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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 risk of AD. We examined the association between single nucleotide polymorphisms (SNPs) tagging common sequence variations in these genes and risk of AD in Caucasian 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, $p=0.01$, $OR=3.41$, $CI=1.77, 6.57$). However, this association was not replicated using another Caucasian sample from the Alzheimer Disease Neuroimaging Initiative. We conclude that the common variations we measured in the 4 microcephaly genes do not affect risk of AD, or that their effect size is small.

Keywords

Alzheimer disease; microcephaly genes; cognitive reserve

Introduction

Genetics has been suggested to play a role in variations in cognitive function in late life ¹. One way that 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

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individual susceptibility to cognitive decline by modulating brain size. This paper examines the role four microcephaly genes, abnormal spindle-like, microcephaly associated (*ASPM*), microcephalin 1 (*MCPH1*), centromeric protein J (*CENPJ*) and cyclin-dependent kinase5 regulatory subunit associated protein 2 (*CDK5RAP2*) play in late-life risk of Alzheimer disease (AD).

Mutations in these four genes cause reduced brain size, head circumference and mental retardation³. The microcephaly genes are expressed in the embryonic brain, especially in the ventricular zone, during cerebral cortical neurogenesis⁴. The timing and location of their expression suggest that they play a role in regulating neurogenic mitosis before birth⁴. Sequence comparisons among primate species suggest that microcephaly genes experienced adaptive evolution due to positive selection^{5–9}. Namely, initially rare variants in these genes increased in frequency over time due to an advantageous phenotype they provide.

There is also evidence that two haplotype-defining single nucleotide polymorphisms (SNPs) in *ASPM* and *MCPH1*, *rs41310927* and *rs930557* respectively, have continued to work under positive selection, beyond emergence of the anatomically modern humans^{6–8}. Previous 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 studies to date have found no association between brain-related phenotypes (brain volumes, general cognitive ability, head circumference, or risk of schizophrenia) and these two polymorphisms. Only two 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 two SNPs in *CDK5RAP2* and schizophrenia, bipolar spectrum disorder, AD and mild cognitive impairment (MCI) and found no association¹⁶.

In this study we examined the association between the microcephaly genes and risk of AD using tag SNPs covering all four genes. To test our hypothesis, we completed a two-step study. In the discovery step two related but separate case-control studies were performed: 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) was used to validate the findings from the discovery step.

Methods

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

Description of Subjects

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

Description of subject evaluations

All subjects are followed semi-annually with standardized clinical examinations. Cognitive and functional assessments are made using the Clinical Dementia Rating (CDR)¹⁸, the Neurobehavioral Cognitive Status Examination¹⁹, the Mini Mental State Examination²⁰ and apsymetric test battery covering key domains²¹. Functional status is determined using the Functional Activities Questionnaire²². A CDR score is assigned to each subject by a neurologist at each semiannual visit based on cognitive and functional exams and collateral history. Subjects underwent routine laboratory tests and imaging for diagnosis of AD. Diagnosis of AD is based on established diagnostic criteria²³. For this study, controls were defined as a CDR = 0 at last evaluation.

Inclusion and exclusion criteria

Included in both discovery analyses were all subjects 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 none of the subjects had an undescribed genetic predilection for AD that could confound the analyses) and, 4) 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 onset of symptoms for cases or at last evaluation for controls. When comparing basic demographics between cohorts to ensure that cohort differences were not confounding the analysis, it became evident that subjects from the memory clinic were younger than the subjects in the longitudinal aging studies. Therefore an age criteria was added. This criteria was reduced to >64 years old 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 study²⁴ and the HapMap CEU (Utah Residents with Northern and Western European Ancestry) population²⁵. In the pilot study coding regions of *ASPM* were re-sequenced in an independent sample of 59 subjects (30 cases and 29 controls) from our study population. We determined that the frequencies of the *ASPM* SNPs in our population were comparable to 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 identical to the program Tagger²⁶. A pair-wise tagging method using single markers with thresholds of 0.8 for r^2 and 3.0 for LOD score was used, and included were SNPs with a minor allele frequency (MAF) of > 5%. A coding SNP (*rs41310927*) was also included in the final tag SNP panel based on our initial pilot study results. In the end, two 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 method²⁸.

