We report a 2000-year Antarctic ice-core record of stable carbon isotope measurements in atmospheric methane ($\delta^{13}$CH$_4$). Large $\delta^{13}$CH$_4$ variations indicate that the methane budget varied unexpectedly during the late preindustrial Holocene (circa 0 to 1700 A.D.) During the first thousand years (0 to 1000 A.D.), $\delta^{13}$CH$_4$ was at least 2 per mil enriched compared to expected values, and during the following 700 years, an about 2 per mil depletion occurred. Our modeled methane source partitioning implies that biomass burning emissions were high from 0 to 1000 A.D. but reduced by almost ~40% over the next 700 years. We suggest that both human activities and natural climate change influenced preindustrial biomass burning emissions and that these emissions have been previously understated in late preindustrial Holocene methane budget research.

Methane is an important greenhouse gas, and measurements of its atmospheric concentration, [CH$_4$], from ice cores demonstrate a slow increase over the late preindustrial Holocene (the LPIH, circa 0 to 1700 A.D.) and a rapid increase to unprecedented levels over recent centuries (1–3). However, the causes of these variations are not well understood (1–3), because emission rates from the diverse methane sources are spatially and temporally variable, sometimes small, and distributed globally. Additionally, methane emissions vary with climate (1) and possibly with preindustrial human activities such as rice cultivation, cattle farming, and biomass burning (4, 5).

The sources of atmospheric methane can be separated into three general categories based on their stable carbon isotope ratios $\delta^{13}$CH$_4$: biogenic (e.g., wetlands, $\delta^{13}$CH$_4$ near ~60 per mil (%o)), fossil (4, 5, $\delta^{13}$CH$_4$ near ~400%), and pyrogenic or biomass burning ($\delta^{13}$CH$_4$ near ~25% for C$_3$ vegetation or ~12% for C$_4$ vegetation). Changes in atmospheric $\delta^{13}$CH$_4$ allow the contributions from each of these source types to be deduced (6). However, very few long $\delta^{13}$CH$_4$ records exist (6), because the small amount of air available from ice cores does not generally meet the large sample size requirements for gas isotope analyses (7). Furthermore, firm-air samples are at most ~100 years old (8–10). To overcome these problems, we used a high-precision technique that was specially adapted (11) to analyze very small volume air samples extracted from Law Dome ice cores with high temporal resolution. We present a 2000-year $\delta^{13}$CH$_4$ and [CH$_4$] record (Fig. 1) that reveal unexpected $\delta^{13}$CH$_4$ features. We used these measurements to evaluate methane sources and enhance our understanding of the LPIH methane budget. Shorter term $\delta^{13}$CH$_4$ variations within the industrial era and the unconfirmed role of preindustrial methane sink variations are investigated elsewhere (12).

On the basis of a greater preindustrial dominance of wetland sources and relatively stable [CH$_4$] (Fig. 1), we expected LPIH atmospheric $\delta^{13}$CH$_4$ to be stable and isotopically depleted relative to the present day [in the range about ~54% to ~49% (13–15)]. However, our $\delta^{13}$CH$_4$ data do not follow those expectations (Fig. 1). Two fundamental conundrums emerge. First, LPIH $\delta^{13}$CH$_4$ is at least 2% more enriched than expected from 0 to 1000 A.D. Second, in contrast to relatively stable [CH$_4$], which varies by no more than ~55 parts per billion (ppb) from 1000 to 1700 A.D., the $\delta^{13}$CH$_4$ measurements reveal a large ~2% depletion.

To quantify the source evolution, we used an atmospheric box model (13) that includes global biogenic, pyrogenic, and fossil sources and accommodates the dynamics of [CH$_4$] and $\delta^{13}$CH$_4$ responses to budget changes. We adopted a weighted average of the kinetic isotope effects of methane sinks ($k_1/k_2$ = 1) of ~7.4‰. This sink fractionation gives consistency between [CH$_4$] and $\delta^{13}$CH$_4$ with the source construction of the EDGAR-HYDE 1.4 data set (16) over 1890 to 1995 A.D. Although OH may have been ~10% higher in the preindustrial compared to the present day (15, 17), it is likely to have been relatively much more stable from 0 to 1700 A.D., and we adopted a constant LPIH methane lifetime of 7.6 years (18). On the basis of previous studies (13–15), we postulated constant LPIH fossil methane emissions of 20 Tg year$^{-1}$, because there is no evidence of elevated fossil emissions from 0 to 1000 A.D. relative to 1000 to 1700 A.D. (even though they may be higher than 20 Tg year$^{-1}$ (19)). Thus, our inverse source partitioning was tightly constrained, because the total methane source strength (over time) was derived from observed [CH$_4$] and the partitioning of biogenic and pyrogenic sources was derived from observed $\delta^{13}$CH$_4$.

