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The ongoing adaptive evolution of *ASPM* and *Microcephalin* is not explained by increased intelligence

Nitzan Mekel-Bobrov[1,2,†], Danielle Posthuma[3,4,*,†], Sandra L. Gilbert[1], Penelope Lind[6], M. Florencia Gosso[3,4], Michelle Luciano[6], Sarah E. Harris[7], Timothy C. Bates[7], Tinca J.C. Polderman[3,10], Lawrence J. Whalley[9], Helen Fox[9], John M. Starr[8], Patrick D. Evans[1,2], Grant W. Montgomery[6], Croydon Fernandes[1], Peter Heutink[3,4,5], Nicholas G. Martin[6], Dorret I. Boomsma[3,4,5], Ian J. Deary[7], Margaret J. Wright[6], Eco J.C. de Geus[3,4] and Bruce T. Lahn[1,*]

[1]Department of Human Genetics and [2]Committee on Genetics, Howard Hughes Medical Institute, University of Chicago, Chicago, IL 60637, USA, [3]Department of Biological Psychology, [4]Center for Neurogenomics and Cognitive Research (CNCR) and [5]Department of Clinical Genetics and Anthropogenetics, Section of Medical Genomics, Vrije Universiteit Medical Center, Amsterdam, The Netherlands, [6]Queensland Institute of Medical Research, Brisbane, Australia, [7]Department of Psychology and [8]Department of Geriatric Medicine, University of Edinburgh, Royal Victoria Hospital, Edinburgh, UK, [9]Department of Mental Health, University of Aberdeen, Clinical Research Centre, Royal Cornhill Hospital, Aberdeen, UK and [10]Department of Child and Adolescent Psychiatry Erasmus MC, Sophia, Rotterdam, The Netherlands

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Recent studies have made great strides towards identifying putative genetic events underlying the evolution of the human brain and its emergent cognitive capacities. One of the most intriguing findings is the recurrent identification of adaptive evolution in genes associated with primary microcephaly, a developmental disorder characterized by severe reduction in brain size and intelligence, reminiscent of the early hominid condition. This has led to the hypothesis that the adaptive evolution of these genes has contributed to the emergence of modern human cognition. As with other candidate loci, however, this hypothesis remains speculative due to the current lack of methodologies for characterizing the evolutionary function of these genes in humans. Two primary microcephaly genes, *ASPM* and *Microcephalin*, have been implicated not only in the adaptive evolution of the lineage leading to humans, but in ongoing selective sweeps in modern humans as well. The presence of both the putatively adaptive and neutral alleles at these loci provides a unique opportunity for using normal trait variation within humans to test the hypothesis that the recent selective sweeps are driven by an advantage in cognitive abilities. Here, we report a large-scale association study between the adaptive alleles of these genes and normal variation in several measures of IQ. Five independent samples were used, totaling 2393 subjects, including both family-based and population-based datasets. Our overall findings do not support a detectable association between the recent adaptive evolution of either *ASPM* or *Microcephalin* and changes in IQ. As we enter the post-genomic era, with the number of candidate loci underlying human evolution growing rapidly, our findings highlight the importance of direct experimental validation in elucidating their evolutionary role in shaping the human phenotype.

*To whom correspondence should be addressed. Tel: +31 7738344393; Fax: +31 7738348470; Email: blahn@bsd.uchicago.edu; Tel: +31 205988814; Fax: +31 205988832; Email: danielle@psy.vu.nl
†The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

INTRODUCTION

The most striking trend in human evolution is the rapid increase in brain size over the past 3–4 million years, and the associated increase in cognitive complexity (1). Recent advances in comparative genomics and population genetics have facilitated rapid progress in studying the genetic basis of human brain evolution. By searching for signatures of adaptive evolution in genes known to function in the mammalian brain, a large number of candidate loci have been identified (2). One of the most intriguing findings thus far has been the prominent role played by genes associated with primary microcephaly (3), a developmental defect in fetal brain growth (4,5). Given the atavistic reduction in brain size associated with their pathological state (6), the finding of strong signatures of positive selection in three of the four primary microcephaly genes identified to date (7–12) has led to many speculations regarding their role in human brain evolution, particularly in terms of cognition (3). As with other candidate loci identified to date, however, the functional role of their adaptive evolution remains speculative, given the technical and ethical limitations inherent to studying human subjects (13). Thus, the next major challenge is to characterize the functional significance of adaptive substitutions at identified candidate loci.