Purified samples of genomic DNA, obtained from 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, bi-directional 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 (Utah Residents with Northern and Western European

Ancestry) population²⁵. 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 LOD score was used, and included were SNPs with an MAF of $> 5\%$. 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 ADNI has been to test 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 subjects 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 subjects with AD who were self-reported “Caucasian”, and were >64 years old (at onset of symptoms for cases or at last evaluation for controls). The mean age of the ADNI subjects meeting these inclusion and exclusion criteria ended up being younger than the discovery sample subjects: $75.92 (\pm 6.06)$ for cases and $78.48 (\pm 5.28)$ for controls in ADNI as opposed to $83.92 (\pm 9.59)$ for cases and $88.55 (\pm 7.28)$ for controls in the discovery sample. Therefore we repeated the validation analysis, this time only including subjects from ADNI matched by age to the subjects from the discovery sample.

Statistical Analysis

JMP (SAS Institute, Cary, NC, US) was used for group comparisons while, R v 2.9.2²⁹(www.R-project.org) PLINK v 1.06³⁰(<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’s T-test. Gender and Apolipoprotein E (*APOE*) genotype differences were compared with chi-square.

Genetics—LD structure and haplotype blocks were determined using the confidence interval method²⁸. 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 was $< 90\%$ of the subjects 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 by multiple logistic regression with AD case/control status as the dependent variable and SNP genotype and additional covariates as independent variables. One model was tested in which the covariates were chosen based on previous knowledge of association with disease risk: cohort, gender, years of education, age (age at onset of symptoms for cases/age at last evaluation for controls) and *APOE* genotype (coded as the presence of one or more $\epsilon 4$ allele versus none). This model tests the additive effects of allele dosage of the minor allele where the Odds Ratio (OR) represents the effect of each extra minor allele controlling for all of the covariates. Parameters were estimated with 95% confidence intervals. Empirical SNP-disease association p-values were obtained by permuting the case/controls status among all

individuals 10,000 times and testing the identical multiple logistic regression to obtain the null distribution.

Results

Subject 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 subjects from the first discovery sample met inclusion/exclusion criteria for the second discovery sample. These 4 were excluded due to change in diagnosis. The validation sample consisted of 276 cases and 217 controls.

Age at onset for cases in both the two discovery samples and validation sample were significantly younger than age at last evaluation for controls. The AD groups in both discovery and validation samples had significantly fewer years of education and more *APOE* $\epsilon 4$ carriers. (Tables 1, 2 and 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, two *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 presence of AD before correction for multiple testing. Neither SNP 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 presence of AD (Table 4).

Validation step

Next, the association between disease status and *MCPH1* in ADNI subjects was assessed. ADNI subjects have already been genotyped using the Illumina Human 610 Quad platform. Genome-wide genotype data is available at the study website. We included all SNPs in *MCPH1* that were already genotyped in the ADNI subjects in our validation analysis. None of the 228 SNPs in *MCPH1* were significantly associated with risk of AD before permutation tests. The SNP 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 two 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 HapMap²⁵ 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 subjects selected for this study (MAF=0.15 for both SNPs). When we limited the validation analysis to subjects matched for age to the subjects in the discovery analysis the result of no association did not change.

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 previous 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 MRI scans and *rs41310927* in *ASPM* and *rs930557* in *MCPHI* ^{11, 14}. Both of these studies found no significant effect of either polymorphism, alone or in combination, on three measures of brain size. This may have been due to lack of power given small sample sizes of *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}.

Several recent studies have reported inconsistent sex-specific relationships between the microcephaly genes and brain volume measures. For example, one study of 867 Han Chinese individuals found no association in their entire sample. However in a gender stratified analysis, they found that males homozygous for the C allele of *rs1057090* in *MCPHI* have larger cranial volumes ¹³. Another study examined the relationship between brain volumes and SNPs in the microcephaly genes in 287 subjects. The authors found an association with 10 SNPs in *CDK5RAP2* and brain volume or cortical area in males only ¹⁶. Four SNPs in *MCPHI* showed an association with brain volume or cortical area in females only. One SNP in *ASPM* showed an association with intracranial volume in females only. The authors replicated their finding for 2 SNPs from *CDK5RAP2* in a validation sample of 657 subjects from ADNI. The authors also examined the association between two 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 subjects in the discovery step by gender and repeating the association analysis. We found no sex-specific effect. The G allele of *rs2442607* remained associated with AD risk in both genders (*p*=0.03 in males-55 cases and 75 controls, and *p*=0.003 in females-105 cases and 93 controls). While 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. Additionally, while one SNP in *MCPHI* was associated with cranial volume in Han Chinese men only, four other SNPs in *MCPHI* 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 four selection criteria to be included in this study for the discovery samples: presence of banked DNA, age, Caucasian 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 subject inclusion to family history. This way we wanted to see if we could validate our findings in a more heterogeneous sample of cases and controls. In the end some subjects in the validation sample had a family history of AD in a first degree relative. Additionally, the validation sample ended up being younger. Because 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 subjects matched for age to the discovery sample subjects. The result of no association did not change. While phenotypic differences may have been a reason for lack of replication, the more likely possibility, 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. While we did not have direct information on this marker, two 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 subjects has been recently shown to be a valid approach³².

