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

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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.

Keywords

primate comparison; phosphocreatine circuit; gene expression; brain metabolism

Introduction

The rapid expansion of brain size that began ~2.5 Ma 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,

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2009). Comparisons of both DNA sequence (Haygood et al., 2007) and mRNA abundance (Uddin et al., 2004; Khaitovich et al., 2006a; Blekhman 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; Leonard and Robertson, 1992, 1994; Milton, 1999, 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, 2007; Tachikawa et al., 2007) makes the phosphocreatine circuit a particularly attractive candidate for beginning to uncover the molecular changes associated with increased encephalization.

The phosphocreatine circuit begins when creatine diffuses across the plasma membrane, through the cytosol, and into the mitochondria (Figure 1A-B), where it is converted to phosphocreatine (Figure 1C), a high-energy compound (Wallimann et al., 1992). Phosphocreatine serves several functions within the brain, skeletal muscle, and other energetically demanding tissues (Brosnan and Brosnan, 2007) (Figure 1). First, phosphocreatine assists the cell in performing energy expensive processes in the cytosol (Figure 1E-F), such as contracting muscle and maintaining membrane potentials (Wyss and Kaddurah-Daouk, 2000). Second, the phosphocreatine circuit serves as an energy shuttle to cellular sites with high ATP utilization (Figure 1D) (Wyss and Kaddurah-Daouk, 2000). Phosphocreatine is able to diffuse across the cytosol faster than ATP, allowing for efficient support of biological processes farther from the mitochondria (Ellington, 2001). Third, by donating a phosphate to ADP, the phosphocreatine circuit also acts to buffer ATP concentrations (Figure 1E) (Wyss and Kaddurah-Daouk, 2000). Without ATP buffering, cellular processes regulated by ATP concentrations would be adversely affected by energy fluctuations during times of rapid energy utilization, leading to cell toxicity (Matthews et al., 1999). These functions are particularly important in the brain.

While skeletal muscle contains large amounts of glycogen for energy storage (Fisher et al., 2002), the brain has few mechanisms for storing energy (Peters et al., 2004), making the phosphocreatine circuit particularly important for maintaining proper brain energy homeostasis (Wallimann and Hemmer, 1994). Given that the phosphocreatine circuit is responsible for critical cellular functions and the association between a meat-rich diet and

elevated creatine levels, we hypothesize that gene regulatory changes occurred during human evolution to increase phosphocreatine circuit gene expression specifically in the brain as a way of increasing ATP energy availability and turnover. This study compares the expression of the genes that encode the phosphocreatine circuit in humans, chimpanzees, and rhesus macaques. The goals are to better understand the role of this circuit in these primates and to infer the functional significance of these differences in the context of human evolution.

Materials and methods

Primate sample collection

Samples were obtained from the Kathleen Price Bryan Brain Bank at Duke University (*Homo sapiens*, frozen tissue), BioChain Institute Incorporated (*Homo sapiens*, total RNA), Southwest National Primate Research Center (*Pan troglodytes* and *Macaca mulatta,* frozen tissue), New England Regional Primate Research Center (*Macaca mulatta*, frozen tissue), and Yerkes National Primate Research Center (*Macaca mulatta*, frozen tissue) (Table 1).

Tissue preparation, total RNA isolation, and cDNA synthesis

All RNA extractions from collected tissue were performed at Duke University's Regional Biocontainment Laboratory (RBL). Special care was taken to section each tissue consistently within and between species. Whole brain tissues maintained natural form in the freezer, allowing for anatomic landmarks, such as major sulci and gyri, to be used for proper brain region identification. Cerebral cortex tissue sections were specifically taken from the frontal lobe, while skeletal muscle tissue samples were taken from the vastus lateralis. All tissue sections were superficially taken with a width of ~4mm, a depth of ~4mm. Tissues were homogenized in QIAzol lysis buffer (Qiagen) using a Tissuelyser II (Qiagen). Total RNA samples were purified using an RNeasy lipid tissue kit (Qiagen) in conjunction with an RNase-free, DNase set (Qiagen). RNA concentration and quality were determined using a Nanodrop spectrophotometer (Thermo Scientific) and an Experion system (BioRad), respectively. Total RNA was converted to cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). All samples were PCR tested for genomic DNA contamination using a *SDHA* primer pair designed to amplify an intronic region (Supplemental Online Material Figure 1).