Our modeled pyrogenic and biogenic source emissions were ~25 ± 1 Tg year$^{-1}$ and ~194 ± 2 Tg year$^{-1}$, respectively, from 0 to 1000 A.D. (Fig. 2) (22). Our preindustrial pyrogenic source is at least a factor of 2.5 larger than expected (14, 15). However, high levels of LPIH burning have been proposed before. Subak (4), using historical land-use records and current estimates of methane emission factors, estimated global pyrogenic emissions in 1500 A.D. at ~26 Tg year$^{-1}$, but well within their estimated upper and lower limits (118 to 325 Tg year$^{-1}$). Our source reconstruction requires that, by 1700 A.D., pyrogenic emissions decrease by ~40% to ~15 ± 1 Tg year$^{-1}$ (relative to emissions from 0 to 1000 A.D.) and that biogenic emissions simultaneously increase by ~10% to ~210 ± 2 Tg year$^{-1}$ (Fig. 2). These methane source variations may be caused by natural climate change or human activities.

Variations in temperature and moisture can influence natural methane emissions from wetlands and wildfires. If warmer temperatures coincide with dryer conditions, then during warm-dry periods we would expect elevated pyrogenic emissions and reduced biogenic emissions compared to those in cool-wet climates. During warm-dry periods, we expect that, even though temperature would increase wetland emission rates (per area), increased evaporation would reduce wetland extent, causing lower net biogenic emissions. Temperature and moisture patterns vary regionally. However, regional records of drought, rainfall, and biomass burning recovered from lakes in Africa (23), Asia (24), Europe (25), Oceania (25), and South, Central, and North America (25–27), together with chemical records of high-latitude Northern Hemisphere-
ic biomass burning from a Greenland ice core (28), provide supporting evidence that moisture is negatively correlated to temperature on a larger scale and that the extent and magnitude of methane emissions from wildfires decreased and that from wetlands increased in response to cooling temperatures and increasing moisture from ~1000 to 1700 A.D. Several of the multicentury and multidecadal anomalies in reconstructed Northern Hemispheric temperatures (29, 30) correlate with δ13CH4 until ~1500 A.D. (Fig. 3A), providing further supporting evidence that natural climate change influenced the methane budget as we propose until ~1500 A.D.

Further evidence for the influence of pyrogenic emissions on the methane budget comes from the Law Dome carbon monoxide concentration ([CO]) record (Fig. 4). CO is a trace gas proxy for preindustrial variations in pyrogenic emissions, especially for combustion of woody biomass, which tends to be more incomplete and productive of CO than the burning of grasslands (31). The close correspondence between [CO] and δ13CH4 from 0 to 1500 A.D. provides supporting evidence that variations in pyrogenic emissions were important between 0 and 1500 A.D. (18). However, from 1500 to 1700 A.D., [CO] remains relatively stable, whereas δ13CH4 declines by another ~1‰ (Fig. 4) and annual pyrogenic methane emissions decline by another ~5 Tg (Fig. 2). A relatively larger decrease in grassland burning from 1500 to 1700 A.D. is consistent with these criteria, because, compared to woody burning, it does not produce as much CO relative to methane and it has stronger δ13CH4 leverage. From ~1500 to 1700 A.D., the weakened Northern Hemisphere temperature–δ13CH4 correlation (Fig. 3A) therefore suggests a reduced influence of climatic change on biomass burning variability during this time.

The factor most likely to have influenced pyrogenic variations from 1500 to 1700 A.D. is human activity. Estimated LPIH human population trends (32, 33) are shown in Fig. 3B. Because grasslands and forests in Europe and China were mostly cleared by 0 A.D. for agricultural or habitable lands (that were not burnt at large scale again) (5), and because relative changes in the African population were small (as compared to the Americas) (32), the relatively small indigenous population of the Americas would have had a disproportionate influence on LPIH anthropogenic methane emissions from fires. Their fires are very likely to have been important because it has been suggested that they burnt very large grassland areas annually (4, 20, 21) and maintained large-scale, smoldering woody fires in the Amazon to produce charcoal for improved soil fertility (34). On the basis of land use practices and present-day methane emission factors, independent studies have estimated pyrogenic CH4 emissions in the Americas at 1500 A.D. to be as large as 10 Tg·year⁻¹ (4) and 8.25 Tg·year⁻¹ (20). However, the indigenous population of the Americas declined by 90% from 1500 to 1600 A.D. (33) because of the introduction of diseases by European explorers. Consequently, pyrogenic emissions
from the Americas must have reduced. The simultaneity from 1500 to 1600 A.D. of the rapid changes in the population of the Americas, $\delta ^{13}$CH$_4$, and [CH$_4$] provides support for our hypothesis that rapid human population decline contributed substantially to the total LPIH biomass burning reduction and global $\delta ^{13}$CH$_4$ depletion (perhaps by as much as a $\sim 5$ Tg reduction in annual emissions).

