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Genetic Differences between Humans and Great Apes

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The remarkable similarity among the genomes of humans and the African great apes could warrant their classification together as a single genus. However, whereas there are many similarities in the biology, life history, and behavior of humans and great apes, there are also many striking differences that need to be explained. The complete sequencing of the human genome creates an opportunity to ask which genes are involved in those differences. A logical approach would be to use the chimpanzee genome for comparison and the other great ape genomes for confirmation. Until such a great ape genome project can become reality, the next best approach must be educated guesses of where the genetic differences may lie and a careful analysis of differences that we do know about. Our group recently discovered a human-specific inactivating mutation in the CMP-sialic acid hydroxylase gene, which results in the loss of expression of a common mammalian cell-surface sugar throughout all cells in the human body. We are currently investigating the implications of this difference for a variety of issues relevant to humans, ranging from pathogen susceptibility to brain development. Evaluating the uniqueness of this finding has also led us to explore the existing literature on the broader issue of genetic differences between humans and great apes. The aim of this brief review is to consider a listing of currently known genetic differences between humans and great apes and to suggest avenues for future research. The differences reported between human and great ape genomes include cytogenetic differences, differences in the type and number of repetitive genomic DNA and transposable elements, abundance and distribution of endogenous retroviruses, the presence and extent of allelic polymorphisms, specific gene inactivation events, gene sequence differences, gene duplications, single nucleotide polymorphisms, gene expression differences, and messenger RNA splicing variations. Evaluation of the reported findings in all these categories indicates that the CMP-sialic hydroxylase mutation is the only one that has so far been shown to result in a global biochemical and structural difference between humans and great apes. Several of the other known genetic dissimilarities deserve more exploration at the functional level. Among the areas of focus for the future should be genes affecting development, mental maturation, reproductive biology, and other aspects of life history. The approaches taken should include both going from the genome up to the adaptive potential of the organisms and going from novel adaptive regimes down to the relevant repercussions in the genome. Also, as much as we desire a simple genetic explanation for the human phenomenon, it is much more probable that our evolution occurred in multiple genetic steps, many of which must have left detectable footprints in our genomes. Ultimately, we need to know the exact number of genetic steps, the order in which they occurred, and the temporal, spatial, environmental, and cultural contexts that determined their impact on human evolution.

More than 100 years ago Huxley and Darwin predicted the close evolutionary relationship of humans with the African great apes (Darwin, 1871; Huxley, 1863). Whereas the precise timing of the speciation events remains uncertain, there is general agreement that *Homo sapiens* shared a common ancestor with *Pan troglodytes* (the chimpanzee) and *Pan paniscus* (the bonobo) ~5–6 million years ago (Mya), with *Gorilla gorilla* (the gorilla) ~7–8 Mya, and with *Pongo pygmaeus* (the orangutan) ~12–13 Mya, with the divergence of chimpanzee and bonobo lineages estimated at ~2 Mya (Sarich and Wilson, 1967; King and Wilson, 1975; Goodman *et al.*, 1983; Caccone and Powell, 1989; Sibley *et al.*, 1990; Arnason *et al.*, 1996; Ruvolo, 1997; Takahata and Satta, 1997; Stewart and Disotell, 1998; Goodman, 1999; Satta *et al.*, 2000). Most intriguing is the fact originally emphasized by King and Wilson, that "the genes of the human and the chimpanzee are as similar as those of sibling species of other organisms" (King and Wilson, 1975). Indeed, if taxonomic classification were based solely upon genomic DNA sequence similarity, the nearly 99% identity of human, chimpanzee, and bonobo genomes would require a reclassification of the latter two into the genus *Homo* (Goodman, 1999). However, while similarities abound in the biology, life history, and behavior of humans and

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great apes, there are also many striking differences that must be explained at the genetic level. We are now at the verge of obtaining the complete sequence of the human genome (Collins and Jegalian, 1999; Venter *et al.*, 1998). In trying to understand what aspects of this massive collection of sequence data relates to "being human," a logical approach would be to use the chimpanzee genome for comparison and the other great ape genomes for confirmation. The call for such a great ape genome project (or Human Genome Evolution Project) has already been made (McConkey and Goodman, 1997; Vigilant and Paabo, 1999; Paabo, 1999; McConkey *et al.*, 2000), but such a project is still far from reality. It has also been emphasized that such a project needs to be complemented by a more detailed understanding of the phenotypes of the great apes, i.e., a "great ape phenome project" (Varki *et al.*, 1998). Until such projects become feasible, the next best approach must be educated guesses of where the genetic differences may lie and a careful analysis of the differences that we already happen to know about.

