ORIGINAL ARTICLE

Similar White Matter Aberrations in Children With Autism and Their Unaffected Siblings

A Diffusion Tensor Imaging Study Using Tract-Based Spatial Statistics

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Context: Autism is a neurobiological condition with a strong genetic component. Recent diffusion tensor imaging (DTI) studies have indicated that white matter structure is aberrant in autism. To date, white matter structure has not been assessed in family members of children with autism.

Objective: To determine whether white matter structure is aberrant in children with autism and their unaffected siblings compared with controls, and to test the hypothesis that white matter structure in autism is correlated with autism spectrum symptomatology.

Design: Cross-sectional, case-control, voxel-based, whole-brain DTI analysis using Tract-Based Spatial Statistics.

Setting: University research center.

Patients: A sample of 37 children: 13 subjects with autism, 13 of their unaffected siblings, and 11 controls. Controls were age- and intelligence quotient–matched to the unaffected siblings; all groups were age matched.

Main Outcome Measure: Fractional anisotropy (FA) and axial and radial diffusivities. In addition, behavioral correlation analyses were conducted using the Autism Diagnostic Interview and Autism Diagnostic Observation Schedule subscales and FA values, as well as axial diffusivity values in the autism group.

Results: Compared with the control group, both the autism and sibling groups had widespread, significantly reduced white matter FA values ($P \leq .05$, corrected) in the frontal parietal and temporal lobes and included, but were not restricted to, regions known to be important for social cognition. Within regions of reduced FA, significant reductions in axial diffusivity, but not radial diffusivity, were observed. There were no significant differences in white matter structure between the autism and sibling groups. There were no significant correlations between autism symptomatology and white matter FA or axial diffusivity.

Conclusions: Our findings suggest that white matter structure may represent a marker of genetic risk for autism or vulnerability to development of this disorder.

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AUTISM IS A NEUROBIOLOGICAL condition with a strong genetic component[1] characterized by impairments in social interaction and communication as well as restricted, repetitive, and stereotyped patterns of behavior, interests, and activities.[2] It is classified in the *DSM-IV TR* as a pervasive developmental disorder, a categorization that also includes Asperger syndrome, Rett syndrome, childhood disintegrative disorder, and pervasive developmental disorder not otherwise specified. Family studies have indicated that concordance rates for autism are 2% to 6% for siblings and dizygotic twins and approximately 60% for monozygotic twins.[3-5] When including the broader autism phenotype, that is, individuals with some impairments in social interaction who do not meet the full criteria for diagnosis of autism,[3,6-8] the number of affected siblings of probands with autism increases to 10% for siblings and dizygotic twins and up to 90% for monozygotic twins.[1,3] Still, most siblings of probands with autism do not have any symptoms of autism despite sharing, on average, 50% of their genes and many environmental influences with their siblings with autism. By studying a population of siblings who are discordant for autism, we gain greater experimental control of the genetic and environmental factors that influence brain development.

Recent studies have suggested that brain connectivity is aberrant in autism.[9-14] Brain connectivity is a general term that can refer to a number of underlying brain properties such as signal transduction and in-

formation transfer at rest or during tasks, the physical structure of neurons, myelin, synapses, and the organization of brain pathways. Minimally invasive studies using functional magnetic resonance imaging (MRI), positron emission tomography, electroencephalography, functional connectivity MRI, and diffusion tensor imaging (DTI) are particularly helpful for providing information about the origins and localization of aberrant brain connectivity in autism.

Early evidence of aberrant white matter structure in autism was found in postmortem and structural MRI studies that showed abnormal white matter volume and development, microscopic evidence of neuroinflammation, and atypical neuronal and minicolumnar structure.[9,11,12,15] Functional MRI analyses have found disrupted functional connectivity in networks important for social emotional and face processing as well as in regions known to be important for social cognition, language processing, and executive function.[10,13,14,16,17] Further, electroencephalographic studies in autism have suggested reduced synchronization,[18] dysfunctional integration of frontal and posterior brain regions, and locally elevated coherence in left hemisphere frontal and temporal regions but globally reduced coherence within frontal regions and between frontal and all other scalp regions.[19]

