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Technical Note

Investigation of *MCPH1* G37995C and *ASPM* A44871G polymorphisms and brain size in a healthy cohort

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Loss-of-function mutations in *MCPH1* and *ASPM* are responsible for some cases of autosomal recessive primary microcephaly. Recent studies have indicated that certain common variants of these genes have been positively selected for during the evolution of modern humans. It is therefore possible that these variants may predispose to an increase in brain size in the normal human population. We genotyped the *MCPH1* G37995C and *ASPM* A44871G polymorphisms in a cohort of 118 healthy people who had undergone structural magnetic resonance imaging analysis. We did not detect significant association of either *MCPH1* G37995C or *ASPM* A44871G genotype with whole brain volume, cerebral cortical volume or proportion of grey matter in this cohort. Nor did we detect an association of combined *MCPH1* 37995C and *ASPM* 44871G allele dosage with these brain measurements. These results were also confirmed in an age-restricted subcohort of 94 individuals. This study suggests that phenotypes other than brain size may have been selected for in *ASPM* and *MCPH1* variants during evolution of modern humans.

Introduction

Autosomal recessive primary microcephaly (MCPH) is a rare genetic disorder characterised clinically by severe reduction in brain size together with mental retardation. Two of the four genes identified for this disorder thus far are *microcephalin* (*MCPH1*, OMIM *607117, responsible for 4% of Northern Pakistani MCPH cases) (Jackson et al., 2002) and *abnormal spindle-like*, *microcephaly associated* (*ASPM*, OMIM *605481, 42% of cases) (Bond et al., 2002; Woods, 2004).

MCPH1 is expressed in human foetal brain and other foetal tissues, as well as some adult tissues. The mouse homologue is highly expressed in the developing cerebral cortex (Jackson et al., 2002). *MCPH1* is implicated in control of chromosome condensation (Trimborn et al., 2004), DNA-damage response (Lin et al., 2005; Xu et al., 2004) and repression of telomerase function (Lin and Elledge, 2003).

ASPM shows high expression in foetal brain and other tissues, lower expression in adult tissues and negligible expression in adult brain (Kouprina et al., 2005). The mouse *ASPM* homologue is preferentially expressed in areas of cerebral cortical neurogenesis (Bond et al., 2002). *ASPM* may regulate the proliferation and/or differentiation of neural stem cells during brain development (Kouprina et al., 2005).

Adult patients with loss-of-function mutations in *MCPH1* have a mean brain size of approximately 400 mL (compared to a normal mean of 1350 mL), and the cerebral cortex is especially small (Evans et al., 2005). Similarly, patients with *ASPM* mutations show a substantial reduction in cerebral cortical size (Bond et al., 2002). As patients have an overall retention of normal brain structure and do not demonstrate abnormalities outside of the nervous system, it was proposed that genes underlying microcephaly are involved in developmental brain size regulation (Mochida and Walsh, 2001; Woods et al., 2005).

MCPH1 and *ASPM* show strong positive selection in the lineage leading to humans, implying that variants in these genes might have played a role in hominid brain evolution (Evans et al., 2004a,b; Kouprina et al., 2004; Wang and Su, 2004; Zhang, 2003). A recent study identified a genetic variant of *MCPH1* that arose approximately 37,000 years ago; that is, since the evolution of

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modern humans. This variant, a 'haplotype' of alleles at several single-nucleotide polymorphisms (SNPs) across the gene is defined by the presence of a guanine to cytosine substitution in exon 8, denoted G37995C (Evans et al., 2005). A similar investigation of *ASPM* revealed a haplotype, defined by the presence of allele G at the SNP A44871G in exon 18, that arose approximately 5800 years ago (Mekel-Bobrov et al., 2005). *MCPH1* 37995C and *ASPM* 44871G worldwide allele frequencies are 70% and 28%, respectively (Evans et al., 2005; Mekel-Bobrov et al., 2005). Both variants have increased in frequency since their origin, implying that they have been selected for due to some evolutionary advantage conferred upon the humans who carried these variants.