Our group recently discovered a human-specific inactivating mutation in the CMP-sialic acid hydroxylase gene, which results in the loss of expression of an otherwise common mammalian cell-surface sialic acid (*N*-glycolyl-neuraminic acid) throughout all cells in the human body (Chou *et al.*, 1998; Muchmore *et al.*, 1998). Evaluating the uniqueness of this finding has led us to explore the existing literature on genetic differences between humans and great apes. The aim of this brief review is to present and discuss a listing of currently known differences and to suggest avenues of research for finding others. In looking for differences it should be kept in mind that the appearance of new phenotypes can result not only from gene duplication and changes in gene regulation, but also from the loss of gene functions (Olson, 1999). The evolution of multigene families, such as the genes for immunoglobulin, olfactory receptors, and major histocompatibility antigens, appears to have been molded by frequent gene loss following duplication, a process known as "birth-and-death" (Nei *et al.*, 1997).

Humans and great apes (collectively known as the hominoids) share many derived characteristics and genetic similarities. We focus here only on uniquely human genetic traits, i.e., features of the genome unique to the human species, which have emerged since humans and chimpanzees/bonobos last shared a common ancestor. Genetic changes that are defining of the human lineage (the hominid lineage, exclusive of the great apes) may include features universally present in all current-day humans, as well as polymorphic systems in which the uniquely human genetic trait is present in only a proportion of all humans. Among the many publications reporting human-specific or uniquely human genetic findings, closer inspection indicates that most represent human-specific polymorphisms rather than genetic traits universally shared by all humans. Because genetic changes involved in producing the human species must by definition be present in all humans, we shall focus more on such differences, of which remarkably few have been reported so far. Of course, relative to the amount of available data about the human genome, there is vanishingly little information about the genomes of great apes (whereas there are almost 3 million entries in GenBank for *H. sapiens*, there are ~1500 for *P. troglodytes*, <200 for *P. paniscus*, and <600 each for *G. gorilla* and *P. pygmaeus*). This bias in favor of human genetic data means that polymorphisms in the apes are underrepresented and that some of the reported differences may not be substantiated if additional great ape individuals are studied. This problem is aggravated by the fact that the genetic diversity of the great apes is significantly higher than that in humans for many genetic loci studied so far (Crouau-Roy *et al.*, 1996; Kaessmann *et al.*, 1999; Gagneux *et al.*, 1999). Much of this genetic diversity is threatened in light of the rapid decline of great ape populations in the wild, which adds further urgency to the study of great ape population genetics.

Human uniqueness does not necessarily consist only of newly derived traits in humans. It is more likely that the retention of ancestral traits in combination with genetic changes unique to the human lineage have produced our species. Because both *Pan* species are more closely related to *Homo* than to other apes, traits that they share with the other apes can reasonably be considered ancestral, rather than convergently evolved. Also, if the rate of genome evolution was constant in recent hominoid evolution, only about half of the genetic differences observed between chimpanzees and humans (i.e., about 0.7% at the level of genomic coding sequences and regulatory elements) would have occurred in humans since the last common ancestor (Saitou, 2000).

