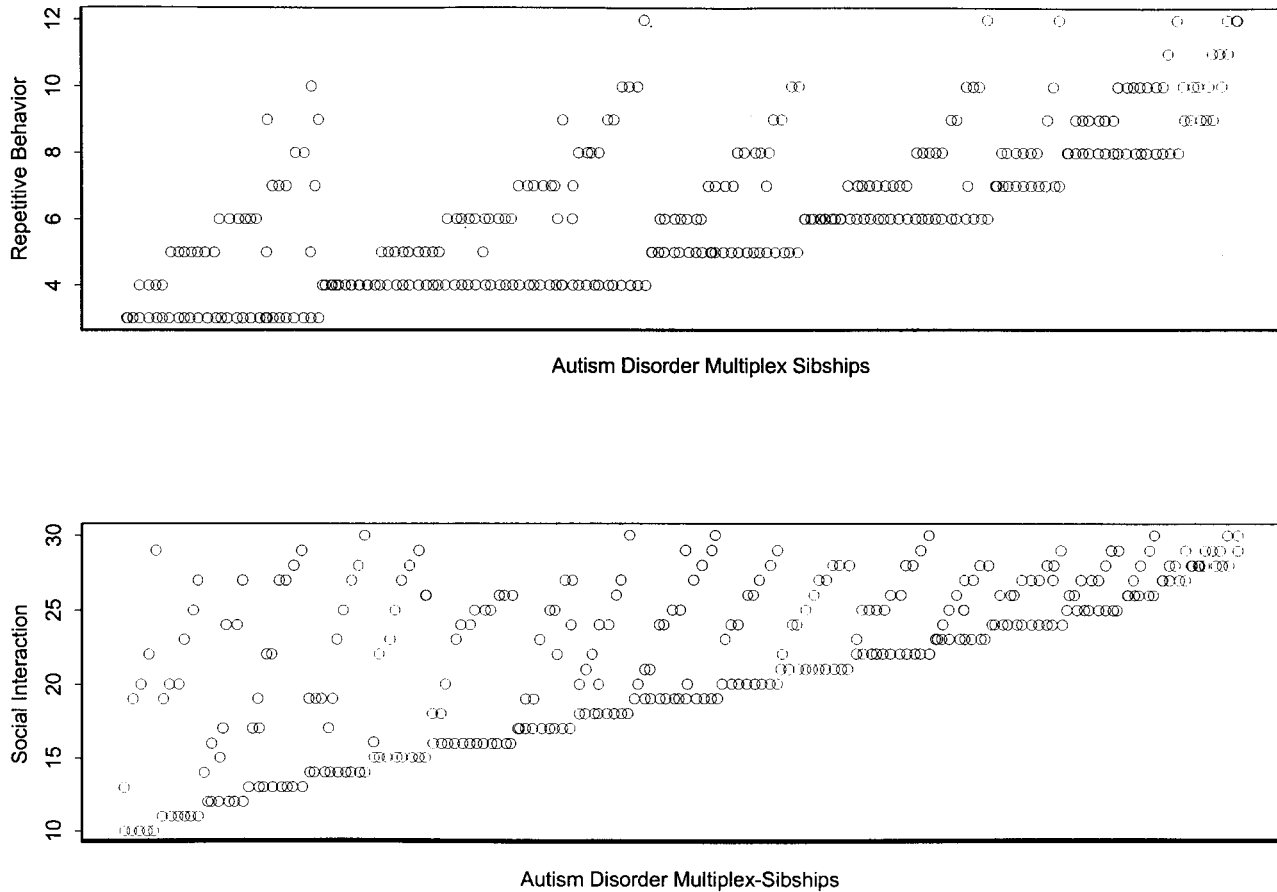


Fig. 1. Distribution of within-siblingship scores (in vertical alignment) for the repetitive behavior and social domains among autism multiplex sibships. The sibships are ordered for visual clarity along the X-axis first by lowest-scoring sibling and then by sibship mean score. Domain scores are slightly jittered horizontally to reveal identical scores when

present in a siblingship. For repetitive behavior, scanning vertically shows within-siblingship scores tend to be similar, indicating familiality. In contrast, the within-siblingship social domain scores are unrelated, indicating the absence of familiality.

were so ranked only in one of the four familial features. We also examined whether there might be significant overlap for any of the specific pairwise combinations. Of the 12 different possible pairwise comparisons (6 for upper tertile and 6 for lower tertile), only the sibships included in the high-tertile age at phrase speech ($n = 27$) and nonverbal communication groups ($n = 20$) were significantly associated (overlap: $n = 13$; chi-square = 12.99, $df = 1$, $P < 0.001$). This sole association was not surprising since delayed speech is a prerequisite to one of the two categories in the nonverbal subdomain.

