Comparative expression analysis of the phosphocreatine circuit in extant primates: Implications for human brain evolution

Adam D. Pfefferle a,b,1, Lisa R. Warner a,b,1, Catrina W. Wang c, William J. Nielsen a,b, Courtney C. Babbitt a,b, Olivier Fedrigo a,b, Gregory A. Wray a,b,c,*

a Biology Department, Duke University, Durham, USA
b Institute for Genome Sciences & Policy, Duke University, Durham, USA
c Department of Evolutionary Anthropology, Duke University, Box 90325 Durham, NC 27708, USA

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Abstract

While the hominid fossil record clearly shows that brain size has rapidly expanded over the last ~2.5 M.yr, the forces driving this change remain unclear. One popular hypothesis proposes that metabolic adaptations in response to dietary shifts supported greater encephalization in humans. An increase in meat consumption distinguishes the human diet from that of other great apes. Creatine, an essential metabolite for energy homeostasis in muscle and brain tissue, is abundant in meat and was likely ingested in higher quantities during human origins. Five phosphocreatine circuit proteins help regulate creatine utilization within energy demanding cells. We compared the expression of all five phosphocreatine circuit genes in cerebral cortex, cerebellum, and skeletal muscle tissue for humans, chimpanzees, and rhesus macaques. Strikingly, SLC6A8 and CKB transcript levels are higher in the human brain, which should increase energy availability and turnover compared to non-human primates. Combined with other well-documented differences between humans and non-human primates, this allocation of energy to the cerebral cortex and cerebellum may be important in supporting the increased metabolic demands of the human brain.

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Introduction

The rapid expansion of brain size that began ~2.5 mya in the lineage leading to modern humans (Schoenemann, 2006; Tattersall, 2008) required substantial metabolic support (Aiello and Wheeler, 1995; Leigh, 2004; Dunbar and Shultz, 2007; Isler and van Schaik, 2009). Comparisons of both DNA sequence (Haygood et al., 2007) and mRNA abundance (Uddin et al., 2004; Khaitovich et al., 2006a; Blekman et al., 2008; Babbitt et al., 2010) indicate that extensive changes in the regulation of metabolic associated genes help distinguish humans from chimpanzees. A dietary shift toward increased meat consumption by early hominids (Stanford, 1999; Stanford and Bunn, 2001; Ungar et al., 2006) may have contributed to some of the bioenergetic modifications necessary to support the human brain (Milton, 1987, 1999; Leonard and Robertson, 1992, 1994, 2003; Leonard et al., 2007). Comparative studies of primate genetics and molecular function provide powerful tools for identifying specific molecular changes associated with a human diet (Luca et al., 2010), an important step in understanding how changes in physiology allowed for the dramatic expansion of our brains.

Creatine, an abundant metabolite of red meat (Williams, 2007), occurs at higher concentrations in the plasma of humans who consume meat (Delanghe et al., 1989; Shomrat et al., 2000) and was likely present at higher quantities in the diet of human ancestors. Interestingly, creatine metabolism has been shown to positively correlate with brain activity (Sauter and Rudin, 1993; Du et al., 2008) and creatine supplements may improve mental performance in humans (Rae et al., 2003; McMorris et al., 2007). Thus, increased meat consumption along the hominin lineage may have influenced brain metabolism by providing additional creatine. The importance of creatine in helping maintain brain energy homeostasis (Wyss and Kaddurah-Daouk, 2000; Brosnan and Brosnan, 2002) has likely been important in supporting the increased metabolic demands of the human brain.

Abbreviations: Cr, creatine; PCR, phosphocreatine; SLC6A8, creatine transporter; CKMT1, creatine kinase mitochondrial 1; CKMT2, creatine kinase mitochondrial 2; CKB, creatine kinase brain; CKB, creatine kinase muscle; UTR, untranslated region; ATP, adenosine triphosphate; ADP, adenosine diphosphate; kb, kilobase; DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid; mya, million years ago.

* Corresponding author.
E-mail address: ggray@duke.edu (G.A. Wray).