The two primary microcephaly genes, *ASPM* (*abnormal spindle-like microcephaly associated*) and *Microcephalin* (*MCPH1*), not only show signatures of adaptive evolution in the lineage leading to humans (7,8,10–12), but have also continued to evolve adaptively since the emergence of anatomically modern humans, with the adaptive alleles rising from one copy, as recently as ~6000 and ~40 000 years ago, to a worldwide frequency of ~30% and ~70%, respectively (14,15). The presence of both the adaptive and neutral alleles of these genes at moderate frequencies provides an ideal opportunity for using natural trait variation within humans for testing hypotheses regarding the phenotypic substrate of their selection. A recent study reported no association between the alleles of *ASPM* and *Microcephalin* and normal variation in brain size (16). These results, however, were based on a very small number of individuals, comprising highly divergent ethnic backgrounds, and whole-brain volume measurements, which may obscure inter-individual variation in specific regions of the brain that are of particular importance to cognitive complexity (17,18). Ultimately, the putative substrate of selection is intelligence, not brain size. Consequently, we chose to focus directly on heritable measures of intelligence, rather than brain size as an indirect proxy. Variation in intelligence, as measured by standard IQ tests, is one of the most heritable behavioral traits identified to date in humans. With heritability estimates ranging from 25–40% in early childhood (19) to 80% in adulthood (20), IQ scores are the best available measures of intelligence for testing genetic associations.

To test the hypothesis that the recent selective sweep at *ASPM* and *Microcephalin* is due to increased intelligence, we genotyped the diagnostic sites that distinguish the adaptive derived allele (D-allele) from the ancestral allele (A-allele) of each gene. We used a large-scale dataset comprising three independent family-based samples (a sample of Dutch children, a Dutch adult sample and an Australian adolescent sample), as well as two independent population-based samples (the LBC1921 sample from the Lothian region of Scotland, and the ABC1936 sample from the Scottish city of Aberdeen). The five replicate samples totaled 2393 individuals for whom several measures of intelligence were available from previous assessments. Our overall findings suggest that intelligence, as measured by these IQ tests, was not detectably associated with the D-allele of either *ASPM* or *Microcephalin*.

Table 1. Frequencies of *ASPM* and *MCPH1* D-alleles in five independent samples

	ASPM			MCPH1		
	Genotype	*N*	Frequency	Genotype	*N*	Frequency
Dutch children	AA	103	0.37	AA	7	0.03
	AD	119	0.43	AD	78	0.27
	DD	58	0.20	DD	203	0.70
Dutch adults	AA	92	0.35	AA	9	0.03
	AD	120	0.45	AD	88	0.30
	DD	53	0.20	DD	201	0.67
Australian	AA	289	0.33	AA	24	0.03
	AD	394	0.45	AD	291	0.33
	DD	190	0.22	DD	559	0.64
Scottish Lothian	AA	168	0.32	AA	16	0.03
	AD	234	0.46	AD	157	0.30
	DD	112	0.22	DD	346	0.67
Scottish Aberdeen	AA	51	0.26	AA	5	0.03
	AD	109	0.54	AD	66	0.32
	DD	39	0.20	DD	130	0.65

N, sample size. The Dutch and Australian samples are family-based samples. In the case of MZ twins, only one individual is included in this table for the purpose of interpretation of allele frequencies—the totals therefore are *lower* than the actual totals available for association tests, and therefore differ from those in Tables 2 and 3; for the association analyses both individuals of MZ twins are included, by taking zygosity status into account.

RESULTS

We genotyped subjects from the five samples for previously identified single nucleotide polymorphisms (SNPs), diagnostic of the *ASPM* and *Microcephalin* D-alleles (14,15). Table 1 lists the occurrence and frequency of each genotype class of *ASPM* and *Microcephalin* for the five replicate samples. Allele and genotype frequencies of *ASPM* and *Microcephalin* were comparable across all samples. Both genes were found to be in Hardy–Weinberg equilibrium (HWE) within each sample, by exact test of random mating with a Markov chain method for unbiased estimation of significance (21,22). Similarly, combining all samples together by Fisher's method (23) showed no violation of HWE. The implication of this finding for the model of adaptive evolution on these genes cannot be determined, given that a lack of deviation from HWE does not mean that its underlying assumptions are not violated (24).

