Microcephaly Genes and Risk of Late-onset Alzheimer Disease

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Abstract: Brain development in the early stages of life has been suggested to be one of the factors that may influence an individual's risk of Alzheimer disease (AD) later in life. Four microcephaly genes, which regulate brain development in utero and have been suggested to play a role in the evolution of the human brain, were selected as candidate genes that may modulate the risk of AD. We examined the association between single nucleotide polymorphisms tagging common sequence variations in these genes and risk of AD in two case-control samples. We found that the G allele of *rs2442607* in microcephalin 1 was associated with an increased risk of AD (under an additive genetic model, $P = 0.01$; odds ratio = 3.41; confidence interval, 1.77-6.57). However, this association was not replicated using another case-control sample research participants from the Alzheimer Disease Neuroimaging Initiative. We conclude that the common variations we measured in the 4 microcephaly genes do not affect the risk of AD or that their effect size is small.

Key Words: Alzheimer disease, microcephaly genes, cognitive reserve

(*Alzheimer Dis Assoc Disord* 2011;25:276–282)

Received for publication June 2, 2010; accepted December 2, 2010.

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Supported by Merit Review Grant and Research Career Development Award, Office of Research and Development, Department of Veterans Affairs, National Institutes of Health (AG08017, MO1 RR000334, UL1 RR024140), Alzheimer Tax Check-off Grant. Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: Abbott; AstraZeneca AB; Bayer Schering Pharma AG; Bristol-Myers Squibb; Eisai Global Clinical Development; Elan Corporation; Genentech; GE Healthcare; GlaxoSmithKline; Innogenetics; Johnson and Johnson; Eli Lilly and Co.; Medpace, Inc.; Merck and Co., Inc.; Novartis AG; Pfizer Inc.; F. Hoffman-La Roche; Schering-Plough; Synarc, Inc.; and nonprofit partners the Alzheimer's Association and Alzheimer's Drug Discovery Foundation, with participation from the US Food and Drug Administration. Private sector contributions to ADNI are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of California, Los Angeles. This research was also supported by NIH grants P30 AG010129, K01 AG030514, and the Dana Foundation.

The Alzheimer's Disease Neuroimaging Initiative: Data used in the preparation of this article were obtained from the ADNI database (www.loni.ucla.edu/ADNI). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in the analysis or the writing of this report. Complete listing of ADNI investigators is available at: www.loni.ucla.edu\ADNI\Collaboration\ADNI\Manuscript\Citations.pdf

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Genetics has been suggested to play a role in variations in cognitive function in late life.[1] One way in which genes may play a role in cognitive function in late life is through providing an "initial endowment" that is more resistant to age-related changes. This initial endowment, or cognitive reserve, may include both functional and structural brain features (such as cerebral size), which may increase the threshold for responses to brain insult.[2] We hypothesize that variations in genes regulating brain size during neurodevelopment may play a role in individual susceptibility to cognitive decline by modulating the brain size. This study examines whether 4 microcephaly genes (abnormal spindle-like microcephaly-associated (*ASPM*), microcephalin 1 (*MCPH1*), centromeric protein J (*CENPJ*), and cyclin-dependent kinase 5 regulatory subunit-associated protein 2 (*CDK5RAP2*)) play a role in late-life risk of Alzheimer disease (AD).

Mutations in these 4 genes cause reduced brain size and head circumference and mental retardation.[3] The microcephaly genes are expressed in the embryonic brain, especially in the ventricular zone, during cerebral cortical neurogenesis.[4] The timing and location of their expression suggest that they play a role in regulating neurogenic mitosis before birth.[4] Sequence comparisons among primate species suggest that microcephaly genes experienced adaptive evolution due to positive selection[5–9]; rare variants in these genes increased in frequency over time due to an advantageous phenotype they provide.