Diffusion tensor imaging is a noninvasive MRI technique that is useful for investigating white matter structure, an important aspect of brain connectivity. Previous DTI studies using region-of-interest analyses, fibertracking, and whole-brain statistical parametric mapping analyses have also reported white matter differences in children, adolescents, and adults with autism compared with controls.[20-29] The current knowledge culled from these DTI studies suggests that white matter structure maturation is altered in autism from a very early age and that aberrant white matter structure may persist into adulthood. The most consistent differences have been found in the corpus callosum, prefrontal white matter, cingulate gyrus, and superior temporal white matter. Thus, it appears that white matter across multiple brain regions is affected, particularly regions involved in social cognition, theory of mind, and sensory processing.[30-32] White matter structure in siblings of probands with autism has not been investigated.

Previous studies have suggested that individuals with autism share some aspects of brain structure and function as well as behavioral and immune profiles with their siblings who do not have autism.[33-39] In this study, we sought to investigate white matter structure in probands with autism as well as their unaffected siblings. We used Tract-based Spatial Statistics[40] (http://www.fmrib.ox.ac.uk/fsl/tbss/), a method specifically designed to allay concerns regarding other methods of whole-brain, voxel-by-voxel analyses of diffusion-weighted data by including reliable registration of subjects to a common space, avoiding the use of a smoothing kernel, minimizing partial volume effects, and using permutation statistics that are not dependent on normal distribution of data. Based on previous studies, we hypothesized that children with autism would have aberrant white matter structure in regions involved in social cognition and theory of mind and that these white matter structural aberrations would be correlated with social impairment severity. We further hypothesized that the unaffected siblings of children with autism would have an intermediate phenotype of white matter aberrations between the autism and control groups.

METHODS

SUBJECTS

The Stanford Institutional Review Board approved this study. Subjects with autism spectrum disorders (n=17), their unaffected siblings (n=17), and typically developing children (controls; n=18) were recruited for this study. These participants were a subset of a sample recruited for an study investigating children with autism and their unaffected siblings.

Sibling pairs discordant for autism were between the ages of 6 and 13 years, sex-matched, fewer than 3 years apart in age, and raised in the same household. Typically developing controls were age- and IQ-matched to the unaffected siblings. Subjects were recruited from the Stanford University Pervasive Developmental Disorders Clinic and the local community. Written informed consent was obtained from parents, and assent from children was obtained when applicable.

ASSESSMENTS

Parents of sibships filled out the Child Behavior Checklist[41] and the Edinburgh inventory for handedness[42] and provided demographic and ethnic information. Parents were interviewed using the Family History Interview for Developmental Disorders of Cognition and Social Functioning[43] for the unaffected siblings to identify any with the broad autism phenotype. Regardless of their original diagnosis (of an autism spectrum disorder), subjects were reassessed and included in the study if the diagnosis of autism was confirmed using the Autism Diagnostic Interview–Revised (ADI-R)[44] and the Autism Diagnostic Observation Schedule (ADOS).[45] Testing was performed by a trained examiner (L.J.L.). Subjects all tested negative for fragile X syndrome. In addition, they underwent a full physical examination by a licensed physician (L.J.L.) to rule out any identifiable genetic or neurologic disorders associated with autism.[46,47] All subjects underwent intelligence testing using the Wechsler Scale of Intelligence in Children, third edition.[48]

EXCLUSION CRITERIA

Sibships were excluded from the study if the unaffected siblings had evidence of certain medical conditions (including premature birth, neurologic or genetic disorders, or central nervous system injury), learning disability (special education services, diagnosis of specific learning disorders), developmental disorder (language and/or motor milestone delay), psychiatric disorder (including pervasive developmental disorder, obsessive-compulsive disorder, Tourette syndrome, or bipolar disorder), or a broader autism phenotype. Sibships also were excluded if the unaffected sibling had a Child Behavior Checklist factor score greater than 1.5 SD from the mean and a full-scale IQ less than 80. Sibships were excluded if the subject with autism had evidence of an identifiable neurologic or genetic disorder, mental age of less than 24 months, and did not meet threshold scores for autistic disorder on the ADI-R and ADOS, or if their height and weight were not within 2 SD of the reference range. Finally, sibships were excluded from the final analysis if one of them could not tolerate the scanning experience or if they had excessive movement artifacts in their scans.