The human genetic differences that have been reported so far are grouped by categories below in a somewhat hierarchical approach, from DNA packaging in chromosomes, to the abundant repeated elements, down to the level of gene families, single genes, and their regulatory sequences. Whereas this is not meant to be an exhaustive listing, we have tried to reflect most findings of potential interest. Furthermore, the presentation is biased toward human–chimpanzee comparisons, because there are more published data on this great ape. The categories also correspond roughly to the mutational processes that would have produced the differences: chromosomal rearrangements by fusion, transposition, and inversion; the amplification and dispersion of transposable elements and endogenous retroviruses (ERVs); the duplication of genes; and insertions, deletions, and point mutations. Many of these types of mutations have been documented in

living humans, in whom they can cause disease ranging from developmental impairment to autoimmune disease and cancer. There is good reason to believe that the same processes sometimes result in new species.

Cytogenetic Differences

Chromosomal differences were the first genetic changes that became directly observable. The great similarity of the karyotypes of humans and the African great apes was pointed out many years ago (Yunis *et al.*, 1980; Yunis and Prakash, 1982). Conventional banding patterns using a variety of dyes as well as fluorescent *in situ* hybridization using large yeast or bacterial artificial chromosomes have been successfully used to detect sites of rearrangement, or "evolutionary breakpoints." The human-specific changes that are currently known are a telomeric fusion between two ancestral chromosomes to form human chromosome 2 (chimpanzee chromosomes 12 and 13, gorilla chromosomes 12 and 14) (Yunis and Prakash, 1982), pericentric inversions on chromosomes 1 and 18, and redistribution of heterochromatin on several chromosomes (Yunis and Prakash, 1982; Nickerson and Nelson, 1998; McConkey, 1997; Meneveri *et al.*, 1995; Archidiacono *et al.*, 1995; Tanabe *et al.*, 1996). There is also evidence for extensive intrachromosomal exchange at the periphery of alpha satellite DNA involving non-alpha satellite portions of the genome during hominoid evolution (Horvath *et al.*, 2000) (alpha satellite DNA consists of highly repetitive tandemly repeated 169- to 172-bp long sequences located at the centromeric region of primate chromosomes). Rearrangements and amplification of gene families have also occurred in the nonrecombining region of the Y chromosomes of hominoids (Glaser *et al.*, 1998), as well as an X–Y transposition followed by Yp inversion through LINE-LINE recombination (Schwartz *et al.*, 1998). A subterminal satellite located adjacent to telomeres in chimpanzees is absent from the human genome (Royle *et al.*, 1994).

Chromosome ends are regarded by some as hotspots for genome evolution (Monfouilloux *et al.*, 1998). An example may be the recent duplication of olfactory receptor genes in those generally gene-rich regions unique to humans (see below). However, a recent claim that telomeres are smaller in humans is confounded by the fact that great ape tissue DNA was compared with DNA from cultured human cell lines (Kakuo *et al.*, 1999). A comparison with similarly collected human tissue DNA seems worthwhile. Studies are currently underway in several laboratories to isolate and sequence the "breakpoint" regions involved in the fusions and inversions mentioned above. The hope is that one or the other of these will affect the structure and/or expression of specific genes that contribute toward human uniqueness. The impact of such chromosome rearrangements on gene activation and regulation remains unknown, but it has been suggested that it could have played a role in reproductive isolation between hominoid species.

