

This Provisional PDF corresponds to the article as it appeared upon acceptance. Copyedited and fully formatted PDF and full text (HTML) versions will be made available soon.

Evolutionary Rate and Gene Expression Across Different Brain Regions

Genome Biology 2008, **9**:R142 doi:10.1186/gb-2008-9-9-r142

Tamir Tuller (tamirtul@post.tau.ac.il)
Martin Kupiec (martin@post.tau.ac.il)
Eytan Ruppin (ruppin@post.tau.ac.il)

ISSN 1465-6906

Article type Research

Submission date 20 August 2008

Acceptance date 23 September 2008

Publication date 23 September 2008

Article URL <http://genomebiology.com/2008/9/9/R142>

This peer-reviewed article was published immediately upon acceptance. It can be downloaded, printed and distributed freely for any purposes (see copyright notice below).

Articles in *Genome Biology* are listed in PubMed and archived at PubMed Central.

For information about publishing your research in *Genome Biology* go to

<http://genomebiology.com/info/instructions/>

Evolutionary rate and gene expression across different brain regions

Tamir Tuller^{1,2}, Martin Kupiec² & Eytan Ruppin^{1,3}

¹ School of Computer Sciences, Tel Aviv University, Ramat Aviv 69978, Israel.

² Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Ramat Aviv 69978, Israel.

³ School of Medicine, Tel Aviv University, Ramat Aviv 69978, Israel.

Correspondence can be addressed to TT (tamirtul@post.tau.ac.il) or ER (ruppin@post.tau.ac.il).

ABSTRACT

Background

The evolutionary rate of a protein is a basic measure of evolution at the molecular level. Previous studies have shown that genes expressed in the brain have significantly lower evolutionary rates than those expressed in somatic tissues.

Results

We study the evolutionary rates of genes expressed in 21 different human brain regions. We find that genes highly expressed in the more recent cortical regions of the brain have lower evolutionary rates than genes highly expressed in sub-cortical regions. This may partially result from the observation that genes which are highly expressed in cortical regions tend to be highly expressed in sub-cortical regions, and thus their evolution faces a richer set of functional constraints. The frequency of mammalian-specific and primate-specific genes is higher in the highly expressed gene sets of sub-cortical brain regions than in those of cortical brain regions. The basic inverse correlation between evolutionary rate and gene expression is significantly stronger in brain *vs* non-brain tissues, and in cortical *vs* sub-cortical regions. Extending upon this cortical/sub-cortical trend, this inverse correlation is generally more marked for tissues that are located higher along the cranial vertical axis during development, giving rise to the possibility that these tissues are also more evolutionary recent.

Conclusions

We find that (a) cortically-expressed genes are more conserved than sub-cortical ones, and that (b) gene expression levels exert stronger constraints on sequence evolution in cortical *vs* sub-cortical regions. Taken together, these findings suggest that cortically-expressed genes are under stronger selective pressure than sub-cortically expressed genes.

Background

The evolutionary rate (ER) of a protein (Throughout the paper when we talk about the ER of a gene we actually refer to the ER of its corresponding protein), the ratio between the rate of its non-synonymous to the rate of its synonymous mutations, dN/dS , is a basic measure of evolution at the molecular level (e.g. see [1-2]). It is affected by many systemic factors including gene dispensability, expression level, the number of protein interactions and the recombination rate [3-7]. Notably, functionally related genes tend to have similar ERs [8-9]. The expression level of yeast genes has been observed to be markedly and negatively correlated with their evolutionary rate (ER) [5,10], even when controlling for the dispensability of the genes [4]. This inverse relation extends to other eukaryotes (including humans and other vertebrates) [11]. Obviously, when considering the relationship between ER and gene expression in multi-cellular organisms, the expression levels of genes in different tissues and cell-types should be considered separately. Indeed, previous studies have shown that genes vary in their rates of evolution according to the tissues in which they are highly expressed, with genes expressed in the brain evolving with significantly slower rates than those expressed in other tissues [12-15]. A general principle arising from such studies has been that tissue-specific genes have higher evolutionary rates than 'housekeeping' genes that are broadly expressed in most tissues [16-18].

To explain this observation, the tissue-driven hypothesis of genomic evolution has been recently put forward, starting from the likely assumption that genes influence phenotypic characters by their expression in specific tissues [19]. Accordingly, if a protein is expressed in several different tissues, the evolution of its sequence may be under multi-tissue-specific constraints, resulting in a slower rate of evolution. Among genes with similar expression broadness (i.e., genes that are expressed in about the same number of tissues), those genes expressed in tissues that are presumably under more stringent evolutionary selection pressure (e.g., neural tissues) generally tend to evolve more slowly than those that are expressed in tissues that presumably are under lesser selection pressure [19]. This hypothesis is concordant with the notion that each tissue is associated with a certain level of evolutionary constraints acting on the genes expressed in it, with the brain imposing more constraints than other tissues [15].

This study aims to go beyond previous investigations and study the tissue-driven hypothesis in higher resolution, in an organ of central importance to human evolution, the brain. To this end, we examine the evolution of genes that are highly expressed in different brain tissues. Our work stems from the basic observation that the transcriptomes of different brain regions differ substantially from each other [20]. These differences are likely to be functionally significant, as they mainly involve genes associated with central functions such as signal transduction and neurogenesis [20]. First, we are interested in examining whether the basic inverse relationship between a gene's tissue specificity and its ER holds also in different brain regions. Second, we examine the ER of highly expressed genes in the more phylogenetically recent cortical brain regions, compared with the ERs of genes highly expressed in older brain regions. It has previously been found that older genes (which have arisen earlier in evolution) tend to evolve more slowly than newer ones [21-22]. Does this finding transcend to the brain tissue/region level? *i.e.*, do genes expressed in older brain regions evolve more slowly than those expressed in new ones? Thirdly, we examine to what extent does the basic correlation between expression level and

sequence conservation vary across brain regions, and learn from its variation about the selection forces that drive sequence evolution of highly expressed genes.

Results

Brain region-specific indices of gene expression and conservation

We analyze a dataset encompassing the expression of 10,594 human genes, across 78 tissues (see Additional data file 1) [23]. Twenty one of these tissues are from different brain regions (Table 1). First, these brain regions can be broadly divided into two major phylogenetic classes: cortical regions, which are primarily characteristic of the mammalian lineage, and sub-cortical brain regions, which have a broad phyletic distribution [24] (No other vertebrates have a structure that clearly resembles the isocortical regions studied here [25]). Second, the brain regions are divided into four major developmental classes, including those that develop from the embryonic forebrain, midbrain, hindbrain and spinal cord [26]. For each brain region we define a gene set, composed of the genes over-expressed in that particular region. A gene is defined as over-expressed in a given brain region if its expression is at least 2 standard deviations higher than the mean of its expression across all the regions. Our dataset encompassed 4,919 genes that are over-expressed in at least one brain region. When this list of genes is analyzed using the GO process category, enrichment for neural functions is found, attesting to their biological relevance (see Additional data file 2 and Additional data file 3). We focus on over-expressed genes, following previous studies of expression signatures of different brain regions [27]. Notably, the enriched GO categories of under-expressed genes do not include neurally-related categories (see Additional data file 4). We additionally define for each brain region a more stringent *specific characteristic set* (SCS) that includes genes that are solely highly expressed in this region and in no other region.

We denote the brain expression specificity T_{max} to be the ratio between the highest expression level of a gene in a brain region and the sum of its expression levels across all 21 brain regions. The CV (coefficient of variance) of a gene is the variance of its expression levels across brain regions divided by its mean expression. The CV thus estimates the expression variability of each gene across regions. The evolutionary rates of all the genes along the human lineage and along a longer, mammalian range (Human-Mouse) were computed (Materials and methods) and were used to extract the median ERs of over-expressed genes in each brain region (columns 4 and 5 in Table 1). As the development of cortical and sub-cortical regions is not a human-specific morphological trait but already a mammalian one, we primarily report the results in the main text using the human-mouse lineage for estimating ERs, and provide the corresponding (qualitatively similar) results using the mammalian ERs in the supplementary material (see Supplementary note 1 in Additional data file 5).

A four-level estimation of gene age was computed following a procedure similar to [21-22], by searching for homologs of each human gene in four sets of organisms

(mammals; fish; insects and worms; and yeast and plants). A gene with a homolog only in mammals is considered a *mammalian* gene, a gene with a homolog only in the primates is considered a *primate* gene (Materials and methods). Table 1 depicts the age group, developmental origin, median ER (Human lineage), median ER (Human-Mouse), frequency of mammalian genes, frequency of mammalian SCS genes, frequency of primate genes, and mean T_{max} for each brain region, computed for the genes over-expressed in each region. The last column in the table includes the correlation between the genes' ER (Human-Mouse), and their expression levels in each region and a corresponding p-value. It can be seen that there is an excellent agreement between the maximally expressed gene set and the more stringent SCS gene set, both in their ER (with both the mammalian and human estimators) and in gene age.

