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Human Uniqueness and Accelerated Storytelling: How Conserved Regulatory Regions in the Genome Challenge Evolution

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Abstract

The Bible clearly states that humans were created in the image of God (Genesis 1:26–27). This makes us distinct in certain ways from the rest of the creatures God created, including primates. In addition to obvious outwardly visible trait differences, it would make sense that we would find certain regions of the genome that are distinctly different between humans and other animals, and this is in fact seen. Secularists postulate that these genetic differences arose from accelerated evolution since the time that humans allegedly diverged from apes; thus they call these regions *human accelerated regions* (HARs). HARs are exceedingly problematic for evolutionists due to the fact that they tend to be highly conserved across vertebrates but are markedly different in humans. However, within supposed vertebrate lineages, many of these regions are taxonomically isolated—they seem to arise suddenly—with no evolutionary history. A new phylogenetic analysis of 105 HAR genes in 10 different vertebrate taxa show that these sequences also display remarkable phylogenic discordance on a broad scale. This is inconsistent with the idea that these genes were generally conserved for tens or hundreds of millions of years but then suddenly evolved into taxonomically restricted forms. The data is more consistent with the creation model, wherein the genes that encode taxonomic distinction were custom designed.

Introduction

At the most fundamental level of objective discernment, even a child can clearly tell the difference between a human and a chimpanzee. However, the secular idea that humans somehow evolved from apes has become a leading icon of the evolutionary paradigm. In a creationist sense, this is one of the most objectionable components of the whole evolutionary paradigm because the Bible not only indicates that God made each creature "after its kind" but also that humans were uniquely created in God's image.

While many creatures exhibit distinct genetic differences, the issue of human relatedness to apes is seemingly bolstered in an evolutionary sense by

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regions of high DNA similarity between humans and great apes (chimpanzees, gorillas, and orangutans), although pervasive inconsistencies, which evolutionists attribute to incomplete lineage sorting, negate a clear path of common ancestry (De et al., 2009; Ebersberger et al., 2007; Hobolth et al., 2007; Patterson et al., 2006). In addition, the DNA similarity paradigm, particularly in regard to human and chimpanzee DNA similarity, tends to be dominated by studies utilizing selective data that excludes genomic regions that are dissimilar (Bergman and Tomkins, 2012; Tomkins and Bergman, 2012).

Another problem in comparing human and ape DNA sequence is that great ape genomes, including chimpanzee, are computationally assembled from small individual sequence reads using the human genome as a reference sequence, and thus they appear to be more humanlike than they really are (Chimpanzee Genome Sequencing Consortium, 2005; Prado-Martinez et al., 2013; Tomkins, 2011). This problem is compounded even further by the fact that the chimpanzee genome is largely still a rough draft with numerous unsequenced gaps. In fact, a large number of studies, based on flow cytometry of nuclei and cytogenetic analyses of banding patterns, estimate that on average the chimpanzee genome is about 8% larger than human with significantly more heterochromatic DNA (Formenti et al., 1983; Koop et al., 1986; Pellicciari et al., 1982; Pellicciari et al., 1988; Pellicciari et al., 1990a; Pellicciari et al., 1990b; Seuanez et al., 1977). At present, it appears the alignable regions of the human and chimpanzee genomes are on average about 88% similar (Tomkins, 2015b). Nevertheless, there are many regions of apparent similarity between the genomes that are about 98% identical. It is these regions that are typically compared by evolutionists because they are conducive to hypothetical analyses regarding selection.

One of the features of the human genome that has been of particular interest to evolutionists during the past decade is termed human accelerated regions (HARs). These regions are a double-edged sword for the evolutionary paradigm in that they are both highly conserved (similar across taxa) yet markedly different in humans compared to other animals (particularly chimpanzees). Therefore, there is interest in finding such sequences and functionally characterizing them, as such sequences may help us understand what makes us uniquely human.

The detection of alleged accelerated regions of evolution assumes that evolution on a grand scale has actually occurred and requires a significant amount of hypothetical modeling. Under this assumption, DNA substitution rates are estimated based upon highly similar genomic regions from humans, great apes, and other vertebrates. These regions are so similar that they generally do not contain many sequence gaps (insertions or deletions) between taxa. In other words, the differences are primarily in single bases, called substitutions.

