A common MECP2 haplotype associates with reduced cortical surface area in humans in two independent populations

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The gene MECP2 is a well-known determinant of brain structure. Mutations in the MECP2 protein cause microencephalopathy and are associated with several neurodevelopmental disorders that affect both brain morphology and cognition. Although mutations in MECP2 result in severe neurological phenotypes, the effect of common variation in this genetic region is unknown. We find that common sequence variations in a region in and around *MECP2* show association with structural brain size measures in 2 independent cohorts, a discovery sample from the Thematic Organized Psychosis research group, and a replication sample from the Alzheimer's Disease Neuroimaging Initiative. The most statistically significant replicated association ($P < 0.025$ in both cohorts) involved the minor allele of SNP rs2239464 with reduced cortical surface area, and the finding was specific to male gender in both populations. Variations in the *MECP2* region were associated with cortical surface area but not cortical thickness. Secondary analysis showed that this allele was also associated with reduced surface area in specific cortical regions (cuneus, fusiform gyrus, pars triangularis) in both populations.

MRI | neuroscience | imaging genetics

The link between genetics and brain structure is a complex and hotly debated area of research (1–3). While rare mutations in several genes result in reduced brain size, common polymorphisms in these genes have shown little or no replicated association with brain structure measures (4, 5). We examined the association of brain structure measures with variants in and around the gene MECP2, chosen because of its role as a determinant of brain morphology in both humans and mice (6–9). Located on Xq28, *MECP2* encodes the methyl CpG binding protein 2, and mutations in this gene cause Rett syndrome (10), and are associated with other neurodevelopmental disorders including autism (11), mental retardation (12), and mild learning disabilities (13). The association of common *MECP2* polymorphisms with brain structure measures has not been investigated.

Studies investigating the relationship between genetic variations and brain structure have suffered from the use of unrefined measures of brain structure, insufficient genetic coverage, small sample sizes, or the failure to assess sex-specific effects. While easy to obtain, measures of head circumference are only rough estimates of brain size and confer no information about discrete brain structures. Methods such as voxel-based morphometry can involve very high dimensional phenotypes and usually require multiple comparison corrections that may reduce power to detect associations. We took advantage of refined measures of brain structure and full genetic coverage of the entire relevant *MECP2* gene region to assess associations between common genetic variants of *MECP2* in 2 large populations: a genetically homogenous, Norwegian discovery sample of healthy controls and patients with psychotic disorders [Thematic Organized Psychosis Research (TOP)], and a more heterogenous, North American replication sample of healthy controls and patients with mild cognitive impairment, and Alzheimer's Disease [Alzheimer's Disease Neuroimaging Initiative (ADNI)].

Although expressed in all cells, MECP2 is developmentally regulated and exists in 2 transcripts of different length in neuronal cells (14). The existence of a 3′UTR in transcripts from neuronal cells, but not other cell types, strongly suggests a more complex level of regulation and possibly a different function for MECP2 in neuronal cells. MECP2 has classically been defined as a transcriptional repressor due to its binding to methylated CpG dinucleotides, resulting in tighter winding of the chromatin coil and reduced transcription. Recent evidence suggests that MECP2 can act as a repressor or transcriptional activator depending on temporal and tissue context (15, 16).

It has been shown that specific MECP2 mutations result in major alterations of brain structure in both mice and humans. Murine Rett syndrome models exhibit a 25% reduction in whole brain volume (17) and show significant reductions in cortical surface area and volume of subcortical structures when Mecp2 exon 3 is either partially or entirely deleted (6). In humans, a classic symptom of Rett syndrome in females is head-growth deceleration (18), and severe MECP2 mutations in males result in neonatal progressive encephalopathy with disproportionate reduction in frontal and temporal lobes (8). Both male (19) and female patients (20) with specific MECP2 mutations also exhibit reduced dendritic tree structure. Variations in the *MECP2* gene have also recently been shown to be associated with autism (11), a disease sharing many phenotypic similarities with Rett syndrome, and characterized by abnormal brain growth in infants at 6–12 months, and premature arrest of growth (21).