Evolutionary rate, gene age and gene expression in cortical vs sub-cortical regions

We computed the correlation between the ER and gene expression levels in each region, and the median ER of its over-expressed genes (see Figures 1-2 for the ER of each tissue separately, and gene Expression in the Prefrontal Cortex vs. their ERs).

The ER of genes highly expressed in cortical brain regions is significantly lower than that of genes highly expressed in non-cortical brain regions (mean ER of 0.1016 vs 0.1378, p-value $< 10^{-16}$). The medians of the SCS genes of the regions in the two sets also show a similar trend (p-value = $6 \cdot 10^{-5}$).

This finding remains robust also after controlling for the total gene expression of genes and their expression breadth (see Supplementary note 2 in Additional data file 5). Similarly, gene compactness and gene essentiality that are additional important determinants of mammalian protein ER (essential genes have lower ERs, compact genes have higher ER values [28]) cannot explain the difference in evolutionary rates between cortical and sub-cortical genes. The frequency of essential genes among cortical genes is lower (15%) than among sub-cortical genes (16%), ruling out the possibility that the lower evolutionary rate of cortical genes is due to the fact that they include higher rate of lethal genes; and the fact that the ER cortical genes is significantly lower than that of non-cortical genes remains robust even after controlling for gene compactness (see Supplementary note 3 in Additional data file 5).

Finally, genes that are over-expressed in both parts of the brain have significantly lower median ERs than genes that are over-expressed in non-brain regions (Figure 2).

The correlation between the ER and expression levels is higher in cortical vs sub-cortical brain regions (p-value=0.038, Figures 1 and 3), and higher in brain tissues than in other tissues. The raw mean expression levels in cortical regions is slightly lower than the sub-cortical regions (514 vs. 518), thus ruling out the possibility that this finding is actually an indirect consequence of lower expression levels in sub-cortical vs. cortical regions (because, hypothetically, lower expression levels could transcribe to a decreased signal-to-noise ratio, and hence to decreased ER/expression correlations).

We repeated our analysis (ER and ER/expression correlations in cortical and sub-cortical regions) in two other organisms, *M. musculus* (mouse) and *P. troglodyte*

(chimp). Although the gene expression measurements of these two organisms is less abundant than in human, in both cases the ER of the cortical genes was lower than that of the sub-cortical genes (see Supplementary notes 4 and 5 in Additional data file 5 and Additional data files 6 and 7).

Interestingly, the prefrontal cortex has the highest correlation between gene expression and ER among all the 78 tissues (see Additional data file 8 and Additional data file 9 for the ER in all the tissues, and the correlation between ER in expression level in each tissue). This cortical region is known to be associated in primates and humans with complex associative cognitive tasks such as those involving delayed response and working memory. Other interesting phenomena are the very low ER of the cerebellum, and the very low correlation between expression level and ER in the various ganglia. We verified that even after removing these tissues the ER of cortical regions is still lower than that of sub-cortical regions ($p\text{-value} = 1.3 \times 10^{-13}$ when considering all the corresponding genes). The very low correlation between expression level and ER observed in the various ganglia may perhaps arise from the very small tissue volume of the latter, which may attenuate their effect in determining the ERs of their highly expressed genes. The high conservation of genes highly expressed in the cerebellum is a quandary – it is not a result of potential lower tissue specificity of these genes -- which is not statistically different from the tissue specificity of the sub-cortical or all brain genes. However, the cerebellar genes have higher mean expression levels than the sub-cortical genes (1.09 vs 0.72, $p\text{-value} < 10^{-16}$), which can partially explain their higher conservation.

The frequency of *mammalian*-specific genes is higher in the highly expressed gene sets of sub-cortical brain regions than in those of cortical regions (with mean frequencies of 0.121 vs 0.086, $p\text{-value}=0.03$). This difference remains similar also when considering primate-specific genes. The mean frequency is 0.0073 in sub-cortical vs 0.0018 in cortical regions ($p\text{-value} = 0.05$). This difference is surprising, but is consistent with the negative correlation found between the genes' age and ER (-0.23 , $p < 10^{-16}$). A similar inverse correlation across all tissues has been previously observed [21-22]. Genes that are expressed in cortical regions have higher mean expression levels across brain tissues than genes expressed in sub-cortical regions (1.6 vs 1, $p\text{-value} = 2.4 \times 10^{-10}$) and across all somatic tissues (0.9 vs 0.7, $p\text{-value} < 10^{-16}$). This fact can partially explain the lower ER values of cortical genes, as these genes are likely to be subjected to diverse simultaneous selective pressures.

Relation between evolutionary rate, expression level and region specificity

Previous work has demonstrated that housekeeping genes tend to evolve more slowly than tissue-specific genes [17]. Gene expression across brain tissues manifests a similar region-specificity relation between gene expression and ER: genes highly expressed in fewer brain regions have higher ER values (the Spearman correlation between T_{\max} and ER along the mammalian lineage is 0.131, $p\text{-value} < 10^{-16}$, and along the human lineage it is 0.0504, $p\text{-value} < 2 \times 10^{-7}$). A similar trend is observed

by noting that genes with higher CV levels have higher ER values (Spearman correlation along the mammalian lineage is 0.1269 p-value $< 10^{-16}$ and along the human lineage it is 0.0447, p-value: 4.1×10^{-6}).

Genes that are expressed in cortical regions are also less region-specific than those expressed in sub-cortical regions; the T_{\max} values of genes expressed in each cortical region are significantly higher than those expressed in each sub-cortical regions (mean T_{\max} of 0.10 vs 0.12, p-value = 0.02). Aggregating all genes expressed in cortical or sub-cortical regions together yields a mean T_{\max} of 0.106 for cortical regions and mean $T_{\max} = 0.116$ for sub-cortical ones (p-value = 3.4×10^{-12}), showing a similar trend. Thus, genes highly expressed in cortical regions have a higher expression breadth that may partly (see supplementary note 6 in Additional data file 5) account for their overall lower ER values. This reduced region specificity of cortical genes may arise due to a 'preferential-attachment' -like process [29], where the genes highly expressed in the more recent cortical regions in the mammalian lineage tend to be those that already have a broad expression breadth in sub-cortical regions. In line with that we find a marked correlation of 0.28 (p-value $< 10^{-16}$) between the number of cortical and sub-cortical regions in which a gene is expressed (Figure 4).

ER and gene expression: a developmental perspective

We divided the brain into five main developmental areas [26]: three forebrain areas, including the pallium, subpallium and the diencephalon, the hind brain and the spinal cord (our data does not include midbrain structures, and additionally includes three cranial nuclei of different developmental origins).

These five developmental areas have an ordered placement along the cranial vertical axis with the spinal cord being the lowest [26, 30], then the hindbrain, followed by the diencephalon, the subpallium and the pallium (the highest). The correlation between evolutionary rate and gene expression levels shows an interesting pattern: their magnitude manifests a significant correlation with the region location on the cranial vertical axis (Spearman ranked correlation of 0.9, p-value = 0.037 when averaging the regions of each developmental area, and Spearman ranked correlation of 0.5 and p-value = 0.034 when considering each region separately) (Figure 5, Additional data file 10, and Additional data file 11). This result reinforces the observations made in the previous sections suggesting that the genes' ERs are under tighter influence of their expression levels in cortical regions (which are of pallial origin).

Discussion

Previous studies have shown that the rate of evolution among brain-expressed genes is probably lower (or at most equal) in humans compared with chimpanzee and old world monkeys (e.g., most recently by [31]). Slower sequence evolution of tissue/region-specific genes is a likely indicator of stronger selective constraints operating on the region in hand. Hence, the overall sequence conservation of highly expressed brain genes makes them an interesting subject for the further study of the basic relation between gene expression and evolutionary rate. To this end, we find that (a) cortically-expressed genes are more conserved than sub-cortical ones, and that (b) gene expression levels exert stronger constraints on sequence evolution in cortical vs

sub-cortical regions. Taken together, these findings testify that cortically-expressed genes are under stronger selective pressure than sub-cortically expressed genes.

One possible mechanism that can partially explain these findings is the overall broader tissue distribution of cortically expressed genes, but other non-exclusive mechanisms may take part. For example, it is possible that there are more frequent genetic and protein interactions among highly expressed genes in the cortical regions, which are known to be correlated with reduced ER levels [7]. The cellular complexity (types of cells and their distribution) of the regions studied is different, which may further determine different and complex evolutionary constraints in each region. Another potential factor influencing these regional differences is the sex-bias of genes, as it has been suggested that the expression of genes that are more pleiotropic (i.e., in terms of our work – have a larger tissue expression breadth) is less sex-biased [32], and that sex-dependent allelic effects cannot maintain polygenic variation [33]. Thus, the exact mechanisms underlying our findings are probably under a quite complex interplay that yet remains to be further explored.