1 These authors contributed equally to this work.
Fig. 1. Schematic representation of the phosphocreatine circuit. A. Creatine enters cells through the membrane transporter SLC6A8. B. Creatine moves across the outer mitochondrial membrane through porin. C. Creatine is phosphorylated within the outer mitochondrial space by CKMT1 or CKMT2. D. Phosphocreatine moves through porin back into the cytosol where it can diffuse to site with high ATPase activity. E. Phosphocreatine interacts with either CKB or CKM to generate ATP. F. The resulting ATP is then available as a source of energy for cytoplasmic ATPases and creatine returns to the mitochondria. ATPases, such as the sodium–potassium pump, are proteins that typically utilize energy from ATP to perform a specific cellular function. SLC6A8: creatine transporter, CKMT1: creatine kinase mitochondrial 1, CKMT2: creatine kinase mitochondrial 2, CKM: creatine kinase muscle, CKB: creatine kinase brain.
Table 1
Primate samples used in this study.

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</table>

Conserved exonic regions among all transcript isoforms and species (Table 2). Because CKMT1A and CKMT1B encode for identical proteins (Ensembl), PCR primers could not be designed to specifically amplify one gene and not the other. As such, a single PCR primer was designed to simultaneously amplify both genes and is referred to as CKMT1. Special care was taken to ensure that the amplified exonic region was found in all known transcript isoforms for each gene. This is important because transcript isoforms may be differentially expressed between species and would complicate our interpretation of the data. This potential confounding factor is controlled by ensuring that all isoforms are captured simultaneously in our expression measurements. Primers were selected using Primer3 Input v0.4.0 (Rozen and Skaletsky, 2000). The primer sequences were blasted to all three species’ genomes using Ensembl BLAST and a test PCR was performed on human cDNA to ensure only one product for each primer pair (SOM Fig. 2).

Quantitative RT-PCR data collection

Quantitative RT-PCR measurements were conducted on a Mastercycler ep realplex machine (Eppendorf) in 10 μl reactions: 5.0 μl 2 × Quantifast SYBR Green PCR Kit (Qiagen), 0.25 μl for each primer (10 μM), 0.5 μl of cDNA template, and 4.0 μl PCR quality water. The following PCR program was used for all reactions: 95 °C for 5 min, 40 cycles of 95 °C for 15 s and 60 °C for 30 s, followed by a melt curve from 60 to 95 °C. A single peak was detected on all melt curves, ensuring a single amplification product size for all species. Ct values were determined using the CalQPllex setting with a baseline drift correction. For each primer pair, a standard curve was setup on human brain or skeletal muscle cDNA over a twelve point, factor of two dilution series to determine the efficiency and working Ct range of each primer set. All primer sets had an efficiency between 94 and 100 percent with r² values greater than 0.99 (Table 2).

Data were collected by running each experimental and control sample in technical triplicate. For genes with medium to high expression (as defined by a mean Ct <33 PCR cycles), only measurements with low standard deviation across replicates (Std Dev <0.4 Ct) were used in the expression analysis (Karlen et al., 2007). All tissue-specific genes fell into this category of medium to high expression (e.g., CKB in both brain regions). For genes with low expression (as defined by a mean Ct >33 PCR cycles), a higher standard deviation threshold was implemented (Std Dev <1.0 Ct) as a result of increased variation of measurements in this Ct range (Karlen et al., 2007). All genes that fell into the category of low expression were genes being expressed in their non-dominant tissue (e.g., CKB in skeletal muscle). Within plates, expression was normalized with two control genes (SDHA and EEF2), selected based on their performance in a geNorm analysis on brain and skeletal muscle tissue for humans and chimpanzees, as well as having a similar expression level to the genes of interest (Vandesompele et al., 2002; Pattyn et al., 2003; Fedrigo et al., 2010).

Between plates, an inter-run calibration was conducted by running the control gene, EEF2, on IMR-32 cell cDNA (Helleman et al., 2007). To convert the raw Ct expression into normalized relative expression, we used a modified delta–delta Ct method (our code is available at: http://www.biology.duke.edu/wraylab/wraylab/Resources.html) (Vandesompele et al., 2002; Helleman et al., 2007; Fedrigo et al., 2010). Raw and normalized data can be found in the supplemental materials (SOM Tables 1 and 2, respectively).

