Human Molecular Genetics, 2008, Vol. 17, No. 9 **1329–1335**
doi:10.1093/hmg/ddn021
Advance Access published on January 19, 2008

A common SNP of MCPH1 is associated with cranial volume variation in Chinese population

Jin-kai Wang[1,2,4], Yi Li[3] and Bing Su[1,2,*]

[1]State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology and [2]Kunming Primate Research Centre, Chinese Academy of Sciences, Kunming, Yunnan, PR China, [3] Department of Chemistry and Life Science, Qujing Normal University, Qujing, Yunnan, PR China and [4]Graduate School of Chinese Academy of Sciences, Beijing, PR China

Received November 17, 2007; Revised December 23, 2007; Accepted January 16, 2008

Microcephaly (MCPH) genes are informative in understanding the genetics and evolution of human brain volume. MCPH1 and abnormal spindle-like MCPH associated (ASPM) are the two known MCPH causing genes that were suggested undergone recent positive selection in human populations. However, previous studies focusing only on the two tag single nucleotide polymorphisms(SNPs) of MCPH1 and ASPM failed to detect any correlation between gene polymorphisms and variations of brain volume and cognitive abilities. We conducted an association study on eight common SNPs of MCPH1 and ASPM in a Chinese population of 867 unrelated individuals. We demonstrate that a non-synonymous SNP (*rs1057090*, V761A in BRCA1 C-terminus (BRCT) domain) of MCPH1 other than the two known tag SNPs is significantly associated with cranial volume in Chinese males. The haplotype analysis confirmed the association of *rs1057090* with cranial volume, and the homozygote males containing the derived alleles of *rs1057090* have larger cranial volumes compared with those containing the ancestral alleles. No recent selection signal can be detected on this SNP, suggesting that the brain volume variation in human populations is likely neutral or under very weak selection in recent human history.

INTRODUCTION

The dramatically increased brain volume plays a pivotal role in the origin of our own species. In humans, brain volume is a quantitative trait with high heritability (1,2). Previous studies indicated that human brain volume is correlated with general intelligence, working memory, perceptual organization and processing speed, which are also highly heritable (1,3,4). To uncover the genetic variants that underlie the variation of human brain volume will contribute to a better understanding of human cognition and human evolution.

The discovery of autosomal recessive primary microcephaly (MCPH), a rare human brain developmental disorder, provided the opportunity of dissecting the genetic makeup in controlling human brain development and human brain size. Patients with MCPH typically have a brain volume of around 400 cm^3, only about one-third to one-fourth of that of normal humans (1200–1600 cm^3) (5). The MCPH patients do not have significant syndromic neurological deficits except for some learning difficulties (6–8). To date, there are four genes reported responsible for MCPH, including microcephalin (MCPH1, MIM 607117), abnormal spindle-like MCPH-associated (ASPM/MCPH5, MIM 605481), cyclin-dependent kinase 5 regulatory associated protein 2 (CDK5RAP2/MCPH3, MIM 608201) and centromere-associated protein J (CENPJ/MCPH6, MIM 609279) (6,7,9).

MCPH1 is located on human chromosome 8p23, encoding a BRCT domain containing protein of 835 amino acids. It has 14 exons, spanning 237 kb in the human genome. It has three BRCA1 C-terminus (BRCT) domains, including a single BRCT domain in the N-terminal and two tandem BRCT domains in the C-terminal. Like other BRCT containing proteins, MCPH1 is involved in the regulation of DNA damage-responsive cell cycle check points although the precise mechanism is not clear (10–12).

*To whom correspondence should be addressed at: State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, 32 East Jiao-Chang Road, Kunming 650223, Yunnan, PR China. Tel: +86 8715120212; Fax: +86 8715193137; Email: sub@mail.kiz.ac.cn

Table 1. Characteristics of the single nucleotide polymorphisms(SNPs) used in the study and their association with cranial volume