Primer design

Gene sequences for *SLC6A8, CKMT1A, CKMT1B, CKMT2, CKB, and CKM* were obtained from the Ensembl genome browser using the *Homo sapiens* 36.3, *Pan troglodytes* 2.1, and *Macaca mulatta* 1.1 builds. PCR primers (Sigma-Aldrich) were designed within completely 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

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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 (Supplemental Online Material Figure 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 2X 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 minutes, 40 cycles of 95 °C for 15 seconds and 60 °C for 30 seconds, 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 CalQPlex 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 r^2 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 (Hellemans 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; Hellemans et al., 2007; Fedrigo et al., 2010). Raw and normalized data can be found in the supplemental materials (Supplemental Online Material Table 1 and Supplemental Online Material Table 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 (Figure 2) using a Mann-Whitney test to

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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 nonhuman 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) (Figure 1A). Since creatine must be transported across the plasma membrane before the cell can harvest its energy potential (Figure 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) (Figure 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) (Figure 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) (Figure 1). Two mitochondrial kinases, creatine kinase mitochondrial 1 (*CKMT1*) and creatine kinase mitochondrial 2 (*CKMT2*), are primarily expressed in the brain and skeletal

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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 (Figure 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 (Figure 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) (Figure 2).

Cytosolic creatine kinases, CKB and CKM

After synthesis by the mitochondrial kinases, phosphocreatine diffuses out of the mitochondria (Figure 1D) to sites with high ATPase activity (Figure 1F) where it is able to interact with cytosolic creatine kinase (Figure 1E) (Figure 1) (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 (Figure 1E). This reaction is coupled with cytosolic ATPases to provide energy for metabolic processes (Figure 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 (Figure 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 rhesus macaque (1.8-fold, p=0.011 and 1.9-fold, p=0.077 respectively) (Figure 2).

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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).

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 larger metabolic demand (Aiello and Wheeler, 1995; Leonard et al., 2007). A shift toward increased meat consumption may have contributed towards 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 (Figure 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 (deGrauw et al., 2003; Schiaffino et al., 2005; Anselm 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 (Figure 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

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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 (Figure 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). The reaction catalyzed by CKB provides energy for a diverse set of enzymatic activities in the cytoplasm (Figure 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 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

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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 and colleagues (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 (M. 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 as 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 (Figure 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 towards 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

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data showing that reducing the amount of normal SLC6A8 and CKB protein produces pathologic phenotypes related to the brain (Jost et al., 2002; deGrauw 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, 2009) 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

Cr	creatine
PCr	phosphocreatine
SLC6A8	creatine transporter
CKMT1	creatine kinase mitochondrial 1
CKMT2	creatine kinase mitochondrial 2
CKB	creatine kinase brain
CKM	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

References

Aiello LC, Wheeler P. The expensive-tissue hypothesis: the brain and the digestive system in human and primate evolution. Curr. Anthropol. 1995; 36:199–221.

Anselm IA, Alkuraya FS, Salomons GS, Jakobs C, Fulton AB, Mazumdar M, Rivkin M, Frye R, Poussaint TY, Marsden D. X-linked creatine transporter defect: a report on two unrelated boys with a severe clinical phenotype. J. Inherit. Metab. Dis. 2006; 29:214–219. [PubMed: 16601897]

Babbitt CC, Fedrigo O, Pfefferle AD, Boyle AP, Horvath JE, Furey TS, Wray GA. Both noncoding and protein-coding RNAs contribute to gene expression evolution in the primate brain. Genome Biol. Evol. 2010; 2:67–79. [PubMed: 20333225]

Blekhman R, Oshlack A, Chabot AE, Smyth GK, Gilad Y. Gene regulation in primates evolves under tissue-specific selection pressures. PLoS Gen. 2008; 4:e1000271.

Brosnan JT, Brosnan ME. Creatine: endogenous metabolite, dietary, and therapeutic supplement. A. Rev. Nutr. 2007; 27:241–261.