Gene expression comparison

Although no generally accepted method exists for overlaying gene expression profiles onto a phylogenetic tree, a few studies have put forth alternatives for identifying natural selection from expression (Gilad et al., 2006; Khaitovich et al., 2006b). We focused on identifying gene expression differences between species (Fig. 2) using a Mann–Whitney test to calculate statistical significance as this approach does not assume a normal data distribution and it works well with smaller sample sets (Table 1). Even though the Mann–Whitney test works well with small sample sets, larger
sample sets have more statistical power to identify significant expression differences and have the potential to give lower p-values. This should be taken into account when comparing p-values across tissues with a higher number of samples (cerebral cortex) to those with a smaller sample set (cerebellum). The motivation behind this research is to identify human-specific expression patterns, as we believe those traits are more likely associated with human-specific phenotypes. As such, two sets of expression comparisons were performed, human versus chimpanzee and human versus rhesus macaque (Table 3).

Results

Comparative expression analysis

To our knowledge, this is the first study to comprehensively report expression measurements of the phosphocreatine circuit genes within non-human primates. Because we are interested in identifying which phosphocreatine circuit genes may be important for human-specific traits, we focused our attention on two comparisons: human versus chimpanzee and human versus rhesus macaque. These two comparisons allow us to identify tissue-specific expression signatures that are unique to humans among these three species.

Creatine transporter, SLC6A8

The phosphocreatine circuit begins with the active transport of creatine through a dedicated transmembrane protein, SLC6A8, into energetically expensive tissues, such as the brain and skeletal muscle (Snow and Murphy, 2001) (Fig. 1A). Since creatine must be transported across the plasma membrane before the cell can harvest its energy potential (Fig. 1B–F), SLC6A8 is a critical protein for fueling the underlying the phosphocreatine circuit. We therefore began by measuring transcript abundance from the SLC6A8 gene that encodes this protein.

Comparisons based on quantitative RT-PCR reveal higher mRNA transcript abundance of the creatine transporter gene SLC6A8 in humans than in chimpanzees and rhesus macaques in both the cerebral cortex (1.7-fold, \( p = 0.028 \) and 2.0-fold, \( p = 0.007 \), respectively) and the cerebellum (1.8-fold, \( p = 0.05 \) and 2.3-fold, \( p = 0.034 \), respectively) (Fig. 2).

Analyzing the skeletal muscle samples reveals almost equal expression of SLC6A8 when comparing human to chimpanzee and rhesus macaque (1.3-fold, \( p = 0.917 \) and 1.1-fold, \( p = 0.807 \), respectively) (Fig. 2).

Mitochondrial creatine kinases, CKMT1 and CKMT2

Once inside the cell, creatine primarily interacts with one family of proteins, the creatine kinases. Each of the four creatine kinase family members has tissue, intracellular, and substrate preferences, allowing for precise metabolic control (Wallimann et al., 1998) (Fig. 1). Two mitochondrial kinases, creatine kinase mitochondrial 1 (CKMT1) and creatine kinase mitochondrial 2 (CKMT2), are primarily expressed in the brain and skeletal muscle, respectively. Coupled with oxidative phosphorylation, these kinases localize within the mitochondria to catalyze the production of phosphocreatine, a high-energy phosphate compound similar to ATP (Fig. 1C) (Vendelin et al., 2004).

Consistent with previous reports in humans (Wyss and Kaddurah-Daouk, 2000), we found that CKMT1 and CKMT2 are also expressed in a tissue-specific fashion in chimpanzees and rhesus macaques, with CKMT1 predominant in the brain regions and CKMT2 in skeletal muscle (Fig. 2). Although we focus our discussion for each gene on their primary tissue of expression, all human to chimpanzee and human to rhesus macaque statistical comparisons were performed (Table 3).