SNP no.	Gene	dbSNP rs number	Physical map location	Alleles	Ancestral allele	SNP type	Location	Minor allele	Minor allele frequency	*P* (All)				*P* (Male)				*P* (Female)			
										Not adjusted	Adjusted by height	Adjusted by weight	Adjusted by height and weight	Not adjusted	Adjusted by height	Adjusted by weight	Adjusted by height and weight	Not adjusted	Adjusted by height	Adjusted by weight	Adjusted by height and weight
SNP1	MCPH1	rs2305022	8: 6259807	A/C	A	Synonymous	BRCT1	C	0.200	0.681	0.833	0.737	0.990	0.742	0.809	0.712	0.990	0.848	0.878	0.990	0.990
SNP2	MCPH1	rs930557	8: 6289591	G/C	G	D314H		G	0.187	0.291	0.788	0.479	0.354	0.618	0.510	0.280	0.354	0.661	0.225	0.354	0.354
SNP3	MCPH1	rs1057090	8: 6466450	C/T	T	V761A	BRCT3	C	0.360	0.645	0.321	0.380	0.888	*0.016*	*0.021*	*0.011*	0.888	0.881	0.905	0.888	0.888
SNP4	MCPH1	rs2912016	8: 6466586	A/C	A	Synonymous	BRCT3	A	0.390	0.212	0.591	0.694	0.692	0.796	0.824	0.513	0.692	0.354	0.985	0.692	0.692
SNP5	MCPH1	rs1057091	8: 6487952	G/A	A	S828P	BRCT3	A	0.184	0.872	0.878	0.915	0.885	0.718	0.700	0.684	0.885	0.820	0.727	0.885	0.885
SNP6	MCPH1	rs2433149	8: 6488159	G/C	G	3′-UTR		G	0.195	0.686	0.772	0.868	0.826	0.299	0.301	0.384	0.826	0.991	0.743	0.826	0.826
SNP7	ASPM	rs3762271	1: 195337065	A/C	C	L2647I	Inter IQ	A	0.155	0.434	0.391	0.212	0.581	0.278	0.244	0.076	0.581	0.482	0.601	0.581	0.581
SNP8	ASPM	rs4915344	1: 195369368	C/T	T	Intron		T	0.322	0.297	0.208	0.316	0.435	0.111	0.109	0.057	0.435	0.464	0.464	0.435	0.435

dbSNP rs number, the rs number in the dbSNP database; the significant *P*-values ($P < 0.05$) are in italics.

The truncated mutations in ASPM are the most common cause of MCPH (13). ASPM is a large gene with 28 exons and a 10.4 kb open reading frame. The 3477 amino acid protein contains a putative N-terminal microtubule binding domain, at least one calponin homology domain, 74 isoleucine-glutamine (IQ) domains, which potentially bind calmodulin (14), and a C-terminal region of unknown function (6). ASPM was suggested playing a role in maintaining symmetric proliferative divisions of neuroepithelial cells (15).

Sequence comparison among extent primate species suggested that all the four MCPH genes experienced adaptive evolution due to Darwinian positive selection (16–21). Further studies in human populations indicated that MCPH1 and ASPM underwent recent (<37000 years ago) adaptive evolution during the origin of modern humans (22), implying that polymorphisms in these two genes may explain the observed variation of brain volume in current human populations.

The most likely driving force of recent positive selection on MCPH1 and ASPM is that the large brain volume is evolutionarily selected in human populations, which is consistent with the positive correlation between the brain volume and intelligence in human (1–4). Recently, there were several association studies in dissecting the relationship between sequence variations of MCPH genes and brain volume (23–25) or IQ score (25,26). All of these studies only tested the two tag SNPs defining the positively selected haplotypes of MCPH1 and ASPM. Surprisingly, none of these studies showed any association between the two tag SNPs and brain volume or IQ score.

In this study, we examined the association between cranial volume and eight common SNPs of MCPH1 and ASPM in a Chinese population of 867 unrelated individuals, and we found that one non-synonymous SNP of MCPH1 (*rs1057090*) other than the two known tag SNPs was associated with cranial volume in Chinese males.

RESULTS

SNP characteristics and cranial volume assessment

In this study, we tested eight SNPs of MCPH1 (six SNPs: *rs2305022, rs930557, rs1057090, rs2912016, rs1057091* and *rs2433149*) and ASPM (two SNPs: *rs3762271* and *rs4915344*) respectively. *G37995C (rs930557) of MCPH1 and C45126A (rs3762271) of ASPM* are the two known tag SNPs defining the two positively selected haplotypes reported (22,27). The information of the eight chosen SNPs is presented in Table 1 and Supplementary Material, Figure S1. Of the eight chosen SNPs, six are located in the coding region and four of them are non-synonymous substitutions. The other two SNPs are *rs2433149* located in the 3′-UTR of MCPH1 and *rs4915344* located in the sixth intron of ASPM. The *rs4915344* was chosen because it was reported undergone recent selection through a genome wide scan (28). The linkage disequilibrium (LD) structure among the SNPs was examined with the program Haploview (29) (Fig. 1), and the selected eight SNPs cover five different LD blocks.