Early Discoveries of HAR and the Enigma of HAR1

The first popularized discovery of a HAR (demarcated HAR1) was a 118 base pair (bp) region that showed 18 base substitutions compared to its counterpart in the chimpanzee genome (Pollard et al., 2006a). When this region was assessed for variability among humans, it was found to be fixed in human populations (nonvariable). Making this discovery even more remarkable was that when the same genomic segment from chimpanzee and chicken were compared, there was only a 2 base difference out of the 118 bases. In the evolutionary mindset, the region clearly was highly conserved across taxa, but why was it so different between humans and chimpanzees? Hence the name human accelerated region is based on the evolutionary belief that it must have changed very rapidly after humans diverged from chimps. While scientists found the data to be especially intriguing, the results defied the evolutionary paradigm of slow and gradual evolution of the genome.

Even more intriguing was the fact that homologs for HAR1 could not be found in frog or any fish genome (Pollard et al., 2006a). Therefore, since it was functional and present in a chicken-like common ancestor (presumably about 310 million years ago according to evolutionary theory), then it originated "suddenly" on the evolutionary scene in some vertebrate ancestor about 400 million years ago. In this light, the HAR1 sequence appeared suddenly in vertebrates with no evolutionary precursor.

However, the homology mystery does not stop here. As it turns out, the HAR1 region is a part of an overlapping set of genes called HAR1A and HAR1B (previously referred to as HAR1F and HAR1R). Whereas the HAR1 region itself is highly conserved across taxa, even in chickens, the larger gene region of which it is only a small part is highly *nonconserved* and is very different between vertebrate taxa. So how could one small isolated segment in this region stay relatively the same during millions of years of evolution, while the surrounding region that it is intimately connected with changed so markedly? In fact, even in rhesus (a monkey) most of the entire HAR1A/B gene region of approximately 9,000 bases is almost completely unalignable to human (Pollard et al., 2006a).

The HAR1A and HAR1B genes produce noncoding RNAs and are expressed in the developing neocortex (Pollard et al., 2006b). As it turns out, the 18-base difference between the human and chimpanzee versions of the HAR1 gene lead to remarkably distinctive secondary structures, as shown in Figure 1 and described in detail as a result of a thorough