Figure 1 shows the mean IQ scores of the three *ASPM* and *Microcephalin* genotypes for each replicate sample. Scores are corrected for age and sex, and z-transformed for comparison across samples. Untransformed values are provided in Table 2. Scores are listed for three different measures of

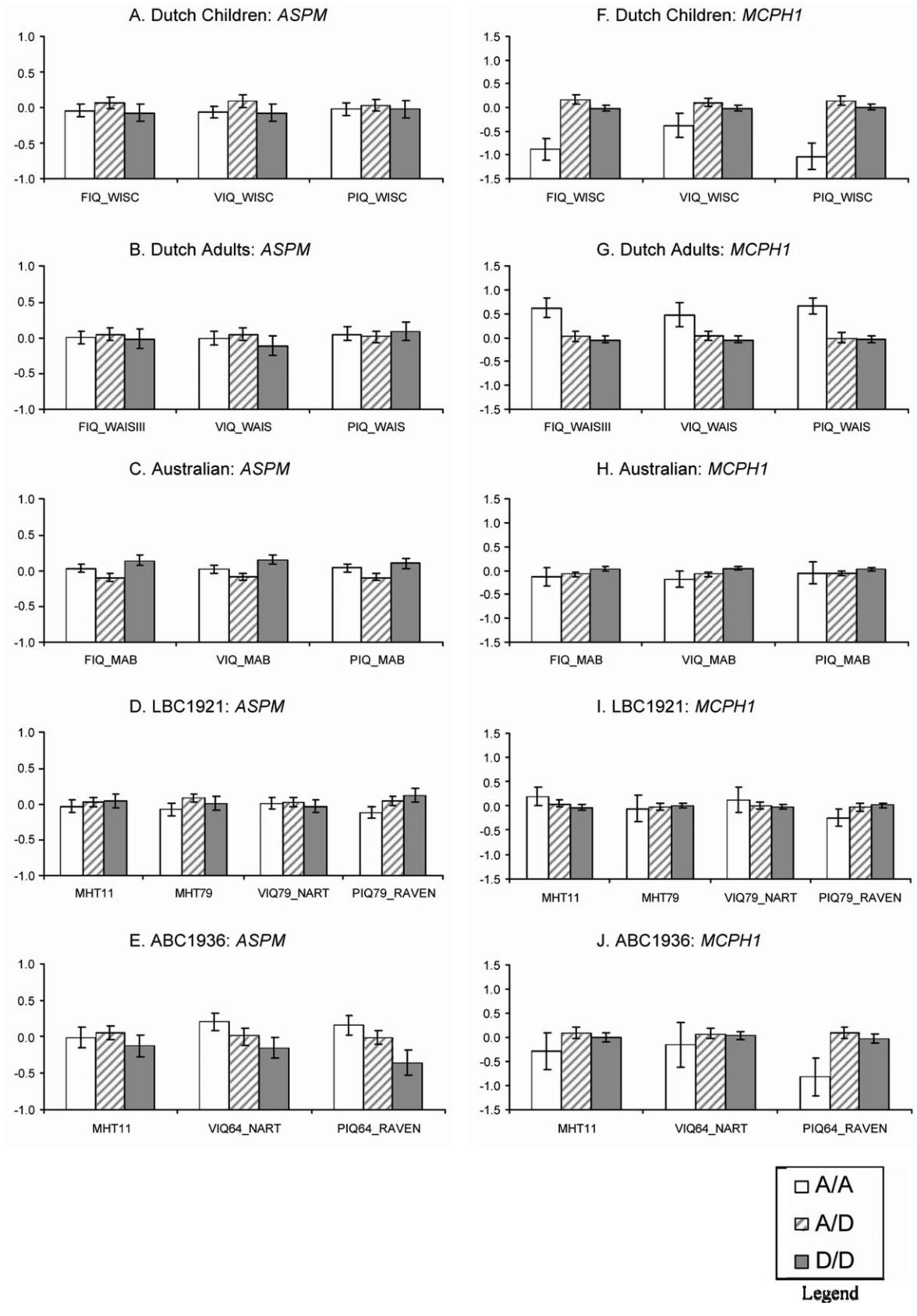

Figure 1. Mean IQ scores with standard errors of *ASPM* and *Microcephalin* (*MCPH1*) genotypes for each sample. Scores are corrected for age and sex, and then Z-transformed for each sample individually for comparison of effect size across samples. Untransformed scores are provided in Table 2.

Table 2. Mean IQ scores (corrected for age and sex) of *ASPM* and *MCPH1* genotypes for each replicate sample