These converging lines-of-evidence suggest that MECP2 may be a promising candidate gene for association studies with commonly-occurring brain structure variation. While major disruptions of MECP2 function and/or regulation result in severe disease phenotypes, it is unclear what role more common variations play in mediating variation in brain morphology, as

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Table 1. Significance levels of SNP by brain structure linear regression for the primary battery of regions in males in TOP and ADNI

				ICV			Brain Volume			Cortical Area			Cortical Thickness		
SNP TOP Study	Major Allele	Position on X	MAF	p-value Nominal	p-value Corrected	Effect Size %	p-value Nominal	p-value Corrected	Effect Size %	p-value Nominal	p-value Corrected	Effect Size, %	p-value Nominal	p-value Corrected	Effect Size, %
rs2266887	A	152892781	0.163	0.0001	0.0036	−6.85	0.0002	0.0038	−6.46	0.0017	0.0279	−5.33	0.3230	0.8334	−0.99
rs2266888	A	152892914	0.159	0.0001	0.0043	−6.78	0.0003	0.0044	−6.37	0.0022	0.0331	−5.23	0.3320	0.8438	−0.97
rs3027898	G	152929084	0.178	1.12E-05	0.0006	−7.64	0.0003	0.0055	−6.19	0.0006	0.0118	−5.71	0.2550	0.7348	−1.12
rs17435	C	152965174	0.173	2.13E-05	0.0009	−7.39	0.0006	0.0081	−5.9	0.0006	0.0117	−5.73	0.3800	0.8834	−0.86
rs7884370	G	152976075	0.061	0.0051	0.0897	−9.03	0.0799	0.4044	−5.58	0.1050	0.5503	−5.01	0.2700	0.7417	−1.98
rs1734791	T	152984114	0.112	0.0023	0.0363	−6.13	0.0053	0.0522	−5.49	0.0041	0.0541	−5.47	0.7490	0.9992	−0.36
rs2239464	A	153001625	0.155	4.03E-05	0.0015	−7.61	0.0004	0.0067	−6.43	0.0005	0.0102	−6.17	0.6590	0.9951	−0.46
rs5987211	C	153029249	0.059	0.0049	0.0852	−9.03	0.0747	0.3863	−5.65	0.0914	0.5094	−5.12	0.2820	0.7602	−1.93
rs5945176	T	153036357	0.246	0.2310	0.7566	−1.88	0.1690	0.6409	−2.11	0.1250	0.5673	−2.28	0.5360	0.9734	0.54
rs7884181	C	153051709	0.129	0.0246	0.2051	−5.11	0.0291	0.2074	−4.85	0.0153	0.153	−5.23	0.7920	0.9998	0.33
rs5987215	T	153053788	0.279	0.1870	0.6804	−2.06	0.1380	0.5841	−2.25	0.1130	0.5373	−2.34	0.5990	0.9879	0.45
ADNI Study					Permuted			Permuted			Permuted				
rs2266888	A	152892914	0.169	0.0942	0.116	−1.68	0.3200	0.3192	−0.46	0.0503	0.0517	−1.62			
rs2239464	A	153001625	0.188	0.1460	0.1697	−1.29	0.2880	0.2852	−0.53	0.0210	0.0219	−1.92			

5 of 11 SNPs in the MECP2 region are associated with 3 out of 4 main summary brain structure measures. For TOP, "corrected" p-values reflect the result of both permutation testing and correction for multiple comparisons in a two-tailed test. For the ADNI replication, p-values reflect permutation testing, but not multiple comparisons correction using a one-tailed test (the direction of the effect is hypothesized from the discovery sample). The association of SNP rs2239464 with cortical surface area in the TOP cohort replicates in the independent ADNI cohort. No significant associations were detected in females.

well as the brain morphology of patients with neurobehavioral, as opposed to severe neurodevelopmental, defects. Here we show that a common haplotype and common polymorphisms in the *MECP2* region are associated with several measures of structural brain size, as defined by whole-brain MRI segmentation procedures in two independent populations.