For cranial volume assessment, we used the indirect method by measuring the external cranial capacity (30,31). The distribution of cranial volumes is shown in Figure 2.

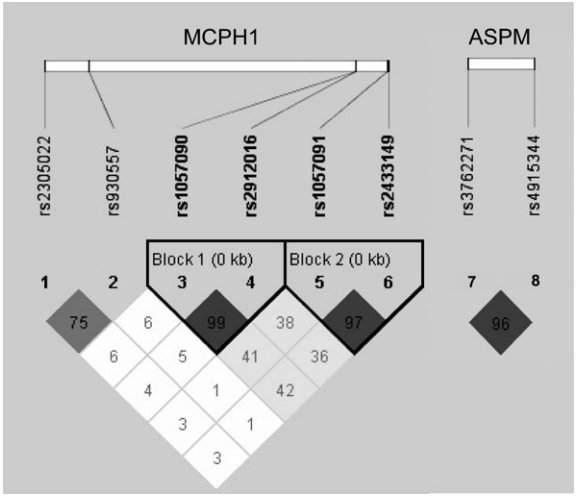

Figure 1. Linkage disequilibrium (LD) plots of the single nucleotide polymorphisms (SNPs) used in this study. We constructed the plots with the program Haploview (29), the triangles are the LD values calculated using the D' measurement. The haplotype blocks were defined using an algorithm which creates the 95% confidence boundaries on D' to define SNP pairs in strong LD.

Single SNP association analysis

We first conduct the single SNP association analysis under an additive model using linear regression. When the males and females are analyzed together as a single data set, none of the eight SNPs show significant association with the cranial volume (Table 1). However, when only the males are considered, *rs1057090* of MCPH1 has a significant association with brain volume ($P = 0.016$, $R = 0.126$), of which the derived C allele correlates with larger cranial volume. In contrast, no significant association is detected in the females (Table 1). Consistent with previous studies, the two reported tag SNPs, *rs930557 (G37995C)* and *rs3762271 (C45126A)* which were suggested undergone strong recent positive selection show no association with cranial volume in either data set (Table 1). We also tested the association between the SNPs and the three cranial dimensions respectively, and no significant association is observed (data not shown).

It is well known that both body height and body weight are correlated with brain volume (32), which is also the case in our samples ($R = 0.522$, $P < 0.001$ for body height; $R = 0.557$, $P < 0.001$ for body weight). To rule out the influence of body height and body weight in our analysis, we conducted the same analysis by correcting the cranial volumes using regressions for body height and body weight, respectively. The same association was observed after the corrections (Table 1). We also tested if the eight SNPs are associated with body height or body weight, and no association was observed (data not shown).

Haplotype association analysis

To confirm the result of single SNP association analysis, we also tested the association by the haplotype-based method (Haplo.stats v1.2.0) (33). Because the first six SNPs are in MCPH1 while the last two SNPs are in ASPM located on a different chromosome, we only consider the first six SNPs of MCPH1 in the haplotype based association analysis. We use a three-SNP sliding window to test if there are haplotypes associated with cranial volume by calculating the global *P*-value of every window. No significance was identified in the female data set (Table 2), which is consistent with the result of single SNP association analysis. In the male data set, we identified a significant *P*-value (global $P = 0.006$) at the window in which the associated SNP (*rs1057090*) is in the middle of the three SNPs (Table 2). We also conducted the two-SNP, four-SNP and five-SNP sliding window analysis, and the same pattern was observed (Table 2). Taken together, the haplotype-based analysis supports the association between *rs1057090* and cranial volume.

We next sought to look into the association between individual haplotypes and cranial volume in males. Indeed, for the three-SNP haplotypes, we observed a significant positive correlation between the CCC haplotype (27.7%) and the cranial volume ($P = 0.004$) while the CTC haplotype (20.4%) has a negative correlation with the brain volume ($P = 0.002$), and the haplotypes are also significant when extended to four SNPs (Table 3). This result indicates that the derived C allele of *rs1057090* contributes to the larger cranial volume in the studied males.