There is also evidence that 2 haplotype-defining single nucleotide polymorphisms (SNPs) in *ASPM* and *MCPH1*, *rs41310927* and *rs930557*, respectively, have continued to work under positive selection, beyond the emergence of the anatomically modern humans.[6–8] Earlier studies imply that the positive selection in the microcephaly genes may be related to an advantageous brain-associated phenotype, such as brain size, cognition, personality, motor control, or susceptibility to neurological or psychiatric disease. Several studies have specifically investigated a relationship between

such phenotypes and some or all of the microcephaly genes.[10–16] Most of the studies to date have found no association between brain-related phenotypes (brain volumes, general cognitive ability, head circumference, or risk of schizophrenia) and these 2 polymorphisms. Only 2 studies have found associations between common SNPs in *MCPH1*, *CDK5RAP2,* or *ASPM* and brain volumes in a sex-specific manner.[13,16] One of these studies also sought for an association between 2 SNPs in *CDK5RAP2* and schizophrenia, bipolar spectrum disorder, AD, and mild cognitive impairment (MCI) and found no association.[16]

In this study, we examined the association between the microcephaly genes and risk of AD using tag SNPs covering all 4 genes. To test our hypothesis, we completed a 2-step study. In the discovery step, 2 related but separate case-control studies were carried out: first the association between SNPs in *ASPM* and AD risk was examined. Later, the association between *MCPH1*, *CDK5RAP2*, and *CENPJ* SNPs and AD risk was examined in a slightly larger case-control sample. Data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) were used to validate the findings from the discovery step.

METHODS

The Portland Veterans Affairs Medical Center Institutional Review Board approved this study.

Description of Participants

Participants were selected from longitudinal aging studies conducted at the Oregon Alzheimer Disease Center (OADC). These studies include the Oregon Brain Aging Study, the Community Brain Donor Program, and the Klamath Falls Exceptional Aging Project.[17] Patients from the memory clinic at Oregon Health and Science University, who were followed longitudinally as part of the OADC, were also included. The Oregon Brain Aging Study enrolls healthy elderly people over the age of 55 years from the community. The Klamath Falls Exceptional Aging Project recruits elderly people who are aged 85 years and older from a rural community in Southern Oregon. The Community Brain Donor Program recruits participants from the community who are aged 55 years and older.

Description of Participant Evaluations

All participants were followed semiannually with standardized clinical examinations. Cognitive and functional assessments were made using the clinical dementia rating (CDR),[18] the neurobehavioral cognitive status examination,[19] the mini-mental state examination,[20] and a psychometric test battery covering key domains.[21] Functional status was determined using the functional activities questionnaire.[22] A CDR score was assigned to each participant by a neurologist at each semiannual visit based on cognitive and functional examinations and collateral history. Participants underwent routine laboratory tests and imaging for diagnosis of AD. Diagnosis of AD was based on established diagnostic criteria.[23] For this study, controls were defined as a CDR = 0 at last evaluation.

Inclusion and Exclusion Criteria

Included in both discovery analyses were all participants from longitudinal studies of the OADC who: (1) had a diagnosis of AD or were cognitively intact on their last examination; (2) identified themselves as "white, not of Hispanic origin"; (3) had banked DNA for genotyping; (4) had no first-degree family history of AD (to ensure that none of the participants had an undescribed genetic predilection for AD that could confound the analyses); and (5) were either >69 years old (for the first discovery case-control sample) or were >64 years old (for the second discovery case-control sample) at the onset of symptoms for cases or at the last evaluation for controls. When comparing basic demographics between cohorts to ensure that cohort differences were not confounding the analysis, it became evident that participants from the memory clinic were younger than the participants in the longitudinal aging studies. Therefore, an age criteria was added. This criteria was reduced to >64 years in the second case-control sample to increase the sample size. Those with a CDR of 0.5 and not meeting diagnostic criteria for AD were excluded.