	ASPM				MCPH1			
	N	FIQ (Mean ± SD)	VIQ (Mean ± SD)	PIQ (Mean ± SD)	*N*	FIQ (Mean ± SD)	VIQ (Mean ± SD)	PIQ (Mean ± SD)
Family-based samples								
Dutch children								
AA	132	99.1 ± 14.6	102.7 ± 11.6	95.9 ± 19.6	9	86.5 ± 10.3	98.5 ± 9.8	76.9 ± 15.8
AD	157	100.9 ± 15.6	104.7 ± 13.2	96.9 ± 18.7	100	102.2 ± 14.6	104.9 ± 12.4	99.2 ± 18.9
DD	79/78	98.7 ± 15.1	102.5 ± 13.9	96.0 ± 19.1	270/269	99.7 ± 15.1	103.3 ± 13.1	96.4 ± 18.6
Total	368/367	99.8 ± 15.1	103.5 ± 12.8	96.4 ± 19.1	379/378	100.0 ± 15.1	103.7 ± 12.8	96.7 ± 18.9
Dutch adults								
AA	107	103.9 ± 10.9	103.5 ± 12.0	106.0 ± 12.0	10	111.0 ± 7.4	109.8 ± 10.6	113.5 ± 6.6
AD	138	104.3 ± 11.3	104.3 ± 12.9	105.6 ± 11.8	97	104.1 ± 11.3	104.2 ± 12.7	105.4 ± 11.9
DD	64	103.7 ± 12.4	102.3 ± 13.9	106.5 ± 13.1	242	103.4 ± 11.5	103.2 ± 13.1	105.0 ± 12.2
Total	309	104.0 ± 11.4	103.6 ± 12.8	105.9 ± 12.1	349	103.8 ± 11.4	103.6 ± 12.9	105.4 ± 12.1
Australian								
AA	328-330	114.7 ± 12.5	111.9 ± 10.9	115.5 ± 15.8	25	112.7 ± 12.2	109.6 ± 9.1	114.1 ± 17.7
AD	448-450	113.0 ± 12.8	110.7 ± 11.4	113.4 ± 15.6	331-333	113.3 ± 12.3	110.8 ± 10.7	114.0 ± 15.6
DD	203	116.1 ± 12.9	113.3 ± 11.3	116.5 ± 16.5	624-626	114.8 ± 13.0	112.2 ± 11.5	115.2 ± 16.0
Total	979-983	114.2 ± 12.7	111.6 ± 11.2	114.7 ± 15.9	980-984	114.2 ± 12.7	111.6 ± 11.2	114.7 ± 15.9

	ASPM					*MCPH1*				
	N	MHT11 (Mean ± SD)	MHT79 (Mean ± SD)	VIQ (Mean ± SD)	PIQ (Mean ± SD)	*N*	MHT11 (Mean ± SD)	MHT79 (Mean ± SD)	VIQ (Mean ± SD)	PIQ (Mean ± SD)
Population-based samples										
LBC1921										
AA	148/167	42.1 ± 12.3	61.3 ± 11.5	36.4 ± 8.4	33.4 ± 8.8	14/16	44.6 ± 8.2	61.4 ± 11.3	37.3 ± 8.5	32.3 ± 6.4
AD	212/231-2	42.9 ± 11.2	62.9 ± 9.4	36.4 ± 8.2	34.8 ± 8.4	147/154–5	43.0 ± 10.6	61.8 ± 10.7	36.3 ± 8.1	34.2 ± 8.9
DD	99/109-11	42.9 ± 11.0	62.2 ± 10.7	36.1 ± 7.3	35.4 ± 8.2	303/341-4	42.1 ± 12.3	62.1 ± 10.4	36.2 ± 8.1	34.5 ± 8.4
Total	459/507-10	42.6 ± 11.5	62.2 ± 10.5	36.3 ± 8.1	34.5 ± 8.5	464/512-15	42.5 ± 11.6	62.0 ± 10.5	36.3 ± 8.1	34.4 ± 8.5
ABC1936										
AA	50/51	41.3 ± 10.6	N/A	33.1 ± 6.1	39.3 ± 7.8	5/5	38.3 ± 9.2	N/A	30.5 ± 7.3	31.4 ± 7.2
AD	109/106-8	42.1 ± 10.4	N/A	31.7 ± 7.5	37.9 ± 7.7	65/63-64	42.4 ± 10.6	N/A	32.0 ± 6.9	38.8 ± 8.1
DD	39/38	40.0 ± 10.1	N/A	30.5 ± 6.3	35.1 ± 8.3	130/129–30	41.4 ± 10.5	N/A	31.9 ± 7.1	37.7 ± 8.1
Total	198/195-7	41.5 ± 10.4	N/A	31.8 ± 7.0	37.8 ± 7.9	200/197–99	41.6 ± 10.5	N/A	31.9 ± 7.0	37.9 ± 8.1

The IQ scores for the Dutch Children sample were measured using the Wechsler Intelligence Scale for Children-Revised; for the Dutch Adults sample using the Wechsler Adult Intelligence Scale III-Revised; and for the Australian sample using the Multi-dimensional Aptitude Battery, as discussed in Materials and Methods. The Scottish IQ scores are on a different scale and do not have a traditional mean of 100 ± 15. MHT-11 and MHT-79 denote the age at which the MHT test was taken (i.e. ages 11 and 79, respectively). The VIQ and PIQ were measured in the LBC1921 and ABC1936 samples at ages 79 and 64, respectively. Sample sizes at ages 11 and either 79 or 64. N/A, not applicable.