Results

Genotyping and brain imaging data were obtained for 289 healthy and psychotic subjects from the TOP study and 655 healthy and demented patients from the ADNI study. In the male population, the minor allele of 5 out of the 11 SNPs tested in TOP were significantly associated ($P < 0.05$) with reduction in 3 out of 4 brain regions of interest from our primary set of summary brain regions: intracranial volume (ICV), parenchymal volume (BV) volume, cortical surface area (CA) and mean cortical thickness (CT) (Table 1). For the two *MECP2*-region SNPs that were assayed on both the TOP and ADNI genotyping platforms (see *Materials and Methods*), we find that the association with CA replicates in the completely independent ADNI study (rs2239464, $P = 0.021$, one-tailed test). There is no hemispheric preference of the effect in either cohort.

Fig. 1 depicts the linkage disequilibrium (LD) structure of the *MECP2* region and the association of each SNP with CA in the TOP cohort. *MECP2* is in linkage with 2 genes, IRAK1 (interleukin receptor-associated kinase 1) and TMEM187 (transmembrane protein of unknown function), neither of which have been shown to play a role in the determination of brain structure.

Statistically significant influences of disease, age, and sex on BV and CA were detected (supporting information (SI) Table S1), and were controlled for in our association analyses via a multiple regression model in both cohorts. A simple case-control association analysis involving *MECP2* SNPs and disease status was performed in PLINK (22), and neither individual *MECP2* variants nor haplotypes were found to be associated with disease status. We also did not detect any significant gene-by-diagnosis or gene-by-age interactions on structural phenotypes via regression modeling. With this in mind, we focused attention on the association between common variants and brain structure measures and not on disease status per se.

Continuous maps of relative cortical surface areal expansion were obtained by computing the area of each vertex in a standardized, spherical atlas space surface tessellation. This

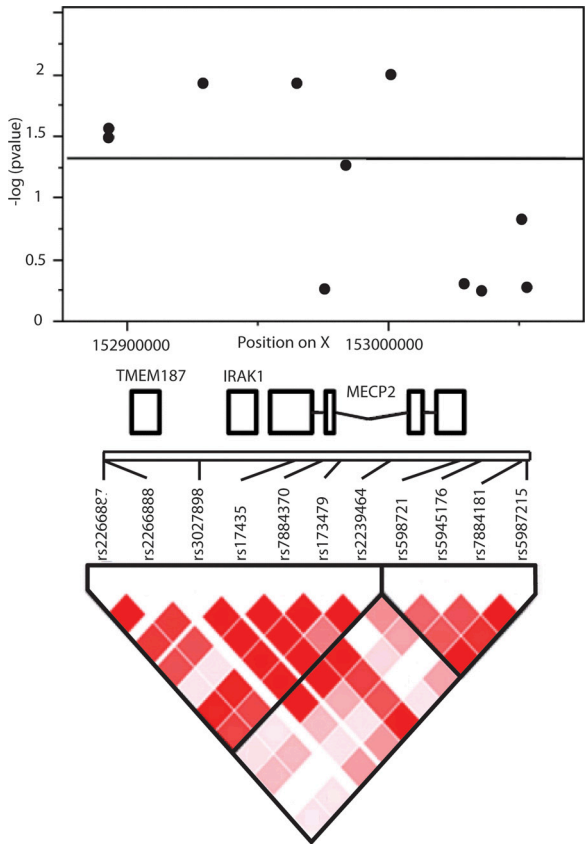

Fig. 1. Linkage disequilibrium map and −log of permuted and multiple comparison-corrected *p*-values for SNP dose vs. CA of MECP2 "expression module" (28) using data from 289 subjects from the TOP cohort.