DISCUSSION

The results provide evidence for reduced variation within families for several key clinical areas associated with autism: severity of repetitive behaviors, the level of deficits in nonverbal communication, the presence of phrase speech, and the age at phrase speech. The categories that drove the repetitive behavior finding were those associated with circumscribed interests, unusual preoccupations, verbal and nonverbal rituals

and compulsions, i.e., symptoms characteristic of obsessive-compulsive disorder; the others involve lower-level stereotypes and a focus on part objects and are perhaps more related to optimizing the level of stimulation.

In contrast to the autism multiplex sibships, using the more broadly defined multiplex sibships led to more attenuated results. This follows, however, from our inclusion requirement of at least one case of autism in the multiplex sibship. When autism is paired with a related disorder, by definition possessing below threshold deficits, the opportunity for scores to be similar is limited. An autism/autism-related disorder multiplex sibship will be most similar when an autistic case has a relatively low score (i.e., just above the threshold level) and an autistic related case has a relatively high score (typically just below the threshold level). An autism/autism-related disorder multiplex sibship in which the autistic had a high score relative to other autistic cases and the other sibling had a high score relative to other autism-related disorder cases (but still low relative to autistic cases) would tend to lower the intraclass correlations, even though in a

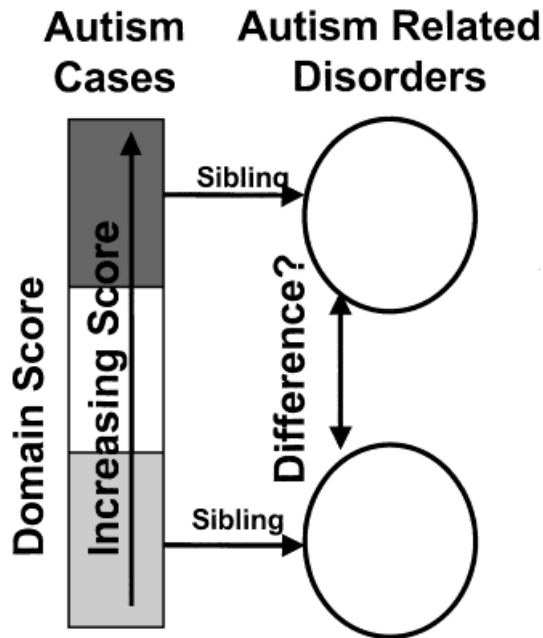


Fig. 2. Design to examine familiarity in siblings of autism probands with autism-related conditions. Autism case tertiles defining the two sibling groups with autism-related conditions were established independently for each domain, communication subdomain, and language variable score.

key sense the familial relationship we are examining would appear to be present: both score high compared with their own reference group.

Hence, the question as to whether the familiarity in core autism extends to related conditions was better and more directly approached using a strategy that strengthened the opportunity to identify familiarity across these disorders if indeed it was present. Interestingly, all the same and only the same symptom domains and language items that showed familiarity in autism when studied alone also showed familiarity in the less severe but phenomenologically related disorders. Nor did these results appear to be attributable to some more broadly defined severity factors extending across symptom domains. Instead, the low level of overlap of siblingships with high (or low) levels across symptom domains or the language variables suggests that the level of these clinical features are largely independent of each other and may have separate underpinnings.

To our knowledge, only two independent collections of siblingships and one series of monozygotic twins have been similarly investigated. In a study of 37 multiple-incidence autism siblingships, few factors showed reduced variance within siblingships (nor did IQ or phrase speech by age 5), but evidence for familiarity was most commonly observed among characteristics in the repetitive behavior domain, especially those related to rituals and repetitive play [Spiker et al., 1994]. A second study, using a broader classification system, examined 46 sibling pairs and found IQ and levels of adaptive socialization and communication showed the most reduced within-siblingship variability [MacLean

et al., 1999]. However, among clinical features measured by the ADI, they found useful phrase speech status (ADI item 19) and the nonverbal communication subdomain were also significantly familial, but not repetitive behaviors or the other domains. Finally, 15 MZ twins and 1 MZ triplet concordant for autism-atypical autism showed increased within-twinship similarity for nonverbal IQ, but not for verbal IQ or a total ADI composite score (comparisons on the individual domains were not reported) [Le Couteur et al., 1996].

