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Genetic correlates of the evolving primate brain

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Abstract

The tremendous shifts in the size, structure, and function of the brain during primate evolution are ultimately caused by changes at the genetic level. Understanding what these changes are and how they effect the phenotypic changes observed lies at the heart of understanding evolutionary change. This chapter focuses on understanding the genetic basis of primate brain evolution, considering the substrates and mechanisms through which genetic change occurs. It also discusses the implications that our current understandings and tools have for what we have already discovered and where our studies will head in the future. While genetic and genomic studies have identified many regions undergoing positive selection during primate evolution, the findings are certainly not exhaustive and functional relevance remains to be confirmed. Nevertheless, a strong foundation has been built upon which future studies will emerge.

Keywords

Genetic evolution; molecular evolution; catarrhine; hominoid; hominin; FOXP2; microcephaly; opsin; olfaction; pleiotropy; gene regulation; divergence; polymorphism

Introduction

Evolution at the most basic level occurs in the genome. In the simplest formulation, mutations occur that give rise to phenotypes upon which selection acts. While overly simplistic, this can form a useful framework to begin our understanding of the genetics of primate brain evolution. If we begin with the premise that the primate brain generally, and the human brain specifically, systematically differ from that of other species then it follows naturally that this must result from a genetic heritage. Understanding the genetic changes that have led to the phenotypic changes that we observe in the primate brain may ultimately lead to a better understanding of the brain itself, the differences between species, and the neuropathologies with which we struggle.

Many studies exist comparing genetic similarities between species for specific classes of genes. In particular it has been shown that genes expressed in the brain show fewer differences between species than genes expressed in other tissues or more ubiquitously (Miyata et al., 1994, Duret and Mouchiroud, 2000). Unsurprising, perhaps, given the enormous complexity of the brain and the hugely deleterious effects of even the most subtle change. And yet, even to an untrained eye, the brain of a mouse, marmoset, chimpanzee and human are immediately and obviously different. Phenotypic change has obviously occurred and this can only mean a genetic underpinning. While there are many contributing factors to this paradox, a significant understanding can be reached simply by noting the different domains in which these observations exist.

When we speak of brain evolution we are conflating at least two disparate concepts. The first is a basic change of brain function, how neurons communicate with one another, the neurotransmitter mechanisms by which the brain operates. The second is a change in

structure and development, neurons continue to function similarly but now there are more of them or they are in different places. It is important to appreciate these differences in order to make sense of the evolutionary studies that focus on brain genetics.

When we compare very divergent classes of organisms, say insects to mammals, the genes and proteins of basic brain function may change dramatically. Mammalian usage of catecholamines, norepinephrine and epinephrine, compared to insect usage of phenolamines, octopamine and tyramine, is an excellent example (Vincent et al., 1998, Caveney et al., 2006). And yet with few exceptions, the basic physiological functionality of the brain is remarkably similar between more closely related species such as mammals generally or primates specifically. Nevertheless the differences that do emerge tend to be correlated with significant change. In primates, change at this basic functional level is felt most strongly in those systems associated with a shift from olfactory perception to visual perception.

Canonical gene evolution studies in primate perception

One of the most salient and extreme examples of genetic evolution in primates is the wholesale loss of olfactory receptors (Young et al., 2002, Gilad et al., 2003a, Gilad et al., 2003b). Mammalian olfactory receptors, a gene superfamily consisting of more than one thousand genes, form a significant portion of the mammalian genome. This extensive diversity is likely the result of olfactory receptors specific binding to odorant molecules. But this specificity that leads to such variety overall also leads to significant losses when a given organism is not exposed to the odorant. In rodents and dogs, only 20% of the olfactory receptor genes are nonfunctional and yet in humans fully 60% of olfactory receptors have undergone pseudogenization (Dong et al., 2009). While initially focused on the human genome, this finding has also held up across other non-human primate species, correlating well with the relative roles of visual and olfactory perception.

Few studies are as dramatic as the evolution of the primate olfactory subgenome, yet we can observe similar findings in other sensory domains. Perhaps unexpectedly, given their similarity to olfactory receptors, taste receptors have also repeatedly shown selective signatures across species including primates (Wooding et al., 2004, Soranzo et al., 2005, Wooding et al., 2006). Divergence between species and polymorphism within species has been widely observed for both the bitter taste receptor gene family as well as the sweet taste receptor genes. Hypothesized to reflect changes in diet and perhaps the ability to distinguish between nutritious and harmful foods, the evolution of taste receptor genes parallels that of the olfactory genes.