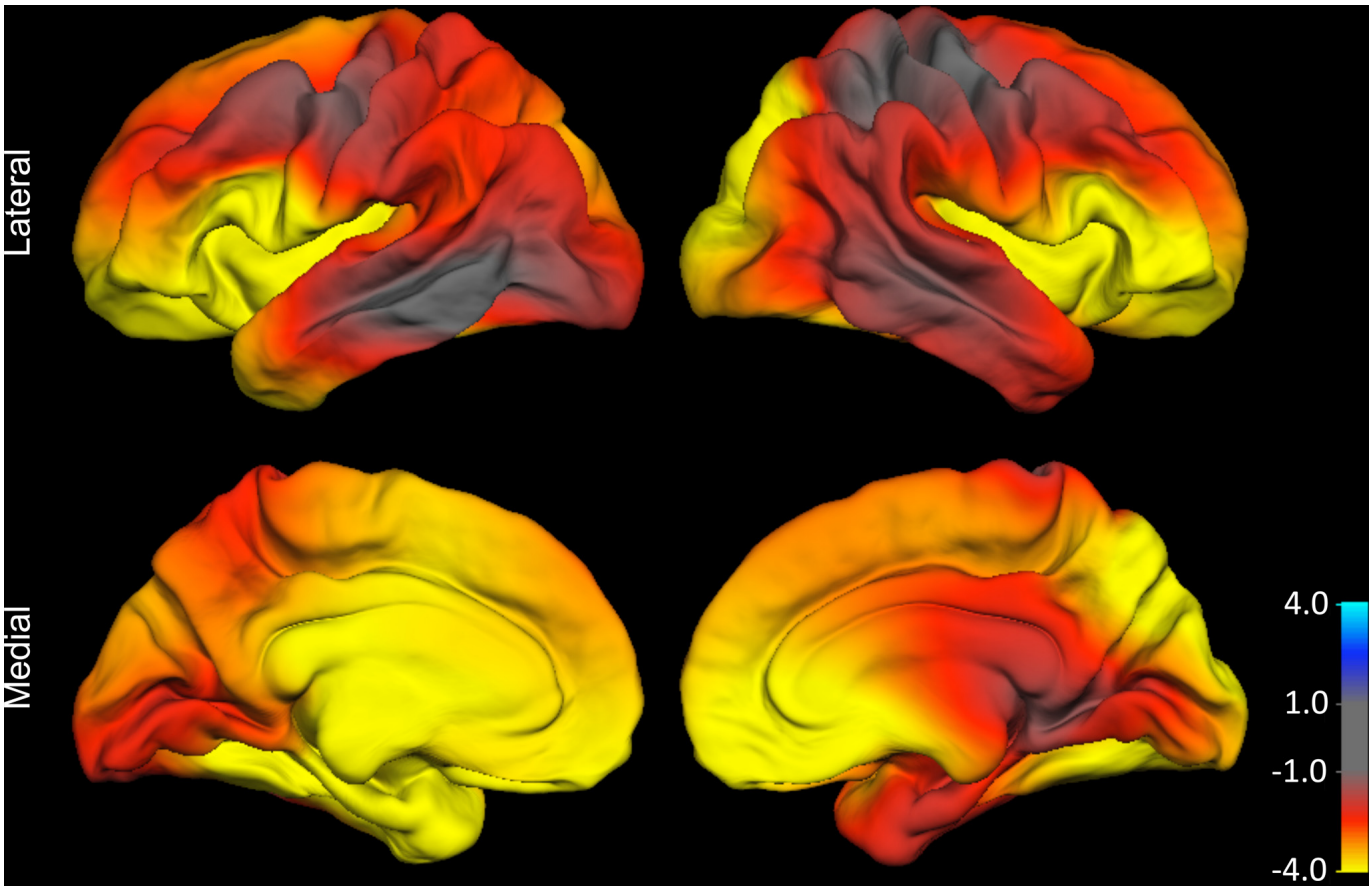

Fig. 2. Association of *MECP2* SNP rs2239464 with cortical surface area in males in the TOP cohort. The map shows the distribution of nominal −log *p*-values (sign indicating direction of effect per copy of minor allele) across the reconstructed cortical surface. (*Top*) Lateral views. (*Bottom*) Mesial views. The corresponding map for females showed no significant effects (Fig. S1).

provides point-by-point estimates of the relative areal expansion or compression of each location in atlas space. Association of genetic variants to cortical surface area could thus be assessed at each surface vertex. A mapping of cortical surface areal expansion in males is shown in Fig. 2; this demonstrates the strength of the association (shown as log *p*-values) for different brain regions. Fig. S1 depicts this mapping in females. It is interesting to note that the association of the rs2239464 variant with reduced cortical surface area is not confined to discrete brain regions, but is distributed throughout the cortex, suggesting the wide-ranging and fundamental influence of MECP2 on cortical surface area. While the association is widespread, there do appear to be regions of increased MECP2 association. Based on mouse and human MECP2 mutation studies, we expected to see reductions in frontal and motor cortex size, and we do indeed observe this (6, 9, 12, 17). Studies of females with Rett syndrome generally show occipital cortex preservation and dorsal-parietal cortical volume reduction (9), and we do not observe this. However, the effect of common polymorphism, rather than specific rare, highly deleterious mutations in these regions, is unknown. Genetic association studies with cortical surface area as the phenotype of interest have not been pursued previously in humans.