Another important limitation of this study is 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 subjects. Practically, it is difficult to have neuropathological and brain volume data on large numbers of subjects. 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. While risk of AD is at best only a crude measure of cognitive reserve, future studies which 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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Table 1

Subject characteristics of the first discovery sample

	AD N=132	Controls N=141	p-value
% 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 <i>APOE</i> ϵ 4 present	34.85%	14.18%	<.0001

Values are frequency or mean (SD)

AD: Alzheimer disease

Table 2

Subject characteristics of the second discovery sample

	AD N=160	Controls N=168	p-value
% 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)	<.0001
Education, years	13.23 (3.45)	14.52 (2.90)	0.0003
One or more <i>APOE</i> ϵ 4 present	39.38%	13.69%	<.0001

Values are frequency or mean (SD)

AD: Alzheimer disease

Table 3

Subject characteristics of the validation sample

	AD N=276	Controls N=217	p-value
% 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)	<.0001
Education, years	15.14 (3.04)	16.06 (2.72)	0.0005
One or more <i>APOE</i> ε4 present	64.86%	25.35%	<.0001

Table 4

Single nucleotide polymorphisms tested in the discovery step

SNP	Gene	Allele Tested	MAF	OR	95% CI	p-value	Permuted p-value
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	MCPI1	A	0.21	0.83	0.50,1.36	0.46	1
rs2442546	MCPI1	G	0.2	0.88	0.54,1.45	0.63	1
rs2305022	MCPI1	C	0.18	0.89	0.54,1.50	0.68	1
rs2254903	MCPI1	G	0.16	1.01	0.59,1.70	0.98	1
rs2440423	MCPI1	A	0.15	0.98	0.58,1.66	0.93	1
rs2440422	MCPI1	T	0.16	0.93	0.55,1.55	0.77	1
rs2442518	MCPI1	G	0.16	0.93	0.55,1.55	0.77	1
rs2442516	MCPI1	G	0.16	0.91	0.55,1.52	0.73	1
rs2053618	MCPI1	A	0.15	1.01	0.59,1.73	0.96	1
rs930557	MCPI1	C	0.16	0.95	0.57,1.59	0.83	1
rs2916750	MCPI1	C	0.15	1.01	0.59,1.73	0.96	1
rs1054073	MCPI1	T	0.16	0.97	0.58,1.62	0.91	1
rs2515576	MCPI1	C	0.16	0.94	0.56,1.57	0.81	1
rs2277136	MCPI1	G	0.11	1.02	0.55,1.92	0.94	1
rs2515591	MCPI1	G	0.09	1.69	0.84,3.40	0.14	0.99
rs13248420	MCPI1	G	0.15	0.98	0.55,1.74	0.93	1