intelligence. Although the specific tests underlying these measures vary among the replicate samples, depending on their geographic origin, they generally correspond to an overall intelligence quotient [full-scale IQ (FIQ) in the family-based samples, and Moray House Test (MHT) in the population-based samples], as well as specific measures of verbal reasoning (VIQ) and nonverbal reasoning (PIQ). The overall intelligence quotient (MHT) for the LBC1921 individuals was measured at age 11 and repeated at age 79, and is consequently given for both ages.

To test for association between the D-alleles of *ASPM* and *Microcephalin* and IQ, multiple non-independent tests were carried out for each replicate sample, modeling both additive and dominant effects. For the family-based samples, we used a quantitative transmission disequilibrium test (qTDT) that is applicable to nuclear families of any size and does not necessitate parental data (25). Based on the orthogonal model developed by Fulker *et al*. (26), genotypic effects are decomposed into between-family and within-family components, such that in the absence of spurious associations due to admixture, both components can be used to implement the more powerful population-based test, but if admixture is detected, a family-based test can still be carried out using the within-family component on its own. Results for the three family-based samples are given in Table 3; where admixture was detected results of the population-based association tests, are omitted, as they cannot be interpreted. Analogous to the family-based samples in the absence of admixture, the two Scottish samples were tested for population-based association by a simple regression model. Results for the two Scottish population-based samples are given in Table 3.

For *ASPM*, the D-allele showed statistically significant association with increased performance in both FIQ and VIQ in the Dutch Adults sample, using the family-based tests of additive effects ($P = 0.04$ for FIQ), and of additive and dominant effects ($P = 0.01$ for FIQ and VIQ). Statistically significant association was also found with increased PIQ, in the population-based test of additive and dominant effects in the LBC1921 sample ($P = 0.05$). These results, however, were not replicated in other samples. Moreover, in the Australian sample, increased FIQ was associated with the A-allele of *ASPM*, rather than the D-allele, using the population-based test of additive and dominant effects ($P = 0.05$); in the second Scottish sample, ABC1936, this same test showed a statistically significant association between the A-allele and increased PIQ. Thus, two of the three measures found to be associated with the D-allele of *ASPM* showed opposite association in other samples.

For *Microcephalin*, the D-allele showed significant association with increased FIQ, VIQ and PIQ in the Dutch Children sample, using the family-based tests of additive effects ($P = 0.02$ for FIQ and VIQ) and of additive and dominant effects ($P = 0.03$ for FIQ and $P = 0.02$ for PIQ), as well as the population-based test of additive and dominant effects ($P = 0.03$ for FIQ and $P = 0.00$ for PIQ). Similar to *ASPM*, however, these results were not replicated in other samples, and in the Dutch Adults sample, VIQ was significantly associated with the A-allele, rather than the D-allele, of *Microcephalin* in the family-based test of additive and dominant effects ($P = 0.03$).

DISCUSSION

Our results do not show a consistent association between the adaptive alleles of *ASPM* and *Microcephalin* and any of the several measures of intelligence we tested. For *ASPM*, significant associations were found between the D-allele and increased FIQ, VIQ, and PIQ in the Dutch Adults and the LBC1921 samples. Each association, however, was observed in only one sample and was not replicated in any of the other datasets. Furthermore, in two of the other datasets, increased FIQ and PIQ were associated with the A-allele instead. For *Microcephalin*, a significant association was seen between the D-allele and all three measures of intelligence in the Dutch Children sample. Similar to *ASPM*, however, these results were not reproducible in any of the other samples, and an opposite pattern of association was seen in the Dutch Adults sample, with increased VIQ showing significant association with the A-allele. Furthermore, for *Microcephalin*, the D-allele is present at a very low frequency (Table 1). Consequently, the observed effect in the Dutch Children sample is driven by a relatively small group of homozygotes.

There are several issues to consider in interpreting these results. On its own, the irreproducibility across different datasets could be attributed to either lack of power in testing for what might be a relatively small effect, or a false positive result. Given, however, that of the three measures of intelligence found to be associated with the *ASPM* and *Microcephalin* D-alleles, all three showed significant association with the A-alleles in other samples, a false positive result appears to be the most parsimonious interpretation. Furthermore, the large size of this dataset suggests that differences in age, environment, genetic background or phenotyping methods, are unlikely to account for the variation in effect, especially since each of these variables is replicated across more than one sample.