We then performed an exploratory analysis with relaxed multiple comparison corrections on 66 surface area measurements of discrete cortical regions assessed by the imaging software suite FreeSurfer using the most significant SNP (rs2239464) common to both studies. Out of 66 surface area measurements, 23 (35%) in TOP and 18 (27%) in ADNI were significantly associated ($P < 0.05$) with variation at this SNP, with 8 common regions significant in both cohorts (Table 2). Using Fisher's combined P value test statistic we found 17 regions were significant in both cohorts (Table 2). Interestingly, several of the regions exhibiting the most reduction with the minor allele have shown involvement in autism. The pars triangularis is a language related region affected in autistic patients (23). MECP2 expression in the fusiform gyrus, and frontal cortex in general, is reduced in autistic patients (24). The fusiform gyrus and cuneus show reduced functional connectivity in autistic patients (25). Our findings are consistent with results from the MECP2 literature, and the replication of association with specific cortical regions in 2 cohorts is strong evidence of a real effect in these populations.

Posthoc region of interest (ROI) association analyses were pursued to examine subcortical, cerebellar, and white matter regions (Table S2). Several regions that have been shown to be affected by MECP2 mutations in mouse models, or as potentially mediating Rett-like or autistic phenotypes in humans, were associated with reduced volumes in subjects with the minor allele [amygdala (17), hippocampus (17), basal ganglia (17), and cerebellum (6)]. For example, Mecp2 knockout mice show reductions in volumes of hippocampus (21%) and cerebellum (approximately 10%). Motor deficits common to autism and Rett syndrome suggest some involvement of basal ganglia and cerebellum (26). Our observation that *MECP2* variations are associated with both cortical and subcortical (albeit in the TOP study data only) brain structures further solidifies the global role of MECP2 in brain development.

Table 2. Significance levels of rs2239464 by cortical sub-region for TOP and ADNI cohorts

SNP rs2293464	TOP						ADNI						Fishers combined	
	Left			Right			Left			Right				
	Nominal	Permuted	Effect size (%)	Nom	Perm	Effect size (%)	Nom	Perm	Effect size (%)	Nom	Perm	Effect size (%)	Left	Right
Fusiform	0.0001	0.0005	−9.8	0.0002	**0.0007**	−8.63	0.1056	0.2389	−1.92	0.0278	**0.03**	−2.55	<.001	<.001
Cuneus	**0.0067**	**0.009**	**−8.01**	**0.0245**	**0.033**	**−6.58**	**0.0381**	**0.038**	**−3.3**	**0.0013**	*0.0014*	**−5.36**	**<.01**	**<.001**
Superior Frontal	0.0047	**0.0099**	−6.47	0.001	0.0034	−7.14	0.0295	**0.0375**	−2.34	0.144	0.2231	−1.35	<.01	NS
Pars Triangularis	0.059	0.06	−7.54	0.0203	**0.04**	−9.25	0.1821	0.3543	−1.93	0.0024	**0.0032**	−5.85	NS	<.01
Lateral Orbitofrontal	0.0029	**0.0082**	−6.55	0.0285	0.045	-4.86	0.0299	**0.0285**	-2.6	0.1881	0.2688	-1.19	<.01	NS
Precentral	0.0032	**0.0088**	-6.31	0.4775	0.6542	-1.57	0.0264	**0.028**	-2.43	0.0246	0.0254	−2.46	<.01	NS
Superior Temporal	0.009	0.022	−6.04	0.0258	0.048	−5.1	0.0714	0.078	−1.96	0.0448	0.0565	−2.24	<.05	<.05
Posterior Cingulate	0.0191	0.031	−6.7	0.0276	**0.049**	−6.52	0.2962	0.3987	−0.86	0.0078	**0.01**	−3.96	NS	<.01
Supramarginal	0.0555	0.07	−5.55	0.0269	0.053	−6.08	0.0333	0.0375	−2.78	0.0402	0.048	−2.78	<.05	<.05
Paracentral	0.0526	0.09	−6.13	0.0154	0.015	−7.49	0.0235	0.0299	−3.35	0.1518	0.2876	−1.78	<.05	NS
Precuneus	0.0999	0.13	−3.95	0.014	**0.028**	−6.26	0.1151	0.2589	−1.62	0.0089	**0.0135**	−3.05	NS	<.01
Pars Orbitalis	0.1931	0.3492	−4.04	0.0011	0.0024	−9.19	0.1106	0.2576	−2.23	0.0846	0.087	−2.32	NS	<.01
Inferior Parietal	0.0804	0.09	−4.69	0.0975	0.16	−4.03	0.0663	0.063	−2.14	0.0938	0.0985	−1.84	<.05	NS
Lateral Occipital	0.1987	0.3567	−3.4	0.0309	0.053	−5.82	0.0441	0.0595	−2.54	0.0708	0.081	−2.14	NS	<.05
Transverse Temporal	0.0129	0.039	−8.2	0.5338	0.6448	−2.33	0.0382	0.0515	−3.46	0.0845	0.104	−2.65	<.05	NS
Lingual	0.1024	0.2386	−4.54	0.0138	0.02	−6.62	0.1513	0.3435	−1.76	0.0854	0.091	−2.27	NS	<.05
Inferior Temporal	0.0151	0.035	−7.31	0.9126	1	−0.33	0.0956	0.095	−2.27	0.237	0.3679	−1.25	<.05	NS