Caretti G, Di Padova M, Micales B, Lyons GE, Sartorelli V. The Polycomb Ezh2 methyltransferase regulates muscle gene expression and skeletal muscle differentiation. Genes Dev. 2004; 18:2627–2638. [PubMed: 15520282]

Deacon TW. What makes the human brain different? Ann. Rev. Anthropol. 1997; 26:337–357.

deGrauw TJ, Cecil KM, Byars AW, Salomons GS, Ball WS, Jakobs C. The clinical syndrome of creatine transporter deficiency. Mol. Cell Biochem. 2003; 244:45–48. [PubMed: 12701808]

Delanghe J, De Slypere JP, De Buyzere M, Robbrecht J, Wieme R, Vermeulen A. Normal reference values for creatine, creatinine, and carnitine are lower in vegetarians. Clin. Chem. 1989; 35:1802–1803. [PubMed: 2758659]

Du F, Zhu XH, Zhang Y, Friedman M, Zhang N, Ugurbil K, Chen W. Tightly coupled brain activity and cerebral ATP metabolic rate. Proc. Natl. Acad. Sci. 2008; 105:6409–6414. [PubMed: 18443293]

Dunbar RI, Shultz S. Evolution in the social brain. Science. 2007; 317:1344–1347. [PubMed: 17823343]

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Ellington WR. Evolution and physiological roles of phosphagen systems. Ann. Rev. Physiol. 2001; 63:289–325. [PubMed: 11181958]

Fedrigo O, Warner LR, Pfefferle AD, Babbitt CC, Cruz-Gordillo P, Wray GA. A pipeline to determine candidate qRT -PCR control genes for evolutionary studies: application to primate gene expression across multiple tissues. PLoS ONE. 2010; 5:e12545. [PubMed: 20824057]

Fisher JS, Nolte LA, Kawanaka K, Han DH, Jones TE, Holloszy JO. Glucose transport rate and glycogen synthase activity both limit skeletal muscle glycogen accumulation. Am. J. Physiol. Endocrinol. Metab. 2002; 282:E1214–E1221. [PubMed: 12006350]

Gibson G. The environmental contribution to gene expression profiles. Nat. Rev. Genet. 2008; 9:575–581. [PubMed: 18574472]

Gilad Y, Oshlack A, Rifkin SA. Natural selection on gene expression. Trends Genet. 2006; 22:456–461. [PubMed: 16806568]

Haygood R, Fedrigo O, Hanson B, Yokoyama KD, Wray GA. Promoter regions of many neural- and nutrition-related genes have experienced positive selection during human evolution. Nat. Genet. 2007; 39:1140–1144. [PubMed: 17694055]

Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. 2007; 8:R19. [PubMed: 17291332]

Hodgins-Davis A, Townsend JP. Evolving gene expression: from G to E to GxE. Trends Ecol. Evol. 2009; 24:649–658. [PubMed: 19699549]

Idaghdour Y, Storey JD, Jadallah SJ, Gibson G. A genome-wide gene expression signature of environmental geography in leukocytes of Moroccan Amazighs. PLoS Genet. 2008; 4:e1000052. [PubMed: 18404217]

Ishikawa J, Taniguchi T, Takeshita A, Maekawa M. Increased creatine kinase BB activity and CKB mRNA expression in patients with hematologic disorders: relation to methylation status of the CKB promoter. Clin. Chim. Acta. 2005; 361:135–140. [PubMed: 15996648]

Isler K, van Schaik CP. The expensive brain: a framework for explaining evolutionary changes in brain size. J. Hum. Evol. 2009; 57:392–400. [PubMed: 19732937]

Jost CR, Van Der Zee CE, In 't Zandt HJ, Oerlemans F, Verheij M, Streijger F, Fransen J, Heerschap A, Cools AR, Wieringa B. Creatine kinase B-driven energy transfer in the brain is important for habituation and spatial learning behaviour, mossy fibre field size and determination of seizure susceptibility. Eur. J. Neurosci. 2002; 15:1692–1706. [PubMed: 12059977]

Karlen Y, McNair A, Perseguers S, Mazza C, Mermod N. Statistical significance of quantitative PCR. BMC Bioinformatics. 2007; 8:131. [PubMed: 17445280]