Thus, our overall findings do not support a detectable association between the D-allele of either *ASPM* or *Microcephalin* and increased IQ. Furthermore, examination of the allelic effects of both genes together shows no evidence of a heterozygous or epistatic effect. This result is consistent with the recent report (16) of a null relationship between *ASPM* and *Microcephalin* variation and brain size—a biological correlate of normal variation in intelligence (27). Nevertheless, we cannot rule out the possibility that our analysis fails to detect a correlation due to the small effect of the correlation. Furthermore, our study is in no way an exhaustive test of brain-related functions. Indeed, given the abundant expression of *ASPM* and *Microcephalin* in the developing brain and the restricted disease phenotype, we would argue that these genes remain very strong candidates for understanding human brain evolution and the D-alleles might confer some advantage in brain function not readily measured by conventional IQ tests.

Primary microcephaly is a particularly interesting disorder from an evolutionary perspective. Based on the phenotype of the human disease, the expression pattern of the underlying genes, their intracellular function in cell cycle progression, and most recently gene knockdown experiments, primary microcephaly is best characterized as a defect in the regulation of neural progenitor cell transition from proliferative to

Table 3. Tests of association with IQ for *ASPM* and *MCPH1* alleles

	ASPM				MCPH1			
	FIQ (*P*-value)	VIQ (*P*-value)	PIQ (*P*-value)	Allele	FIQ (*P*-value)	VIQ (*P*-value)	PIQ (*P*-value)	Allele
Family-based samples								
Dutch Children								
Family-based test of additive effects	0.67	0.61	0.94		0.02*	0.02*	0.09	D
Population-based test of additive effects	0.89	0.89	0.89		N/A	N/A	0.34	
Family-based test of additive and dominant effects	0.45	0.12	0.98		0.03*	0.06	0.02*	D
Population-based test of additive and dominant effects	0.97	N/A	0.99		0.03*	N/A	0.00†	D
Dutch adults								
Family-based test of additive effects	0.04*	0.06	0.13	D	0.85	0.47	0.69	
Population-based test of additive effects	0.30	0.45	0.42		0.41	0.56	0.34	
Family-based test of additive and dominant effects	0.01†	0.01†	0.08	D	0.29	0.03*	0.91	A
Population-based test of additive and dominant effects	N/A	N/A	0.69		0.28	N/A	0.44	
Australian								
Family-based test of additive effects	0.45	0.35	0.09		0.71	0.67	0.85	
Population-based test of additive effects	0.22	N/A	0.21		0.16	0.12	0.34	
Family-based test of additive and dominant effects	0.37	0.39	0.11		0.76	0.73	0.90	
Population-based test of additive and dominant effects	0.05*	N/A	0.09	A	0.36	0.29	0.63	

	ASPM					*MCPH1*			
	MHT11 (*P*-value)	MHT79 (*P*-value)	VIQ (*P*-value)	PIQ (*P*-value)	Allele	MHT11 (*P*-value)	MHT79 (*P*-value)	VIQ (*P*-value)	PIQ (*P*-value)
Population-based samples									
LBC1921									
Test of additive effects	0.53	0.37	0.80	0.05*	D	0.31	0.73	0.73	0.40
Test of additive and dominant effects	0.78	0.31	0.93	0.13		0.59	0.94	0.87	0.58
ABC1936									
Test of additive effects	0.61	N/A	0.09	0.02*	A	0.86	N/A	0.92	0.86
Test of additive and dominant effects	0.58	N/A	0.23	0.04*	A	0.64	N/A	0.90	0.13

All tests were carried out using QTDT and include correction for age and sex, as described in Materials and Methods. Allele denotes the allele showing the statistically significant association with increased IQ. N/A is marked where spurious association due to admixture was statistically significant such that the results of the population-based tests cannot be interpreted.
*$P \leq 0.05$.
†$P \leq 0.01$.

neurogenic division (5,28). Over a decade ago, Rakic proposed that genes regulating the timing of this transition may explain evolutionary modifications in brain size (29). Indeed, the phenotype of primary microcephaly is atavistic: characterized by severe reduction in brain volume, a simplified gyral pattern of the cortex, and a hypoplastic skull vault, yet no gross abnormality in cortical architecture, it bears a unique resemblance to the early hominid condition (6). Moreover, the decrease in brain volume is strongly correlated with decreased cognitive capacity (5,30). Thus, the identification of signatures of adaptive evolution at three of the four primary microcephaly genes identified to date, and the extremely rapid selective sweeps at *ASPM* and *Microcephalin* in the recent history of our species, makes them prime candidates for studying the genetic basis of human brain evolution.