SNP	Gene	Allele Tested	MAF	OR	95% CI	p-value	Permutated p-value
rs12677501	MCPHI	C	0.15	0.99	0.56,1.74	0.96	1
rs2979664	MCPHI	G	0.25	1.24	0.79,1.93	0.35	1
rs2440432	MCPHI	C	0.24	1.24	0.79,1.94	0.36	1
rs2442487	MCPHI	C	0.10	1.54	0.81,2.94	0.19	0.99
rs2442485	MCPHI	G	0.24	1.22	0.77,1.92	0.39	1
rs2920681	MCPHI	A	0.17	0.93	0.55,1.57	0.78	1
rs2442475	MCPHI	A	0.31	0.62	0.39,0.97	0.04	0.77
rs2916716	MCPHI	A	0.33	0.87	0.57,1.31	0.49	1
rs2916715	MCPHI	G	0.33	0.83	0.55,1.26	0.38	1
rs2515432	MCPHI	G	0.46	1.49	1.2,2.4	0.05	0.87
rs2922895	MCPHI	C	0.38	1.30	0.87,1.94	0.19	0.99
rs2515464	MCPHI	A	0.36	0.79	0.53,1.20	0.28	1
rs3824310	MCPHI	G	0.32	0.88	0.58,1.34	0.56	1
rs3824312	MCPHI	A	0.31	0.89	0.58,1.38	0.63	1
rs2442608	MCPHI	G	0.44	1.53	1.02,2.29	0.04	0.81
rs1868554	MCPHI	T	0.26	0.92	0.59,1.44	0.71	1
rs2442607	MCPHI	G	0.12	3.41	1.77,6.57	0.0002	0.01
rs734701	MCPHI	G	0.27	0.96	0.62,1.51	0.87	1
rs4841224	MCPHI	A	0.32	0.87	0.57,1.34	0.53	1
rs2959809	MCPHI	A	0.42	1.13	0.76,1.70	0.55	1
rs2442600	MCPHI	A	0.42	1.13	0.75,1.69	0.56	1
rs17552444	MCPHI	G	0.32	0.85	0.56,1.30	0.46	1
rs13255574	MCPHI	A	0.21	0.89	0.55,1.46	0.65	1
rs17553089	MCPHI	A	0.15	0.48	0.26,0.86	0.01	0.43
rs2442592	MCPHI	G	0.22	0.59	0.37,0.98	0.04	0.83
rs2515493	MCPHI	C	0.15	0.72	0.41,1.26	0.25	1
rs17623771	MCPHI	C	0.05	0.42	0.16,1.13	0.09	0.99
rs2442591	MCPHI	A	0.09	0.48	0.23,1.01	0.05	0.89
rs2253560	MCPHI	A	0.31	1.15	0.77,1.73	0.50	1

SNP	Gene	Allele Tested	MAF	OR	95% CI	p-value	Permutated p-value
rs1057090	MCPHI	A	0.43	1.19	0.80,1.76	0.39	1
rs2912016	MCPHI	A	0.38	1.15	0.77,1.71	0.49	1
rs3020264	MCPHI	A	0.32	1.07	0.72,1.59	0.74	1
rs2912064	MCPHI	A	0.39	1.06	0.71,1.57	0.78	1
rs1974946	MCPHI	A	0.32	1.07	0.72,1.59	0.75	1
rs2433148	MCPHI	A	0.11	0.67	0.34,1.29	0.23	0.99
rs1057091	MCPHI	A	0.34	1.11	0.73,1.68	0.62	1
rs2433149	MCPHI	G	0.35	1.13	0.75,1.71	0.56	1
rs11994063	MCPHI	A	0.24	1.27	0.82,1.95	0.28	1
rs2433150	MCPHI	A	0.23	1.29	0.83,1.99	0.26	1
rs17570753	MCPHI	T	0.08	0.72	0.34,1.53	0.39	1
rs11774231	MCPHI	A	0.08	0.71	0.33,1.51	0.38	1
rs1055749	MCPHI	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

SNP	Gene	Allele Tested	MAF	OR	95% CI	p-value	Permuted p-value
rs9553464	<i>CENPJ</i>	A	0.47	1.33	0.88,2.02	0.18	0.99
rs12871627	<i>CENPJ</i>	C	0.39	0.85	0.57,1.28	0.44	1
rs17402892	<i>CENPJ</i>	C	0.16	1.14	0.67,1.94	0.63	1
rs10220033	<i>CENPJ</i>	A	0.17	1.15	0.67,1.96	0.62	1
rs2290277	<i>CENPJ</i>	A	0.15	1.25	0.73,2.17	0.42	1
rs9511510	<i>CENPJ</i>	A	0.15	1.13	0.67,1.94	0.64	1
rs1029113	<i>CENPJ</i>	C	0.31	0.97	0.64,1.46	0.87	1
rs4769388	<i>CENPJ</i>	A	0.29	0.99	0.64,1.56	0.99	1

SNP: Single nucleotide polymorphism, ASPM: abnormal spindle-like microcephaly associated (chromosome 1), MCPH1: microcephalin (chromosome 8), CDK5RAP2: cyclin-dependent kinase5 regulatory subunit associated protein 2 (chromosome 9), CENPJ: centromere protein J (chromosome 13), MAF: minor allele frequency, OR: odds ratio, CI: confidence interval.