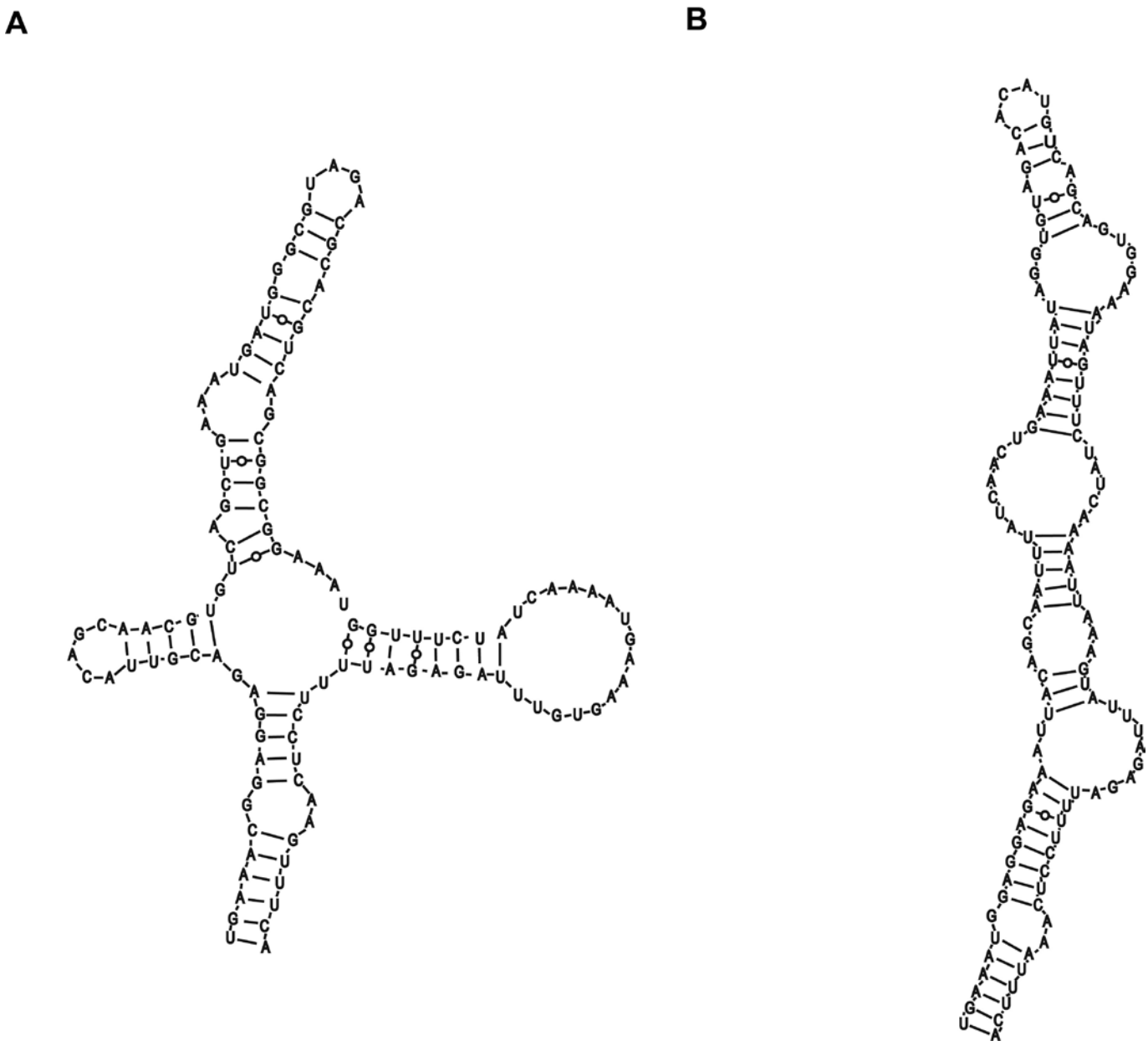

Figure 1. The secondary structures for the human HAR1 RNA (A) and the chimpanzee HAR1 (B). Notice that the difference in sequence results in a molecule with a significantly different shape. Based on their assumption of universal common ancestry, evolutionists believe this region of the genome underwent rapid evolution after humans split from chimps. Figure was adapted from Beniaminov, et al., 2008.

biochemical investigation performed several years after the original discovery of the gene (Beniaminov et al., 2008). The chimpanzee HAR1 RNA adopts a long hairpin structure, while the human HAR1 RNA forms a completely different cloverleaf structure. These dramatically different configurations are clearly associated with taxonomic specificity and function.

Other HAR Discoveries

At about the same time the discovery of HAR1 was being announced, several other reports were published describ-

ing larger genome-wide investigations of accelerated noncoding regions in humans and other vertebrates (Pollard et al., 2006a; Prabhakar et al., 2006). Prabhakar et al. (2006) compared the conserved noncoding regions (CNS) in humans, chimpanzees, and mice with the result that none of the overall patterns across lineages conformed to the grand evolutionary paradigm (inferred evolutionary trees). They also found that the CNS regions were heavily enriched near neuronal cell adhesion genes (cadherins, protocadherins, contactins, and neurologins) in chimpanzees and humans, but they were not in mice—a clear anomaly for the overall mammalian evolutionary model. Furthermore, disparate evolutionary results were obtained between humans and chimpanzees, to which the authors responded, "This suggests independent accelerated evolution of neuronal cell adhesion functions in the human and chimpanzee lineages" and "It is unlikely that acceleration of neuronal adhesion CNSs in humans and chimpanzees resulted in the same neuronal phenotypes, because the CNSs accelerated in the two lineages are largely disjoint and would therefore have had different consequences for brain development and cognitive function." In the end, they finally identified 992 CNS regions that were human-specific and attributed to advanced neural capabilities in humans versus other primates.

In the study by Pollard et al. (2006a), the researchers focused specifically on 202 carefully selected candidate regions they claimed had been under strong negative selection, which is defined as the removal of alleles that are deleterious (also referred to as purifying selection). However, it should be noted that selection is not actually observed in cases like this but merely inferred based on the variability of the compared sequences in question. These regions are essentially nonvariant in humans but are significantly different from their counterparts in chimpanzees. Thus, it is thought they evolved quickly and then became indispensable to the human lineage and further evolution was shut down (constrained) in these regions due to the newly acquired functional importance of the sequence. This is essentially the mindset of the evolutionist in evaluating such sequences in a comparative sense.

The closest genes to these CNS regions in the Pollard et al. (2006a) study were enriched for transcription factors, DNA-binding proteins, and regulators of nucleic acid metabolism; they were shown to be statistically correlated with high levels of association to cellular processes involved with development, neurogenesis, and morphogenesis.