The magnitudes of some of the ER/expression correlations reported here are lower than in yeast (see figure 1 for gene Expression in the Prefrontal Cortex vs their ERs), even though these correlations are highly significant. In the case of the yeast (e.g. [5]) the respective correlation found is around 1.5 times higher. There are three main reasons that may explain this difference. First and foremost, in contrast to the yeast, humans are multi-cellular organisms with hundreds of distinct cell types and diverse tissues; thus, gene ER in humans is likely to be under a large variety of (sometimes perhaps counteracting) selection forces, resulting in a lower correlation with gene expression in any single specific cell/tissue type [28]. Second, since this study focuses on human brain regions, we have estimated ER values along shorter evolutionary time periods (the last 6.5-10 million years of the human lineage, after the human-chimp split [34,35], and the 50-100 million years corresponding to the human mouse split), in contrast to the much longer time spans employed for estimating ER in the yeast studies. Indeed, when using ER estimates using the human-mouse lineage we obtain ER/expression correlations that are 2 times higher than those obtained when using ER estimates from the shorter period, human-chimp lineage. Thirdly, the sets of genes studies differ markedly, with the number of genes included in this study being 2-3 times higher than the number of genes examined in previous yeast studies (larger datasets usually increase the correlations but may decrease their significance).

Cortical regions, at least in their extensive mammalian form, are more recent than sub-cortical regions, which have a broader phyletic distribution. The evolutionary rate of cortically-expressed genes is yet slower than that of sub-cortically expressed genes. This is in contrast to the findings at the gene level, where the evolutionary rate of younger genes is higher than that of older ones [21]. This seems paradoxical at first, as one would perhaps expect that genes highly expressed in the more recently evolving cortical brain regions would be younger than the genes highly expressed in sub-cortical regions. Yet, this is not the case; highly expressed cortical genes tend to be also highly expressed in many sub-cortical regions, and thus both types of regions are composed of both younger and older highly expressed genes (with cortical areas being actually composed of older genes than sub-cortical regions, on the average). Furthermore, although we find that cortically-expressed genes are more conserved than sub-cortical ones, this does not necessarily imply that cortical regions offer more

stringent 'environments' for gene evolution than sub-cortical regions, as this excess conservation may arise from their broader, somatic, tissue-distribution. However, the tighter correlation between ER and expression levels that characterizes cortically-expressed genes does point to the fact that the cortex may form a more stringent environment for gene evolution than other brain and somatic tissues, as one may intuitively expect [19] [obviously, in turn, it is also possible that the rates of gene evolution may play an important role in shaping their expression profiles in the cortex].

There are many definitions for the tissue/region specificity of genes [for example, based on Expressed Sequence Tags (EST) data, Serial Analysis of Gene Expression (SAGE) data, literature [36,37], or based on gene expression, as was adopted here]. Each of the definition may give rather different sets of genes. Currently, there are no available datasets based on EST/SAGE/or literature that provide information about brain regional specificity. Hence we have focused on the gene expression definition of region specificity. A comparison of our results with those based on other tissue specificity definitions will have to be deferred until the corresponding biological information is available.

Finally, the results reported in Figure 5 are intriguing, generalizing in a way the results reported in Figures 1-3. While the latter reports that cortical regions exhibit a correlation between evolutionary rates and tissue gene expression levels in cortical vs sub-cortical regions, the former shows that this correlation tends to be stronger for vertically higher regions in the developmental axis. As each point in Figure 5 corresponds to the correlation between ER and expression levels of 10,594 genes in all the regions in a developmental area, the reported correlation values are highly robust. Thus, drawing an analogy from the observation that cortical regions are evolutionary more recent than sub-cortical ones [24], one may (perhaps boldly) speculate that regions located higher on the vertical axis at brain development are also more evolutionary recent. However, as even the basic claim that cortical regions are more recent is not accepted overboard, care should obviously be taken with such hypotheses. Their examination should await the accumulation of additional gene expression samples from more brain tissues and from more mammalian species.

Conclusions

Our findings testify that cortically-expressed genes are under stronger selective pressure than sub-cortically expressed genes. They may also suggest that regions located higher on the vertical axis at brain development are also more evolutionary recent. These findings should be re-examined when additional biological data (*e.g.* gene expression samples from more brain tissues and from more mammalian species) will become available.

Materials and methods

Computation of evolutionary rates

We used two estimations of human evolutionary rates: (1) Long term evolutionary rates along the mammalian lineage (human-mouse dN/dS) that were downloaded from EBI – BioMart (BioMart July 2008).

(2) Short term evolutionary rates along the human lineage (human-chimpanzee dN/dS) whose computations is described in the following subsection.

Computing gene evolutionary rates along the human and chimp lineages

We downloaded the orthologous groups of *Homo sapiens* (humans), *Pan troglodytes* (chimp) and *Macaca mulatta* (macaque) from EBI – BioMart Homology (BioMart November 2007). We considered only sets that include orthologs in all three species. Sets of homologs that did not include exactly one representative in each organism were removed from our dataset, to filter out paralogs and avoid potential errors in evolutionary rate estimation due to duplication events (today there are no reported cases of horizontal gene transfer) between *Primates*; see e.g. [38]). This procedure resulted in a total of 15,176 orthologous gene sets.

In the next step, stop codons were removed from each gene and the genes were translated to sequences of amino acids. The corresponding amino acid sequences of each orthologous gene set were aligned by CLUSTALW 1.83 [39], with default parameters. By using amino acids as templates for the nucleotide sequences and by ignoring gaps we generated gap-free multiple alignments of the three orthologous proteins in each orthologous set and their corresponding coding sequences.

Given the alignments of each set of orthologs and given the phylogenetic tree of the three primates (see Figure 6 A.), we used the codeml program in PAML for the joint reconstruction of ancestral codons in the internal nodes of the phylogenetic tree [40] (*i.e.* the ancestor of the human and chimp, see Figure 6 A.). This reconstruction induced the sequence of the ancestral proteins and their corresponding ancestral DNA coding sequences. We hence obtained sets of 4 sequences; 3 from the previous step (corresponding to the 3 leaves of the phylogenetic tree) plus 1 reconstructed sequences of the internal node of the phylogenetic tree. We denote such a set of 4 sequences a *complete orthologous set*. For each *complete orthologous set*, we computed the *dN* (the rate of nonsynonymous substitutions) and *dS* (the rate of synonymous substitutions) along the lineage to the human (the branch between the internal node and the human node, see Figure 6A.) by the y00 program in PAML [41-42]. The ER of a gene is the *dN* divided by the *dS* of its corresponding *complete orthologous set* along the human lineage, *i. e.* the *dN/dS* along the human lineage. Similarly, we computed that *dN* and *dS* and a corresponding ER (*dN/dS*) along the chimp lineage.

Computing evolutionary rates of tissues

The ER of a tissue/region is the median ER of all the genes that are over-expressed in that tissue (*i.e.* genes that are 2 standard deviations higher than their mean expression

across all the tissues). We used median instead of average since the analyzed set of genes included genes with $dS = 0$ (*i.e.* ER equals infinity).

It is important to note that all the results reported here are remarkably robust to changing the cutoff of 2 standard deviations. For example if we choose a cutoff of 3 standard deviations, the average median ER of cortical brain regions is 0.1532 *vs* 0.2387 in sub-cortical brain regions, and 0.2914 in non brain tissues (p-values 0.014 and 0.002 respectively) .

Gene expression data

The gene expression of 78 tissues (including 21 brain regions) was downloaded from the work of [23]. All the analyzed gene expression measurements were from the same technology (Affymetrix GeneChip Human Genome U133 Array Set HG-U133A) and included two technical repeats (that we averaged). A list of all the tissue names and other properties appears in Additional data file 12 . The human tissue samples were obtained from several sources: Clinomics Biosciences (Pittsfield, MA), Clontech, AllCells (Berkeley, CA), Clonetics/BioWhittaker (Walk-ersville, MD), AMS Biotechnology (Abingdon, Oxfordshire, U.K.), and the University of California at San Diego. When samples from four or more subjects were available, equal numbers of male and female subjects were used to make two independent pools; when fewer than four samples were available, RNA samples were pooled, and duplicate amplifications were performed for each pool; more details appear in supporting Table 1 in [23]. We averaged the signals of all the probes of each gene to obtain a final set of 10,594 genes with both ER measurements and gene expression measurements across all tissues.