In the absence of dependable preindustrial values, we postulate constant natural fossil emissions (20 Tg/year$^{-1}$) and a constant C$_3$/C$_4$ ratio of burnt biomass (60:40). Even if the C$_3$/C$_4$ plant type mix in the biomass fuel changed (as [CO] suggests from 1500 to 1700 A.D.), the overall shape of the inferred pyrogenic source evolution would be unchanged (Fig. 2). Thus, our linkage between LPIH global biomass burning and $\delta ^{13}$CH$_4$ is robust, leading to our conclusion that biomass burning substantially affected the LPIH methane budget.

Our modeled methane source partitioning implies that a $\sim 10 \pm 1$ Tg reduction in annual global biomass burning emissions of methane is the main cause of the $\sim 2\%$ $\delta ^{13}$CH$_4$ depletion from 1000 to 1700 A.D. Simultaneously, a compensatory growth in biogenic methane emissions causes a small $\sim 50$ ppb [CH$_4$] increase. Both natural and anthropogenic sources have likely contributed to the changes, such that (i) from 1000 to 1500 A.D., natural climatic change (becoming cooler and wetter) is the most likely cause for a reduced incidence of wildfires and an increased wetland area, and (ii) from 1500 to 1700 A.D., regional human population variations are the most likely causes of reduced pyrogenic emissions. Anthropogenic expansion of rice and ruminant agriculture ($4$, $5$) may have also contributed to increasing natural wetland emissions from 0 to 1700 A.D. We therefore suggest that humans played a much larger than expected role in the evolution of the LPIH methane budget. Our work corroborates independent assessments that preindustrial anthropogenic pyrogenic emissions approximated those of today ($4$, $20$) and therefore suggests that pyrogenic emissions have been previously understated in LPIH methane budget research ($14$, $15$).

Fig. 3. Natural climate and human population variations. (A) The relationship between $\delta ^{13}$CH$_4$ (dark blue circles) and Northern Hemisphere (NH) temperature anomaly reconstructions [relative to the 1961 to 1990 mean; Jones and Mann (29), blue line and shading (1σ error); Moberg et al. (30), green line and shading (largest errors at 95% confidence interval)]. Because atmospheric methane is short-lived ($\sim 10$ years), the Jones and Mann record, which mainly uses tree ring data of decadal resolution and provides information on both temperature and moisture, is likely to better represent methane budget changes until $\sim 1500$ A.D., when the relationship with $\delta ^{13}$CH$_4$ diminishes (cf. multicentennial changes that the Moberg record better incorporates by use of low-resolution proxies). (B) Regional human population variations ($32$, $33$).

By incorporating land-use practices of different regions (i.e., biomass burning in the Americas), we investigate the relationship between population and anthropogenic source variation. Of most significance is the substantial population decline in the Americas from $\sim 1500$ A.D. "Indian & Other" refers to the remainder of the world population (which is mainly Indian).

Fig. 4. Law Dome $\delta ^{13}$CH$_4$ (black circles) and [CO] (gray triangles) ($35$). CO is a short-lived trace gas proxy for biomass burning, especially of woody biomass in the pre-industrial era. The [CO]–$\delta ^{13}$CH$_4$ relationship extends over 0 to 1500 A.D. but less so during 1500 to 1700. Measurements of [CO] in the ice core have been corrected for an extraction system contamination of 6 ppb. The error bars reflect the uncertainty in the measurement and in the storage of CO in the ice.