Eight cortical regions show significant ($P < 0.05$) area reductions with the minor allele of rs2239464 in both the TOP and ADNI cohort in at least one brain hemisphere (text in bold, table sorted by combined *P* value from all hemispheres). Using the Fisher's combined statistic, the number of significantly associated cortical regions increases to 17. Simulated *P* values are based on a 100k permutation-based test. TOP *P* values are based on a 2-tailed test, whereas ADNI values are based on a 1-tailed test.

To further explore the relationship between *MECP2* sequence variations and brain structure, we performed haplotype analysis using 2–5 SNP sliding windows across the *MECP2* region (Fig. S2). We used 100,000 permutations using the method implemented in the Haplo.Stats computer package to assess the statistical significance of the associations (27). Males and females were analyzed separately, as the proper model to investigate their effects together is unknown due to X-chromosome inactivation. Consistent with the single SNP analysis results, no female haplotypes were significantly associated with brain structure measures. In males, haplotypes exhibited similar effect sizes to the single SNP analyses, with the exception of the 2-marker haplotype comprised of the first 2 SNPs in the region (permutation P value = .0002 in TOP) indicating that either there exists a combined *cis*-acting effect of the alleles at this locus, or there is an additional variant in linkage disequilibrium with the SNPs used to construct the haplotype. Interestingly, this haplotype contains SNPs in the gene TMEM187, approximately 40 kb downstream of *MECP2*. This is a site of predicted MECP2 enhancer activity as determined experimentally by Liu and Francke (28). While a direct involvement of TMEM187 cannot be ruled out, there is no previously reported role of TMEM187 in determining brain structure. The 2 main haplotypes at this locus exist at frequencies of 83% and 16%, with the minor haplotype exhibiting association with reduced brain-structure size in males.

Since brain structure is likely to be under selection pressure, we looked for evidence of positive selection in this genetic region. Allele frequencies for SNPs in this region are largely different between the HapMap populations (29). In fact, the minor allele in Caucasians is actually the major allele in both Han Chinese (approximately %80) and Yoruban populations (approximately %60). While this is suggestive of selection, the Haplotter website (30), which uses data from Hapmap populations to estimate FST, shows no evidence for positive selection in this region. A haplotype-based test, the linkage-disequilibrium decay test, did not identify MECP2 as a gene under selection pressure (31).

Discussion

Strikingly, no *MECP2* SNPs were found to be associated with any brain structure measures in the female population. Under an additive model, we would expect the dose effect to be approximately half of that for females, due to X-inactivation in females. We observe the slope derived from a regression of CA on genotype in females to be less than half the slope in males, suggesting some sort of compensatory affect by which the single activated female allele is robust to *MECP2* sequence variations that might otherwise influence cortical surface area. The biology of this effect is unknown, but skewed X chromosome inactivation or hormonal effects may be involved.