Khaitovich P, Enard W, Lachmann M, Paabo S. Evolution of primate gene expression. Nat. Rev. Genet. 2006a; 7:693–702. [PubMed: 16921347]

Khaitovich P, Tang K, Franz H, Kelso J, Hellmann I, Enard W, Lachmann M, Paabo S. Positive selection on gene expression in the human brain. Curr. Biol. 2006b; 16:R356–R358. [PubMed: 16618540]

King MC, Wilson AC. Evolution at two levels in humans and chimpanzees. Science. 1975; 188:107–116. [PubMed: 1090005]

Kushmerick MJ. Energy balance in muscle activity: simulations of ATPase coupled to oxidative phosphorylation and to creatine kinase. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 1998; 120:109–123. [PubMed: 9787781]

Leigh SR. Brain growth, life history, and cognition in primate and human evolution. Am. J. Primatol. 2004; 62:139–164. [PubMed: 15027089]

Leonard WR, Robertson ML. Nutritional requirements and human evolution: A bioenergetics model. Am. J. Hum. Biol. 1992; 4:179–195.

Leonard WR, Robertson ML. Evolutionary perspectives on human nutrition: The influence of brain and body size on diet and metabolism. Am. J. Hum. Biol. 1994; 6:77–88.

Leonard WR, Robertson ML, Snodgrass JJ, Kuzawa CW. Metabolic correlates of hominid brain evolution. Comp. Biochem. Physiol. A. 2003; 136:5–15.

Leonard WR, Snodgrass JJ, Robertson ML. Effects of brain evolution on human nutrition and metabolism. A. Rev. Nutr. 2007; 27:311–327.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Luca F, Perry GH, Di Rienzo A. Evolutionary adaptations to dietary changes. A. Rev. Nutr. 2010; 30:291–314.

Matthews RT, Ferrante RJ, Klivenyi P, Yang L, Klein AM, Mueller G, Kaddurah-Daouk R, Beal MF. Creatine and cyclocreatine attenuate MPTP neurotoxicity. Exp. Neurol. 1999; 157:142–149. [PubMed: 10222117]

McMorris T, Mielcarz G, Harris RC, Swain JP, Howard A. Creatine supplementation and cognitive performance in elderly individuals. Neuropsychol. Dev. Cogn. B Aging Neuropsychol. Cogn. 2007; 14:517–528. [PubMed: 17828627]

Milton, K. Primate diets and gut morphology: implications for hominid evolution. In: Harris, M.; Ross, EB., editors. Food and Evolution: Toward a Theory of Human Food Habits. Temple University Press; Philadelphia: 1987. p. 93-115.

Milton K. A hypothesis to explain the role of meat-eating in human evolution. Evol. Anthropol. 1999; 8:11–21.

Milton K. The critical role played by animal source foods in human (*Homo*) evolution. J. Nutr. 2003; 133:3886S–3892S. [PubMed: 14672286]

Pattyn F, Speleman F, De Paepe A, Vandesompele J. RTPrimerDB: the real-time PCR primer and probe database. Nucleic Acids Res. 2003; 31:122–123. [PubMed: 12519963]

Peters A, Schweiger U, Pellerin L, Hubold C, Oltmanns KM, Conrad M, Schultes B, Born J, Fehm HL. The selfish brain: competition for energy resources. Neurosci. Biobehav. Rev. 2004; 28:143–180. [PubMed: 15172762]

Rae C, Digney AL, McEwan SR, Bates TC. Oral creatine monohydrate supplementation improves brain performance: a double-blind, placebo-controlled, cross-over trial. Proc. Biol. Sci. 2003; 270:2147–2150. [PubMed: 14561278]

Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. Methods Mol. Biol. 2000; 132:365–386. [PubMed: 10547847]

Sauter A, Rudin M. Determination of creatine kinase kinetic parameters in rat brain by NMR magnetization transfer: Correlation with brain function. J. Biol. Chem. 1993; 268:13166–13171. [PubMed: 8514755]

Schiaffino MC, Bellini C, Costabello L, Caruso U, Jakobs C, Salomons GS, Bonioli E. X-linked creatine transporter deficiency: clinical description of a patient with a novel SLC6A8 gene mutation. Neurogenetics. 2005; 6:165–168. [PubMed: 16086185]

Schoenemann PT. Evolution of the size and functional areas of the human brain. Ann. Rev. Anthropol. 2006; 35:379–406.