The evolution of trichromatic vision in primates is also a prominent example of molecular evolution (Shyue et al., 1995, Zhou and Li, 1996). Humans, apes, and old-world monkeys are tri-chromatic because they have three opsin genes each of which detects light at a different wavelength. The blue (short-wave) opsin is shared across primates and is located autosomally, while the green (middle-wave) and red (long-wave) opsins are located on the X chromosome. While bi-chromatic platyrrhines only possess one X-chromosome opsin gene, trichromatic catarrhines have two. Starting from this observation, it has been possible to reconstruct the molecular evolution of the X-chromosome opsins, beginning with a gene duplication event approximately 35-40 million years ago, after the divergence of catarrhines from new world monkeys, and the subsequent functional divergence of the two genes allowing them to separately recognize the red and green wavelengths (Figure 1). Interestingly, new world monkeys have multiple alleles at the X-chromosome opsin gene, allowing female homozygotes to effectively have trichromatic vision while all males are obligatorily color-blind. The exception here is in howler monkeys where a duplication, akin

to what is observed in catarrhines, has fixed the two platyrrhine alleles (Boissinot et al., 1998).

These examples, though, are the exception rather than the rule; changes are large and obvious. In the olfactory system hundreds of genes are inactivated, a not-so-subtle change at a level that cannot be overlooked. The visual system represents an extreme phenotypic change and a strong single gene effect. Neither of these situations generalize. The evolution of most genes is subtle, with changes that affect function hiding amongst large numbers of obfuscatory neutral differences. Most phenotypes, especially when dealing with the brain, are the result of many interacting genes and proteins and associating specific genetic changes with observable phenotypic differences is extremely difficult. It is to this, however, that we set our mind when we seek to understand the genetic differences underlying primate brain evolution.

Gene gain and loss

Before applying genetic and molecular evolution to questions of the brain it is useful to discuss it in a more general sense. Despite inferences elsewhere there is no reason to believe that the molecular evolution of the primate, or human, brain is inherently any different than the genetic evolution of any other character or trait. The brain, as a complex system, may be inclined towards certain categories of change, but it is governed by the same factors that are seen elsewhere.

As demonstrated in the emergence of primate tri-chromatic vision, gene gain through duplication can be a major source of evolutionary novelty (Ohno, 1970, Zhang, 2003b). Following the initial duplication event, selective pressures are temporarily relaxed as genetic redundancy can hide evolutionary missteps. By far the most common fate following a duplication event is the most evolutionarily uninteresting, the pseudogenization of one copy and the perseverance, in a largely unaltered form, of the second. However, in rare instances both copies of the duplicated gene can persist either through neofunctionalization (Hughes, 1994), where one copy evolves a new functionality separate from the original, or by subfunctionalization (Force et al., 1999), where the two copies divide among each other the functional characteristics of the original. In this latter case, multiple functions of a single gene can thus be disjoined allowing for the separate evolution of each.

Yet while the most evolutionarily satisfying from a theoretical point of view, it is unclear the degree to which novel genes actually contribute to short time scale evolutionary events, such as primate evolution. The number of gene gains found along any primate lineage is fairly small, despite the relative simplicity in identifying them. This combination of their paucity and their potential to generate major evolutionary change has made gene gains a major focus for evolutionary inquiry. Yet despite this fact there are relatively few examples of gene gains with functional effects in primates and even fewer that involve brain development or function.

Among the more interesting cases are those of the DUF1220 domain family of genes (Popesco et al., 2006). This gene family has expanded greatly in primates and seems to be uniquely and specifically expressed in neurons, but the overall function of the gene remains unclear. Other gene families, such as the *morpheus* genes (Johnson et al., 2001) and the KRAB-ZNF genes (Nowick et al., 2010), have also showed rapid evolution in primates, though again their functional relevance is unclear.

The duplication of glutamate dehydrogenase (*GLUD*), on the other hand, represents a more clear functional picture (Burki and Kaessmann, 2004). In old world monkeys, like most mammals, there exists only a single *GLUD* gene. It encodes for a protein that recycles the

excitatory neurotransmitter glutamate. In species with only one *GLUD* gene, this protein is ubiquitously expressed. In apes, however, a duplication event has led to the emergence of a second glutamate dehydrogenase, *GLUD2*. This gene is expressed uniquely in the nerve tissues and testis (Shashidharan et al., 1994) and appears to have undergone positive selection to optimize its function in environments, like the brain, with high GTP concentrations (Plaitakis et al., 2003). The difficulty here, however, is that while the specific protein function is understood, a phenotypic consequence is less clear.

The other side of the coin to gene gain is gene loss. Like gene gain, gene loss is relatively simple to identify and the change in function is easy to interpret; the functional gene is either there or it is not. Typically gene loss is envisioned as a loss of a trait. The pseudogenization of the olfactory system is a good example. In species with particular intact olfactory receptors, individuals have the capacity to recognize certain odors; individuals in species where these receptors have been lost cannot. The gene loss corresponds to a trait loss. Of course, genes and their products operate in complex systems and their loss can have far reaching effects that may ultimately result in a phenotypic trait gain.