Functionality of HARs

To help determine the functionality of HAR sequences, a recent study compiled a comprehensive list of 2,649 noncoding HARs, combining data from over five studies (Capra, et al., 2013). They then determined functionality for these regions using data from the ENCODE project for transcription factor binding, histone modifications, and other indicators of chromatin state. They also analyzed positional data to determine the genomic landscape in which these sequences were situated. Using this combinatorial data, they found that at least 30% were clearly functional enhancer elements, with more than half (~60%) of the elements showing enhancer activity in at least one type of cellular context. Thus, well over half of these types of sequences appear to function as enhancers.

Enhancers are short 50 to 1500 bp regions that bind with transcription factors to activate transcription of a gene (Capra, et al., 2013). They are generally cis-acting, and can be located up to 1 million bp away from a gene that they regulate, upstream or downstream from the gene's start site and in either the plus or minus strand orientation. Over 40,000 enhancers have been catalogued in the human genome, and many are related to developmental processes (Andersson et al., 2014). Enhancer HARs have been found to be enriched in both intergenic regions across the genome and intragenic regions inside introns (Capra et al., 2013). The HARs in the Capra et al. (2013) study were on average 257 bp long, and most were within 1 Mb of a known gene, with 19% of these genes encoding transcription factor binding sites. So clearly these are important regulatory sequences in the overall scheme of gene and genome regulation.

Interestingly, the researchers of the Capra et al. (2013) study also tested a small number of enhancers from both human and chimpanzees in transgenic mice. While this effort was not exhaustive in scope, a significant number of enhancers from both human and chimpanzee drove markedly different expression patterns in developing mouse embryos, indicating significant differences in functionality. At present, ten different HAR sequences have been tested in functional assays such as this in a variety of studies. Most were implicated in brain development, while two enhancer HARs were implicated in limb and eye development (Kamm et al., 2013a; Kamm et al., 2013b; Lindblad-Toh et al., 2011; Pollard and Franchini, 2015; Rossant, 2015; Sumiyama and Saitou, 2011). Of course, a limitation for studies like this (testing foreign constructs in transgenic mice) is that they cannot truly recapitulate the true function of a human or chimpanzee DNA regulatory element - they can only show how differences in the sequence produce different functional outcomes, and in some cases, what types of tissues their expression may correspond with (Pollard and Franchini, 2015).

The developmental process itself is orchestrated through complex regulatory networks that are tightly regulated and highly constrained (Davidson and Erwin, 2006). All types of DNA sequences, both developmental genes (e.g., transcription factors) and regula-

tory sequences (like HARs) play major roles in development. Transcription factor genes are highly pleiotropic. In other words, they participate in multiple independent processes, both spatially and temporally. In contrast, noncoding regulatory sequences, such as enhancers, tend to function in a more limited number of cell types and processes. They also tend to operate more in an additive manner—combining together to control the complex expression patterns of developmental genes such as transcription factors (Noonan and McCallion, 2010).

Evolutionists seem to think this highly efficient, yet complex system of regulatory and developmental gene modules is somehow conducive to evolution (Carroll, 2008), despite the fact that the evolutionary model cannot account for their origin and disrupting these sequences often leads to serious problems, including catastrophic system failure. The most obvious and parsimonious explanation is that this type of complex modularity in code is analogous to human-engineered computer software that is both modular and often object oriented in its construction, where methods (functions) can be called in an additive fashion to instances of an object, thereby controlling and altering its output in the overall program. The ingenious design patterns in the genome are truly spectacular, but the significance of the implications are generally missed by those with the mindset of an evolutionist entrenched in naturalistic thinking.

Deleted Accelerated Regions in Humans?

Not only are the presence of HARs an enigma for the evolutionary paradigm, but so is the absence of such regions when comparing taxa. One must keep in mind that within the evolutionary mindset, these regions are allegedly under strong selective constraint and thus differ very little in their sequence between taxa. Thus, their sudden "disappearance" from a genome in the grand evolutionary tree of life is difficult to account for.