SNP Selection and Genotyping

A tag SNP panel was generated using data obtained from a pilot study[24] and the HapMap CEU (Utah Residents with Northern and Western European Ancestry) population.[25] In the pilot study, coding regions of *ASPM* were resequenced in an independent sample of 59 participants (30 cases and 29 controls) from our study population. We determined that the frequencies of the *ASPM* SNPs in our population were comparable with the HapMap CEU population. Therefore, tag SNPs spanning the entire *ASPM* gene were selected using Haploview 3.2 based on data obtained from the HapMap CEU population. Haploview uses a method that is identical to the program Tagger.[26] A pair-wise tagging method using single markers with thresholds of 0.8 for r^2 and 3.0 for logarithm of the odds score was used, and SNPs with a minor allele frequency (MAF) of $>5\%$ were included. A coding SNP (*rs41310927*) was also included in the final tag SNP panel based on our initial pilot study results. In the end, 2 coding SNPs previously suggested to have undergone positive selection were included (*rs41310927* and *rs3762271*).[27,28] The final SNP panel consisted of 11 SNPs. Linkage disequilibrium (LD) structure was examined using Haploview, and the haplotypes were defined using the confidence interval (CI) method.[28]

Purified samples of genomic DNA, obtained from the blood or brain, were used for genomic analysis. SNPs were genotyped using a polymerase chain reaction (PCR)-based DNA sequencing method. Primer design, PCR amplification, bidirectional sequencing of PCR products on the VariantSEQr Resequencing system (Applied Biosystems), and polymorphism analyses using a customized version of Agent Software (Paracel Inc.) were performed by Polymorphic DNA Technologies (www.polymorphicdna.com).

In the second analysis, tag SNPs were selected from *MCPH1*, *CENPJ*, and *CDK5RAP2* using data from the HapMap CEU population.[25] A similar tag SNP selection method described above was used; a pair-wise tagging method using single markers with thresholds of 0.8 for r^2 and 3.0 for logarithm of the odds score was used, and SNPs with an MAF of $>5\%$ were included. Sixty tag SNPs from *MCPH1*, 13 tag SNPs from *CDKRAP2*, and 11 tag SNPs from *CENPJ* were genotyped using an Illumina GoldenGate Custom Array.

Validation Sample

Data used to validate the findings from the discovery sample were obtained from the ADNI (www.loni.ucla.edu\ADNI). The primary goal of the ADNI has been to test

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whether biological markers, such as serial imaging and clinical and neuropsychological assessments, can be combined to measure the progression of MCI and early AD. Currently, approximately 200 cognitively normal older, 400 people with MCI, and 200 people with early AD are enrolled. ADNI participants have been genotyped using the Illumina Human 610-Quad Bead Chip. For up-to-date information see www.adni-info.org.

From the ADNI cohort, we only included controls or participants with AD who were self-reported, "White", and were > 64 years old (at onset of symptoms for cases or at the last evaluation for controls). The mean age of the ADNI participants meeting these inclusion and exclusion criteria ended up being younger than the discovery sample participants: 75.92 (± 6.06) for cases and 78.48 (± 5.28) for controls in ADNI as opposed to 83.92 (± 9.59) for cases and 88.55 (± 7.28) for controls in the discovery sample. Therefore, we repeated the validation analysis, this time only including participants from ADNI matched by age with the participants from the discovery sample.

Statistical Analysis

JMP (SAS Institute, Cary, NC) was used for group comparisons, whereas R version 2.9.2[29] (www.R-project.org), PLINK version 1.06[30] (http://pngu.mgh.harvard.edu/~purcell/plink/), and Haploview 3.2 (www.broad.mit.edu/mpd/haploview) were used for genetic analysis and SNP-disease association analysis.

Demographics

Differences in education, age at onset (cases), or age at last evaluation (controls) were compared between cases and controls with student *t* test. Sex and Apolipoprotein E (*APOE*) genotype differences were compared with χ^2 test.