We hypothesised that if this evolutionary advantage conferred was increased brain size, present carriers of the derived variants of *MCPH1* and/or *ASPM* might have larger brains, relative to age and sex, than carriers of the ancestral variants. We tested this hypothesis by genotyping *MCPH1* G37995C and *ASPM* A44871G in a cohort of 118 healthy people who had undergone structural magnetic resonance imaging as part of the Brain Resource International Database (Gordon et al., 2005).

Subjects and methods

Subjects

One hundred and eighteen healthy participants of European ancestry (range 9–79 years; 69 males) took part in the Brain Resource International Database (BRID) study (http://www.brainresource.com). Informed written consent was provided in accordance with local human research ethical requirements. Participants were excluded if they demonstrated a family history of a genetic disorder or a personal history of mental illness, drug or alcohol addiction, physical brain injury, neurological disorder or other serious medical condition.

MRI acquisition

In order to generate structural images of participant brains for volume estimation, magnetic resonance imaging was performed on 1.5-T Siemens Vision Plus and Siemens Sonata systems (Siemens, Erlangen, Germany). High-resolution T1-weighted images were acquired using a 3D magnetisation-prepared rapid acquisition gradient echo (MPRAGE) sequence in the sagittal plane, with 180 slices, 1 mm cubic voxels, 256×256 matrix, repetition time (TR)=9.7 ms, echo time (TE)=4 ms, TI=200 ms and flip angle=12°. Images were screened for gross anatomical abnormalities by a radiologist.

MRI analysis

Segmentation and spatial normalisation of MRI data was performed using voxel-based morphometry in SPM2 (http://www.fil.ion.ucl.ac.uk/spm), using a protocol described previously (Ashburner and Friston, 2000; Good et al., 2001). Images were spatially normalised by transforming each brain to a standardised stereotactic space based on the ICBM 152 template (Montreal Neurological Institute). Images were segmented into grey, white, CSF and non-brain portions based on a cluster analysis method to separate pixels based on intensity differences, together with *a priori* knowledge of spatial tissue distribution patterns in normal subjects (Friston et al., 1996). Customised templates created from 223 individuals in the BRID were used for normalisation and segmentation processes (Grieve et al., 2005). A correction was made to preserve quantitative tissue volumes following the normalisation procedure (Ashburner and Friston, 2000; Whitford et al., 2007). The regions of interest for volumetric analysis were defined using neuroanatomical masks for the frontal, parietal, temporal and occipital cortices. These were based on a previously published anatomical parcellation of the MNI single-subject brain (Tzourio-Mazoyer et al., 2002). We determined cerebral cortical volume by summing frontal, parietal, temporal and occipital grey and white matter. The proportion of grey matter measure was derived by dividing absolute grey matter volume by total brain volume.

Spot the Word test

Participants performed the Spot the Word test as part of a standardised touch-screen-based battery of neuropsychological tests, via the BRID (Gordon, 2003; Gordon et al., 2005). This task is similar to the Spot the Real Word test (Baddeley et al., 1993). On each trial, a valid English word was presented simultaneously with a non-word foil, and participants selected the valid word via the touch screen. Test score is highly correlated (r=0.8) with the WAIS test of intellectual ability (Paul et al., 2005) and is a good indicator of premorbid IQ.