One of the most interesting examples of gene loss and the role it may play in primate brain evolution also demonstrates this circuitry. *MYH16* is a protein from the gene family that makes up the myosin strands in skeletal muscle. It was identified in 2004 as having been lost in humans (Stedman et al., 2004). Further investigation found that *MYH16* was expressed uniquely in masticatory muscles, those muscles that attach the jaw to the skull and allow for a greater bite strength and chewing ability. The loss of this gene in *Homo sapiens* also seemed to correlate with the dietary transition to cooked meat and the loss of a need for a heavy masticatory apparatus. This also released structural constraints for attaching these muscles to the skull and allowed for an enlargement of the human brain. Naturally much of this latter is conjecture based upon circumstantial evidence that has subsequently been challenged (Perry et al., 2005). As we will see repeatedly, the basic functional change is undebatable, the gene and protein were there in chimpanzees but lost in humans, but the question of how this change fits into an evolutionary framework is more difficult.

So far our discussions have focused on the most obvious, yet rare, molecular evolutionary events. By far the most common of evolutionary changes are much more subtle; the functional element is still there as before but it is changed slightly. This can occur in either protein coding regions or in regulatory regions and by many different kinds of genetic changes (Figure 2). In protein coding regions we often think of point mutations that can change the encoded amino acid. If the amino acid is important and/or the change is drastic enough the proteins function can be changed. In regulatory regions point mutations can have similar effects, but so too can insertions or deletions or other mutational events that would have the singular effect in protein coding regions of pseudogenization. Because regulatory regions seem to be much more labile in their structural and functional organization, they can tolerate changes that coding regions cannot.

These latter changes occur much more commonly than gene gain or gene loss. Small changes to an individual's genome occur in every generation. Most times these changes are nearly neutral, that is to say they have little to no effect on the overall selective fitness of an organism. Changes that do have a noticeable effect are almost always deleterious, again think of genetic diseases. It is upon this background of changes that evolution operates. Identifying changes that have impacted the observable differences between species thus becomes a major challenge to understanding the genetic basis behind evolutionary change.

Detecting adaptive genetic change

When we compare two species at the genetic level we find many fixed differences. Of course, the more evolutionarily distant the two species the greater the genetic difference, but even closely related species show millions of differences. Indeed, even populations within a single species will show fixed differences relative to each other. The issue becomes separating out changes that matter, functionally relevant changes, from those that don't. Ideally, this would be accomplished via direct functional assay: If a receptor is exactly the same except for this specific change does it bind its ligand differently? If this promoter region is exactly the same except for this mutation is the expression pattern of its gene changed? This is the gold standard for an unequivocal statement of functional change, yet it is obviously not tenable on large scales. Further, it can be difficult, if not impossible, to find the relevant assay for detecting a functional effect. Because of this, we have developed computational and bioinformatic approaches to identify likely candidates.

One of the most common and widespread ways to detect functionally important differences between species is to look for mutations that have fixed at a faster rate than they would have if they were behaving neutrally. This positive selection on the mutations argues that they were functionally important. Many algorithms have been developed around this basic premise (Li et al., 1985, Nei and Gojobori, 1986, Goldman and Yang, 1994, Yang and Nielsen, 2000). In proteins this approach measures the rate of fixation of nonsynonymous or replacement changes, those that do change an amino acid, to the rate of fixation of synonymous changes, those that don't change an amino acid. The synonymous rate is alternatively called K_S or d_S and is assumed to represent the neutral mutation rate while the nonsynonymous rate is called K_A or d_N and is reflective of protein change. The ratio of these rates (K_A/K_S , d_N/d_S , or ω) can be used to infer the selective pressure on the gene. A neutrally evolving gene, one under no selective pressure, would have a K_A/K_S rate equal to one. Most genes are under negative selection, the protein has an evolved function that it cannot stray from, and have K_A/K_S rates less than 1. (The average across genes in primates hovers around 0.2 (The Chimpanzee Sequencing and Analysis Consortium, 2005, Gibbs et al., 2007).) Positive selection, genes whose function has changed during evolution, shows K_A/K_S rates above 1.

One of the findings of the early genomic studies was that genes expressed in the brain tended to have lower K_A/K_S rates than genes expressed in other tissues (Miyata et al., 1994, Duret and Mouchiroud, 2000). These results applied across multiple brain regions (Tuller et al., 2008) and across multiple species comparisons including among primates where selection on the brain was anticipated (Khaitovich et al., 2005). In some ways this is unsurprising, the brain is extremely complicated and changes that disturb this delicate balance are likely to have deleterious effects. On the other hand this conservation should not be taken as a lack of selection either. Rather, it highlights the complexities of detecting positive selection in this manner.