Genetics

LD structure and haplotype blocks were determined using the CI method.[28] The Hardy-Weinberg Equilibrium (HWE) was calculated for all tag SNPs. DNA samples with call rates < 95% were excluded. SNPs were excluded from any further analysis if: (1) the MAF was < 5%; (2) individual SNP call rate of the participants was < 90%; and (3) the SNP genotype distribution departed from the HWE in the controls using a threshold corrected for multiple comparisons.

Each SNP was tested for SNP disease trait association using multiple logistic regression with AD case/control status as the dependent variable and the SNP genotype and additional covariates as independent variables. One model was tested in which the covariates were chosen based on earlier knowledge of association with disease risk: cohort, sex, years of education, age (age at onset of symptoms for cases or age at last evaluation for controls), and APOE genotype (coded as the presence of one or more ε4 allele versus none). This model tests the additive effects of allele dosage of the minor allele in which the odds ratio (OR) represents the effect of each extra minor allele controlling all the covariates. Parameters were estimated with 95% CI. Empirical SNP-disease association *P*-values were obtained by permuting the case/control status among all individuals 10,000 times, and testing the identical multiple logistic regression to obtain null distribution.

RESULTS

Participant Characteristics

In the end, 132 cases and 141 controls were included in the first discovery analysis and 160 cases and 168 controls in the second discovery analysis. All but 4 participants from the first discovery sample met inclusion/exclusion criteria for the second discovery sample. These 4 participants were excluded due to the change in diagnosis. The validation sample consisted of 276 cases and 217 controls.

Age at onset for cases in the 2 discovery samples and validation sample were significantly younger than age at the last evaluation for controls. The AD groups in both discovery and validation samples had significantly fewer years of education and more *APOE ε4* carriers (Tables 1–3).

Discovery Steps

Two SNPs, *rs12674488* and *rs17623747* in *MCPH1*, were not in HWE in cases and controls, and were excluded from further analyses. All remaining SNPs were in HWE in both cases and controls.

In the first discovery analysis, 2 *ASPM* SNPs showed significant association with disease status. Presence of the C allele in *rs36004306* and the presence of the T allele in *rs12116571* were significantly associated with the presence of AD before correction for multiple testing. None of the SNPs remained significant after correction for multiple testing (Table 4).

In the second discovery analysis, 5 *MCPH1* SNPs, *rs2442475*, *rs2442608*, *rs2442607*, *rs17553089*, and *rs2442592*, were significantly associated with disease status before correction for multiple testing. After correction for multiple testing, presence of the G allele in *rs2442607* remained significantly associated with the presence of AD (Table 4).

Validation Step

Next,the association between disease status and *MCPH1* in ADNI participants was assessed. ADNI participants have already been genotyped using the Illumina Human 610 Quad platform. Genome-wide genotype data are available at the study website. We included all SNPs in *MCPH1* that were already genotyped in the ADNI participants in our validation analysis. None of

TABLE 1. Participant Characteristics of the First Discovery Sample

	AD (N = 132)	Controls (N = 141)	*P*
Women	65.91%	55.32%	0.07
Age at onset (AD) or age at last evaluation (controls), years	85.26 (8.13)	88.59 (5.44)	< 0001
Education, years	13.21 (3.41)	14.48 (2.92)	0.001
One or more *APOE* ε4 present	34.85%	14.18%	< 0.0001

Values are frequency or mean (SD).
AD indicates Alzheimer disease

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TABLE 2. Participant Characteristics of the Second Discovery Sample

	AD (N = 160)	Controls (N = 168)	*P*
Women	65.63%	55.36%	0.06
Age at onset (AD) or age at last evaluation (controls), years	83.92 (9.59)	88.55 (7.28)	< 0.0001
Education, years	13.23 (3.45)	14.52 (2.90)	0.0003
One or more *APOE ε4* present	39.38%	13.69%	< 0.0001

Values are frequency or mean (SD).
AD indicates Alzheimer disease.