Similar to the unaffected siblings' exclusion and inclusion criteria, control subjects were included in the study if they had a Child Behavior Checklist score of less than 1.5 SD from the mean, full-scale IQ higher than 80, no evidence of medical problems, learning disabilities, developmental disorders, or psychiatric symptoms. In addition, control subjects were included only if their height and weight were within 2 SD of the reference range and if they were able to remain still in the scanner.

IMAGING

All subjects were trained in the scanning procedure using a mock scanner. Subjects were not sedated for the scan; however, some scans were acquired late in the evening when the child was asleep. Magnetic resonance images were acquired using a GE-Signa 1.5-Tesla scanner (General Electric, Milwaukee, Wisconsin). A diffusion-weighted image sequence was based on a single-shot, spin-echo, echo-planar imaging sequence with diffusion sensitizing gradients applied on either side of the 180° refocusing pulse.[49,50] Imaging parameters for the diffusion-weighted sequence were field of view, 24 cm; matrix size, 128 × 128; echo time, 106 milliseconds; time to repetition, 6000 milliseconds; 19 axial-oblique slices; slice thickness, 5 mm; skip, 1.5 mm. The diffusion gradient duration was $\delta = 32$ milliseconds, and diffusion weighting was $b = 900$ s/mm^2. In addition, T2-weighted images were acquired by removing the diffusion sensitizing gradients. Diffusion was measured along 6 noncollinear directions: XY, XZ, YZ, –XY, –XZ and –YZ. This pattern was repeated 4 times for each slice with the sign of all diffusion gradients inverted for odd repetitions.

STATISTICAL ANALYSIS

The variables of interest included fractional anisotropy (FA) and radial and axial diffusivities. Fractional anisotropy is a measure that reflects the degree of diffusion anisotropy within a voxel (ie, how diffusion varies along different directions). Anisotropy within a given white matter voxel is determined by fiber diameter and density, degree of myelination,[51] extracellular diffusion, interaxonal spacing,[52] and intravoxel fiber-tract coherence.[53] Axial diffusivity is the diffusivity of water molecules along the axis of the fiber (largest diffusivity, $\lambda 1$), and radial diffusivity is the mean of the diffusivities perpendicular to the largest diffusivity ($\lambda 2 + \lambda 3/2$). Axial diffusivity has been shown to change with changes in fiber coherence, whereas radial diffusivity is thought to represent fiber integrity and myelinization.[54]

Diffusion-weighted images were corrected for eddy current distortions and head motion using linear image registration (Automated Image Registration algorithm).[55] DtiStudio[56] (https://www.mristudio.org/) was used to generate FA, axial, and radial diffusivity maps. First, all individual images were visually inspected to discard images with artifacts; no more than 2 images were discarded per direction within a slice. The remaining images were averaged; the pixel intensities of the multiple diffusion-weighted images were then fitted to obtain the 6 elements of the symmetric diffusion tensor. The diffusion tensors at each pixel were diagonalized to obtain pixel eigenvalues and eigenvectors. The FA, axial, and radial diffusivity values were calculated in DtiStudio for each voxel according to Basser and Pierpaoli[53] to produce FA, axial, and radial diffusivity maps. These maps were further processed using tract-based spatial statistics (Tract-based Spatial Statistics 1.2),[40] an automated, observer-independent, voxelwise, whole-brain, between-group analysis. Tract-based Spatial Statistics was implemented in FSL 4.1[57] (http://www.fmrib.ox.ac.uk/fsl/). Fractional anisotropy maps were analyzed first; FA values from each individual were coregistered using nonlinear registration (FNIRT in FSL; Analysis Group FMRIB, Oxford, England) to align every FA image to every other one. Data from this process were used to identify the most typical subject, which was then used as a target image. The target image was affine aligned to MNI152 standard space. Subsequently, all other subjects were nonlinearly transformed to the target and then affine transformed to the MNI152 space. After image registration, FA maps were averaged to produce a group mean FA image. A skeletonization algorithm was applied to the group mean FA image to define a group template of the lines of maximum FA. This skeleton corresponds to centers of white matter tracts; thus, it ignores voxels at the edges of tracts, which are susceptible to partial volume effects. Fractional anisotropy values for each individual subject were then projected onto the group template skeleton by searching along perpendiculars from the skeleton to find local maxima. The FA skeleton was thresholded to an FA of 0.30 or greater to include the major white matter pathways but avoiding peripheral tracts, which are more vulnerable to intersubject variability and/or partial volume effects with gray matter. Each subject's aligned FA data were then projected onto this skeleton and the resulting data for each between-group analysis (comparing probands with autism, their unaffected siblings, and control subjects, as well as unaffected siblings and controls) were fed into voxelwise cross-subject statistics ($P < .05$) using "randomize" (v.1.2 in FSL4.1), a permutation program used for inference (thresholding) on statistic maps when the null distribution is not known.[58] All analyses were corrected for multiple comparisons, familywise error, and used threshold-free cluster enhancement,[59] with default parameters (height, 2; extent, 1; connectivity, 26). The original nonlinear registration from the FA maps was then applied to the axial and radial diffusivity maps, which were further analyzed as described above.