When comparing human CKMT1 expression to chimpanzee in the brain regions, we observe about equal transcript abundance in the cerebral cortex (0.9-fold, \( p = 0.242 \)) but an increase in the cerebellum (1.7-fold, \( p = 0.028 \)). A more consistent pattern was measured between the human and the rhesus macaque brain samples, with humans having lower CKMT1 expression in both the cerebral cortex (0.3-fold, \( p = 0.007 \)) and the cerebellum (0.7-fold, \( p = 0.289 \)).

The other mitochondrial kinase, CKMT2, is expressed primarily in skeletal muscle. Human to chimpanzee comparisons show decreased expression (0.7-fold, \( p = 0.754 \)) of CKMT2 in the skeletal muscle samples, but an analysis of human to rhesus macaque shows increased expression (1.6-fold, \( p = 0.327 \)) (Fig. 2).

Cytosolic creatine kinases, CKB and CKM

After synthesis by the mitochondrial kinases, phosphocreatine diffuses out of the mitochondria (Fig. 1D) to sites with high ATPase activity (Fig. 1F) where it is able to interact with cytosolic creatine kinase (Fig. 1E) (Wallimann et al., 1998). Two cytosolic kinases, creatine kinase brain type (CKB) and creatine kinase muscle type (CKM), are predominantly expressed in the brain and skeletal muscle, respectively. Complementary to their mitochondrial counterparts, the cytosolic kinases drive the production of ATP (Fig. 1E). This reaction is coupled with cytosolic ATPases to provide energy for metabolic processes (Fig. 1F), such as muscle contractions and maintaining plasma membrane potentials (Kushmerick, 1998; Wyss and Kaddurah-Daouk, 2000).

Similar to previous reports in humans (Wyss and Kaddurah-Daouk, 2000) and to the mitochondrial kinases measured in this study, expression analysis of both cytosolic creatine kinases reveals a corresponding tissue-specific expression pattern in chimpanzee and rhesus macaque (Fig. 2). CKB is dominant in both brain regions of interest while CKM is dominant in skeletal muscle. As with the mitochondrial kinases, for each of these genes we concentrate on the tissue in which they are most highly expressed.

We found that CKB expression has undergone a human-specific increase in the cerebral cortex and cerebellum compared to chimpanzee (2.0-fold, \( p = 0.008 \) and 2.5-fold, \( p = 0.014 \), respectively) and

---

**Table 2**

<table>
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<th>Gene</th>
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Figure 2. Phosphocreatine circuit gene expression comparisons among species. Quantitative PCR measurements for the creatine transporter and kinases in humans, chimpanzees, and rhesus macaques. Individuals are each represented by a point, the horizontal bar is the mean, and the spread of the bar from the mean represents one standard deviation. SLC6A8: creatine transporter, CKMT1: creatine kinase mitochondrial 1, CKMT2: creatine kinase mitochondrial 2, CKM: creatine kinase muscle, CKB: creatine kinase brain, Hsap: Homo sapiens, Ptro: Pan troglodytes, Mmul: Macaca mulatta.
respectively) (Wallimann et al., 2007). The reaction catalyzed by CKB provides energy for a diverse set of enzymatic activities in the cytoplasm (Fig. 1E–F), potentially providing humans with the ability to support a greater number of simultaneous enzymatic reactions in the brain than either chimpanzee or rhesus macaque.

Thus, gene expression underlying two key components of the phosphocreatine circuit are elevated in the brains of humans relative to chimpanzees and rhesus macaques. Interestingly, these observations are tissue-specific, suggesting differential allocation of a key metabolite between two metabolically demanding tissues in the body. Furthermore, the expression changes in these two genes are likely to be synergistic by transporting more creatine into cells and increasing the capacity to utilize phosphocreatine as a source of energy for ATP-dependent enzymatic reactions. These changes in

### Discussion

In an influential paper published in 1975, King and Wilson posited that changes in gene regulation generated many of the phenotypic differences that distinguish humans from chimpanzees (King and Wilson, 1975). Controlling the abundance of mRNA is one of the most important aspects of gene regulation, as fluctuations in the expression of specific genes are known to produce a wide variety of phenotypic consequences (Wray, 2007). Comparing gene expression between primate species provides an initial approach for understanding observed physiological differences.