In a large genome-wide survey for highly conserved sequences absent in human but present in chimpanzee and other mammals, researchers found 510 such sequences, all of which (except for one) mapped to noncoding regions of the human genome (McLean et al., 2011). Several of these allegedly deleted regions in humans corresponded to apparent enhancer elements present in the genomes of other mammals. The conserved chimp and mouse elements, along with deletions of them, were tested in transgenic mice. It was found that in transgenic mouse embryos, one of the deletions removed sensory vibrissae (tactile hair on the head, e.g., whiskers) and a penile spine enhancer element from a homolog to the human androgen receptor gene. The alleged deletion of this element in humans is quite large and corresponds to about 60,000 base pairs. Another supposed deletion was found to correspond to the removal of a forebrain subventricular zone (paired brain structure situated throughout the lateral walls of the lateral ventricles) enhancer element in transgenic mice.

This original study of these two specific highly conserved enhancer elements (present in other mammals but mysteriously missing in humans) were followed up several years later in another study (Reno et al., 2013). Using a combination of large-scale database sequence analyses and direct DNA analysis of the genomes in question, researchers demonstrated that the penile spine/vibrissa enhancer element was missing in all human genomes surveyed, and also in the archaic human genomes of Neandertal and Denisovan, but present in DNA samples of chimpanzees and the other great apes and other primates that exhibit some form of penile spine and facial vibrissae. The other 508 conserved elements supposedly deleted during evolution in a common ancestor of humans and chimps remain to be functionally characterized.

Another major evolutionary anomaly with overall patterns of these conserved noncoding elements in regard to their alleged mysterious deletion in major animal lineages is that the patterns are erratic and the supposed sudden absence of these elements are said to represent "independent losses" and are "not uniform" (Hiller et al., 2012). In other words, they do not form consistent evolutionary trees regarding their presence and absence across lineages. Hiller et al. (2012) explained the majority of these aberrant patterns by claiming that many of the lost elements were slightly less evolutionarily constrained and shorter and thus must have been less pleiotropic. Enhancer elements for the most part do appear to be less pleiotropic on average than protein-coding developmental genes (Carroll, 2008; Wray, 2007). But this is not really a satisfactory reason for their erratic presence or absence across major lineages, given their functional importance and the alleged evolutionary constraint ascribed to them.

Materials and Methods

To supplement the literature review in this report and to fill in a glaring gap within the HAR research community, the phylogenetic analysis of 105 different HAR sequences was undertaken for the following taxa: human, chimpanzee, gorilla, orangutan, macaque, mouse, elephant, cow, and chicken. The approach to acquiring the data was as follows: (1) Using url links at <docpollard.com/HARs.html>, each individual HAR sequence was followed to its respective hg17 "Vertebrate Multiz Alignment & Conservation" view at the UCSC genome browser (http://genome.ucsc.edu). (2) I then went to "View" then "Other genomes (Convert)"and used the more current hg19 version for my alignment data (adjusting the browser view for the species listed above). (3) I clicked on the

alignment link for each respective HAR gene and downloaded the subsequent alignment view as a plain text file. (4) I processed each downloaded text file with a Python script I wrote that converted it into standard FASTA file format.

The phylogenetic analysis pipeline was performed as follows: (1) The MUSCLE v3.8.31 program (Edgar, 2004) was used on the set of 105 FASTA files produced as described above, yielding multiple DNA alignment output files in FASTA format (MUSCLE default parameters). (2) The MUSCLE program was used again to produce neighbor-joining trees (parameters: -maketree, -cluster neighbor joining). (3) These individual tree files were further text-processed and combined into a single multitree specialized Newick-style file required by the tree comparison program topd_v3.3.pl (Puigbo et al., 2007). Steps 1 through 3 were performed via a Python pipeline script written by this author with MUSCLE being employed as system calls within Python. The resulting Newick-style, multitree file processed with the same Python pipeline script was analyzed with topd_v3.3.pl but also evaluated for commonalities in topology by basic UNIX shell programs such as uniq and grep, the latter was employed with a variety of different regular expressions for pattern matching. Phylogenetic trees, including those for this publication were drawn and printed to file using the Phylodendron Phylogenetic tree printer program (http://iubio.bio.indiana.edu/treeapp/treeprint-form.html). The two python scripts for processing the UCSC "Vertebrate Multiz Alignment & Conservation" text files and implementing the MUSCLE-tree pipeline, along with the FASTA files used in this study have been posted at github (https://github.com/jt-icr/har_code.git).