Behavioral correlation analyses also were conducted using FA values and the ADI-R and ADOS subscale scores. The subjects with autism were tested with different ADOS modules appropriate to their age and language abilities. Thus, this analysis was conducted on 10 of the 13 autistic subjects, who were assessed on the same ADOS module (module 3). Fractional anisotropy values were correlated with the ADOS subscale scores, social impairment, communication impairment, and combined, as well as the ADI-R subscale scores, social and communication. All behavioral analyses were conducted in randomize, correcting for multiple comparisons, familywise error, and using threshold-free cluster enhancement as described above.

RESULTS

Thirteen same-sex sibships (2 female) and 11 control subjects (2 female subjects) successfully completed the study. There were no significant differences in age between the groups (mean [SD] sibling age, 8.9 [1.9] years; mean [SD] control age, 9.6 [2.1] years; mean [SD] autism group age, 10.5 [2] years; $F_2 = 1.2$; $P = .3$). There was no significant difference between mean full-scale IQ (FSIQ) scores of the unaffected siblings and control subjects (mean [SD] FSIQ score for unaffected siblings, 118.8 [13.6]; mean [SD] FSIQ score for controls, 119.9 [13.3]; $P = .63$). However, there was a significant difference in FSIQ scores between the autism and control groups, with the autism group scoring lower than the control group (mean [SD] FSIQ score for autism group, 85.9 [17.4]; mean FSIQ score for controls, 119.9 [13.3]; $P < .001$). Similarly, there was a significant difference in FSIQ score between the autism and unaffected siblings groups, with the autism group scoring lower than the unaffected siblings group (autism mean [SD] FSIQ

score, 85.9 [17.4]; unaffected sibling mean [SD] FSIQ score, 118.8 [13.6]; $P < .001$). To account for possible effects of differences in FSIQ between the autism group compared with the sibling and controls groups, we repeated the analysis using FSIQ as a nuisance covariate using the same analysis described above.

VOXELWISE WHOLE-BRAIN ANALYSIS

Individuals With Autism vs Control Subjects

Subjects with autism had significant widespread reductions in FA values compared with control subjects (**Figure 1**, red and yellow); these differences were observed in 52% of voxels in the FA skeleton mask. Specifically, these reductions were observed throughout the medial prefrontal white matter, frontal corona radiata, genu and anterior forceps of the corpus callosum, body of the corpus callosum, left splenium, internal capsules bilaterally, external capsules bilaterally (possibly in the uncinate fasciculus bilaterally), bilateral superior longitudinal fasciculus (SLF), bilateral mid/posterior cingulate gyrus, within the thalamus, bilateral superior temporal gyrus (STG) approaching the hippocampus and the amygdala, bilateral temporo-parietal junctions, and bilateral fronto-parietal centrum semiovale. There were no significantly increased FA values in the autism group compared with controls. When using IQ as a nuisance covariate, fewer significant clusters emerged; however, significant clusters of between-group differences remained in all the major pathways including medial prefrontal white matter, frontal corona radiata, the body of the corpus callosum, the right internal capsule, external capsules bilaterally, the right SLF, bilateral STG, bilateral temporo-parietal junctions, and bilateral fronto-parietal centrum semiovale (eFigure; www.archgenpsychiatry.com).

Significant differences were observed between individuals with autism and controls in axial diffusivity in the medial prefrontal regions bilaterally (more on the right), right anterior forceps, right internal capsule, right SLF, right corona radiata, and the right STG. Twenty percent of the full skeleton showed significant differences in axial diffusivity between subjects with autism and controls. No significant differences were seen between these groups in radial diffusivity.