In this study, we measured expression of the phosphocreatine circuit genes to determine if they are differentially expressed between primate species. Because the brain is such an energetically expensive organ, the approximately two-fold increase in cranial capacity that occurred during the past ~2 M.yr. of human evolution (Schoenemann, 2006) imposed a substantially large metabolic demand (Aiello and Wheeler, 1995; Leonard et al., 2007). A shift toward increased meat consumption may have contributed toward meeting that increased demand (Milton, 1999; Stanford, 1999; Stanford and Bunn, 2001; Ungar et al., 2006). Knowing that creatine is an abundant nutrient in red meat (Williams, 2007) and that phosphocreatine is critical to metabolically active cells (Wyss and Kaddurah-Daouk, 2000; Brosnan and Brosnan, 2007; Tachikawa et al., 2007), we hypothesized that a brain-specific increase in of phosphocreatine circuit gene expression arose in the lineage leading to humans. Higher expression of this circuit in humans would provide additional ATP energy to brain cells by increasing ATP turnover and transport efficiency (Wyss and Kaddurah-Daouk, 2000; Snow and Murphy, 2001).

Our results show that there is higher expression of genes encoding two key components of the phosphocreatine circuit in the cerebral cortex and cerebellum of humans (Fig. 2). The first of these genes, SLC6A8, encodes a protein that mediates creatine transport across the plasma membrane (Snow and Murphy, 2001). The importance of intracellular creatine for normal brain anatomy, physiology, and cognition is revealed by creatine transporter deficiency syndromes (MIM ID #300352), which involve impaired transport of creatine across the blood brain barrier and lead to serious health consequences in humans, including mental retardation, language impairment, seizures, and microcephaly (de Grauw et al., 2003; Schiaffino et al., 2005; Ansell et al., 2006). These phenotypes indicate that the transport of creatine into the brain is important within humans and suggest that differences in intracellular creatine concentrations between primate species may also be significant.

Expression comparisons reveal that SLC6A8 is expressed at about twice the level in the cerebral cortex and cerebellum of humans as it is in chimpanzees (Fig. 2). Given that SLC6A8 expression is positively correlated with intracellular creatine concentrations (Wyss and Kaddurah-Daouk, 2000), the observed increase in SLC6A8 expression, combined with increased meat intake, would likely increase the transport of creatine into the brain. In contrast, SLC6A8 expression levels in skeletal muscle are not significantly different between human and chimpanzee (Fig. 2). Thus, not only did more creatine become available to the body from the dietary shift toward meat consumption, but a greater proportion of this creatine is likely transported into the brain as opposed to another metabolically demanding tissue, skeletal muscle. Higher intracellular creatine concentrations in the brain would fuel the phosphocreatine circuit (Brosnan and Brosnan, 2007).

The second phosphocreatine circuit gene whose expression differs between humans and chimpanzees is CKB. This gene encodes a kinase that generates ATP from ADP using phosphocreatine as a source of high-energy phosphate in the cytosol. CKB protein plays a critical role in maintaining proper brain energy homeostasis (Wyss and Kaddurah-Daouk, 2000) and brain activity positively correlates with CKB function (Sauter and Rudin, 1993; Du et al., 2008). In rats, creatine kinase regenerates ATP twelve times faster than through oxidative phosphorylation (Wallimann et al., 1992). This rapidly available energy is important in regulating neurotransmitter release, maintaining membrane potentials, assisting growth cone migration, and restoring energy homeostasis (Wallimann et al., 1992; Wallimann and Hemmer, 1994). Further evidence for CKB’s importance comes from CKB−/− knockout mice. In addition to other neurological conditions, these mice show decreased spatial learning and decreased habituation behavior (Jost et al., 2002).

Our expression comparisons show that humans have approximately twice as much CKB mRNA in both the cerebral cortex and cerebellum compared to chimpanzees and rhesus macaques. The corresponding gene that is expressed in muscle, CKM, shows no difference in transcript abundance between these three species. Because CKB expression positively correlates with CKB protein activity (Ishikawa et al., 2005), higher CKB transcript abundance in humans may allow for more efficient ATP regeneration during energy utilization, helping support the increased metabolic demands of the human brain (Wallimann et al., 1992; Wallimann and Hemmer, 1994).