Genotyping

Genomic DNA was extracted from cheek swab samples by standard proteinase K digestion and chloroform extraction. *MCPH1* G37995C and *ASPM* A44871G genotypes were determined using primer extension followed by mass spectrometry analysis on the Sequenom MassARRAY system (Sequenom, San Diego, CA) by the Australian Genome Research Facility (http://www.agrf.org.au/). Genotypes of four samples unsuitable for analysis by mass spectrometry were determined by polymerase chain reaction (PCR) amplification of DNA under standard conditions, followed by direct sequencing of PCR products. Primers used for PCR amplification of *MCPH1* G37995C were 5′-TTTCAAAGGAAGAAATAAACTTGC-3′ and 5′-GAGGTGAATGGGAGCCATGT-3′; *ASPM* A44871G was amplified using primers 5′-AGGGCTGCAGTTCTCATTCAG-3′ and 5′-GCCCACTGAAGCTTTTGGTAG-3′. Direct sequencing was performed using the BigDye Version 3.1 reaction kit and capillary electrophoresis on the 3730 DNA Analyzer (both Applied Biosystems, Foster City, CA).

Data analysis

We performed between-group comparisons of demographic data by using independent t tests, one-way analysis of variance, or χ^2 tests, as appropriate. Whole brain volume, cerebral cortical volume and proportion of grey matter were analysed using analysis of covariance with *ASPM* or *MCPH1* genotype as the between-group factor and age and sex as covariates. The effect of increasing 'derived allele' (*MCPH1* 377995C and *ASPM* 44871G) dosage was investigated in a multiple regression model. Age, sex and derived allele loading (1=*MCPH1*/*ASPM* genotype GC/AA; 2=GC/AG or CC/AA; 3=GC/GG or CC/AG; 4=CC/GG) were

Table 1
Demographics of *MCPH1*, *ASPM* and derived allele genotype groups

	MCPH1 genotype, $n=117$		*ASPM* genotype, $n=118$			No. of derived alleles[a], $n=114$			
	GG or GC	CC	AA	AG	GG	1	2	3	4
n	38[b]	79	56	45	17	12	56	36	10
Gender (M/F)	(24/14)	(45/34)	(33/23)	(25/20)	(11/6)	(9/3)	(33/23)	(19/17)	(7/3)
Age[c]	41.4±20.8	35.9±15.9	36.8±19.6	36.8±14.9	43.3±17.9	39.8±24.9	34.2±15.7	38.7±16.4	42.2±16.5
VI score[c,d]	50.3±6.8	49.8±4.4	50.4±5.3	48.8±5.3	51.6±4.2	49.2±7.3	49.6±5.2	50.1±4.8	50.1±3.7

M: no. of males; F: no. of females; VI: verbal intelligence.
[a] Genotypes of derived allele groups (derived alleles underlined): 1, *MCPH1*/*ASPM* genotype GC/AA; 2, GC/AG or CC/AA; 3, GC/GG or CC/AG; 4, CC/GG.
[b] GG, $n=3$; GC, $n=35$.
[c] Mean±standard deviation.
[d] Score from Spot the Word test.

used as predictors. Given the explicit hypotheses for the study, data were analysed using an α level of $p<0.05$.

Results

Genotype frequencies for *MCPH1* G37995C and *ASPM* A44871G and demographic characteristics are presented in Table 1. For *MCPH1*, G denotes the ancestral allele and C denotes the derived allele. For *ASPM*, the ancestral and derived alleles are A and G, respectively. Genotype frequencies were in Hardy–Weinberg equilibrium (*MCPH1* G37995C, GG=2.6%, GC=29.9%, CC=67.5%, $\chi^2_{(2)}=0.143$, $p=0.931$; *ASPM* A44871G, AA=47.5%, AG=38.1%, GG=14.4%, $\chi^2_{(2)}=2.439$, $p=0.295$). *MCPH1* derived allele frequency (193/234 chromosomes, 82.5%) was not significantly different from that calculated from European populations reported by Evans et al. (2005) (191/230, 83.0%) ($\chi^2_{(1)}=0.026$, $p=0.872$). However, *ASPM* derived allele frequency (79/236, 33.5%) did differ significantly from that reported by Mekel-Bobrov et al. (2005) (96/214, 44.9%) ($\chi^2_{(1)}=6.121$, $p=0.013$).