Shomrat A, Weinstein Y, Katz A. Effect of creatine feeding on maximal exercise performance in vegetarians. Eur. J. Appl. Physiol. 2000; 82:321–325. [PubMed: 10958375]

Snow RJ, Murphy RM. Creatine and the creatine transporter: a review. Mol. Cell Biochem. 2001; 224:169–181. [PubMed: 11693194]

Somel M, Creely H, Franz H, Mueller U, Lachmann M, Khaitovich P, Paabo S. Human and chimpanzee gene expression differences replicated in mice fed different diets. PLoS One. 2008; 3:e1504. [PubMed: 18231591]

Stanford, CB. The hunting apes: meat eating and the origins of human behavior. Princeton University Press; New Jersey: 1999.

Stanford, CB.; Bunn, HT. Meat-eating and human evolution. Oxford University Press Inc.; New York: 2001.

Tachikawa M, Hosoya K, Ohtsuki S, Terasaki T. A novel relationship between creatine transport at the blood-brain and blood-retinal barriers, creatine biosynthesis, and its use for brain and retinal energy homeostasis. Subcell. Biochem. 2007; 46:83–98. [PubMed: 18652073]

Tattersall I. An evolutionary framework for the acquisition of symbolic cognition by Homo sapiens. Comp. Cog. Beh. Rev. 2008; 3:99–114.

Uddin M, Wildman DE, Liu G, Xu W, Johnson RM, Hof PR, Kapatos G, Grossman LI, Goodman M. Sister grouping of chimpanzees and humans as revealed by genome-wide phylogenetic analysis of brain gene expression profiles. Proc. Natl. Acad. Sci. 2004; 101:2957–2962. [PubMed: 14976249]

Ungar PS, Grine FE, Teaford MF. Diet in early Homo: A review of the evidence and a new model of adaptive versatility. Ann. Rev. Anthropol. 2006; 35:209–228.

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Uzawa OT, Endo Y, Bukawa H, Yokoe H, Shibahara T, Tanzawa H. Ubiquitous mitochondrial creatine kinase downregulated in oral squamous cell carcinoma. Br. J. Cancer. 2006; 94:698–709. [PubMed: 16479256]

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002; 3:RESEARCH0034. [PubMed: 12184808]

Vendelin M, Lemba M, Saks VA. Analysis of functional coupling: mitochondrial creatine kinase and adenine nucleotide translocase. Biophys. J. 2004; 87:696–713. [PubMed: 15240503]

Wallimann T, Dolder M, Schlattner U, Eder M, Hornemann T, O'Gorman E, Ruck A, Brdiczka D. Some new aspects of creatine kinase (CK): compartmentation, structure, function and regulation for cellular and mitochondrial bioenergetics and physiology. Biofactors. 1998; 8:229–234. [PubMed: 9914824]

Wallimann T, Hemmer W. Creatine kinase in non-muscle tissues and cells. Mol. Cell Biochem. 1994; 133:193–220. [PubMed: 7808454]

Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. Biochem. J. 1992; 281(Pt 1):21–40. [PubMed: 1731757]

Williams P. Nutritional composition of red meat. Nutr. Diet. 2007; 64:S113–S119.

Wray GA. The evolutionary significance of cis-regulatory mutations. Nat. Rev. Genet. 2007; 8:206–216. [PubMed: 17304246]

Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. Physiol. Rev. 2000; 80:1107–1213. [PubMed: 10893433]