the 228 SNPs in *MCHP1* were significantly associated with risk of AD before permutation tests. The SNP that significantly associated with disease status in the OADC cohort, *rs2442607*, was not included in the set of SNPs already genotyped in the ADNI cohort as part of the Illumina 610 Human Quad platform. However, 2 SNPs, *rs1868553* and *rs2515477*, that were in strong LD with *rs2442607* ($r^2 = 0.94$ and $r^2 = 1$, respectively) based on data from the CEU population in the HapMap[25] showed no association with disease status in the ADNI cohort (OR = 0.95; CI, 0.66-1.40 and OR = 0.97; CI, 0.66-1.42, respectively). The minor allele frequencies of *rs1868553* and *rs2515477* were very similar in the HapMap CEU population (MAF = 0.16 for both SNPs) and ADNI participants selected for this study (MAF = 0.15 for both SNPs). Repeating the validation analyses with participants matched for age to the discovery sample participants did not change the results.

DISCUSSION

Our results suggest that variations in the 4 microcephaly genes are not associated with AD risk or that their effect size is small. This finding is consistent with many earlier studies that have failed to show a relationship between brain-related phenotypes and microcephaly genes. Two studies investigated the association between brain volumes measured by magnetic resonance imaging and r*s41310927* in *ASPM* and *rs930557* in *MCPH1*.[11,14] Both these studies found no significant effect of either polymorphism, alone or in combination, on the 3 measures of brain size. This may have been due to the lack of power, given the small sample sizes (n = 120 and n = 118, consecutively). Two larger studies, with sample sizes of n = 2393 and n = 644, examining the association between intelligence and these same gene variations were also negative.[10,12]

TABLE 3. Participant Characteristics of the Validation Sample

	AD (N = 276)	Controls (N = 217)	*P*
Women	43.84%	45.62%	0.69
Age at onset (AD) or age at last evaluation (controls), years	75.92 (6.06)	78.48 (5.29)	< 0.0001
Education, years	15.14 (3.04)	16.06 (2.72)	0.0005
One or more *APOE* ε4 present	64.86%	25.35%	< 0.0001

Several recent studies have reported inconsistent sex-specific relationships between the microcephaly genes and brain volume measures. For example, 1 study of 867 Han Chinese individuals found no association in their entire sample. However in a sex-stratified analysis, they found that male individuals homozygous for the C allele of *rs1057090* in *MCPH1* have larger cranial volumes.[13] Another study examined the relationship between brain volumes and SNPs in the microcephaly genes in 287 participants. The researchers found an association with 10 SNPs in *CDK5RAP2* and brain volume or cortical area in male patients only.[16] Four SNPs in *MCPH1* showed an association with brain volume or cortical area in female patients only. One SNP in *ASPM* showed an association with intracranial volume in female patients only. The researchers replicated their finding for 2 SNPs from *CDK5RAP2* in a validation sample of 657 participants from ADNI. The researchers also examined the association between 2 SNPs from *CDK5RAP2* and AD or MCI and did not find an association. Although our a priori hypothesis and analyses were not to investigate a sex-specific effect, we conducted a post hoc analysis and sought a sex-specific effect for *rs2442607* by stratifying participants in the discovery step based on their sex and repeating the association analysis. We found no sex-specific effect. The G allele of *rs2442607* remained associated with AD risk in both the sexes ($P = 0.03$ in male participants—55 cases and 75 controls; and $P = 0.003$ in female participants—105 cases and 93 controls). Although sex-specific effects are plausible, the underlying mechanism is not clear. To our knowledge, mutations in these genes have not been reported to cause sex-specific phenotypes. In addition, 1 SNP in *MCPH1* was associated with cranial volume in Han Chinese men only, whereas 4 other SNPs in *MCPH1* were associated with brain volumes in women only in the Rimol study. It is difficult to interpret these findings or to propose a plausible biological mechanism leading to these different observations.