This method for detecting positive selection is very sensitive to dilution, either spatial or temporal. The first is the result of parts of genes being under differential selective pressures. In the context of the brain, a G-protein coupled receptor is likely to be under strong negative selection in its transmembrane domains while the intra-or extra-cellular domains may be under less strong selection or even positive selection (Figure 3). If the entire gene is used as the unit of selection then the positive selection in, say, the ligand binding site may be obscured by the negative selection in the transmembrane domains. Time can also dilute the effects of selection. If the selective event was specific and short-lived then K_A/K_S levels may be higher during those periods but lost overall. Pairwise species comparisons necessarily entail the entirety of the evolutionary history separating the species, so if the

selective event only happened for a short period then it can be obscured. This is particularly important when considering brain evolution in primates because encephalization events are generally short and discreet rather than long and pervasive. Methodologies have been developed that can take these factors into account (Yang, 2007), but the smaller the physical region or time frame, the smaller the K_S , the greater the noise in the system.

There are many examples of genes putatively involved in the evolution of the primate brain that show signatures of positive selection. Some of the most extensive evidence is found in genes associated with primary microcephaly. Primary microcephaly is a disease in which the brain is reduced in size, but without any other gross abnormalities in structure (Dobyns, 2002). This malformation has been of interest in the evolutionary community because the condition seemingly recapitulates ancestral primate brain characters, the suggestion being that the same genes that cause the disease may also have been those genes under selection to produce modern brain phenotypes (Woods et al., 2005, Kaindl et al., 2010). Over the years K_A/K_S -based methods have been used to identify positive selection on multiple genes implicated in the disorder: *ASPM* (Zhang, 2003a, Evans et al., 2004b, Kouprina et al., 2004), *MCPHI* (Evans et al., 2004a, Wang and Su, 2004), *CDK5RAP2* and *CENPJ* (Evans et al., 2006), and *CEP152* (Guernsey et al., 2010). In each of these cases, however, the evidence for positive selection has come from comparative genomics and specific attributable functional change remains elusive.

Protein coding changes can be more or less straightforward to identify bioinformatically but difficult to ascertain function. Non-coding changes are the inverse, with functionality yielding easily, but identification much more slowly. The difficulty in regulatory regions lies in identifying which changes are functionally relevant and which are not. Initial studies used either protein coding synonymous sites (Wong and Nielsen, 2004) or surrounding intronic regions (Haygood et al., 2007) to calibrate for neutral mutations. While there was some limited success with these methods, functional sites in the test regions are likely to be swamped out by neutral sites, significantly reducing power. As our understanding of gene regulation improves these test regions can be better defined and better tools developed. Indeed more recent attempts incorporate transcription factor binding motifs to define regions (Hoffman and Birney, 2010).

The specific successes in regulatory regions, especially regarding the brain, are few, the gene encoding the opioid neuropeptide precursor, *PDYN*, being the significant exception (Rockman et al., 2005). For this gene an identified *cis*-regulatory element was shown to have accumulated an exceptionally high number of mutations since the divergence of humans from chimpanzees consistent with a hypothesis of positive selection. More importantly, however, functional assays employed showed that the humanized regulatory element drove much higher levels of expression compared to the chimpanzee element *in vitro*. Interestingly this finding has recently been extended to other members of the opioidergic system (Cruz-Gordillo et al., 2010), though it remains unclear if the fact that both of these findings arise in a single system is biologically meaningful or it simply represents a relative paucity of study elsewhere.

Implications of genetic change

Our ability to detect genetic change has significantly impacted the way that we pursue questions of genetic evolution. It has long been appreciated that regulatory change is likely to play a major role in brain evolution. Building on the work of others (Ohno, 1972), in 1975 King and Wilson (King and Wilson, 1975) noted that “The organismal differences between chimpanzees and humans would then result chiefly from genetic changes in a few regulatory systems, while amino acid substitutions in general would rarely be a key factor in major

adaptive shifts.” Little has occurred in the intervening years that would force us to reassess this basic belief. Rather, it has been buttressed by subsequent work and the emergence of the field of evolutionary developmental biology (Carroll, 2008). Yet the preponderance of work to date has focused on protein change rather than regulatory change, largely because of the tools at our disposal.