Yet, how much can we learn from loss-of-function mutations about a gene's specific role in a species' evolutionary history? In fact, we can ask more broadly, how informative is the general function of a gene for understanding the phenotypic consequences of specific nucleotide substitutions? A number of recent reviews on the genetic basis of human evolution have called for the use of data from human disease, model organisms and *in vitro* systems to relate human-specific substitutions to the unique aspects of our anatomy and physiology (2,3,31,32). Because of the technical and ethical limitations of studying human subjects, the need to rely on this type of inductive reasoning is much greater than in evolutionary studies of model organisms. Nonetheless, as our findings suggest, the relationship between a gene's known function and its role in shaping a species' evolutionary history may be far more complex. Thus, although general functional classification is a necessary and powerful first step for identifying candidate genes and forming hypotheses regarding their evolutionary significance, ultimately, phenotypic characterization of evolutionary signatures will require developing new deductive approaches based on direct experimental observation.

Functional characterization of adaptive evolution in model organisms has made tremendous progress in the past two decades by capitalizing on intraspecies variability and the increasing number of molecular markers to identify adaptive mutations (33–35). In studies of other species, for which experimental crosses are not possible, analysis of variation in natural populations has shown great promise (36). Whereas older selective sweeps in humans have often gone to completion, and consequently are no longer polymorphic in reference to the selective event, the adaptive alleles of genes with a recent history of positive selection are more likely to have not reached fixation. These loci provide a unique opportunity to use natural trait variation within humans for phenotypic characterization of signatures of positive selection. Thus, we propose the use of candidate gene-based association studies as a method for identifying the phenotypic substrate underlying signatures of ongoing adaptive evolution.

MATERIALS AND METHODS

Human subjects

The Dutch children cohort consisted of 384 subjects (181 males) from 170 families. There were 91 MZ pairs, 76 DZ pairs and 47 of their non-twin siblings. Mean age at the time of testing was 12.4 (SD = 0.9). The Dutch children were part of an ongoing study on the genetics of attention (37). Participation in this study included a voluntary agreement to provide buccal swabs for DNA isolation and genotyping. The Dutch adult cohort consisted of 361 subjects (168 males) from 174 families. There were 42 MZ pairs, 61 DZ pairs, 1 DZ triplet and 152 siblings. Mean age at the time of testing was 36.4 (SD = 12.4). The Dutch adults were part of an ongoing study on the genetics of brain function (38). Participation in this study included a voluntary agreement to donate a blood sample for DNA isolation and genotyping. The Australian adolescent cohort consisted of 985 subjects (482 males) from 460 families. There were 94 MZ pairs, 298 DZ pairs and 201 siblings. Mean age at the time of testing was 16.4 (SD = 0.7). The Australian adolescents were part of an ongoing study on the genetics of cognition (39). Participation in this study included a voluntary agreement to donate a blood sample for DNA isolation and genotyping. Parental genotypes but no parental IQ scores were available. Recruitment of LBC1921 has been described previously (40). Briefly, the LBC1921 consisted of 526 subjects (219 males) who took part in the Scottish Mental Survey of 1932 at the age of 11 years, and were retested recently at age 79. All participants lived independently in the community. For this study, inclusion criteria were no history of dementia and a Mini-Mental State Examination score of 24 or greater. Recruitment of ABC1936 has been described previously (40), and consisted of 205 subjects (109 males) who took part in the Scottish Mental Survey of 1947 at the age of 11 years, and were retested recently at age 64. All participants lived independently in the community. Inclusion criteria were as for LBC1921.

IQ phenotyping

For the Dutch Children sample, cognitive ability was assessed with the Dutch adaptation of the Wechsler Intelligence Scale for Children-Revised (WISC-R) (41). For the Dutch Adult sample, cognitive ability was assessed with the Dutch adaptation of the Wechsler Adult Intelligence Scale III-Revised (WAIS-IIR) (42). VIQ, PIQ and FIQ were calculated following the WAIS-III guidelines and were normally distributed. For the Australian sample, the Multi-dimensional Aptitude Battery (MAB) (43) was used. Scaled scores for VIQ, PIQ and FIQ were calculated following the manual and were normally distributed. Although the scaled scores were based upon Canadian normative data, this does not affect the validity of the scale for testing genetic effects within samples. All LBC1921 and ABC1936 subjects took a version of the MHT, No. 2 (44,45), a general mental ability test at age 11 years, in the Scottish Mental Surveys of 1932 and 1947, respectively. LBC1921 repeated the same test at about age 79 years. The test was previously described in detail (40). The ABC1936 were re-tested at about age 64 years. Nonverbal reasoning was examined in LBC1921 and ABC1936 using Raven's Standard Progressive Matrices (Raven) (46), and is referred to as Performance IQ (PIQ) in the Scottish samples. The National Adult Reading Test (NART) assessed premorbid or prior cognitive ability (47–49), and is taken as

an index of Verbal IQ (VIQ) in the Scottish samples. Although the MHT scores, VIQ, PIQ and FIQ are standardized measures with respect to age and sex, we still found small significant effects of age and sex. We therefore conducted all analyses on the residuals (i.e. corrected for age and sex). Corrections were carried out separately within each sample.