This study has some limitations. First, it is possible that phenotypic differences between the discovery and validation samples may be the reason that we could not replicate our initial findings. We set forth 4 selection criteria to be included in this study for the discovery samples: presence of banked DNA, age, White ethnicity, and no first-degree relative with AD. These selection criteria may have introduced some bias, mainly impacting our ability to generalize our findings. When selecting the validation sample, we purposely did not limit participant inclusion to family history. This way we wanted to see whether we could validate our findings in a more heterogeneous sample of cases and controls. In the end, some participants in the validation sample had a family history of AD in a first-degree relative. In addition, the validation sample ended up being younger. As age-varying associations have been suggested as a reason for failure to replicate findings from genetic association studies,[31] we repeated the validation analysis this time with participants matched for age with the discovery sample participants. The result of no association did not change. Although phenotypic differences may have been a reason for lack of replication, the more likely possibility,

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TABLE 4. Single Nucleotide Polymorphisms Tested in the Discovery Step

SNP	Gene	Allele Tested	MAF	OR	95% CI	*P*	Permuted *P*
rs41310927	*ASPM*	G	0.43	1.09	0.69,1.7	0.71	0.99
rs121383361	*ASPM*	G	0.08	1.29	0.57,2.9	0.55	0.99
rs14126401	*ASPM*	T	0.1	1.61	0.75,3.43	0.22	0.79
rs36004306	*ASPM*	C	0.06	3.08	1.06,8.92	0.04	0.19
rs3762271	*ASPM*	A	0.43	1.09	0.69,1.73	0.69	0.99
rs10922162	*ASPM*	T	0.16	1.31	0.71,2.44	0.38	0.98
rs10801589	*ASPM*	T	0.46	0.67	0.42,1.07	0.09	0.50
rs12085377	*ASPM*	A	0.16	1.31	0.70,2.42	0.39	0.98
rs12116571	*ASPM*	T	0.12	0.49	0.25,0.98	0.04	0.19
rs6676084	*ASPM*	T	0.31	0.65	0.40,1.04	0.07	0.44
rs17550662	*ASPM*	G	0.08	0.64	0.29,1.41	0.27	0.84
rs1550697	*MCPH1*	A	0.21	0.83	0.50,1.36	0.46	1
rs2442546	*MCPH1*	G	0.2	0.88	0.54,1.45	0.63	1
rs2305022	*MCPH1*	C	0.18	0.89	0.54,1.50	0.68	1
rs2254903	*MCPH1*	G	0.16	1.01	0.59,1.70	0.98	1
rs2440423	*MCPH1*	A	0.15	0.98	0.58,1.66	0.93	1
rs2440422	*MCPH1*	T	0.16	0.93	0.55,1.55	0.77	1
rs2442518	*MCPH1*	G	0.16	0.93	0.55,1.55	0.77	1
rs2442516	*MCPH1*	G	0.16	0.91	0.55,1.52	0.73	1