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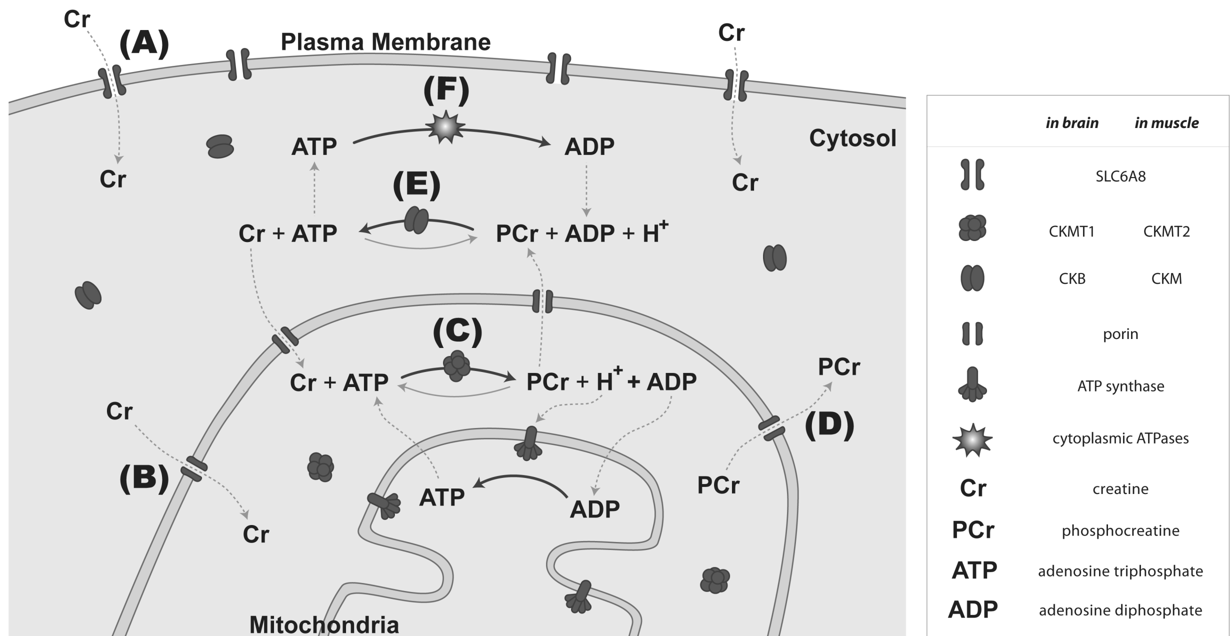

Figure 1. Schematic representation of the phosphocreatine circuit

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

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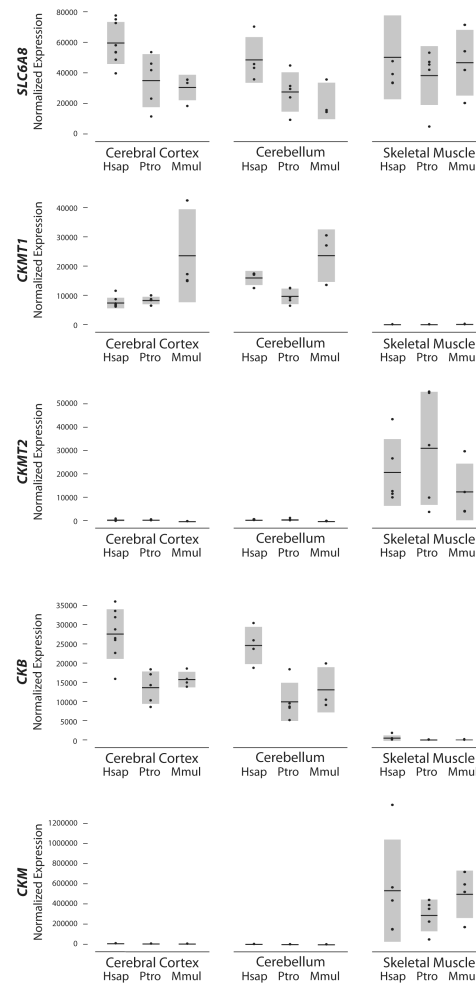