Repetitive Genomic DNA and Transposable Elements

More than 40% of the human genome consists of transposable elements known as long (6- to 8-kb) and short (130- to 300-bp) interspersed nuclear elements (LINE and SINE, respectively) (Bonner *et al.*, 1982; Holmes *et al.*, 1994; Zhu *et al.*, 1994a; Kim *et al.*, 1999; Smit, 1999). SINEs share 3′ sequence homology with LINEs. LINEs are thought to be older and carry their own reverse transcriptase, on which SINEs also depend for transposition (Kim *et al.*, 1999; Smit, 1999). The most abundant class of SINEs are the primate-specific *Alu* elements of which there are more than 500,000 copies scattered throughout the human genome (Jurka and Smith, 1988) (these elements are derived from tRNA genes and are so called because they can be excised by the use of an *Arthrobacter luteus Alu I* restriction enzyme). One particular subset of *Alu* elements with very little sequence divergence and a common ancestor estimated at less than 2 Mya is unique to humans (Hamdi *et al.*, 1999; Leeflang *et al.*, 1993). A uniquely human insertion of an *Alu* element occurred in an intron of the alpha-fetoprotein-encoding gene (Nishio *et al.*, 1995). Another human-specific insertion is that of SINE R.C2, a member of the human endogenous retrovirus-K family (HERV-K) in an intron of the complement gene C2 (Zhu *et al.*, 1994b). Differences in alphoid repeat DNA in the centromere region of homologous chromosomes have also been reported (Jorgensen *et al.*, 1992). There are several sporadic examples of disruption of gene function in humans due to the insertion of repetitive elements, such as neurofibromatosis type 1, where an *Alu* insertion in an intron results in a splice site alteration, loss of an exon, and shift in reading frame of the NF1 gene (Wallace *et al.*, 1991). It is becoming evident that in addition to their so-called parasitic characteristics and their ability to cause disease, transposable elements (and endogenous retroviruses, see below) can play important roles in genome evolution of their hosts by affecting the structure and expression of endogenous genes (Kidwell and Lisch, 2000; Hamdi *et al.*, 2000). There doubtlessly are more insertions with effects on human gene expression—however, a specific change due to such elements affecting all humans and not great apes has yet to be found.

Endogenous Retroviruses

Endogenous retroviruses are derived from ancient germline infections by exogenous retroviruses. The fact that endogenous retroviruses may convey partial resistance to novel exogenous retrovirus infection (Larsson and Andersson, 1998) may explain why germline infections have become fixed by natural selection in ancestral populations. At least 1% of the human genome consists of endogenous retroviruses which have a size

typical of retroviruses (9–12 kb) with each end consisting of a 1-kb long-terminal repeat (LTR), also characteristic of retroviruses. Numerous copies are found in primate genomes, both full-length sequences and a much higher number of solitary long-terminal repeats (solo LTRs) (Nei *et al.*, 1997; Leib-Mosch *et al.*, 1993). The potential importance of endogenous retroviruses and their high-copy-number solo LTRs for primate genome rearrangements and the spread of *Alu* repeats has recently been highlighted (Sverdlov, 1998, 2000). Transpositions of LTR and ERV are ongoing in all hominoid lineages today and the importance of the process for speciation is not well understood. Humans are hosts to certain human-specific integrations of human retrovirus-K endogenous retrovirus family (HERV-K cluster 9). Judging from the low sequence divergence between elements, these have entered the human genome quite recently (Medstrand and Mager, 1998; Costas and Naveira, 2000). Similarly, LTR13 elements have been shown to be uniquely human (Liao *et al.*, 1998). The different great ape lineages seem to have experienced independent amplifications of HERV-K retroviruses and LTRs. As with repetitive elements, the insertion of endogenous retroviruses in new locations can disrupt gene function. Since retroviral sequences, and LTRs in particular, carry powerful enhancer signals, they can also have an impact on the regulation of nearby genes. To date, however, no single retroviral insertion site unique to humans has been shown to affect the expression of a relevant gene. The role played by ERVs during placental development and embryogenesis seems particularly worthy of comparative exploration for human-specific ERV activity (Kjellman *et al.*, 1999). A human placental syncytiotrophoblast protein called syncytin, is encoded by the envelope gene of HERV-W (Mi *et al.*, 2000). The data indicate that syncytin may be important in human placental morphogenesis, and information concerning the situation in great apes would be very interesting. Several retroviruses are found in all or some of the great apes but only to a limited extent in humans. These include lentiviruses such as the Simian Immunodeficiency viruses (SIV), oncoviruses such as primate T-cell lymphotropic Virus (HTLV), and foamy viruses (Spumaviruses). Whereas retroviruses of primate origin such as HIV and HTLV are pathogenic and increasingly widespread in humans, foamy virus has been found in only a small number of humans with a history of contact with great apes and could so far not be shown to be pathogenic in humans (Schweizer *et al.*, 1995; Goepfert *et al.*, 1996). Retroviruses insert a DNA copy of their genome into host cells and become transiently if not permanently part of the host genome. The absence of foamy virus (and until recently HIV) from humans indicates that our species, by an unknown mechanism, has evaded these viruses that are otherwise found in the African great apes, where they seem to be of low pathogenicity. There must be underlying genetic differences that determine the difference in response of humans and great apes to these viruses.