Additionally, we compared the cranial volume between the homozygotes of CCC and CTC haplotypes by an independent *t*-test. The *P*-value is highly significant ($P = 0.001$, two-tailed *t*-test) in males, and the average cranial volume of CCC homozygotes is 97.8 ml (7.5%) larger than that of the CTC homozygotes. No difference was detected in females ($P = 0.77$, two-tailed *t*-test).

To exclude the possibility that the significance was due to the formula we used in calculating the cranial volume, we employed a different formula derived by Dekaban and Lieberman (34): $0.5238 \times L \times B \times H$. Similar results were obtained for all the above analyses (data not shown) though the formula was derived to calculate the cranial volume of deceased humans.

Test for recent selection on *rs1057090*

The increased brain volume is one of the most fundamental change during human evolution in the past 5–6 million years since human and chimpanzee split (35), which is considered resulting from strong selection. The derived C allele of *rs1057090* is correlated with the increased cranial volume in males, which begs the question whether this SNP is under positive selection in human populations. We conducted a neutrality test using the HapMap data (36). If a newly derived allele increased in frequency rapidly due to recent positive selection, the haplotypes with this allele would extend much longer as compared with the haplotypes containing the ancestral allele (37,38). This can be evaluated by the iHS (integrated haplotype score) statistic, which detects recent positive selection at a locus (38). The iHS value of the three HapMap populations (36) is 0.735 (Ease Asians (ASN), Chinese + Japanese), −0.581 northern and western Europeans (CEU) and −0.192 Yoruba people from Nigeria (YRI) respectively, and none of them show deviation from neutral expectation (38). The EHH (extended haplotype homozygosity) (37) decay plot of ASN is shown in Figure 3. Hence, *rs1057090*

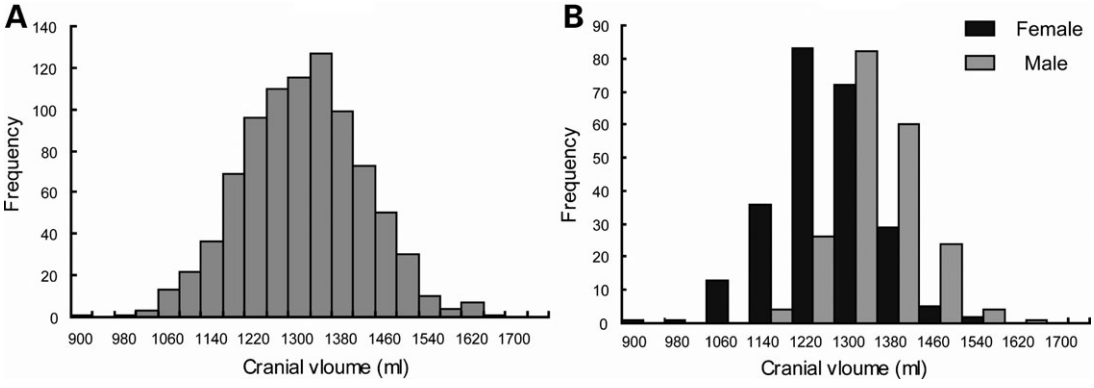

Figure 2. Distribution of the cranial volumes. (**A**) The overall distribution of the cranial volumes. (**B**) The sex distribution of the cranial volumes.

Table 2. Summary of the haplotype association analysis

Two-SNP windows			Three-SNP windows			Four(five)-SNP windows		
SNP no.	Global *P*		SNP no.	Global *P*		SNP no.	Global *P*	
	Male	Female		Male	Female		Male	Female
1 2	0.809	0.773	1 2 3	0.082	0.434	1 2 3 4	*0.018*	0.676
2 3	*0.021*	0.318	2 3 4	*0.006*	0.522	2 3 4 5	*0.002*	0.819
3 4	*0.014*	0.644	3 4 5	0.129	0.803	3 4 5 6	0.107	0.736
4 5	0.750	0.590	4 5 6	0.427	0.770	1 2 3 4 5	*0.036*	0.839
5 6	0.335	0.991				2 3 4 5 6	1.000	0.762

SNP no., the serial number of the SNPs, for example, '1 2 3' means the window constituted of SNP1, SNP2 and SNP3; the global *P*-values were calculated by Haplo.stats (33); the significant *P*-values ($P < 0.05$) are in italics.