Proteins, seemingly especially brain proteins, have been optimized for their specific roles; there simply is not a lot of room for them to change without causing major effects. This is especially true because of genetic pleiotropy. In essence, proteins serve many functions simultaneously and any change that might be made affecting one of these functions necessarily affects all of them. While it is true that not all proteins are pleiotropic, it seems likely that many of the developmental proteins that would most likely account for the evolutionary differences that we see in the primate brain are. As we have seen, one way in which these pleiotropic effects can be overcome is through gene duplication, yet this is rare. One major finding that has come following the publication of the non-human primate genomes is that there simply are not a tremendous number of species-unique genes. The gene complement of each of the primate species is largely the same enough so the exceptions warrant significant attention even in the absence of any functional understanding. Again, while a theoretically satisfying method to generate novelty, gene duplication does not seem to be a major mechanism for adaptation in primates.

Because of what is currently feasible, the focus so far has been predominantly on protein change. While it may only represent a portion of the genetic underpinnings of primate brain evolution, it has been tractable and significant changes have been identified. As we move forward, however, it will be increasingly important to expand our studies of regulatory regions. Many attempts are being made to address this disparity with varying degrees of success, yet it is clear that the next major step forward in evolutionary genetics will follow our ability to solve this problem.

Phenotypic change in the primate brain

When speaking of the evolution of the primate brain we are, by and large, talking about developmental differences. The most obvious of these is simply an overall expansion in size. The volume of the brain in humans is roughly eight times the size of that of large new world monkeys, six times that of old world monkeys and three to four times that of apes (Jerison, 1973). Generally speaking, an enlargement of the brain in its entirety, or at least the whole brain excluding the olfactory regions, seems to be a common mechanism upon which selection acts in mammals generally including primates (Finlay and Darlington, 1995). It has also been observed, however, that neocortex increases in the brains of apes and humans are particularly pronounced (Semendeferi et al., 2002, Kaas, 2005). Of course, brain size, or neocortex size, is not the direct phenotypic output upon which selection is acting. Rather we are assuming that behavioral complexity or intelligence is somehow correlating with the increase in size. It is this behavioral output that selection is ultimately acting upon, introducing another obfuscatory layer to evolutionary analysis.

In primate evolution we see several major bouts of brain growth, encephalization events. In fact, each major divergence event, Haplorrhini and Strepsirrhini, Catarrhini and Platyrrhini, Cercopithecoidea and Hominoidea, seems to correlate with a brain size expansion. In more recent human evolution we also see brain size expansions, from *Australopithecus* to *Homo*, in the emergence of *H. erectus*, and finally in the emergence of *H. sapiens*. Each of these encephalization events has presumably left marks in the genome. It isn't known, however, if each of these events was the result of succeeding changes to the same genes or if each event utilized novel genes.

It is also important not to overlook other sources of phenotypic differences. While large-scale structural differences can be the most obvious to observe, connectivity changes can have significant impacts as can more subtle microstructural changes. We also have numerous examples from human and rodent literature of variation associated with neurotransmission having significant effects on behavior and, in humans at least, this variation seemingly under variable selective regimes.

Implications of phenotypic change for genetic evolution

The context of brain evolution has several important implications for genetic studies: what we look for and when we look for it. In general when we are considering primate brain evolution, we are focusing on developmental changes: changes in growth rates, changes in developmental timing, changes in patterns of expression. As previously mentioned, developmental change can seemingly be more easily effected through changes in gene regulation than by changes in protein sequence and function (Carroll, 2008). This may help explain why studies of primate evolution generally can have power to detect selection in other systems, but not in the brain despite its relative importance to adaptation.

When we do undertake studies of protein evolution it is informative to consider where we find our best signals of selection, at least relative to the brain. We have already mentioned the positive selection detected in the genes associated with primary microcephaly. Although undoubtedly functionally complex, it is interesting to note that these genes, when mutated in humans, have a decidedly non-pleiotropic effect. Indeed their initial selection for consideration as relevant substrates for selection was precisely because the disease that they associated with resulted in a decrease in brain size without any other significant pathologies. In other words, the focus, even in proteins, has been on genes most likely to have singular functions.

The other effect that this emphasis on developmental change has had on primate brain evolution can be seen in the genes identified. In several of the early pseudo-genomic studies an accelerated rate of evolution in brain genes was dominated by genes of predominantly developmental function (Dorus et al., 2004, Khaitovich et al., 2005). Other studies have also broadly identified “transcription factors” as a category of genes rapidly evolving in primates relative to rodents (Gibbs et al., 2007). In both cases it is primarily by gathering together genes of like function that these patterns emerge. If this primate phenotypic evolution is occurring primarily by many changes of very small effect then this is the sort of pattern that we would anticipate.