Thus, no measure (including IQ, useful phrase speech, overall symptom severity, or any specific autistic domain) has been consistently found to have reduced variance within families. In light of the present study, however, this lack of consistency across studies may be explained by their modest size, reducing statistical power. Here, using a substantially larger sample and focusing exclusively on the hallmark clinical features of autism (IQ assessments and adaptive behavior measures are currently being conducted in our sample), the three most positive findings related to autism domains (repetitive behaviors [Spiker et al., 1994] and nonverbal communication [MacLean et al., 1999]) and the language distinction [MacLean et al., 1999] were replicated. Similar to the twin study, there was no evidence for familiarity using the composite ADI total score (data not shown), but the rather different scoring ranges for each domain measured led us to exclude this score from our primary analyses at the outset. Hence, with respect to the clinical symptoms of autism, this study confirms and ties together all the major but formerly disparate findings in the smaller, earlier studies. Furthermore, while two of the earlier studies included siblings with subthreshold autism conditions, this is the first study to examine directly whether the patterns observed with autism multiplex siblingships similarly hold for autism-related disorders. Finally, the fourth familial feature identified, age at phrase speech, was not previously studied for familiarity, but a closely related distinction (speech delay beyond 36 months) has recently led to increased evidence for linkage on chromosome 7q and chromosome 2 [Bradford et al., 2001; Buxbaum et al., 2001]. The present results support the validity of linkage strategies making such family distinctions for stratified analysis and suggests that similarly useful strategies might be independently employed for repetitive behavior, deficits in nonverbal communication, and the overall absence of phrase speech.

Even the features with the strongest within-siblingship correlations were of moderate strength or less. However, weak correlations do not always imply unimportant sources of influence [Abelson, 1985], nor does it mitigate evidence for heterogeneity. If, for example, the presence or absence of a disease-associated allele for a gene leads to modest but detectable differences in a disease-associated trait, the crucial aspect of the difference is how detectable it is, not how slight; stratifying families by those trait differences, however modest, would provide increased homogeneity

TABLE III. ADI Domain, Subdomain, Category, Language, and Item Scores in Autism-Related Disorder Cases in Families With Autistic Cases in the Low- and High-Domain Tertile

	Low autism tertile		High autism tertile		Test	
	n (cut scores) ^a	mean \pm SD	n (cut scores)	mean \pm SD	<i>t</i> (df)	<i>P</i>
Qualitative impairments in reciprocal social interaction	25 (< 21)	13.0 \pm 6.7	20 (> 25)	13.3 \pm 8.6	-0.13 (43)	NS
Repetitive behaviors and stereotyped patterns	20 (< 5)	2.1 \pm 2.0	23 (> 7)	3.7 \pm 2.9	-2.08 (41)	< 0.05
D1, encompassing preoccupation/circumscribed pattern of interest	20 (< 5)	0.5 \pm 0.8	23 (> 7)	1.1 \pm 1.2	-1.96 (41)	0.06
D2, apparently compulsive adherence to nonfunctional routines/rituals	20 (< 5)	0.3 \pm 0.6	23 (> 7)	1.0 \pm 1.4	-2.16 (41)	< 0.05
25, verbal rituals	20 (< 5)	0.2 \pm 0.5	23 (> 7)	0.4 \pm 0.7	-1.04 (41)	NS
75, compulsion/rituals	20 (< 5)	0.1 \pm 0.3	23 (> 7)	0.6 \pm 0.8	-2.73 (41)	< 0.01
D3, stereotyped and repetitive motor mannerisms	20 (< 5)	0.6 \pm 0.8	23 (> 7)	0.7 \pm 0.9	-0.58 (41)	NS
D4, preoccupation with parts of objects or nonfunctional elements of materials	20 (< 5)	0.8 \pm 0.8	23 (> 7)	0.9 \pm 0.9	-0.47 (41)	NS
Communication	20 (< 14)	8.1 \pm 4.3	27 (> 16)	9.8 \pm 4.0	-1.21 (45)	NS
Nonverbal communication	24 (< 11)	5.5 \pm 3.1	27 (> 13)	8.2 \pm 5.2	-2.18 (49)	< 0.05
C1, lack of/delay in spoken language and failure to compensate through gesture	24 (< 11)	2.2 \pm 2.1	27 (> 13)	4.4 \pm 3.6	-2.73 (49)	< 0.01
30, pointing to express interest	24 (< 11)	0.7 \pm 0.9	27 (> 13)	1.0 \pm 1.0	-1.29 (49)	NS
31, conventional instrumental gestures	24 (< 11)	0.4 \pm 0.8	27 (> 13)	1.2 \pm 0.9	-3.26 (49)	< 0.005
32, nodding	24 (< 11)	0.5 \pm 0.8	27 (> 13)	1.1 \pm 0.9	-2.45 (49)	< 0.05
33, headshaking	24 (< 11)	0.5 \pm 0.9	27 (> 13)	1.0 \pm 1.0	-1.93 (49)	0.06
C4, lack of varied spontaneous make-believe or social imitative play	24 (< 11)	3.3 \pm 1.9	27 (> 13)	3.7 \pm 2.1	-0.64 (49)	NS
Verbal communication ^b	7 (< 6)	3.7 \pm 2.4	10 (> 7)	3.1 \pm 1.9	0.59 (15)	NS
Useful phrase speech ^c	38 (0)	0.2 \pm 0.6	32 (2)	0.8 \pm 0.9	-3.10 (68)	< 0.005
Age (months) at phrase speech	24 (< 44)	33 \pm 11	33 (\geq 72)	52 \pm 19	-4.38 (55)	< 0.001