rs2053618	*MCPH1*	A	0.15	1.01	0.59,1.73	0.96	1
rs930557	*MCPH1*	C	0.16	0.95	0.57,1.59	0.83	1
rs2916750	*MCPH1*	C	0.15	1.01	0.59,1.73	0.96	1
rs1054073	*MCPH1*	T	0.16	0.97	0.58,1.62	0.91	1
rs2515576	*MCPH1*	C	0.16	0.94	0.56,1.57	0.81	1
rs2277136	*MCPH1*	G	0.11	1.02	0.55,1.92	0.94	1
rs2515591	*MCPH1*	G	0.09	1.69	0.84,3.40	0.14	0.99
rs13248420	*MCPH1*	G	0.15	0.98	0.55,1.74	0.93	1
rs12677501	*MCPH1*	C	0.15	0.99	0.56,1.74	0.96	1
rs2979664	*MCPH1*	G	0.25	1.24	0.79,1.93	0.35	1
rs2440432	*MCPH1*	C	0.24	1.24	0.79,1.94	0.36	1
rs2442487	*MCPH1*	C	0.10	1.54	0.81,2.94	0.19	0.99
rs2442485	*MCPH1*	G	0.24	1.22	0.77,1.92	0.39	1
rs2920681	*MCPH1*	A	0.17	0.93	0.55,1.57	0.78	1
rs2442475	*MCPH1*	A	0.31	0.62	0.39,0.97	0.04	0.77
rs2916716	*MCPH1*	A	0.33	0.87	0.57,1.31	0.49	1
rs2916715	*MCPH1*	G	0.33	0.83	0.55,1.26	0.38	1
rs2515432	*MCPH1*	G	0.46	1.49	1,2.24	0.05	0.87
rs2922895	*MCPH1*	C	0.38	1.30	0.87,1.94	0.19	0.99
rs2515464	*MCPH1*	A	0.36	0.79	0.53,1.20	0.28	1
rs3824310	*MCPH1*	G	0.32	0.88	0.58,1.34	0.56	1
rs3824312	*MCPH1*	A	0.31	0.89	0.58,1.38	0.63	1
rs2442608	*MCPH1*	G	0.44	1.53	1.02,2.29	0.04	0.81
rs1868554	*MCPH1*	T	0.26	0.92	0.59,1.44	0.71	1
rs2442607	*MCPH1*	G	0.12	3.41	1.77,6.57	0.0002	0.01
rs734701	*MCPH1*	G	0.27	0.96	0.62,1.51	0.87	1
rs4841224	*MCPH1*	A	0.32	0.87	0.57,1.34	0.53	1
rs2959809	*MCPH1*	A	0.42	1.13	0.76,1.70	0.55	1
rs2442600	*MCPH1*	A	0.42	1.13	0.75,1.69	0.56	1
rs17552444	*MCPH1*	G	0.32	0.85	0.56,1.30	0.46	1
rs13255574	*MCPH1*	A	0.21	0.89	0.55,1.46	0.65	1
rs17553089	*MCPH1*	A	0.15	0.48	0.26,0.86	0.01	0.43
rs2442592	*MCPH1*	G	0.22	0.59	0.37,0.98	0.04	0.83
rs2515493	*MCPH1*	C	0.15	0.72	0.41,1.26	0.25	1
rs17623771	*MCPH1*	C	0.05	0.42	0.16,1.13	0.09	0.99
rs2442591	*MCPH1*	A	0.09	0.48	0.23,1.01	0.05	0.89
rs2253560	*MCPH1*	A	0.31	1.15	0.77,1.73	0.50	1
rs1057090	*MCPH1*	A	0.43	1.19	0.80,1.76	0.39	1
rs2912016	*MCPH1*	A	0.38	1.15	0.77,1.71	0.49	1
rs3020264	*MCPH1*	A	0.32	1.07	0.72,1.59	0.74	1
rs2912064	*MCPH1*	A	0.39	1.06	0.71,1.57	0.78	1
rs1974946	*MCPH1*	A	0.32	1.07	0.72,1.59	0.75	1
rs2433148	*MCPH1*	A	0.11	0.67	0.34,1.29	0.23	0.99
rs1057091	*MCPH1*	A	0.34	1.11	0.73,1.68	0.62	1
rs2433149	*MCPH1*	G	0.35	1.13	0.75,1.71	0.56	1
rs11994063	*MCPH1*	A	0.24	1.27	0.82,1.95	0.28	1
rs2433150	*MCPH1*	A	0.23	1.29	0.83,1.99	0.26	1
rs17570753	*MCPH1*	T	0.08	0.72	0.34,1.53	0.39	1
rs11774231	*MCPH1*	A	0.08	0.71	0.33,1.51	0.38	1

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TABLE 4. (*continued*)