Figure 2. Phosphocreatine circuit gene expression comparisons among species

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*

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Table 1

Primate samples used in this study

	Sample	identifier	Tissue	Obtained From
HUMANS	Hsap1	1412	Cerebral Cortex	Kathleen Price Bryan Brain Bank
	Hsap2	1320	Cerebral Cortex	Kathleen Price Bryan Brain Bank
	Hsap3	99	Cerebral Cortex	Kathleen Price Bryan Brain Bank
	Hsap4	A803148	Cerebral Cortex	BioChain Institute Incorporated
	Hsap5	A803146	Cerebral Cortex	BioChain Institute Incorporated
	Hsap6	A803159	Cerebral Cortex	BioChain Institute Incorporated
	Hsap7	A507293	Cerebral Cortex	BioChain Institute Incorporated
	Hsap8	A509243	Cerebral Cortex	BioChain Institute Incorporated
	Hsap9	A508112	Cerebral Cortex	BioChain Institute Incorporated
	Hsap10	A508285	Cerebellum	BioChain Institute Incorporated
	Hsap11	A510131	Cerebellum	BioChain Institute Incorporated
	Hsap12	A611366	Cerebellum	BioChain Institute Incorporated
	Hsap13	B104083	Skeletal Muscle	BioChain Institute Incorporated
	Hsap14	A508352	Skeletal Muscle	BioChain Institute Incorporated
	Hsap15	B104053	Skeletal Muscle	BioChain Institute Incorporated
	Hsap16	A811244	Skeletal Muscle	BioChain Institute Incorporated
	Hsap17	B207204	Skeletal Muscle	BioChain Institute Incorporated
	Sample	identifier	Tissue	Obtained From
CHIMPANZEES	Ptro1	4X0327	Cerebral Cortex Cerebellum Skeletal Muscle	Southwest National Primate Research Center
	Ptro2	4X0391	Cerebral Cortex Cerebellum Skeletal Muscle	Southwest National Primate Research Center
	Ptro3	4X0505	Cerebral Cortex Cerebellum Skeletal Muscle	Southwest National Primate Research Center
	Ptro5	4X0519	Cerebral Cortex Cerebellum Skeletal Muscle	Southwest National Primate Research Center
	Ptro5	4X0523	Cerebral Cortex Cerebellum Skeletal Muscle	Southwest National Primate Research Center
	Sample	identifier	Tissue	Obtained From
MACAQUES	Mmul1	A08-296	Cerebral Cortex Cerebellum Skeletal Muscle	New England Regional Primate Research Center
	Mmul2	A08-298	Cerebral Cortex Cerebellum Skeletal Muscle	New England Regional Primate Research Center
	Mmul3	19222	Cerebral Cortex Cerebellum Skeletal Muscle	Southwest National Primate Research Center

Mmul4	RFc8	Cerebral Cortex Cerebellum Skeletal Muscle	Yerkes National Primate Research Center

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Table 2

Primer sequences for quantitative RT-PCR

	Forward Primer 5′ to 3′	Reverse Primer 5′ to 3′	Efficiency	R^2
EEF2	AGAAGCTGTGGGGTGACAG	GATCAGCTGGCAGAAGGTG	97.80%	0.996
SDHA	TGGGAACAAGAGGGCATCTG	CCACCACTGCATCAAATTCATG	98.50%	0.998
SLC6A8	GGGACCCAGATTTTCTTTTCTTAC	CCACCTTGGAGATGTGCAC	97.10%	0.991
CKMT1	AGAGTCAGAACTGGCCGAAG	CTGCTGTTCAGCCTCTGTCA	98.29%	0.991
CKB	CCTTCTCCAACAGCCACAAC	CAGCTCCGCGTACAGCTC	94.90%	0.990
CKMT2	ATCACCCAAGGGCAGTTC	GTGATGGCCACGTTCTC	96.60%	0.999
CKM	CAAGCCTGAGGAGGAGTACC	GGGTTGTCCACTCCTGTCTG	98.13%	0.996

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Table 3

p-values for human-specific expression comparisons (Figure 2) using a Mann-Whitney test. Significant values are highlighted (p 0.05)

	Human to Chimpanzee		
	Cerebral Cortex	**Cerebellum**	**Skeletal Muscle**
SLC6A8	0.0281	0.0500	0.9168
CKMT1	0.2416	0.0275	0.9168
CKB	0.0084	0.0143	0.0163
CKMT2	1.0000	0.3272	0.7540
CKM	0.1432	0.0275	0.4647

	Human to Macaque		
	Cerebral Cortex	**Cerebellum**	**Skeletal Muscle**
SLC6A8	0.0066	0.0339	0.8065
CKMT1	0.0066	0.2888	0.0500
CKB	0.0108	0.0771	0.1416
CKMT2	0.0066	0.0339	0.3272
CKM	0.01742	0.0339	0.4624