Gene expression of *M. musculus* (mouse) was downloaded from [43]. It includes gene expression from 61 tissues. 14 of these tissues are brain tissues (12 cortical and 12 subcortical). The mouse gene expression appears in Additional data file 13. The gene expression of *P. troglodytes* (chimp) was downloaded from Gene Expression Omnibus (GEO) database ([48]; GDS2678 record). It included 12 brain tissues, one of them was sub-cortical and all the others were cortical.

Estimating gene age

An estimation of gene age was obtained following the procedure described in [21,22] (see Figure 6B). First, we used the homology search engine of BioMart (BioMart November 2007) to find all the orthologs of each of the human genes in a set of 34 organisms (the list of organisms appear in Additional data file 14 , and phyletic patterns of all the human genes appear in Additional data file 15). Next, the organism set was divided into four groups: 1) Mammals (youngest group), 2) Fish, 3) Insects, Worm, Ciona , 4) Plants, Yeast (oldest group). The age of each of the human genes was determined according to the oldest organism group with a homolog of the gene (see Figure 6B.). Accordingly, a gene that has homolog(s) only in group 1 is named a mammalian gene while a gene with homolog(s) in group 4 is very old. We also performed an additional study of primate specific genes, defined as human genes that have homolog(s) only in the primates (*Pan troglodytes* or *Macaca mulatta*).

GO Enrichments

Hyper-geometric functional GO enrichment of an over-expressed set of genes, including a correction for multiple testing, was computed by the FuncAssociate [49].

Gene length and gene essentiality

Information about gene and protein lengths was downloaded from EBI – BioMart (BioMart August 2008). The information about gene essentiality was based on the mouse phenotypic data, and was downloaded from Mouse Genome Informatics (MGI) database [50]. Human genes whose mouse orthologs have knockout phenotype of lethality or sterility were defined as essential. That is, those entries possessing embryonic lethality (MP: 0002080), prenatal lethality (MP: 0002081), survival postnatal lethality (MP: 0002082), premature death or induced morbidity (MP: 0002083), reproductive system phenotype (MP: 0002161, MP:0005389), lethality-postnatal (MP:0005373), or lethality-prenatal/perinatal (MP:0005374).

Abbreviations

ER - Evolutionary Rate.
SCS - Specific Characteristic Set.
CV - Coefficient of Variance.
GEO - Gene Expression Omnibus.
MGI - Mouse Genome Informatics.
EST -Expressed Sequence Tags.
SAGE - Serial Analysis of Gene Expression.

Authors' contributions

TT carried out all the analysis. All authors participated in the design of the study. All authors have been involved in drafting and writing the manuscript. All authors read and approved the final manuscript.

Additional data files

The following additional data are available with the online version of this paper:

Additional data file 1 is a table listing various parameters of the analyzed genes.

Additional data file 2 is a table listing the GO enrichments for the genes that are over-expressed in the cortical regions.

Additional data file 3 is a table listing the GO enrichments for the genes that are over-expressed in the sub-cortical regions.

Additional data file 4 is a table listing the GO enrichment categories for the genes that are under expressed in the cortical and in the sub-cortical brain regions.

Additional data file 5 includes the supplementary notes (supplementary note 1 - supplementary note 6).

Additional data file 6 is a table that includes the ER/expression correlation and ER in each mouse tissue.

Additional data file 7 is a table that includes the ER/expression correlation in the chimp brain tissues.

Additional data file 8 is a figure that depicts: A. Median ER (Human lineage) in brain tissues and other tissues. B. Median ER in each brain region. C. The correlation between ER (Human lineage) and expression level in each tissue. D. The correlation between ER and the expression levels in each brain region.

Additional data file 9 is a figure that depicts: A. Median ER (Human-Mouse dN/dS) in brain tissues and other tissues. B. The correlation between ER (Human-Mouse dN/dS) and expression level in each tissue.

Additional data file 10 is a figure that depicts the mean correlation of expression levels with ER (Human lineage), for regions belonging to five different embryonic developmental origins.

Additional data file 11 is a figure that depicts the mean ER (mouse - human), for regions belonging to five different embryonic developmental origins.

Additional data file 12 is a table listing various properties of the analyzed tissues.

Additional data file 13 is a table that includes the mouse gene expression and ER.

Additional data file 14 is a table with the set of organisms that was used for estimating gene ages.

Additional data file 15 is a table that includes the phyletic patterns of all the human genes.

Acknowledgments

TT is supported by the Edmond J. Safra Bioinformatics program at Tel Aviv University. MK's research is supported by grants from the Israel Science Foundation (ISF) and the Israeli Ministry of Science and Technology. ER's research is supported by grants from the Israel Science Foundation (ISF), the Yishaya Horowitz center for complexity science, and the Tauber fund.

References

1. Hartl DL, Jones EW: *Genetics: Analysis of Genes and Genomes* (Jones and Bartlett, Mississauga), 2005, pp: 718-724.
2. Kimura M, Ota T: Free in PMC On some principles governing molecular evolution. *Proc. Natl. Acad. Sci. U. S. A.* 1974, 71:2848-52.
3. Pál C, Papp B, Lercher MJ: An integrated view of protein evolution *Nat. Rev. Genet.* 2007, 7: 337-348.
4. Wall DP, *et al.*: Functional Genomic Analysis of the Rate of Protein Evolution. *Proc. Natl. Acad. Sci. U.S.A.* 2005, 102:5483-5488.
5. Drummond DA, Bloom JD, Adami C, Wilke CO, Arnold FH: Why highly expressed proteins evolve slowly. *Proc. Natl. Acad. Sci. U. S. A.* 2005, 102:14338-43.
6. Hirsh AE, Fraser HB: Protein dispensability and rate of evolution. *Nature* 2003, 421: 497-498.
7. Fraser HB, Hirsh AE, Steinmetz LM, Scharfe C, Feldman MW: Evolutionary rate in the protein interaction network. *Science* 2002, 296: 750-752.
8. Chena Y, Dokholyana NV: The coordinated evolution of yeast proteins is constrained by functional modularity. *Trends in Genet.* 2006, 22:416-419.
9. Marino-Ramirez L, Bodenreider O, Kantz N, Jordan IK: Co-evolutionary Rates of Functionally Related Yeast Genes. *Evol. Bioinformatics* 2006, 2295–300.
10. Pál C, Papp B, Hurst LD: Highly expressed genes in yeast evolve slowly. *Genetics* 2001, 158: 927–931.
11. Subramanian S, Kumar S: Gene expression intensity shapes evolutionary rates of the proteins encoded by the vertebrate genome. *Genetics* 2004, 168:373-81.
12. Kuma K, Iwabe N, Miyata T: Functional constraints against variations on molecules from the tissue level: slowly evolving brain-specific genes demonstrated by protein kinase and immunoglobulin supergene families. *Mol. Biol. Evol.* 1995, 12:123-30.
13. Miyata T, Kuma K, Iwabe N, Nikoh NA: possible link between molecular evolution and tissue evolution demonstrated by tissue specific genes. *Jpn. J. Genet.* 1994, 69:473-80.
14. Duret L, Mouchiroud D: Determinants of substitution rates in mammalian genes: expression pattern affects selection intensity but not mutation rate. *Mol Biol Evol.* 2000, 17:68-74.
15. Khaitovich P, Hellmann I, Enard W, Nowick K, Leinweber M: Parallel Patterns of Evolution in the Genomes and Transcriptomes of Humans and Chimpanzees. *Science* 2005, 309:1850 – 1854.