One last gene set worthy of mention is the class of genes involved in the apoptotic pathways, particularly the extrinsic apoptosis pathways. These genes were first identified as undergoing positive selection in a large scale scan comparing humans and chimpanzees (Nielsen et al., 2005). Focused follow-up studies confirmed and extended the findings (Vallender and Lahn, 2006, da Fonseca et al., 2010). Apoptosis, programmed cell death, is important in many developmental processes including brain growth. Knockouts of many of these genes in mice result in dramatic brain phenotypes (Putchá et al., 2002). Again, however the question of pleiotropy is significant. The role of apoptosis in neuronal development is undisputed, yet so too is the role of apoptosis in numerous other processes including immune response, a known major driver of selection.

Another important confound that this introduces revolves around the specific encephalization event implicitly under study. Many studies of recent evolution, almost always in humans, focus on polymorphism levels and have an effective resolution of only the last several million years. In humans this means at best the changes in the brain since the Australopithecines. In reality, demographic effects, including bottlenecks and the “Out-of-

Africa” migration of *Homo sapiens* (and possibly *Homo erectus*), likely create an even shorter timeframe of effective resolution.

Studies that focus on the differences between chimpanzees and humans, with or without an outgroup to stratify changes, encompass all the successive encephalization events in the terminal *Homo* lineage. The primary difficulty with these studies is that stochastic noise in the neutral rate of evolution is great, often dominating the selective signal. This is a pervasive problem with short lineages and likely extends across all ape pairwise comparisons. Some power will be gained when the gorilla and orangutan genomes become widely available and all four primary great ape lineages can be integrated, though it seems likely that stochastic effects will continue to dominate.

When old world monkeys are included in the analyses this short lineage effect is largely ameliorated. The cost of this, however, is that the time period that dominates the selective effects seen corresponds to the lineage separating the apes from the last common catarrhini ancestor. In effect this means that many studies focusing on the human-old world monkey (rhesus macaque) comparison may actually be more generally studies of the evolution of the ape brain rather than the human brain.

To date few studies have included new world monkeys or other more basal primate lineages, though this is certain to change in the future. These studies will be informative, but particularly for understanding brain evolution during the Catarrhini-Platyrrhini or Haplorrhini-Strepsirrhini events. Perhaps the most important of the genomes yet to be sequenced for understanding brain evolution, particularly in the neocortex, is the gibbon. This lineage separated from the apes approximately ten million years after the old world monkeys and may help isolate and elucidate the changes involved in the emergence of the ape brain.

Surveys of genetic evolution

There have been several large scale surveys of genetic evolution that have emerged over the last decade. Yet while there has been significant comment arising from these studies, it has been difficult to translate them into biological understanding. This is in part because of the large differences in their findings. The initial focus of many of these surveys was on divergence between human and chimpanzee (Clark et al., 2003, Bustamante et al., 2005, Nielsen et al., 2005, The Chimpanzee Sequencing and Analysis Consortium, 2005, Arbiza et al., 2006) and indeed much of the confusion lies in the nature of the human-chimpanzee comparison itself. The short lineage effects can make small differences in methodologies, including the species used as outgroup, alignment method, divergence algorithm, and universe of genes under study, have large impact.

At the same time there have been studies focusing on polymorphism within humans as a means to detect more recent positive selection (Carlson et al., 2005, International HapMap Consortium, 2005, Kelley et al., 2006, Voight et al., 2006, Sabeti et al., 2007, Williamson et al., 2007). Again there have been few consensus findings. Like the human-chimpanzee comparisons, variability in methodologies may have a large effect on these polymorphism based studies. Many of these approaches are only recently being pioneered as data developed from large-scale human diversity studies becomes available. In addition to methodological uncertainty, however, polymorphism studies suffer from biological difficulties. Demographic effects complicate any study and range of resolution can have major effects on results. Recent selection study designs may capture only the most recent *Homo erectus* to *Homo sapiens* speciation or may extend further. This general issue is a common one when comparing genome wide surveys; different study designs focus on

different time frames. Perhaps the most noteworthy finding that can be drawn from this is that the same genes do not seem to be underlying each adaptive event.

Across genomic studies, genes involved in any aspect of brain or nervous system biology regularly fail to appear. Several possible interpretations exist for this. In human-chimp and human-old world monkey studies, the focus is almost exclusively, and necessarily so, on protein coding sequence. A lack of brain genes in these studies seems to confirm a primacy of regulatory substrates for evolution. Polymorphism studies, however, are ambivalent to substrate and still don't offer significant insight into brain evolution. An explanation for this could be that we simply don't understand enough about gene regulation and our annotation of positively selected regions is deficient, but perhaps more likely is that selective pressures are not sufficient to reach significance levels required for genome-wide detection. It is for this reason that candidate gene studies continue to be the most useful.