Due to the low frequency of the *MCPH1* ancestral G allele, GG and GC genotype groups were grouped together for all statistical analyses. *MCPH1* and *ASPM* genotype groups did not differ significantly in mean age (*MCPH1*, $t_{(58)}=1.45$, $p=0.152$; *ASPM*, $F_{(2,115)}=0.99$, $p=0.376$) sex distribution (*MCPH1*, $\chi^2_{(1)}=0.407$, $p=0.523$; *ASPM*, $\chi^2_{(2)}=0.435$, $p=0.805$) or Spot the Word score, a measure of verbal intelligence (*MCPH1*, $t_{(49)}=0.37$, $p=0.715$; *ASPM*, $F_{(2,113)}=2.01$, $p=0.139$) (Table 1).

To assess the impact of microcephaly-associated gene variants on structural brain characteristics, we compared *MCPH1* and *ASPM* genotype groups for three different measures: total brain volume, cerebral cortical volume and proportion of grey matter in the brain. As expected, age and sex had significant ($p<0.05$) effects on all three measures (Table 2). Mean total brain volume did not significantly differ between genotype groups for *MCPH1* ($p=0.959$) or for *ASPM* ($p=0.995$) (Table 2; Figs. 1a, d). Mean cerebral cortical volumes of *MCPH1* and *ASPM* genotype groups are presented in Table 2 and Figs. 1b and e. We did not detect a significant effect of genotype (*MCPH1*: $p=0.555$; *ASPM*: $p=0.973$). The proportion of grey matter to whole brain volume was calculated for *MCPH1* and *ASPM* genotype groups (Table 2; Figs. 1c, f). Again, no significant genotype effects were detected (*MCPH1*: $p=0.610$; *ASPM*: $p=0.325$).

Given that the effect of sex on brain size is well established (Gur et al., 1991; Witelson et al., 2006), it is possible that *MCPH1*

Table 2
Univariate ANCOVA of *MCPH1* or *ASPM* genotype and brain size characteristics

	MCPH1: Mean±SE[a,b]			F[c]	p	Partial η^2[d]	ASPM: Mean±SE[a,e]				F[f]	p	Partial η^2[d]
	GG or GC	CC					AA	AG	GG				
Total brain volume (mL)	1543±19	1542±13	*MCPH1*	0.00	0.959	<0.001	1541±16	1539±18	1540±29	*ASPM*	0.00	0.995	<0.001
			Age	4.73	0.032	0.040				Age	4.93	0.028	0.042
			Sex	85.71	<0.001	0.431				Sex	87.94	<0.001	0.438
Cerebral cortical volume (mL)	720±10	728±7	*MCPH1*	0.35	0.555	0.003	726±8	723±9	723±15	*ASPM*	0.03	0.973	<0.001
			Age	24.80	<0.001	0.180				Age	26.03	<0.001	0.187
			Sex	60.55	<0.001	0.349				Sex	61.57	<0.001	0.353
Proportion GM (% of total brain volume)	49.2±0.3	49.3±0.2	*MCPH1*	0.26	0.610	0.002	49.5±0.2	49.3±0.2	48.8±0.4	*ASPM*	1.14	0.325	0.020
			Age	190.62	<0.001	0.628				Age	187.70	<0.001	0.624
			Sex	10.20	0.002	0.083				Sex	11.14	0.001	0.090

GM: grey matter.
[a] Estimated marginal means, adjusted for age and sex.
[b] *MCPH1* G37995C genotype groups: G is the ancestral allele, C is the derived allele.
[c] Degrees of freedom: 1, 113.
[d] Estimate of effect size. $\eta^2=0.5$ indicates that effect accounts for 50% of the variance in the dependent variable.
[e] *ASPM* A44871G genotype groups: A is the ancestral allele, G is the derived allele.
[f] Degrees of freedom: (*ASPM*) 2, 113; (other variables) 1, 113.