^aADI domain and subdomain scores used to identify approximate low and high tertiles in autism cases with autism-related disorder siblings.

^bRequiring that only families with all autistic cases having useful phrase speech led to a relatively reduced number of autism related disorder cases.

^cThe restricted range of the level-of-language item and the relatively few autism cases scoring 1 led to a larger number of autism-related disorder cases.

over using the entire sample and strengthen the opportunity to identify the locus.

Are there other plausible explanations beyond true familiarity for the results? Raters assessed all affected members within a family and cases were ascertained in two different countries. However, controlling for these two factors did not change the evidence for familiarity. In addition, with respect to repetitive behaviors, our data replicate an earlier study that used different raters within families [Spiker et al., 1994]. More

problematic is the possibility that interviewed mothers, in describing their affected children, provide inaccurately similar clinical pictures. Such a bias cannot be discounted, but we note, as others have [MacLean et al., 1999], that, if anything, mothers generally appear rather to accentuate the differences between their affected children. Furthermore, a maternal reporting bias, if present, would appear to have surprising specificity for certain domains and not others. For one consistent finding across siblingship studies is that

TABLE IV. Of the Four Familial Features, the Number of Times Mixed (i.e., Autism Plus Autism-Related Disorder) Siblingships (n = 84) Were Ranked in the Low and High Tertiles

Tertile	Siblingships ranked more than one times (ever ranked), n (%)	Ever ranked siblingships classified by the number of times ranked			
		1, n (%)	2, n (%)	3, n (%)	4, n (%)
Low	57 (68)	36 (63)	16 (28)	5 (9)	0 (0)
High	58 (69)	33 (57)	12 (21)	13 (22)	0 (0)

there is little to no evidence of familiarity in the social domain or the verbal communication subdomain. Finally, on a heuristic basis, we again note that stratifying by a concordant language feature determined by maternal report with one rater has led to promising molecular genetic results [Buxbaum et al., 2001].

Beyond genetic factors, other unknown groupings that tend to work in a family-wise manner may explain our results. Increased within-family exposure to specific phenotypic-affecting factors (such as, perhaps, treatment) constitutes true familiarity, but of a form due to environmental rather than genetic factors. Such exposures, however, would have to account for the familiarity of these features in the siblings with more mild autism-related phenotypes and for the particular and independently identified domains observed. Nevertheless, highlighting such alternative possibilities underscores the point that familiarity cannot be interpreted as providing direct evidence for different genes operating in different families. That requires the identification of a gene or tightly linked genetic marker that varies across families.

Whether of genetic or nongenetic origin, these findings suggest that specific symptom dimensions (i.e., repetitive behavior, nonverbal communication, useful phrase speech, and age at phrase speech) tend to run within families and independently so, both in subjects affected with autism and those with related conditions. Assuming that genetic heterogeneity is present in autism, our results suggest that making distinctions with regard to the severity of these symptom areas may identify more homogeneous groups for molecular genetic studies.

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APPENDIX

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