Individuals With Autism vs Unaffected Siblings

There were no significant FA differences in the whole-brain voxelwise *t* test analysis (**Figure 2**). We repeated the analysis with a paired *t* test design to account for the expected similarities in brain structure between family members; however, no significant white matter differences were observed.

A post hoc paired *t* test analysis was conducted using regions of FA differences between subjects with autism and controls as a mask. Again, no regions of significant difference in FA were observed in this analysis. Similarly, there were no differences in axial or radial diffusivities between subjects with autism and their unaffected siblings. Covarying for FSIQ did not reveal any significant differences in FA, axial, or radial diffusivities in this analysis. The analysis was repeated with false discovery rate correction at thresholds of 5%, 10%, and 20%; however, no between-group differences emerged.

Unaffected Siblings of Individuals With Autism vs Controls

Fractional anisotropy values were significantly reduced in the unaffected siblings compared with control subjects, primarily in overlapping regions in the right hemisphere, though not as extensive as those observed in the probands with autism/controls comparison. Twenty-one percent of the voxels in the FA skeleton mask were different in this contrast compared with 52% in the autism/controls analysis. Specifically, significant FA reductions in unaffected siblings were observed in the right medial prefrontal white matter, right anterior forceps, and throughout the corpus callosum (body and splenium) in right midposterior cingulate, right SLF, internal and external capsules, STG, and temporoparietal junctions. After covarying for FSIQ (nuisance covariate), the primary results remained, though they were less extensive (eFigure).

Within regions of significant FA differences between unaffected siblings and controls, significant differences in axial diffusivity were observed only in the right hemisphere in the internal capsule, external capsule, corona radiate, SLF, body of the corpus callosum, and STG. Ten percent of the whole skeleton had significant differences in axial diffusivity when comparing subjects with autism to controls. No differences in radial diffusivity were observed between the unaffected siblings and control subjects.

BEHAVIORAL CORRELATIONS

In the autism group, we computed correlations with ADOS subscale scores (combined, communication, and social scores) and ADI-R subscale scores (communication and social scores). The ADOS analyses were repeated while covarying for age and FSIQ, and the ADI-R analyses were repeated while covarying for FSIQ only (as the questionnaire relates to early childhood in all subjects). No significant correlations were observed between these behavioral measures and FA values within regions of significant FA differences generated from the between-group analyses.

COMMENT

In this study we investigated white matter structure in children with autism and their unaffected siblings compared with control children. Our results indicate that, for both the autism and the unaffected sibling groups, white matter structure differs significantly from controls in numerous brain regions. Significant white matter differences from controls included (but were not restricted to) regions that have been implicated in social cognition, theory of mind, and face processing (eg, medial prefrontal, and superior temporal regions, the temporo-parietal junctions).[32,61-63] We also found white matter aberrations in children with autism and their siblings encom-