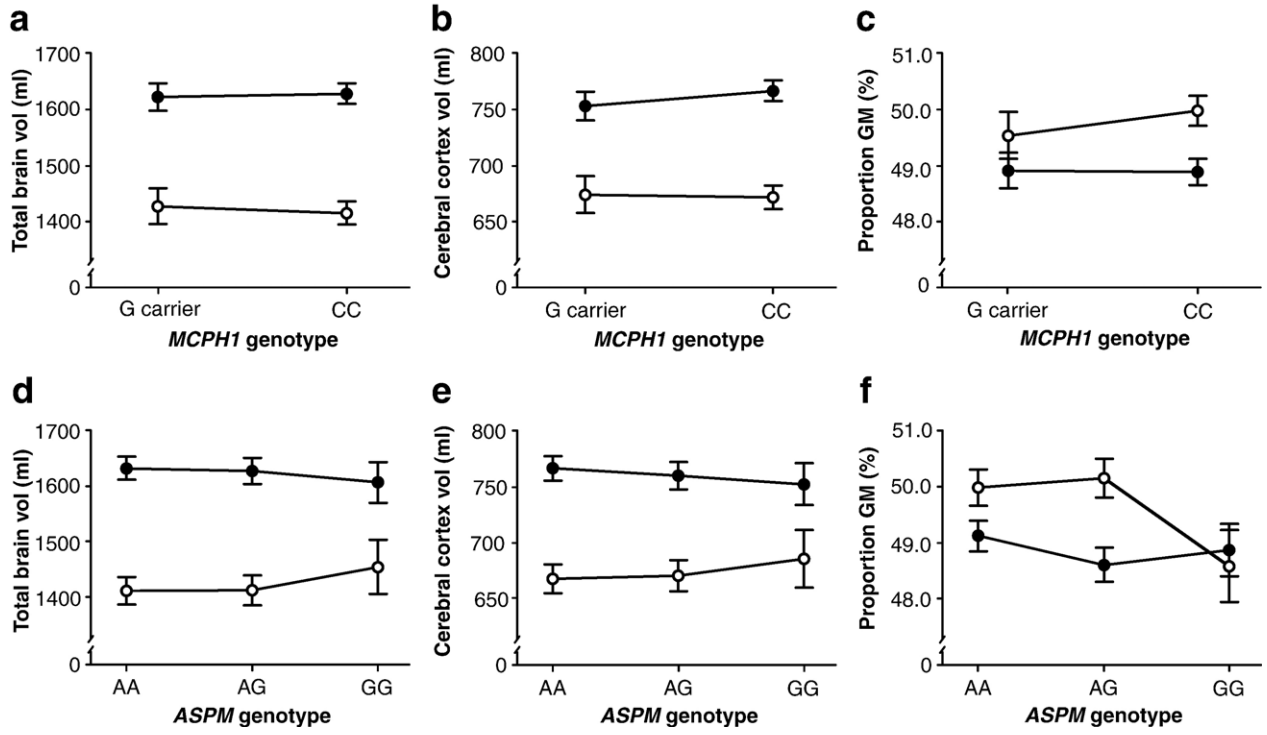

Fig. 1. Comparison of brain characteristics for *MCPH1* and *ASPM* genotype groups. Estimated marginal means, adjusted for age, of (a, d) total brain volume, (b, e) cerebral cortical volume and (c, f) proportion of grey matter (percentage of total brain volume), organised by (a–c) *MCPH1* G37995C genotype or (d–f) *ASPM* A44871G genotype. Black circles denote males, white circles denote females. Error bars indicate standard error of the mean.

and/or *ASPM* variants have differential effects on brain size according to sex. However, separate ANCOVAs using sex as a between-group factor demonstrated no significant sex-by-genotype interaction for total brain volume (sex × *MCPH1*: $p=0.700$; sex × *ASPM*: $p=0.595$), cerebral cortical volume (sex × *MCPH1*: $p=0.526$; sex × *ASPM*: $p=0.667$), or proportion of grey matter (sex × *MCPH1*: $p=0.464$; sex × *ASPM*: $p=0.130$).