16. Winter EE, Goodstadt L, Ponting CP, Elevated rates of protein secretion, evolution, and disease among tissue-specific genes. *Genome Res.* 2004, 14:54-61.
17. Zhang L, Li WH: Mammalian housekeeping genes evolve more slowly than tissue-specific genes. *Mol Biol Evol.* 2004, 21:236-9.
18. Liao BY, Zhang J: Low Rates of Expression Profile Divergence in Highly Expressed Genes and Tissue-Specific Genes During Mammalian Evolution. *Mol. Biol. Evol.* 2006, 23:1119–1128.
19. Gu X, Su Z: Tissue-driven hypothesis of genomic evolution and sequence-expression correlations. *Proc. Natl. Acad. Sci. U. S. A.* 2007, 104: 2779–2784.
20. Khaitovich P: Regional Patterns of Gene Expression in Human and Chimpanzee Brains. *Genome Res.* 2004,14:1462-1473.
21. Albá MM, Castresana J: Inverse relationship between evolutionary rate and age of mammalian genes. *Mol. Biol. Evol.* 2005, 22:598-606.
22. Albá MM, Castresana J: On homology searches by protein Blast and the characterization of the age of genes. *BMC Evol. Biol.* 2007, 7:53.
23. Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA: A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc. Natl. Acad. Sci. U. S.* 2004, 101:6062-7.
24. Jarvis ED, et al.: Avian brains and a new understanding of vertebrate brain evolution. *Nat. Rev. Neurosci.* 2005, 6:151-159.
25. Kaas JH., Preuss TM: Human Brain Evolution, in Fundamental Neuroscience, eds Squire LR, Bloom FE, McConnel SK, Roberts JL, N.C. Spitzer NC, Zigmond MJ.(Amsterdam: Academic Press), 2003, pp 1147-1166.
26. Cochard LR: Netter's Atlas of Human Embryology. The Feinberg School of Medicine, Northwestern University, Chicago, IL, USA. 2002.
27. Preuss TM, Cáceres M, Oldham MC, Geschwind DH: Human brain evolution: insights from microarrays. *Nat. Rev. Genet.* 2004, 5:850-860.
28. Liao BY, Scott NM, Zhang J. Impacts of gene essentiality, expression pattern, and gene compactness on the evolutionary rate of mammalian proteins. *Mol Biol Evol.* 2006 23:2072-80.
29. Albert R and Barabási A: Statistical mechanics of complex networks. *Rev. Mod. Phys.* 2002, 74: 47--97.

30. Drews U: Color Atlas of Embryology. Thieme, New York 1995.
31. Wang HY, *et al.*: Rate of evolution in brain-expressed genes in humans and other primates. *PLoS Biol.* 2007, 5:e13.
32. Mank JE, Hultin-Rosenberg L, Zwahlen M, Ellegren H. Pleiotropic constraint hampers the resolution of sexual antagonism in vertebrate gene expression. *Am Nat.* 2008 171:35-43.
33. Turelli M, Barton NH. Polygenic Variation Maintained by Balancing Selection: Pleiotropy, Sex-Dependent Allelic Effects and GxE Interactions. *Genetics.* 2004 166:1053-79.
34. Benton MJ, Ayala FJ. Dating the tree of life. *Science* 2003, 300:1698-700.
35. Benton MJ, Donoghue PC: Paleontological evidence to date the tree of life *Mol Biol Evol.* 2007, 24:26-53.
36. Sémon M, Lobry JR, Duret L: No evidence for tissue-specific adaptation of synonymous codon usage in humans. *Mol Biol Evol.* 2006. 23:523-9.
37. Dorus S, Vallender EJ, Evans PD, Anderson JR, Gilbert SL, Mahowald M, Wyckoff GJ, Malcom CM, Lahn BT: Accelerated evolution of nervous system genes in the origin of Homo sapiens. *Cell.* 2004, 119:1027-40.
38. Bapteste E, *et al.*: Do orthologous gene phylogenies really support tree-thinking? *BMC Evol. Biol.* 2005, 5.
39. Cahenna R, *et al.* (2003) Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Res.* 31: 3497-3500.
40. Pupko T, Pe'er I, Shamir R, and Graur D: A fast algorithm for joint reconstruction of ancestral amino acid sequences. *Mol. Biol. Evol.* 2000, 17:890-896.
41. Yang Z: PAML: a program package for phylogenetic analysis by maximum likelihood. *Comput. Appl. Biosci.* 1997,13:555.
42. Yang Z, Nielsen R: Estimating synonymous and nonsynonymous substitution rates under realistic evolutionary models. *Mol. Biol. Evol.* 2000, 17:32-43.
- 43 SU AI, Cooke MP, Ching KA, Hakak Y, Walker JA, Wiltshire T, Orth AP, Raquel G. Vega, Sapinoso LM, Moqrich A, Patapoutian A, Hampton GM, Schultz PG, and Hogenesch JB. Large-scale analysis of the human and mouse transcriptomes. *Proc. Natl. Acad. Sci. U. S. A.* 2002, 99:4465-70.

44. Tanomtong A, Khunsook S, Chaveerach A, Kaensa W and Banjongrat R. Comparative Phylogenetic Studies of Rhesus Monkey (*Macaca mulatta*) and Human (*Homo sapiens*) using G-Banding Pattern. *Cytologia*. 2006, 71: 87–92.
45. Janeka JE, Miller W, Pringle TH, Wiens F, Zitzmann A, Helgen KM, Springer MS, Murphy WJ. Molecular and Genomic Data Identify the Closest Living Relative of Primates. *Science*. 2007, 318: 792 – 794.
46. Peterso KJ, Lyons JB, Nowak KS, Takacs CM, Wargo MJ, and McPeck MA. Evolution Estimating metazoan divergence times with a molecular clock. *Proc. Natl. Acad. Sci. U. S. A.* 2004, 101: 6536-6541.
47. A Wray GA. Dating branches on the Tree of Life using DNA. *Genome Biol.* 2001, 3.
48. (Gene Expression Omnibus (GEO) database [<http://www.ncbi.nlm.nih.gov/geo/>])
49. The Gene Set Functionator [<http://llama.med.harvard.edu/cgi/func/funcassociate>]
50. Mouse Genome Informatics database.[<http://www.informatics.jax.org/>].

Figure Legends

Figure 1. Expression in the Prefrontal Cortex (x-axis, log scale) vs ER (y-axis; human-mouse, log scale).

Figure 2. Median ER in each brain region. Top-left corner: Comparison of the (aggregated) medians of ER in cortical brain regions, sub-cortical brain regions, and somatic, non-brain tissues.

Figure 3. The correlation between ER and the expression levels in each brain region. Top-right corner: The mean correlation between ER and expression in cortical brain regions, sub-cortical brain regions, and somatic, non-brain tissues.

Figure 4. The mean number of cortical brain regions in which a gene is highly expressed (y-axis), given the number of sub-cortical brain regions in which the same gene is highly expressed (x-axis). Genes that are highly expressed in more sub-cortical regions tend to be highly expressed in more cortical regions.

Figure 5. The mean correlation of expression levels with ER (human-mouse), for regions belonging to five different embryonic developmental origins. The latter are ordered on the x-axis in accordance with their height on the cranial vertical axis during early embryonic stages (Spinal Cord is the lowest, and Forebrain (pallium) is the highest). As evident, these ER/expression correlations are ordered by their cranial vertical location (Spearman rank correlation of 0.9, p-value = 0.037). Similar result was observed when computing the ER using the human lineage (human-chimpanzee, Additional data file 10).

Figure 6. A. Phylogeny of the three primates whose genes were used for computing the ERs along the human lineage. **B.** Illustration of the procedure and the phylogenetic tree used for estimating gene age.

Tables

Index	Brain Tissue	Developmental Origin	Median ER (Human lineage)	Median ER (Mouse - human)	Frequency of mammalian genes	Frequency of mammalian for SCS	Frequency of Primate genes	Brain Region-specificity index (T_{max})	Correlation (and p-value) between ER and expression level (Human-Mouse)
1	Dorsal Root Ganglion	Spinal cord	0.31	0.167	0.17	0.12	0.016248	0.12	-0.0747 ($1.4*10^{-14}$)
2	Medulla Oblongata	Hindbrain	0.28	0.102	0.12	0.14	0.005525	0.1	-0.1844 ($p < 10^{-16}$)
3	Pons	Hindbrain	0.27	0.13	0.17	0.13	0.013158	0.11	-0.1442 ($p < 10^{-16}$)
4	Spinal cord	Spinal cord	0.25	0.127	0.13	0.16	0.003704	0.11	-0.1664 ($p < 10^{-16}$)
5	Olfactory Bulb	subpallium (Forebrain)	0.28	0.126	0.09	0.08	0	0.14	-0.1475 ($p < 10^{-16}$)
6	Trigeminal Ganglion	Different developmental origin	0.31	0.161	0.17	0.17	0.021223	0.12	-0.0445 ($4.7*10^{-6}$)
7	Ciliary Ganglion	Different developmental origin	0.29	0.154	0.14	0.12	0.015152	0.12	-0.0710 ($2.6*10^{-13}$)
8	Superior Cervical Ganglion	Different developmental origin	0.31	0.161	0.16	0.13	0.013664	0.12	-0.0335 ($5.7*10^{-4}$)
9	Cerebellum	Hindbrain	0.14	0.083	0.08	0.09	0.004357	0.11	-0.1799 ($p < 10^{-16}$)
10	Cerebellum Peduncles	Hindbrain	0.17	0.096	0.08	0.09	0.005102	0.12	-0.1846 ($p < 10^{-16}$)
11	Hypothalamus	diencephalon (Forebrain)	0.27	0.12	0.08	0.08	0	0.11	-0.1922 ($p < 10^{-16}$)