Table 3. Result of the specific haplotype association analysis in males

SNP1	SNP2	SNP3	SNP4	SNP5	Frequency	Hap-score	*P*-value	Simulated *P*-value
	C	T	C		0.204	−2.92	*0.003*	*0.002*
A	C	T	C		0.201	−2.46	*0.014*	*0.016*
	C	T	C	G	0.120	−2.06	*0.039*	*0.049*
	C	T	C	A	0.083	−1.97	*0.049*	*0.042*
	C	C	C		0.277	2.93	*0.003*	*0.004*
A	C	C	C		0.255	3.03	*0.002*	*0.003*
	C	C	C	G	0.248	2.48	*0.013*	*0.014*

The significant *P*-values ($P < 0.05$) are in italics. The Hap-scores were obtained by Haplo.stats v1.2.0. The positive values indicate positive correlation with cranial volume, while the negative values indicate negative correlation with cranial volume; *P*-value, the *P*-value calculated from the Hap-score; simulated *P*-value, the *P*-value calculated by simulation.

is probably neutral or under very weak selection in human populations.

DISCUSSION

The SNP *rs1057090* is a T to C change, corresponding to a valine to an alanine change at amino acid residue 761, which is located at the C-terminal BRCT domain. The two tandem BRCT domains in the C-terminal of MCPH1 are necessary for the IRIF (ionizing radiation induced nuclear foci) formation, which is required for DNA damage-responsive S and G2-M-phase checkpoints (39). Through alignment of MCPH1 protein sequences among human, chimpanzee and rhesus macaque, we found that the BRCT domains are much more conserved than the other regions, suggesting that the amino acid change at residue 761 may have a functional consequence (Supplementary Material, Fig. S2). In fact, it was reported that an isoleucine to serine mutation of human MRCA1, a close paralog of MCPH1, at the same position causes the instability of the protein with reduced ability to recognize the phosphopeptide (40–43).

The observed gender dependent association of cranial volume is intriguing, suggesting that the manifestation of genetic variations for brain development is likely different

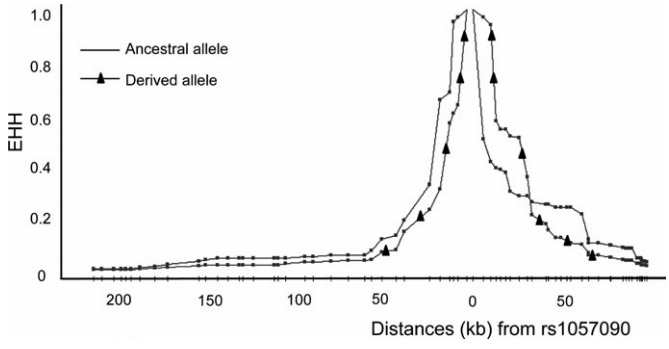

Figure 3. EHH (extended haplotype homozygosity) decay plot around *rs1057090* in HapMap ASN (Chinese + Japanese) population. The figure was created by using Haplotter (38). The line with triangles represents the derived C allele, while the line without triangles represents the ancestral T allele. Each dot on the line represents a single nucleotide polymorphism(SNP). The distances (kb) from the *rs1057090* is displayed in the *x*-axis, and the EHH values are shown in the *y*-axis. The EHH of the derived allele decays as rapidly as that of the ancestral allele, showing no recent selection signal.

between males and females. It was shown that brain sexual differentiation occurs in the early stage of development and is regulated by sex hormones (44), which might result from varied selection pressures during the origin of humans (45,46).The social brain hypothesis suggested that selection has favored larger brains and more complex cognitive capacities to cope with the challenges of social life (47). Thus, the different social roles of males and females may explain the brain sexual differentiation. For example, it was suggested that the hunting activity likely favors the males having larger brains because hunting requires communication and coordination of actions among the hunters (48,49). Alternatively, sexual selection may also play a role in the differential selection pressure between males and females (45,46).

The length of the haplotypes with an allele is also an indicator of the allele age (37). The EHH decay patterns are similar between the haplotypes with the derived C allele and the ancestral haplotypes, and both of them show rapid EHH decay in a short distance from *rs1057090* (Fig. 3), suggesting that the derived C allele arose from a relatively ancient substitution in human.