To investigate whether the combination of *MCPH1* and *ASPM* alleles has an additive effect on brain size characteristics, we predicted mean total brain volume, proportion of grey matter and cerebral cortical volume using 'derived allele dosage' (i.e., the total number of derived alleles possessed (*MCPH1* 37995*C* and/or *ASPM* 44871*G*)), age and sex as regressors. Subjects were divided into groups containing one (*MCPH1*/*ASPM* genotype GC/AA), two (GC/AG or CC/AA), three (GC/GG or CC/AG) or four (CC/GG) derived alleles (Tables 1 and 2). Subjects with *MCPH1* GG genotype ($n=3$) were excluded to simplify analysis. Multiple regression analysis indicated no significant effect of increasing derived allele dosage on any of the measures examined (Table 3).

To determine if differences in very young or old subjects were masking genotype effects in our full cohort, we also calculated mean total brain volume, cerebral cortical volume and proportion

Table 3
Regression analyses to assess effect of derived allele dosage on brain size characteristics

Dependent variable	Model summary	Explanatory variable	β [a]	t	p
Total brain volume	$F_{(3,110)}=29.38$, $p<0.001$, $R^2_{adj}=0.430$	Age	−0.158	−2.21	0.029
		Sex [b]	−0.642	−9.03	<0.001
		No. derived alleles [c]	0.002	0.03	0.973
Cerebral cortical volume	$F_{(3,110)}=27.40$, $p<0.001$, $R^2_{adj}=0.412$	Age	−0.333	−4.59	<0.001
		Sex	−0.551	−7.63	<0.001
		No. derived alleles	0.001	0.01	0.991
Proportion GM	$F_{(3,110)}=63.80$, $p<0.001$, $R^2_{adj}=0.625$	Age	−0.780	−13.49	<0.001
		Sex	0.176	3.05	0.003
		No. derived alleles	−0.035	−0.61	0.546

GM: grey matter; R^2_{adj}: adjusted R square value.

[a] Standardised β coefficient. The size and sign of β indicate the magnitude and direction, respectively, of the relationship between explanatory and dependent variables.

[b] Coded as follows: 0=male, 1=female.

[c] Coded as follows (derived alleles underlined): 1=*MCPH1*/*ASPM* genotype GC/AA, 2=GC/AG or CC/AA, 3=GC/GG or CC/AG, 4=CC/GG.

of grey matter of *MCPH1*, *ASPM* and derived allele genotype groups in an age-restricted subcohort (18–59 years, $n=94$) (Supplementary Table 1). No significant effects of genotype were detected.

Discussion

In this study, we did not detect a significant effect of *MCPH1* G37995C or *ASPM* A44871G, either alone or in combination, on three measures of brain size. This study had >80% power to detect an effect accounting for 6.5% of the variance (Purcell et al., 2003): e.g., a 4.0% mean difference in total brain volume due to *ASPM* genotype or 4.3% mean difference due to *MCPH1* genotype (Lenth, 2006). During completion of this study, Woods et al. (2006) also reported no significant effect of *MCPH1* G37995C or *ASPM* A44871G genotype on brain size in a similarly sized cohort. These negative findings in two studies indicate that, if common *MCPH1* and/or *ASPM* variants do affect brain size, their effect is too small to be detected without using substantially larger cohorts than are routinely used for structural MRI analyses. The low partial η^2 estimates for effect of *ASPM* and *MCPH1* (<0.001–0.003, Table 2) and low β coefficients for the effect of derived allele dosage (−0.035–0.002, Table 3) suggest that this is indeed the case.