Candidate gene studies

Candidate gene studies are often driven first by phenotype. This can mean either that functional studies, often in rodents, or diseases in humans have implicated a gene in brain development. Indeed it is often the neurodevelopmental research that drives the evolutionary studies rather than vice-versa. This has not only allowed for a better focus on genes with phenotypic effects of interest, but it also lets us focus more carefully on the evolutionary questions. Significance thresholds for candidate gene studies needn't account for multiple testing to the same degree as whole genome studies. Also the increased scrutiny lends itself to more subtle effects and nuanced interpretations. Researchers on candidate gene studies also, in general, have the greater personal investment required to follow up and understand the functional implications of the evolutionary changes. When so often the functionally relevant changes are hidden amongst selectively and functionally neutral changes, this increased motivation is crucial.

To this end it is useful to compare several recent candidate gene studies (Figure 4), beginning by reconsidering the situation of the genes implicated in primary microcephaly and their evolutionary history. The first two primary microcephaly genes identified and for which evolutionary studies were undertaken were *ASPM* and *microcephalin* (*MCPHI*). For each of these genes the first studies, conducted independently in multiple labs, focused on inter-species comparisons. For *microcephalin* the findings indicated positive selection in the lineages leading from old world monkeys to the great apes (Evans et al., 2004a, Wang and Su, 2004). While elevated rates of protein evolution were found elsewhere, notably in the lineages following the divergence between gorillas and the hominins, they are not significant. In *ASPM*, on the other hand, significant positive selection is seen both in the human terminal lineage and the lineage separating the great apes from the lesser apes (Zhang, 2003a, Evans et al., 2004b, Kouprina et al., 2004).

Several early studies of polymorphism in humans on these same genes also found evidence of non-neutral evolution (Evans et al., 2005, Mekel-Bobrov et al., 2005). This implication led to an interpretation that these genes were also under more recent selection. Later findings, however, suggested that the patterns of polymorphism seen in *ASPM* and *microcephalin*, while not expected under neutrality, were not uncommon in the *Homo sapiens* genome (Currat et al., 2006, Yu et al., 2007). This suggested that demographic effects, rather than selection effects, were primarily responsible for the observed patterns of variation. Additionally, studies were unable to identify any phenotypic effect for these polymorphisms (Woods et al., 2006, Dobson-Stone et al., 2007).

The difficulty in conducting functional studies of these genes has played a significant role in interpreting evolutionary genetic findings. Like most genes implicated in developmental

processes, *in vitro* studies of function are difficult to do. While *ex vivo* or *in vivo* models can be used to understand their roles and functions, it is difficult to assess the impact of specific mutations in a shared context or environment. This ultimately has led to a difficulty in attributing definitive and unambiguous meanings to the explicit and unequivocal patterns in the genomes.

Another candidate gene study worthy of mention is *FOXP2*. Like the genes implicated in primary microcephaly, *FOXP2* initially garnered interest because of its association with a pathological disorder, in this case a speech and language deficit (Lai et al., 2001). Evolutionary studies followed that showed a remarkable conservation of the gene across mammals, but significant multiple amino acid changes in the human lineage (Enard et al., 2002, Zhang et al., 2002). Since these studies, several additional findings have driven interest in the gene. The first was evidence that *FOXP2* plays an important role in song-learning in birds (Haesler et al., 2004). The second used “humanized” mice, transgenic mice with the human version of *FOXP2*, to demonstrate “qualitatively different ultrasonic vocalizations” (Enard et al., 2009). *In vitro* and *in vivo* studies were then developed that showed that human and chimpanzee *FOXP2* differentially regulated downstream gene expression (Konopka et al., 2009). Most recently, a study has shown that differentially expressed genes in the developing cerebral cortex, particularly in the Broca and Wernicke areas, show accelerated evolution in humans and are enriched for transcriptional targets of *FOXP2* (Lambert et al., 2011).

Perhaps most notable about *FOXP2* is how completely its evolutionary importance is driven first by functional concerns. Of all the genes considered here, and indeed perhaps among all the genes implicated in primate brain evolution, the case for *FOXP2* is perhaps the strongest yet the evidence at the genetic level is the weakest. Contrast *FOXP2* with *ADCYAP1*. *ADCYAP1* encodes a protein that has been shown to play a part in the transition from proliferative to differentiated states during neurogenesis (Dicicco-Bloom et al., 1998). It is one of the most divergent genes between humans and chimpanzees and has strong genetic evidence for positive selection in humans (Wang et al., 2005). In essence the pattern of evolution for these genes two is the same, yet for one the functional evidence dominates while for the other the genetic evidence dominates. It is informative, and yet not surprising, where the balance of confidence lies. It also provides an instructive guideline for evolutionary studies moving forward.