Early autism studies that screened exonic regions of *MECP2* failed to find causative mutations (32, 33). More recent studies have identified mutations in *MECP2* regulatory regions in autistic boys (13, 34), rather than protein coding mutations found in female Rett syndrome patients. These results may be explained on the basis of the extra (inactive) copy of the X chromosome providing redundancy or buffering to severe protein-coding mutations in females. Thus, the specific MECP2 protein coding mutations in males, in the absence of the buffering effects of an extra X chromosome, may be either lethal in the embryo or cause severe abnormalities at birth. In this light, mutations in regulatory regions may be tolerable to the brain of the developing male but induce phenotypic variations. Studies in rat brain have shown significantly less Mecp2 mRNA and protein expression in the amygdala of male rat brain during the steroid-sensitive period of brain development (35). Ultimately, there is ample evidence suggesting that regulation of MECP2 is different between genders, consistent with our observations of the differential influence of *MECP2* variations on brain structure in males and females.

Interestingly, our results suggest that *MECP2* DNA sequence variations are associated with cortical surface area, but not thickness. Cortical volume and area have been shown to be significantly reduced in Mecp2 mutant mice (6), although thickness was not measured in these studies and could not be tested. Recent evidence points to independent cellular determinants of cortical thickness and area, namely intermediate (IPC) and radial (RPC) progenitor cells (36). Mouse mutations of the genes Pax6, Lrp6, and Ngn1/2 modify IPC abundance and result in parallel changes in cortical thickness, but not area. In humans, homozygous mutations in PAX6 (37) or TBR2 (38) result in disproportionate reductions of cortical thickness relative to surface area. On the other hand, promotion of RPC proliferation in mice by transgenic expression of B-catenin (39) or caspase-9 (40) knockout results in expansion of cortical surface area and gyral folding, but has no apparent effect on thickness. While MECP2 activity is usually associated with the newly mature neuron, neuronal precursors do stain for MECP2 (41). The role of MECP2 in RPC proliferation is worthy of examination in light of our finding that *MECP2* variants associate with cortical surface area, but not thickness.

Microencephalopathy patients with head-growth deceleration show largely N-terminal MECP2 mutations (18). While we show common polymorphism association in the C-terminal region and more downstream regions, these findings are not mutually exclusive, as C-terminal, 3′UTR, or downstream variations may be better tolerated than N-terminal mutations, which likely cause severe developmental brain undergrowth. Although our results indicate associations primarily with variations in the downstream region of *MECP2*, the high LD prevents us from further distinguishing whether intronic, exonic, or 3′UTR, IRAK1, or TMEM187 variants are driving the association. Furthermore, the entire 150 kb region contains many experimentally verified regulatory elements (enhancers, silencers) that control MECP2 expression (28).

Effect sizes in the ADNI cohort are generally smaller than those observed in the TOP cohort. This may be due to the inclusion of several subjects of Southern European descent in the ADNI study. These subjects may possess slightly different linkage structures than the Northern Europeans, which may work to reduce the effect size of SNPs that are farther from the actual functional variant. The inclusion of subjects with dementia, and therefore more pronounced brain degeneration, may act to cloud the signal as well.

In summary, we find that there is a cluster of linked polymorphisms in a region associated with brain structure phenotypes, and that *MECP2* is one of the candidate genes within this region. Other genes in this linkage block (IRAK1, TMEM187) have no known influence on brain structure. The minor haplotype, existing in approximately 15% of the Norwegian and North American study populations, is strongly associated with reduced cortical surface area, but not cortical thickness, in males. It appears that females may be more robust to the effect of common genetic variations in this region. While our results suggest an association between common variants in and around *MECP2* and brain size measures, MECP2 is a massive transcriptional hub, and there are likely subcomponents of this network that are responsible for individual variance in distinct brain regions. Recent work (15) suggests that thousands of genes are activated or repressed by MECP2. Dissection of the MECP2 network is likely to yield fundamental insights into brain development in both normal and disease cohorts.