Table 3

<table>
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Table 3: p-values for human-specific expression comparisons (Fig. 2) using a Mann–Whitney test. Significant values are highlighted (p ≤ 0.05).

Consistent with the other skeletal muscle genes (SLC6A8 and CKMT2), CKM lacks a species-specific expression pattern in our skeletal muscle samples. Average CKM expression in skeletal muscle shows an increase in abundance in humans compared to chimpanzee (1.9-fold, p = 0.465), but about equal expression compared to rhesus macaque (1.1-fold, p = 0.462).

### Human to Chimpanzee

<table>
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<th>Skeletal muscle</th>
</tr>
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<tbody>
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<td>0.0500</td>
</tr>
<tr>
<td>CKB</td>
<td>0.0108</td>
<td>0.0771</td>
<td>0.1416</td>
</tr>
<tr>
<td>CKMT2</td>
<td>0.0066</td>
<td>0.0339</td>
<td>0.3272</td>
</tr>
<tr>
<td>CKM</td>
<td>0.1742</td>
<td>0.0339</td>
<td>0.4624</td>
</tr>
</tbody>
</table>

### Human to Macaque

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cerebral cortex</th>
<th>Cerebellum</th>
<th>Skeletal muscle</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.8065</td>
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<tr>
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<td>0.0771</td>
<td>0.1416</td>
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<tr>
<td>CKMT2</td>
<td>0.0066</td>
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</tr>
<tr>
<td>CKM</td>
<td>0.1742</td>
<td>0.0339</td>
<td>0.4624</td>
</tr>
</tbody>
</table>

rhesus macaque (1.8-fold, p = 0.011 and 1.9-fold, p = 0.077 respectively) (Fig. 2).

The importance of phosphocreatine circuit gene expression pattern arose in the lineage leading to humans. Higher expression of this circuit in humans would likely increase the transport of creatine into the brain as opposed to another metabolically demanding tissue, skeletal muscle. Higher intracellular creatine concentrations in the brain would fuel the phosphocreatine circuit (Brosnan and Brosnan, 2007).

Thus, gene expression underlying two key components of the phosphocreatine circuit are elevated in the brains of humans relative to chimpanzees and rhesus macaques. Interestingly, these observations are tissue-specific, suggesting differential allocation of a key metabolite between two metabolically demanding tissues in the body. Furthermore, the expression changes in these two genes are likely to be synergistic by transporting more creatine into cells and increasing the capacity to utilize phosphocreatine as a source of energy for ATP-dependent enzymatic reactions. These changes in
gene expression are consistent with the hypothesis that humans utilize the phosphocreatine circuit more heavily than chimpanzees and rhesus macaques to support our energy demanding brain (Peters et al., 2004).

An important concern for this study and follow-up experiments is whether gene expression differences are the result of genetic changes between species or the result of environmental effects, such as different diets. It is clear that environmental factors can influence gene expression (Idaghdour et al., 2008; Somel et al., 2008; Gibson, 2008; Hodgins-Davis and Townsend, 2009). Of direct relevance to this study, creatine levels and creatine metabolism are influenced by dietary intake of creatine (Wyss and Kaddurah-Daouk, 2000; Snow and Murphy, 2001; Brosnan and Brosnan, 2007). Although it is not possible to carry out studies in chimpanzees and humans that fully control for dietary differences, these kinds of studies can readily be conducted with mice. Somel et al. (2008) investigated the effects of diet on gene expression in the liver and brain of mice. After feeding adult mice four different diets for eight weeks (chimpanzee diet, cafeteria food diet, McDonald’s diet, and pellet diet), the authors measured transcript abundance using microarrays (Somel et al., 2008). Importantly for the present study, they observed no significant difference in the expression levels of either SLC6A8 or CKB among the four diets (Somel, pers. comm.). While these data do not rule out the possibility that diet can influence the expression of these two genes, they do support the interpretation that the differences in transcription levels we observe are unlikely to be exclusively a result of diet. An interesting question for future studies will be parsing the relative influences of genetic factors, environmental influences, or a combination of both, on creatine distribution and utilization in primates.