12	Thalamus	diencephalon (Forebrain)	0.19	0.103	0.1	0.04	0	0.10	-0.1903 ($p < 10^{-16}$)
13	Subthalamic Nucleus	diencephalon (Forebrain)	0.23	0.11	0.14	0.2	0.005362	0.10	-0.1811 ($p < 10^{-16}$)
14	Caudate Nucleus	subpallium (Forebrain)	0.26	0.105	0.09	0	0	0.11	-0.1817 ($p < 10^{-16}$)
15	Globus Pallidus	subpallium (Forebrain)	0.23	0.107	0.12	0.1	0.010753	0.1	-0.1733 ($p < 10^{-16}$)
16	Amygdala	subpallium (Forebrain)	0.21	0.084	0.09	0.11	0.002151	0.1	-0.2294 ($p < 10^{-16}$)
17	Cingulate Cortex	pallium (Forebrain)	0.2	0.094	0.07	0.05	0	0.1	-0.1931 ($p < 10^{-16}$)
18	Occipital Lobe	(pallium) (Forebrain)	0.2	0.089	0.05	0	0	0.09	-0.2199 ($p < 10^{-16}$)
19	Parietal Lobe	pallium (Forebrain)	0.22	0.119	0.1	0.14	0	0.1	-0.1893 ($p < 10^{-16}$)
20	Temporal Lobe	pallium (Forebrain)	0.21	0.104	0.13	0.1	0.006897	0.11	-0.2214 ($p < 10^{-16}$)
21	Prefrontal Cortex	pallium (Forebrain)	0.22	0.089	0.08	0.06	0.002299	0.1	-0.1747 ($p < 10^{-16}$)

Table 1. The 21 brain regions examined in this research and their characteristics.