Non-traditional substrates of evolution

In the past, evolutionary studies have focused primarily on point mutations and primary DNA sequences. As our understanding of biological function grows, so too have the possibilities for evolutionary action. Perhaps not surprisingly most of these revolve around gene regulation. Some of these novel substrates are natural extensions of existing concepts, such as the emergence of non-coding RNA genes. Though they operate in a manner unlike proteins or *cis*-regulatory sequences, positive selection on these regulatory nucleic acids can be identified in a similar manner. Indeed already a human non-coding RNA has been identified that shows signatures of positive selection (Pollard et al., 2006). While the precise function of this gene is unknown, it is found in the neurons of the developing neocortex. Again, functional studies lag behind genetic implications but the prospect of the finding is there.

Three other substrates for evolution recently under exploration similarly represent outgrowths from existing studies. Selection on regulatory regions may occur through selection on epigenetic markers (Enard et al., 2004) and early studies have identified differences in methylation patterns between humans and nonhuman primates in the brain

(Farcas et al., 2009). Whole genes may not be emerging or vanishing as rapidly as we once thought, but the intriguing possibility is arising that alternative splice forms may be more variable between species than previously thought. Again one early piece of evidence for this was a comparison between the human and non-human primate brain (Lin et al., 2010). Finally plasticity in post-translational modifications on proteins may represent another source of regulatory evolution.

The auto-catalytic domain of the sonic hedgehog (*SHH*) gene was shown to be under positive selection in the lineage separating old world monkeys from apes (Dorus et al., 2006). This was peculiar as *SHH* is one of the most preeminent developmental regulator genes with many functions across many tissues and developmental stages. A potential explanation may be found in the nature of those changes, a statistically non-random excess of serines and threonines, residues often the targets of post-translational modifications. This raises the intriguing possibility that it is these regulatory effects that underlie the observed phenomena.

The future of primate brain evolution genetics

When viewing these studies from afar one cannot help but notice the proliferation coinciding with the emergence and publication of the human, chimp, and rhesus genomes as well as the major human polymorphism surveys. The amount of data generated by these studies provided tremendous fodder for evolutionary geneticists eager to expand our understanding of brain evolution. Yet too often these studies were “one-and-done”; genetic evidence implicating many genes in brain evolution has gone without follow-up. This will be the next major hurdle faced by those seeking to understand primate brain evolution.

The mantra of evolutionary genetics, particularly regarding the primate brain, going forward will be function, function, function. Descriptive genetic studies have demonstrated both their utility and their drawbacks. Leads have emerged by the dozens, if not hundreds, but until an unambiguous functionality can be associated with the putative selective mutations little will advance. This recognition is setting in and is now driving studies. Increasingly we are seeing more studies focused on gene expression, a combined result of its likely importance in developmental regulation and the practicality of assaying these changes. This is not to say that there are not other approaches still to be taken or substrates to be found, simply that research has transitioned to the next lowest hanging fruit.

Perhaps the most important thing to emerge during this decade of explosive growth in primate evolutionary genetics is the increasing appreciation for the interconnectedness between fields. Researchers studying neurodevelopment can more easily and with more confidence interpret their findings through a comparative evolutionary framework. Evolutionary geneticists have an increasing appreciation and focus on the basic functional work of developmental neurobiologists. As we come to view primate brain evolution within a more complete and gestalt framework we are increasingly likely to understand both its genetic and phenotypic roots as well as its implications for human health, biology, and our understanding of ourselves.

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Figure 1.

Molecular evolution of opsin genes in the primates. The blue opsin, on autosomes has remained conserved across primate species. In the ancestral primate, and in extant strepsirrhines only a green opsin is present on the X chromosome. In catarrhines, this gene has been duplicated and been functionally altered to detect both red and green. In most new world monkeys, and seemingly the ancestral platyrrhine, only one opsin exists on the X chromosome though it has multiple alleles and female heterozygotes may have trichromatic vision. A separate duplication event in howler monkeys has fixed red and green opsins on the X chromosome as well.

Figure 2.
Schematic demonstrating the various effects of regulatory and coding mutations on genes.

Figure 3.

Hypothetical sliding window analysis of a seven transmembrane domain G-protein coupled receptor (after Choi and Lahn, 2003). K_A/K_S ratios in transmembrane (TM) and intracellular (IC) domains are low indicative of negative selection while K_A/K_S ratios for extracellular (EC) domains are above 1, indicative of positive selection. The dashed line indicates the gene average K_A/K_S , under 1 and evidence of overall negative selection.

Figure 4. Cladograms representing consensus findings on evolutionary histories of specific genes. Lineages in bold have been identified as undergoing positive selection for the gene noted.