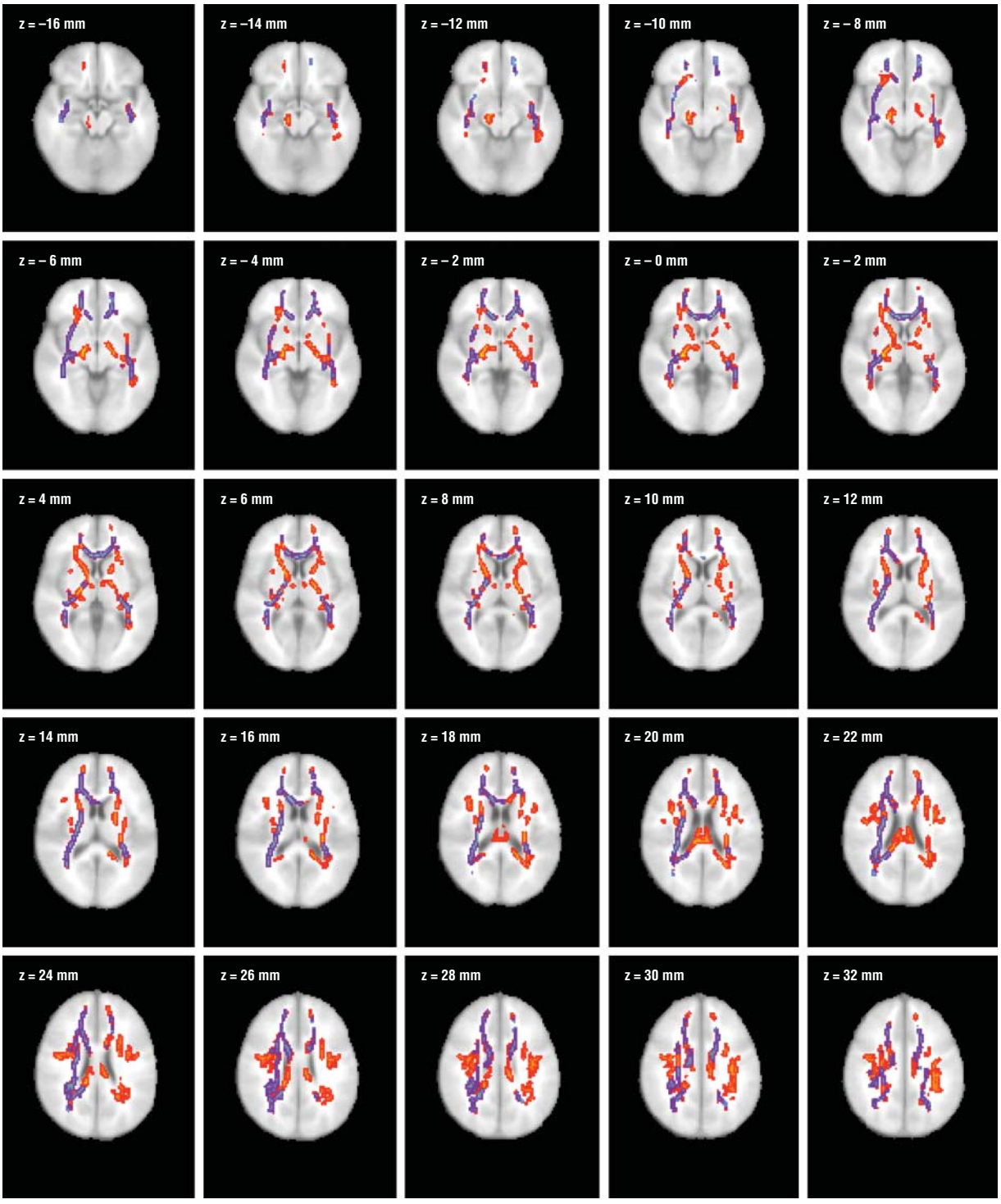

Figure 1. Superimposed results of voxels that showed significant reduction ($P<.05$, corrected) in white matter fractional anisotropy (FA). Group differences were "thickened" (for visualization purposes) by expanding the significant white matter skeleton cluster to the full extent of the local FA map. Children with autism were compared with control subjects (shown in yellow, corresponding to the actual results within the FA skeleton, and red). Siblings of children with autism were compared with controls (shown in light blue corresponding to the actual results within the FA skeleton, and dark blue), with overlap shown in purple. Results are mapped onto an average T1 Montreal Neurological Institute template.[60]

passing pathways that are important for cognitive, motor, and sensory functions. White matter differences were only significant when comparing children with autism with unrelated controls and when comparing unaffected siblings with unrelated controls; we found no significant differences in white matter structure when comparing children with autism with their unaffected siblings. Finally, our study suggests that, despite the inherent

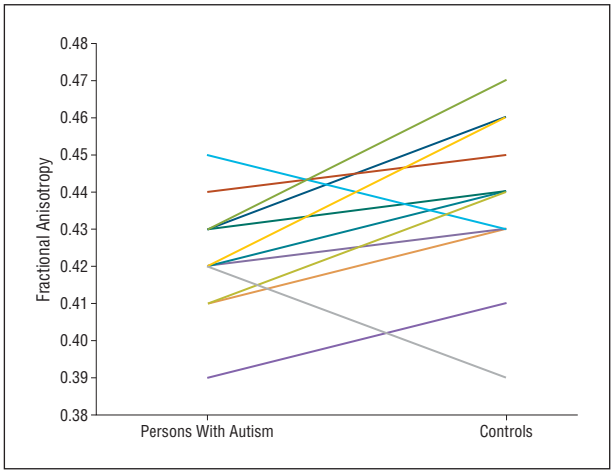

Figure 2. Fractional anisotropy values (extracted from regions of significant differences between subjects with autism and controls) in participants with autism and in their unaffected siblings. Colored lines connect fractional anisotropy values within sibling pairs. Only 12 pairs are shown because 2 pairs had overlapping values.