References and Notes
11. Materials and methods are available as supporting material on Science Online.
18. Even if LPIH variations in OH were as large as 10%, the weighted average fractionation of all sinks would only vary by $\sim 0.15\%$, which is equivalent to $\delta ^{13}$CH$_4$ measurement uncertainty, and [CH$_4$] would vary by $\sim 50$ ppb, which is equivalent to observed [CH$_4$] variability. The CO variations present in Fig. 4 over 0 to 1500 A.D. could cause OH abundance to vary by up to $\sim 10\%$. The 0.3°C temperature variations (Fig. 3) would cause a small change in the OH rate constant; however, $\delta ^{13}$CH$_4$ would vary by less than 0.01 and [CH$_4$] would vary by $\sim 4$ ppb.
22. The modeled emission uncertainties reflect the different $\delta ^{13}$CH$_4$ leverage of each source and result from [CH$_4$]$_3$ and $\delta ^{13}$CH$_4$ uncertainties during ice-core extraction, analysis, and calibration.
**Microcephalin, a Gene Regulating Brain Size, Continues to Evolve Adaptively in Humans**

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The gene *Microcephalin* (MCPH1) regulates brain size and has evolved under strong positive selection in the human evolutionary lineage. We show that one genetic variant of *Microcephalin* in modern humans, which arose ~37,000 years ago, increased in frequency too rapidly to be compatible with neutral drift. This indicates that it has spread under strong positive selection, although the exact nature of the selection is unknown. The finding that an important brain gene has continued to evolve adaptively in anatomically modern humans suggests the ongoing evolutionary plasticity of the human brain. It also makes *Microcephalin* an attractive candidate locus for studying the genetics of human variation in brain-related phenotypes.

The most distinct trait of *Homo sapiens* is the exceptional size and complexity of the brain (1, 2). Several recent studies have linked specific genes to the evolution of the human brain (3–12). One of these is *Microcephalin* (7, 8); mutations in this gene cause primary microcephaly [MCPH; Online Mendelian Inheritance in Man (OMIM) accession 251200] (13, 14). MCPH is defined clinically as severe reductions in brain size coupled with mental retardation, but remarkably, an overall retention of normal brain structure and a lack of overt abnormalities outside of the nervous system (15–17). This led to the notion that the brains of MCPH patients function normally for their size and that genes underlying MCPH are specific developmental regulators of brain size (15–17).

*Microcephalin* is one of six known loci, named MCPH1 through MCPH6, for which recessive mutations lead to MCPH (14, 18–23). For four of these, the underlying genes have been identified as *Microcephalin* (MCPH1), *CDK5RAP2* (MCPH3), *ASPM* (MCPH5), and *CENPJ* (MCPH6) (14, 21, 23). Patients with loss-of-function mutations in *Microcephalin* have cranial capacities about 4 SD below the mean at birth. As adults, their typical brain size is around 400 cm³ (whereas the normal range is 1200 to 1600 cm³), and the cerebral cortex is especially small (13, 14). *Microcephalin* is suggested to control the proliferation and/or differentiation of neuroblasts during neurogenesis. This postulate was consistent with several observations. First, mouse *Microcephalin* is expressed prominently in the proliferative zones of the embryonic brain (14). Second, the *Microcephalin* protein contains several copies of the BRCT domain that is found in cell cycle regulators, such as BRC1 (14, 24). Finally, cell culture studies indeed suggested a role of *Microcephalin* in regulating cell cycle (25–27).

The finding that *Microcephalin* is a critical regulator of brain size spurred the hypothesis that it might have played a role in brain evolution (16, 28). Consistent with this hypothesis, phylogenetic analysis of *Microcephalin* revealed signatures of strong positive selection in the lineage leading to humans (7, 8). Here, we examine the possibility that positive selection has continued to operate on this gene after the emergence of anatomically modern humans.

The human *Microcephalin* locus has 14 exons spanning about 236 kb on chromosome 8p23 (14) (Fig. 1). We previously sequenced all the exons in 27 humans (8). When reanalyzing the data, we noticed that one haplotype had a much higher frequency than the other haplotypes. Additionally, this haplotype differed consistently from the others at position 37995 of the genomic sequence (counting from the start codon) or position 940 on the open reading frame. This polymorphism falls in exon 8 and changes amino acid residue 314 from an ancestral aspartate to a histidine. (This polymorphism is described as G37995C with G denoting the ancestral allele.)

To investigate whether positive selection has acted on the high-frequency haplotype, we resequenced 23.4 kb of a 29-kb region centered around the G37995C polymorphism (Fig. 1). Sequencing was performed on a panel of 89 individuals from the Coriell Institute, which broadly represents human diversity (see SOM). To assign the ancestral state of polymorphisms, we also sequenced the common chimpanzee. Several GC-rich segments were not sequenced because of technical difficulties. The resulting sequence data contained 220 polymorphic sites, including 213 single-nucleotide polymorphisms (SNPs) and 7 insertion/deletion polymorphisms (indels) (table S1).

Haplotypes were inferred using the PHASE 2.1 program (29, 30). A total of 86 haplotypes

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References and Notes

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Materials and Methods

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