DNA collection and genotyping

Zygosity was assessed using 11 polymorphic microsatellite markers (Het > 0.80) in the Dutch samples and nine in the Australian sample with P (DZ|conc) $< 10^{-4}$. Genotyping was performed blind to familial status and phenotypic data. Genotyping of *ASPM* for all samples except for ABC1936 was performed by automated sequencing of A44871G, as described previously (14), using the primer pair TCAGACAATGGCATTCTGCT and CTGCCTGAACACAAGTCTCT. In the ABC1936 sample, genotyping was performed using the C45126A SNP instead. Each PCR product was sequenced on both strands. Genotyping of *Microcephalin* was performed by automated sequencing of the diagnostic G37995C SNP, as described previously (15), using the primer pair AGAAATTTCTGAGTAATCTTTCAAAGG and ACTGAGGAACTCCTGGGTCT. Each PCR product was sequenced on both strands. In the Dutch Children sample, *ASPM* genotyping failed for 16 subjects and *Microcephalin* for five subjects. In the Dutch Adults sample, *ASPM* failed for 53 subjects and *Microcephalin* for 12 subjects. In the Australian sample, *ASPM* failed for one subject. In the LBC1921 sample, *ASPM* failed for 13 subjects and *Microcephalin* for eight subjects. In the ABC1936 sample, *ASPM* failed for 19 subjects and *Microcephalin* for 17 subjects. Genotyping was performed at the Howard Hughes Medical Institute (LBC1921: *ASPM, Microcephalin*), Vrije Universiteit Medical Center, Amsterdam (Dutch Children and Adults samples: *ASPM, Microcephalin*), Queensland Institute of Medical research (Australian sample: *ASPM, Microcephalin*) and by KBiosciences (Herts, UK, http://www.kbioscience.co.uk) using KASPar chemistry (ABC1936: *ASPM, Microcephalin*).

Statistical analyses

For the family-based samples, qTDTs were conducted using the program QTDT, which implements the orthogonal model developed by Abecasis *et al*. (25) as an extension of the of Fulker *et al*. (26). This model allows one to decompose the genotypic effect into orthogonal between- (βb) and within- (βw) family components, and also models the residual sib-correlation as a function of polygenic or environmental factors. MZ twins can be included and are modeled as such, by adding zygosity status to the data file. They are not informative to the within family component (unless they are paired with non-twin siblings), but are informative for the between-family component. The between-family association component is sensitive to population admixture, whereas the within-family component is unaffected by spurious associations due to population structure. Thus, if population structure creates a false association, the test for association using the within-family component is still valid, though usually less powerful. The full model including additive effects as well as dominance for each sample is represented as: $E(y_{ij}) = \mu + \beta_{abi}a_b + \beta_{dbi}d_b + \beta_{awij}a_w + \beta_{dwij}d_w$, where $E(y_{ij})$ represents the expected phenotypic value of sib j from the ith family, μ denotes the overall trait mean (equal for all individuals), β_{abi} is the coefficient for the *between families* additive genetic effect for the ith family, β_{dbi} is the coefficient for the *between families* dominant genetic effect for the ith family, β_{awij} denotes the coefficient as derived for the *within families* additive genetic effects for sib j from the ith family, β_{dwij} denotes the coefficient as derived for the *within families* dominant genetic effects for sib j from the ith family, a_b and a_w are the estimated additive *between* and *within* parameters, d_b and d_w are the estimated dominance *between* and *within* family parameters. The test for spurious association tests whether $a_b = a_w$ and $d_b = d_w$. If this test is significant, association can be tested by H_o: $a_w = d_w = 0$. If there is no population stratification, the expectation simplifies to population-based association represented by a simple regression model $E(y_{ij}) = \mu + \beta_{ai}a + \beta_{di}d$, where a denotes the overall or total additive genetic effect and d denotes the total dominance effect. The test for 'total' association is then H_o: $a = d = 0$. As the Scottish samples are population based, the within-family test cannot be conducted, and in these samples only the latter simple regression test for association was performed.

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Conflict of Interest statement. None declared.

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