At this point, it is not possible to conclude that the gene expression differences we observe in the phosphocreatine circuit serve as adaptions. Comparisons of gene expression have been used to infer adaptation by concluding that interspecies differences that are significantly larger than intraspecies variation are more likely to result from positive selection than drift (Blekhman et al., 2008). To date, these techniques have been optimized and applied on a genome-wide scale, making it difficult to apply these same methods to our study. However, the pattern of expression changes in both SLC6A8 and CKB loosely meet these criteria (Fig. 2). The false positive rate using this test for selection is not well understood, and we do not draw any firm conclusions from it. A second line of evidence regarding adaptation comes from analysis of DNA sequences. We sought evidence of positive selection on all five phosphocreatine circuit genes by examining three regions that can house gene regulatory elements (5′ flanking region, 5′ UTR and 3′ UTR) and one that encodes the protein function (coding region) (SOM Appendix). We found no evidence of positive selection for mutations in or around any of the five genes (SOM Table 3). It is important to bear in mind, however, that these methods are generally underpowered, and are unable to identify selection on single point mutations, any other kind of mutation, or epigenetic modifications, any of which could influence gene expression. (In fact, expression of CKMT1, CKM, and CKB can be influenced by epigenetic regulation [Caretti et al., 2004; Ishikawa et al., 2005; Uzawa et al., 2006]).

Although our data do not speak directly to the question of adaptation, they do focus attention on changes in specific molecular processes that may have contributed to a shift in energy allocation toward the brain during human evolution. Energy trade-off hypotheses predict that metabolic reallocation from other energetically demanding tissues to the brain allowed for greater encephalization in humans (Aiello and Wheeler, 1995; Leonard et al., 2003; Isler and van Schaik, 2009). The tissue- and species-specific differences in SLC6A8 and CKB expression we report here are consistent with these predictions. Perhaps the most convincing evidence that these expression differences are functionally important comes from genetic data showing that reducing the amount of normal SLC6A8 and CKB protein produces pathologic phenotypes related to the brain (Jost et al., 2002; de Grauw et al., 2003; Schiaffino et al., 2005; Anselm et al., 2006). The implication to our study being that elevated expression of these genes would have increased the metabolic scope of the brain.

Comparative gene expression studies in primates provide exciting opportunities to complement the extensive body of work investigating energetic trade-offs at the level of tissue mass (Aiello and Wheeler, 1995; Leonard et al., 2003; Isler and van Schaik, 2005) by giving molecular insight into the physiological underpinnings of those tissues. It seems highly unlikely that only a small set of molecular changes accounted for differential energy allocation among tissues during human evolution. Indeed, our earlier genome-wide analysis of noncoding sequences in the same three species examined here suggested that diverse genes involved in carbohydrate metabolism experienced positive selection on regulatory sequences during human origins (Haygood et al., 2007). Large-scale surveys of gene expression have begun to identify numerous genes whose expression differs among primate species (Uddin et al., 2004; Khaitovich et al., 2006a; Blekhman et al., 2008; Babbitt et al., 2010), greatly expanding our view of the specific molecular changes that accompanied the origin of humans as a distinct species. An important challenge for the coming years will be identifying which of these changes were associated with the evolution of uniquely human traits.

Conclusions

While it is well known that the anatomy and physiology of the human brain differs from other great apes in numerous regards (Deacon, 1997; Schoenemann, 2006), the underlying molecular mechanisms responsible for those differences have remained elusive. Gene expression analysis provides a rapid and powerful tool for identifying functional differences among primate species (Khaitovich et al., 2006a). Our analysis of the phosphocreatine circuit has revealed two genes, SLC6A8 and CKB, within the phosphocreatine circuit that are consistently and differentially expressed between humans, chimpanzees, and rhesus macaques specifically within the cerebral cortex and cerebellum. Given the bioenergetic importance of this circuit and its association with dietary intake, increased expression of SLC6A8 and CKB in the human brain may have a profound influence on brain energy homeostasis today and during human origins by increasing ATP energy availability and turnover.

Acknowledgements

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Appendix. SOM

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.jhevol.2010.10.004.