Results

The results of the phylogeny analyses of the 105 HAR sequences tested were inconsistent with the grand evolutionary paradigm, in complete accordance with all of the other data discussed above. Based on analyses with the topd_v3.3.pl program, which exhaustively compares tree topologies to each other using a variety of algorithms, there was no evidence for a unified evolutionary tree in this data set. These trees did not support the inferred evolutionary phylogeny for the species tested.

A sampling of the discordant trees is shown (incorporating genetic distance) in Figure 2. Most notable among these trees are those for HAR1 and HAR2, several of the best-studied HAR genes that are also noted for their evolutionarily unsupportive sequences (alleged acceleration in humans compared to chimpanzees). For HAR1, human and mouse cluster together in the same branch, as does elephant and chicken. The tree for HAR2 likewise is completely discordant with evolution, as human clusters with elephant.

As a whole, the different HAR genes gave widely different topologies. This is frequently observed and is attributed to incomplete lineage sorting, a rescuing device used by evolutionists to explain incongruent data. This type of evolution-negating pattern has been a common finding of studies analyzing many different genes, genomics regions, or even protein sequences (Degnan and Rosenberg, 2009; Hobolth et al., 2011; Pisani et al., 2012; Tomkins and Bergman, 2013). It appears the phylogenetic discordance for HAR sequences greatly exceeds that for other types of regions, such as protein-coding gene exons.

Even when analyzing subtrees within the data set, humans and chimpanzees clustered together on the same branch only on 15 occasions (14% of the trees). Gorilla and human clustered together in only 8 instances, and orangutans, supposedly more distant to humans than gorillas, clustered directly with human on 11 trees. Furthermore, a two-branch cluster with human and at least two apes (e.g. "[chimp, human] gorilla," "[gorilla, human] chimp," etc.) was only seen on 13 occasions. In other words, if two great apes occupied a branch with humans, it was typical for the other to be located on a completely separate branch.

Summary

Human accelerated regions (HARs) are noncoding DNA sequences in the genome that, according to evolutionary reasoning, changed very little over the course of animal evolution but mysteriously and quite suddenly experienced a "burst" of change since the alleged divergence of humans from chimpanzee. These HAR-type sequences also appear suddenly in assumed vertebrate lineages with no prior evolutionary history, while others disappear and then reappear.

In humans and several other mammals, many of these HARs are being functionally characterized as enhancer elements, developmental gene regulatory elements, and even noncoding RNA genes. Many of them are also associated with a wide variety of important neurological traits unique to the humans. Evolutionists claim that the lack of variation in these sequences among other animals is due to "conserved function." Of course, very little is actually known about what these sequences are doing in the different kinds of animals in which they are found. In reality, we are just beginning to discover what they are doing even in humans.

In addition to the alleged "accelerated" evolution of these sequences within the human genome, this study shows that HAR genes show a pattern inconsistent with evolutionary predictions about the common ancestry of vertebrate lineages; this pattern is typically explained away as incomplete lineage sorting. The experimental data presented in this report shows that these alleged highly conserved sequences are discordant with classic evolutionary phylogenetic analyses. The analysis of 105