The increased brain volume is thought to be crucial in the origin of modern human. But we detect no recent selection signal around *rs1057090* though its significant association with brain volume in males. Interestingly, according to the fossil records, although the absolute brain size increased dramatically over the past several million years after human split with chimpanzee, it decreased over the past 35 000 years (50,51), suggesting that the brain volume might be evolutionarily neural or under very weak selection in recent human history. However, we cannot rule out the possibility that the derived allele of *rs1057090* might experience selection during the early stage of modern human evolution because selection was indeed detected for other SNPs of MCPH1 (16,19). On the other hand, the genetic variant associated with larger brains could be slightly deleterious with effects only apparent in the more genetically susceptible males. One reported example is the association of larger head circumference with human mental disorders such as autism (52).

MATERIALS AND METHODS

Samples and genotyping

A total of 867 unrelated individuals including 387 males and 480 females were included. Most of the samples are Han Chinese (90.0%), and the others (10.0%) are from 14 ethnic populations of southwestern China. The identities of the subjects were self-declared and confirmed by their registered ID profiles. All the sampled individuals are from Yunnan province of southwestern China. Informed consents for this study were obtained from all the subjects. The ages of the 867 individuals range from 19 to 28 years with 98% of them being 21–26 years old. The DNA samples were prepared by extracting DNA from blood. Genotyping of the eight SNPs were conducted using the SNaPShot method on an ABI 3130 sequencer. For each SNP, direct sequencing for about 30 samples was conducted to confirm the results of genotyping using the SNaPShot method.

Hardy–Weinberg equilibrium tests

Hardy–Weinberg equilibrium (HWE) of all the eight SNPs were tested by the software PEDSTATS v0.6.8 (53) (http://www.sph.umich.edu/csg/abecasis/). All the SNPs are in HWE except for *rs3762271*, which deviates from HWE due to heterozygote deficit ($P = 0.036$). Double check with sequencing of 32 individuals for *rs3762271* ruled out the possibility of genotyping errors. In addition, the genotype frequencies of *rs3762271* are similar with that of the HapMap HCB (Chinese) population (36).

Measurement of cranial volume

Three principal dimensions of the cranium were measured in a blinded fashion. (i) Maximum antero-posterior length (*L*, measured between glabella and the inion). (ii) Maximum breadth (*B*, biparietal diameter; measured between two parietal eminences). (iii) Cranial height (*H*, basi-bregmatic height, measured between the internal acoustic meatus to the highest point of the vertex). The first two measurements were carried out using the spreading caliper and the cranial height is measured using the Todd's head spanner. Then the cranial volumes were computed using the following formula derived by Williams *et al.* (31): Male, $0.337\ (L-1.1)\ (B-1.1)\ (H-1.1) + 406.01$cc; Female, $0.400\ (L-1.1)\ (B-1.1)\ (H-1.1) + 206.60$cc. To rule out the potential bias of the formula, we also employed a different formula derived by Dekaban and Lieberman (34): $0.5238 \times L \times B \times H$.

Association analysis

The single SNP association analysis was conducted under an additive model using linear regression, in which the values of 1, 2 represent the homozygotes and 1.5 for the heterozygotes, and cranial volume is used as dependent.

Haplotype-based association analysis was done using the Haplo.ststs Package v1.2.0 (http://mayoresearch.mayo.edu/mayo/research/schaid_lab/software.cfm) in the R environment (R project for Statistical Computing, http://www.r-project.org).

Cranial volume adjustment by body height and body weight

Before adjusting the cranial volume by body height (weight), in each data set, a linear regression formula was inferred with the cranial volume as the dependent and the body height (weight) as the independent, the adjusted cranial volume by height (weight) = (cranial volume − the constant of the regression formula)/body height (weight).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

ACKNOWLEDGEMENTS

We thank Dr Ning Wang for her insightful suggestion on data analysis. We are also grateful to Hui Zhang, Shan Li, Xiao-dong Shi and Wei Zhang for their technical assistance in DNA extraction and cranial volume measurement.

Conflict of Interest statement. None declared.

FUNDING

This study was supported by grants from the National 973 project of China (*2006CB701506, 2007CB815705*), the Chinese Academy of Sciences (*KSCX1-YW-R-34*), the National Natural Science Foundation of China (*30623007, 30525028* and *30630013*), and the Natural Science Foundation of Yunnan Province of China (*2006C0083M*).

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