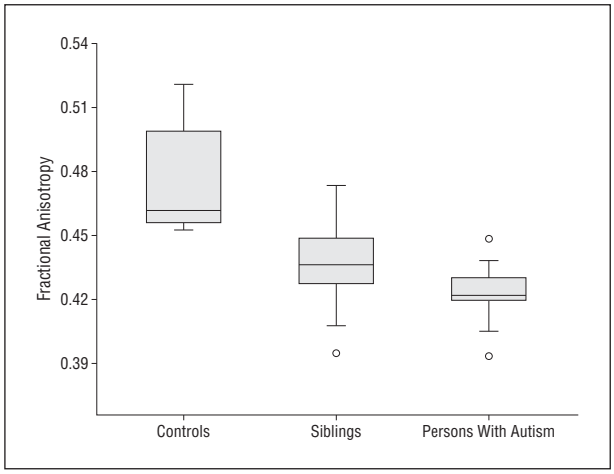

Figure 3. Box plot of average fractional anisotropy values extracted from regions of significant differences between subjects with autism and controls. Median fractional anisotropy, interquartile range, and outliers are shown in the autism, sibling, and control groups.

heterogeneity in a sample of individuals with autism, reduced FA is a consistent finding. In our sample, there is very little overlap in FA values between the autism and control groups (**Figure 3**).

Despite the lack of significant voxelwise differences in white matter structure between subjects with autism and their unaffected siblings, there was a qualitative difference in the extent of white matter differences between these groups and controls. Specifically, the autism/controls comparison showed more widespread and bilateral white matter differences compared with the unaffected siblings/controls contrast (which were more lateralized to the right). This finding suggests that the extent and laterality of white matter impairment may play a role in the development of the autistic phenotype.

A new finding in this study is reduced axial diffusivity in some white matter pathways with no change in radial diffusivity between the control group and subjects with autism or their unaffected siblings. This finding suggests that white matter aberrations in autism may arise primarily from changes in fiber coherence and not changes in myelination.[54] However, changes in axial diffusivity did not completely overlap with FA changes, suggesting that reduced axial diffusivity combined with increased radial diffusivity may play a role in some brain pathways in autism, whereas aberrant coherence (affecting axial diffusivity) dominates in other circuits. This finding is in contrast with 2 previous studies that investigated a mixed-age group of children, adolescents, and adults and reported reduced FA and increased radial diffusivity but not axial diffusivity in the corpus callosum and the temporal lobe in autism.[24,25] These conflicting results may represent different subtypes of autism or changes in white matter structure in autism with development.

The SLF showed aberrant white matter structure in autism in the current study as well as in one recent study.[29] The SLF is a major intrahemispheric fiber tract that traverses the frontal, parietal, and temporal lobes and is composed of 4 subpathways: the SLF I, II, and III, and the arcuate fasciculus. It appears that the differences we found in this study are located primarily in SLF II and/or SLF III,[64] though this must be confirmed in future fibertracking studies using higher-resolution data. Interestingly, both the SLF II and III are thought to contain neurons that may be relevant to the phenotype seen in autism. The SLF II connects Brodmann areas 6, 8Ad, 9/46, and 46[65]; premotor area 6 contains mirror neurons that have recently been implicated in social deficits observed in autism.[66,67] Area 8Ad is important for spatial awareness and the orienting aspects of attention,[68] and area 46 is involved in spatial working memory[69] maintenance of attention and engagement in the environment. These all are cognitive functions known to be impaired in autism.[70-73] The SLF III also contains fibers connecting mirror neurons and may be important in gestural communication that facilitates language development.[74]

In this context, mirror neurons deserve special consideration. The mirror neuron system (MNS) was shown to be involved in action recognition,[75,76] imitation empathy, social competence,[77,78] and possibly in language processing.[79,80] Given the social and language attributes of the mirror neuron system, it is not surprising that this system became a focus of interest in autism research.[66,81-83] Findings of differences in the mirror neuron system in autism relative to controls include local decreases in gray matter in regions subserving the mirror neuron system in adults with autism, which were correlated with severity of symptoms as assessed by the ADI-R social and communication scores[84] and a consistent delay and reduced response in the mirror neuron system during imitation of lip postures in adults with Asperger disorder when compared with controls.[85] Our current finding of reduced FA in the SLF provides additional evidence suggesting aberrant connectivity may underlie mirror neuron dysfunction in autism.