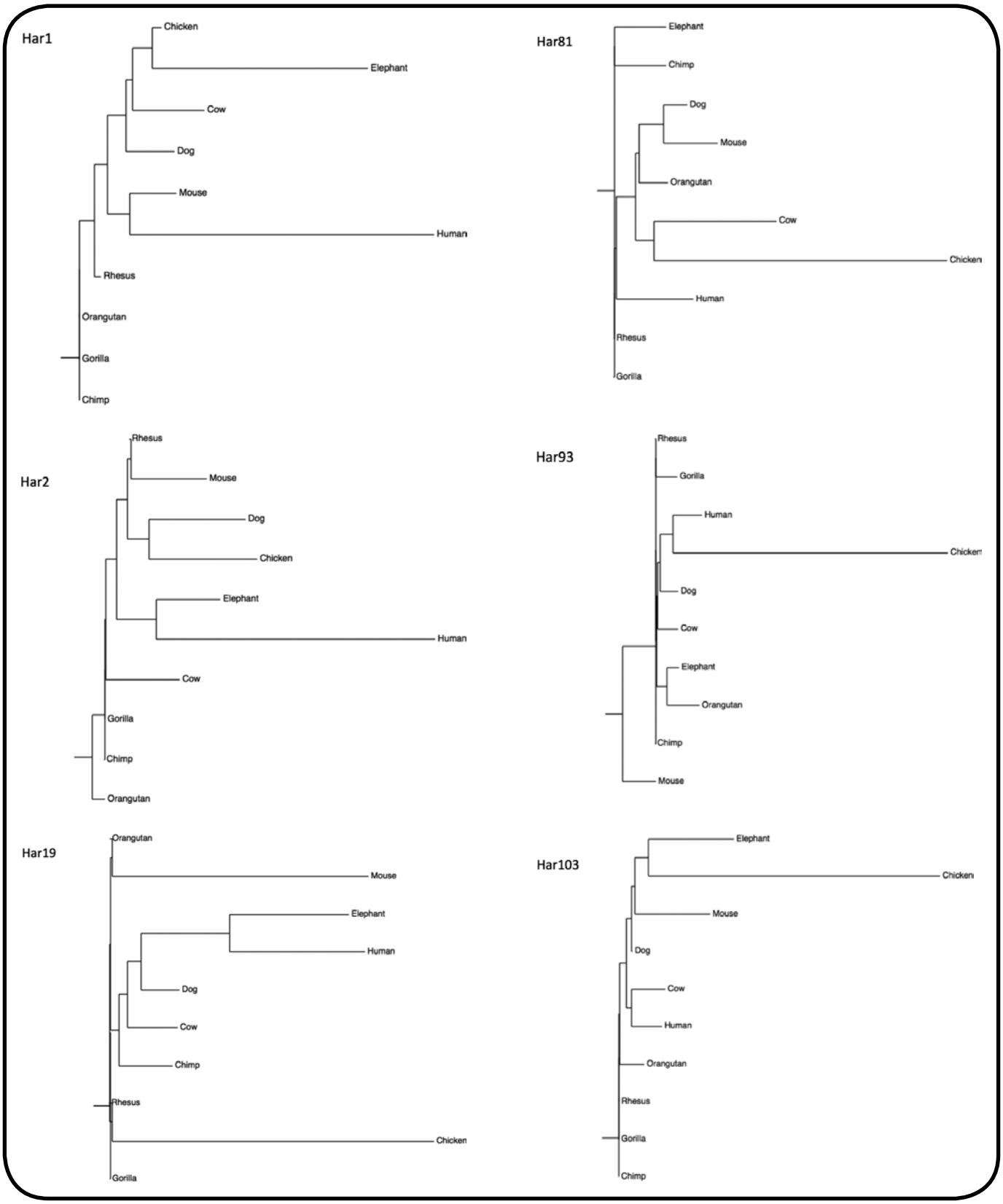

Figure 2. A selection of six different neighbor–joining trees from the 105 HAR phylogenies produced using MUSCLE program. Philodendron was used to draw the trees. Notice how inconsistent the results are, showing that the hypothesis of universal common ancestry is not supported by these genetic data.

different HAR genes from 10 different vertebrate taxa, including humans and the great apes, show extremely discordant evolutionary trees.

So what can we make of all this evolutionarily incongruent data surrounding HARs? Cleary, the most parsimonious answer is that they represent designed functional mammalian genetic elements that encode the novel phenotype of mankind. This is consistent with humans being uniquely created in the image of God, as clearly stated in the Bible. There is no evidence that these human-specific sequences evolved at any level or that they experienced a "burst of changes in humans since divergence from chimpanzees."

The standard explanation for HARs is also clearly falsified by recent research that has shown that for any mammalian species there is a *profound waiting time problem* associated with establishing new traits that require multiple new mutations (Sanford et al., 2015). Even establishing two codependent mutations in a hominin population is extremely problematic—requiring tens of millions of years. Since HAR genes are different at many nucleotide positions, the hypothesis that HAR genes arose very suddenly in just a few million years due to accelerated evolution is not even remotely credible.

In Psalm 139:14, 16, it is stated: "I will praise thee; for I am fearfully and wonderfully made ... and in thy *book* all my members were *written*." The Hebrew word for book is *siphrah*, which means a writing or document and by implication, a book, letter, or scroll. The Hebrew word for written is kâtab, which means to write, describe, inscribe, prescribe, or subscribe. We now know from the study of genetics and genomics that the genome is, in fact, a highly complex multidimensional document written in multiple codes and languages that we are now only beginning to understand (Tomkins, 2015a). Needless to say, this kind of handiwork far exceeds the abilities of even humans to engineer (or even fully understand). It clearly points to the Creator described in the Bible.

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