Materials and Methods

Subjects—TOP. Two hundred and thirty-five healthy controls and 501 subjects with severe mental disorders were included in the study. There were 289 subjects with both successful MRI scans and genotyping, including 102 healthy control participants, 80 individuals with schizophrenia disorders, 70 individuals with affective disorders, and 37 individuals with severe psychotic disorders. *SI Methods* contains additional details on TOP subjects.

Subjects—ADNI. One hundred and eighty-seven healthy controls, 322 subjects with mild cognitive impairment, and 146 subjects with Alzheimer's disease were included in the study. The ADNI study participant general eligibility criteria are described in the ADNI Protocol Summary page of the ADNI-Info Web site at http://www.adni-info.org/index.php?option = com_content&task = view&id = 9&Itemid = 43. Briefly, subjects were 55–90 years old, did not suffer from major depression, had a modified Hachinski score of 4 or less, and had a study partner able to provide an independent evaluation of functioning. Up-to-date information is available from the home page of the ADNI-Info Web site at http://www.adni-info.org.

Genotyping. For the TOP study, DNA was extracted from blood and genotyped by Expression Analysis with the Affymetrix 6.0 array. To detect genetic stratification in the group of self-reported, ethnic Norwegian TOP subjects, PCA was performed with the EIGENSOFT smartpca tool (42). No evidence was found for multiple genetic clusters, so no subjects in the TOP study were removed. ADNI samples were genotyped with the Illumina Human610-Quad BeadChip. Smartpca was used to identify outlier ADNI subjects relative to a genetic cluster composed almost exclusively of subjects whose self-reported race was ''White.'' The self-reported race for the majority of the 53 outliers was ''Black/African American,'' and for the remainder was ''White,'' ''Asian,'' or ''Other.'' ADNI outliers were not included in subsequent analyses. Discrepancies between annotated and predicted gender were removed. (n = 15 samples removed). Samples with more than 10% missing calls were removed (n = 27 samples removed). *MECP2* SNPs were defined by Liu and Francke (28) with a call rate of >95% and Minor Allele Frequency (MAF) of >0.05, resulting in 11 SNPs in TOP and 2 SNPs in ADNI.

Brain Imaging Protocol and Morphometric Parameters. For the TOP study, MRI scans were performed with a 1.5T Siemens Magnetom Sonata scanner equipped with a standard head coil. Structural image acquisitions included a conventional 3-plane localizer and 2 T1-weighted volumes that were acquired with the same pulse sequence (TE = 3.8 ms, TR = 10.7 ms, TI = 1000 ms, flip angle = 8°, TD = 750 ms, bandwidth = 31.25 Hz/pixel, FOV = 24 cm, matrix = 192 × 192, slice thickness = 1.2 mm) and subsequently averaged together to increase the signal to noise ratio. Acquisition parameters were optimized for increased gray/white matter image contrast. The imaging protocol was identical for all subjects studied. For the ADNI study, data were collected from studies with a variety of 1.5-T MRI scanners. Protocols are described in detail on the ADNI Research Cores page of the LONI Web site (http://www.loni.ucla.edu/ADNI/Research/Cores/index.shtml) published in 2007. Raw Digital Imaging and Communications in Medicine MR images were downloaded from the ADNI Data page of the public ADNI site at the LONI Web site (http://www.loni.ucla.edu/ADNI/Data/index.shtml) published in 2007.

MR Image Processing. MRI scans from both studies (TOP and ADNI) were analyzed using identical procedures, with software developed at the University of California at San Diego MultiModal Imaging Laboratory, based on the freely available FreeSurfer software package (http://freesurfer-software.org). Please refer to *SI Methods* for additional detail.

Statistical Procedures. Genotype was modeled as minor allele SNP-dosage (homozygote of minor allele = 2, heterozygote = 1, homozygote of major allele = 0), and was separated into 2 regressor variables for SNP-dosage for male and females separately. SNP-wise univariate General Linear Models (GLMs) were fitted with morphometry phenotypes as the dependent variables and genotype as the independent variable, with age, sex, and diagnosis categories (TOP: schizophrenia, bipolar disorder, and ''other diagnoses,'' ADNI: mild cognitive impairment, Alzheimer's) as covariates. Nonparametric p-values for each SNP-phenotype regression were calculated from the nominal p-values estimated by the model, via permutation using 100,000 iterations. A more detailed description of the permutation method is available in *SI Methods*.

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