Previous studies have also found similarities between individuals with autism, their twins or siblings, and their parents. These similarities include increased head circumference in probands with autism as well as their unaffected siblings and their parents[86] and increased autoimmune reactivity in the cerebellum, cerebellar deep nuclei, and cingulate gyrus[37] in both probands with au-

tism and their siblings. However, unlike their affected siblings, unaffected siblings did not have increased levels of autoimmune antibodies to human brain tissue in the thalamus and hypothalamus.[34] In addition, elevated levels of particular amino acids important for brain development and reduced plasma glutamine were observed in children with autism, their unaffected siblings, and their parents.[33] Brain volumes were recently investigated between 14 monozygotic twins, with varying levels of discordance for autism. The study found no neurovolumetric or area differences between the twin pairs; however, most brain structure volume measures of the less-affected co-twins tended to be midrange between twins with autism and typically developing comparison subjects.[87] Finally, a functional MRI study of unaffected siblings of individuals with autism showed that unaffected siblings had significantly reduced gaze fixation and right fusiform activation in response to images of human faces when compared with controls.[35] In the same study, amygdala volume in the sibling group was found to be similar to the autism group and was significantly reduced compared with the control group. Interpretation of some of these studies is difficult, as not all excluded siblings with the broader autism phenotype, as in our study.

Physical characteristics including altered brain structure and function in unaffected siblings have been described for other mental health disorders with complex genetic and environmental risk factors. It has been long known that unaffected family members of individuals with schizophrenia share potential endophenotypic features with their affected family members. Most classically described are smooth pursuit eye movement and sensory gating P50 deficit.[88,89] More recently, imaging studies have shown that, similar to their affected siblings with schizophrenia, unaffected siblings have aberrant basal ganglia shape, reduced cortical gray matter and hippocampal volume, and reduced brain activation in response to a serial reaction time task.[90-94] In attention-deficit/hyperactivity disorder, activity in both the prefrontal cortex and cerebellum was altered in affected and unaffected siblings when compared with controls.[95]

Changes in brain structure and function in complex mental health disorders may be related to genetic influences that predispose individuals to develop these disorders and serve as a marker of genetic risk for autism or vulnerability to the development of disease. Alternatively, these brain changes also could be traits that cosegregate with the disorder in families but are not directly related to the actual psychopathology. Results from these studies in schizophrenia and attention-deficit/hyperactivity disorder, as well as our current results, suggest that neuroimaging studies may provide clues to endophenotypes in mental health disorders[96] that are more sensitive than neuropsychological testing.[97]

Limitations of the study include a small sample size, which may have affected power to find group differences and brain/behavior correlations. In addition, because the DTI acquisition sequence had gaps between slices, the scans were not well suited for fibertracking and exact fiber localization and fibertracking-based analyses could not be carried out. Further, the IQ discrepancy between subjects with autism and controls may have accounted for some of the white matter structure differences between the 2 groups. However, we found significant white matter differences between unaffected siblings and controls, who were IQ-matched. In addition, we did not find significant white matter differences between unaffected and affected siblings despite significant differences in IQ. Thus, these white matter differences are likely not attributable to IQ alone. Finally, the behavioral measures we included are noncontinuous variables and may not be sufficiently sensitive for the investigation of continuous variables representing the brain structure underlying these behaviors. Future studies with larger samples, fibertracking analyses, a longitudinal design, and the use of more sensitive behavioral measures could improve our understanding of how white matter is affected in the families of children with autism. Additional studies could also include comparisons of individuals with low- and high-functioning autism to investigate whether there are white matter differences between these groups, as well as comparing siblings with the broader phenotype to siblings who meet full criteria for autism. Finally, an imaging genomics approach may help identify loci that are involved in white matter development in autism.

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