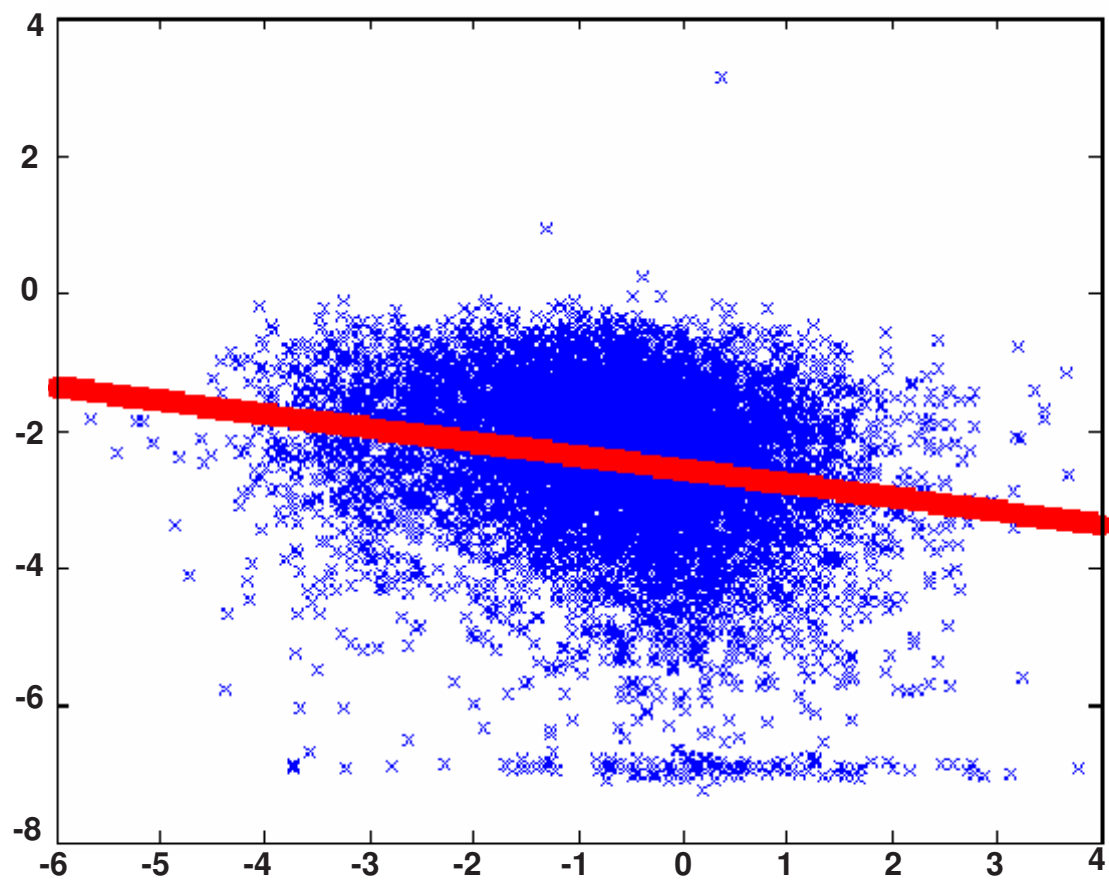


Figure 1

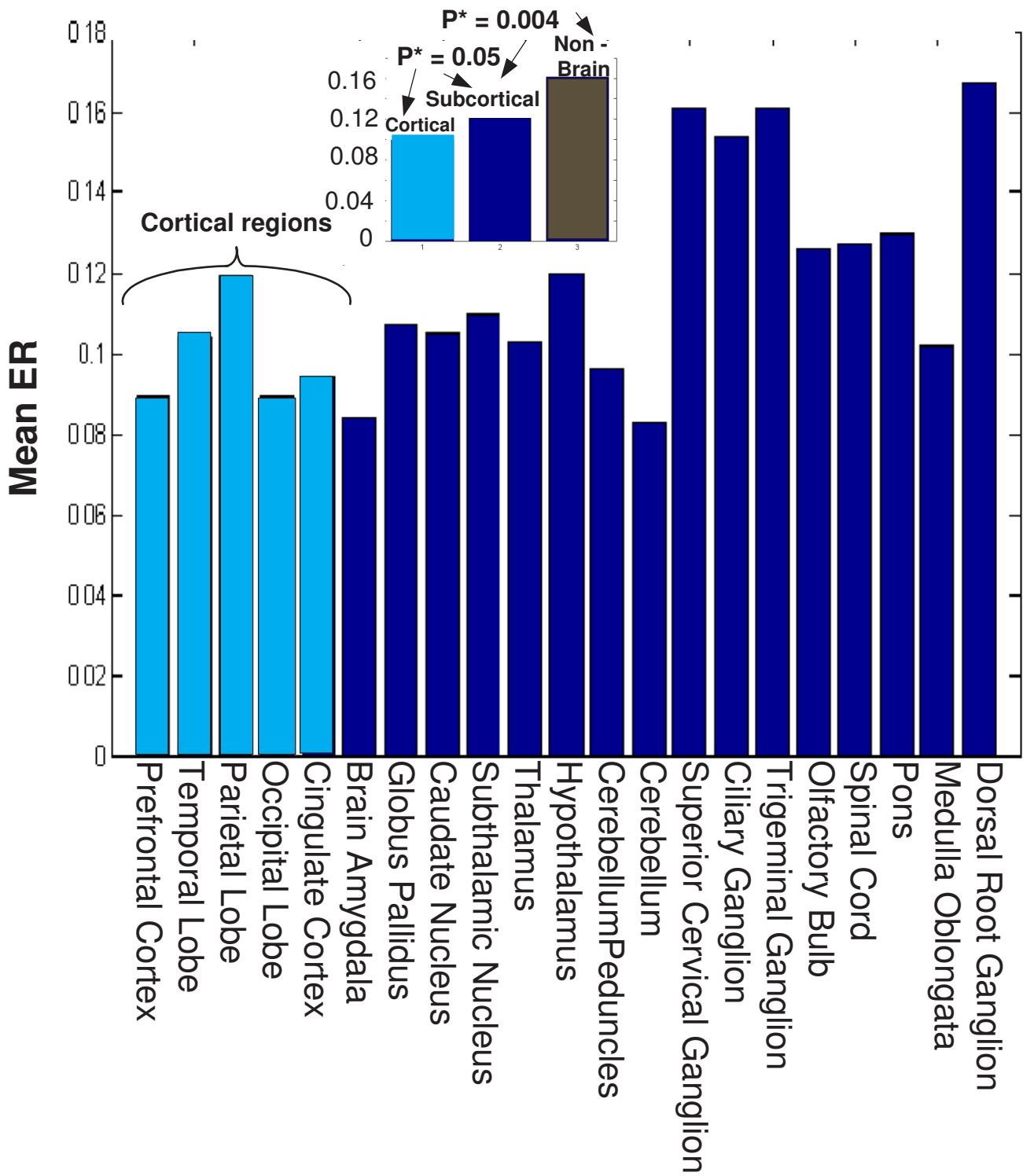


Figure 2

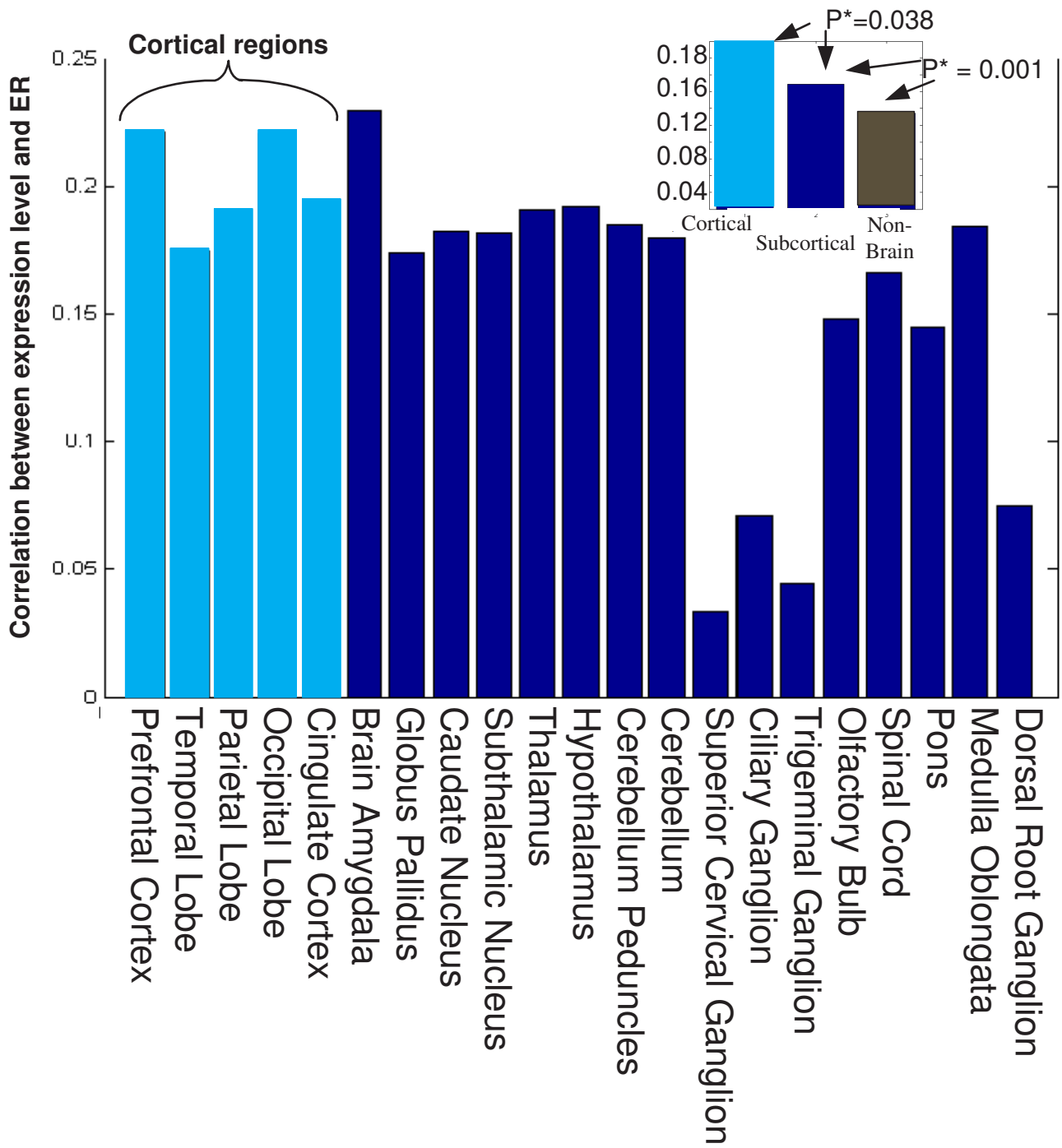


Figure 3

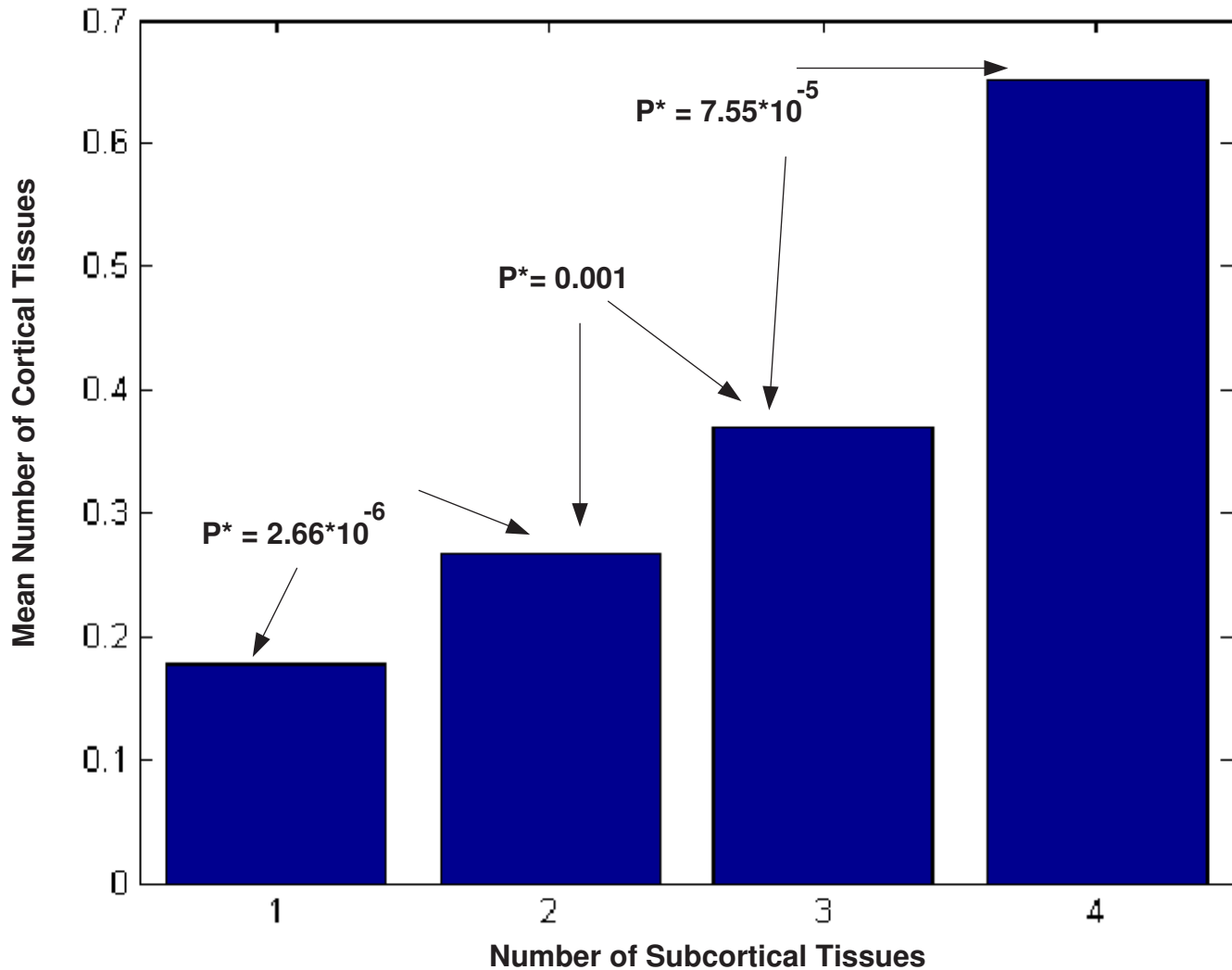


Figure 4

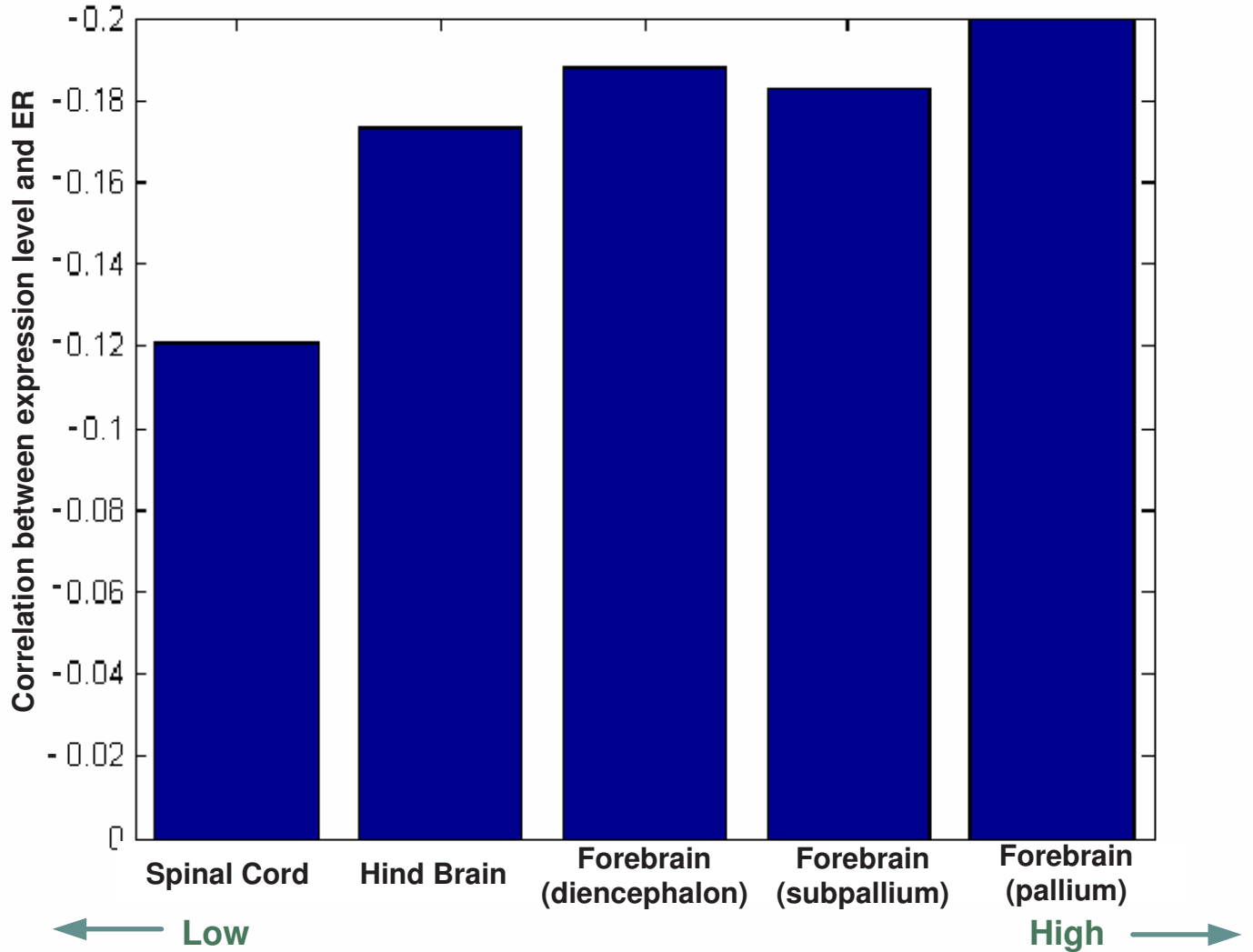


Figure 5

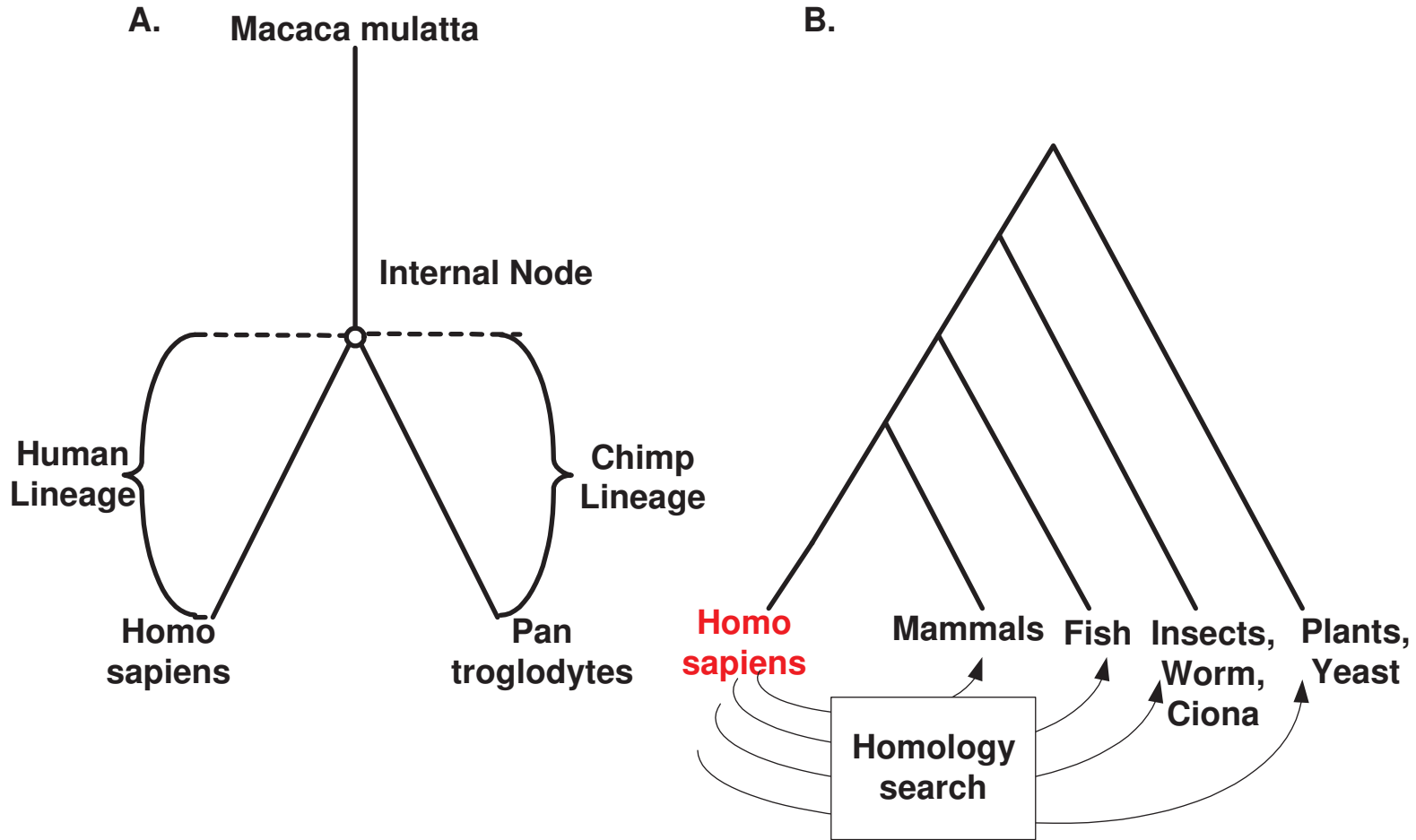


Figure 6

Additional files provided with this submission:

Additional file 1: add1_suppl_table1.xls, 17083K

<http://genomebiology.com/imedia/1555518103223544/supp1.xls>

Additional file 2: add2_suppl_table2.xls, 371K

<http://genomebiology.com/imedia/1765243155223544/supp2.xls>

Additional file 3: add3_suppl_table3.xls, 1031K

<http://genomebiology.com/imedia/1299304120223544/supp3.xls>

Additional file 4: add4_suppl_table4.xls, 32K

<http://genomebiology.com/imedia/1514520427223544/supp4.xls>

Additional file 5: add5_supplementary_notes.doc, 37K

<http://genomebiology.com/imedia/6741295302235441/supp5.doc>

Additional file 6: add6_suppl_table9.xls, 20K

<http://genomebiology.com/imedia/2649826652235441/supp6.xls>

Additional file 7: add7_suppl_table10.xls, 14K

<http://genomebiology.com/imedia/9222059442235442/supp7.xls>

Additional file 8: add8_suppl_figure1.doc, 8434K

<http://genomebiology.com/imedia/1438587891223544/supp8.doc>

Additional file 9: add9_suppl_figure2.doc, 10463K

<http://genomebiology.com/imedia/1199106362235442/supp9.doc>

Additional file 10: add10_suppl_figure3.doc, 1334K

<http://genomebiology.com/imedia/1934629804223544/supp10.doc>

Additional file 11: add11_suppl_figure4.doc, 1348K

<http://genomebiology.com/imedia/2717170222354422/supp11.doc>

Additional file 12: add12_suppl_table5.xls, 29K

<http://genomebiology.com/imedia/7855118022235442/supp12.xls>

Additional file 13: add13_suppl_table6.xls, 4193K

<http://genomebiology.com/imedia/5417304052235442/supp13.xls>

Additional file 14: add14_suppl_table7.doc, 86K

<http://genomebiology.com/imedia/1059562627223544/supp14.doc>

Additional file 15: add15_suppl_table8.xls, 8735K

<http://genomebiology.com/imedia/6236559962235442/supp15.xls>