MCPH1 and *ASPM* are expressed predominantly in foetal tissues (Bond et al., 2002; Jackson et al., 2002), and abnormalities in patients with loss-of-function mutations are apparent from 32 weeks of gestation (Woods et al., 2005). We therefore anticipated that if common variants of these genes had an effect on brain size, this effect would be evident from birth and could be detected in our full cohort. Analysis of an age-restricted subcohort of 18- to 59-year-old adults also indicated that the genetic effects were not being masked by either developmental factors in children or degeneration in elderly subjects (Supplementary Table 1).

In the absence of a detectable effect on brain size *per se*, it is feasible that more specific phenotypes of brain structure could detect an influence of *MCPH1* and *ASPM* genotypes. Primate brain evolution has been characterised by a relative increase in the size of the cerebral cortex (Clark et al., 2001): one phenotype that could have been selected for during the evolution of modern humans is increased cerebral cortical size relative to total brain volume. The cerebral cortex is especially small relative to brain size in microcephaly patients with *MCPH1* or *ASPM* mutations (Bond et al., 2002; Evans et al., 2005). It is therefore not unreasonable to assume that any effects on brain size by variation in *MCPH1* and *ASPM* variants may be more detectable when considering the cerebral cortex alone. However, this was not the case in our cohort (Figs. 1b and e; Table 2). As *MCPH1* and *ASPM* are involved in neuronal proliferation (Kouprina et al., 2005; Lin et al., 2005; Xu et al., 2004), we also considered that the proportion of grey matter in the brain might be more influenced by variation in these genes than brain size alone. However, we could not detect such an effect in this study (Figs. 1c and f; Table 2). Future studies could consider using diffusion tensor imaging analyses to distinguish between *MCPH1* and *ASPM* genotype groups, as this may reveal differences in cortical connectivity that are not detectable as volume changes.

It is possible that, in contrast to *MCPH1* and *ASPM* null mutations, common polymorphisms in these genes only have an effect in small regions of the brain. As such, they might affect more subtle functional phenotypes, such as cognition and temperament, without producing a measurable increase in brain size. We did not see any differences for an estimate of verbal intelligence as deduced from the Spot the Word score (Table 1). However, as cognitive performance and personality scores are substantially less heritable than brain size (Baare et al., 2001; Bouchard and McGue, 2003; Wright et al., 2001), larger cohorts are required to assess these effects. Since completion of this study, two reports of large cohorts have been published that found no effect of *ASPM* or *MCPH1* polymorphisms on general mental ability, altruism (Rushton et al., 2007) or IQ (Mekel-Bobrov et al., 2007).

The evolution of *MCPH1* and *ASPM* in modern humans may even have been driven by selection of a non-neurological phenotype. *MCPH1* is expressed in foetal liver and kidney at similar amounts to foetal brain, and at lower levels in other foetal tissues and adult tissues (Jackson et al., 2002). *ASPM* is also expressed in several foetal tissues in addition to brain (Kouprina et al., 2005). *ASPM* is upregulated and *MCPH1* is downregulated in cancers of varying origin (Kouprina et al., 2005; Rai et al., 2006) – relative susceptibility to cancer might therefore be an interesting phenotype to compare in carriers of ancestral and derived variants of these genes. Lastly, we note that some authors find no evidence for recent positive selection in *MCPH1* and/or *ASPM* (Currat et al., 2006; Yu et al., 2007). If they are correct, people with *MCPH1* 37995C or *ASPM* 44871G variants may not differ at all from those who carry the ancestral alleles.

In summary, despite recent reports of evolutionary expansion of certain *MCPH1* and *ASPM* haplotypes in modern humans (Evans et al., 2005; Mekel-Bobrov et al., 2005), we did not detect significant association of either *MCPH1* G37995C or *ASPM* A44871G genotype with whole brain volume, cerebral cortical volume or proportion of grey matter as determined by structural MRI in this cohort.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2007.05.011.

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