SNP	Gene	Allele Tested	MAF	OR	95% CI	*P*	Permuted *P*
rs1055749	*MCPH1*	A	0.34	1.03	0.68,1.56	0.91	1
rs10984901	*CDK5RAP2*	C	0.35	0.79	0.54,1.18	0.26	1
rs16909739	*CDK5RAP2*	G	0.09	1.19	0.62,2.34	0.59	1
rs1888893	*CDK5RAP2*	G	0.24	0.87	0.56,1.36	0.54	1
rs4837768	*CDK5RAP2*	C	0.24	0.86	0.55,1.34	0.51	1
rs7853439	*CDK5RAP2*	A	0.24	1.19	0.77,1.85	0.43	1
rs944642	*CDK5RAP2*	C	0.08	0.57	0.26,1.2	0.15	0.99
rs10818456	*CDK5RAP2*	A	0.08	1.36	0.67,2.75	0.39	1
rs7869290	*CDK5RAP2*	G	0.36	1.19	0.81,1.77	0.37	1
rs10984922	*CDK5RAP2*	A	0.15	1.03	0.59,1.76	0.92	1
rs10818464	*CDK5RAP2*	C	0.26	0.86	0.56,1.33	0.50	1
rs10984956	*CDK5RAP2*	G	0.07	0.67	0.31,1.44	0.31	1
rs12348950	*CDK5RAP2*	G	0.41	1.02	0.68,1.53	0.92	1
rs13299371	*CDK5RAP2*	A	0.20	0.99	0.63,1.58	0.99	1
rs12864973	*CENPJ*	A	0.14	1.24	0.72,2.12	0.44	1
rs9511504	*CENPJ*	G	0.27	0.90	0.59,1.39	0.64	1
rs11616836	*CENPJ*	A	0.07	1.01	0.49,2.08	0.97	1
rs9553464	*CENPJ*	A	0.47	1.33	0.88,2.02	0.18	0.99
rs12871627	*CENPJ*	C	0.39	0.85	0.57,1.28	0.44	1
rs17402892	*CENPJ*	C	0.16	1.14	0.67,1.94	0.63	1
rs10220033	*CENPJ*	A	0.17	1.15	0.67,1.96	0.62	1
rs2290277	*CENPJ*	A	0.15	1.25	0.73,2.17	0.42	1
rs9511510	*CENPJ*	A	0.15	1.13	0.67,1.94	0.64	1
rs1029113	*CENPJ*	C	0.31	0.97	0.64,1.46	0.87	1
rs4769388	*CENPJ*	A	0.29	0.99	0.64,1.56	0.99	1

ASPM indicates abnormal spindle-like microcephaly associated (chromosome 1); *CDK5RAP2*, cyclin-dependent kinase 5 regulatory subunit-associated protein 2 (chromosome 9); *CENPJ*, centromere protein J (chromosome 13); CI, confidence interval; MAF, minor allele frequency; *MCPH1*, microcephalin (chromosome 8); OR, odds ratio; SNP, single nucleotide polymorphism.

given the uncommonly high OR, is that the initial finding was spurious.

The second limitation is that *rs2442607* in *MCPH1* was not genotyped in the ADNI population. Although we did not have direct information on this marker, 2 SNPs in strong LD with this marker were genotyped in ADNI and did not show an association with risk of AD. Using LD data from the HapMap CEU population for selection of SNPs in the ADNI participants has been recently shown to be a valid approach.[32]

Another important limitation of this study was the dichotomous outcome used. Our initial hypothesis was that variations in the microcephaly genes affect cognitive reserve. Ideally, to be able to study cognitive reserve, one needs to have information on the amount of brain injury (such as amount of AD neuropathology and vascular changes) in relation to reserve (such as brain size, brain function, and synapse number) and the cognitive status of the participants. Practically, it is difficult to have neuropathologic and brain volume data on large numbers of participants. Thus, a less direct way of studying cognitive reserve has been to look at the risk of AD in cases and controls.[33,34]

We conclude that the common variations we measured in the 4 microcephaly genes do not affect risk of late-onset AD or that their effect size is small. As risk of AD is at best only a crude measure of cognitive reserve, future studies that will include additional information on the amount of neuropathology and brain size or function in relation to cognitive status and polymorphisms in these genes will likely be needed to definitively answer the question of whether these genes may influence cognitive reserve.

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