Polymorphisms

There are several well-known polymorphisms present in human populations but apparently absent in the chimpanzee. These include aspects of the MN blood groups (Onda *et al.*, 1993; Huang *et al.*, 1995; Xie *et al.*, 1997) (not fully polymorphic in chimpanzees) and ABO blood groups (Saitou and Yamamoto, 1997) (chimpanzees express only the A and O blood group, gorillas only B), inactivation of the chemokine receptor CCR5 in some humans (Voevodin *et al.*, 1998), differences in polymorphisms in the TCR VA and B loci in the two species (Jaeger *et al.*, 1998), and a haptoglobin 2 allele coding for a uniquely human alpha2 polypeptide (Maeda *et al.*, 1984; Maeda and Kim, 1990; Erickson *et al.*, 1992; Erickson and Maeda, 1994). There is a uniquely human polymorphic LTR insertion in the intronic region of HLA DQB1 gene (DQLTR3) (Donner *et al.*, 1999) and uniquely human *Alu* polymorphisms are also known (Arcot *et al.*, 1995). The HLA Class II DRB1 alleles (Bergstrom *et al.*, 1998) and the apolipoprotein E2 and E3 alleles (Hanlon and Rubinsztein, 1995) also seem to have arisen after the *Homo/Pan* divergence (chimpanzees express only the ancestral ApoE4 allele). The melanocortin 1 receptor (MC1R) is a regulator of eu- and phaeomelanin production in melanocytes, and mutations in this gene cause coat color changes in many mammals. This gene also shows considerable polymorphism amongst human populations, whereas the corresponding great ape gene seems to be identical to a "human consensus sequence" (Rana *et al.*, 1999).

As mentioned above, the apparent lack of many of these polymorphisms in great apes could result from a sampling bias in favor of humans. Regardless, as polymorphic genes, these loci show variability among humans and are therefore unlikely candidates for explaining differences between humans and other species. However, the processes generating such polymorphisms could be clues to what steps may have generated the species-defining differences. Of course, polymorphisms may indicate genetic systems that have diverged, and a nonfunctional allele in a population could rapidly get fixed if the loss of function of that gene was to become advantageous. As such, these polymorphic systems may be more relevant for the future evolution of humans and apes. Alternatively, the relative absence of polymorphisms can be an indication of purifying selection on a gene that has acquired increased importance in humans.

Gene Inactivation Events

We found only three examples of inactivation of functional genes in humans. The V10 variable gene of human T cell receptor gamma locus is inactive in humans

and is intact in great apes (Zhang *et al.*, 1996). A single point mutation in the V10 leader donor splice site converts this into a pseudogene in humans. In contrast, the functional splice site is present in the chimpanzee and the gorilla. Thus, V10+ gamma/delta T cells would be selectively missing in humans. However, this is one of several such V genes, and no specific consequence to immune function is known from the loss of this particular subclass of gamma-delta T cells. Similarly, one of the many olfactory receptor genes has been inactivated in humans but still carries an open reading frame in chimpanzees, while a different mutation has inactivated the same gene in gorillas (Rouquier *et al.*, 1998). The loss of this functional gene is thus not uniquely human, even if the specific mutation that caused it is. The only other known genetic change giving a human-specific loss of function is a 92-bp exon deletion in the human CMP-sialic acid hydroxylase gene (Irie *et al.*, 1998; Muchmore *et al.*, 1998). This results in a frameshift mutation that explains the lack of enzyme activity in humans. Genomic analysis indicates that the mutation occurred sometime after the divergence of hominids from great apes (~5–7 Mya), but before the common origin of all modern humans. Studies are currently underway to try and obtain a better timing of the event. The loss of enzyme activity leads to the absence of a particular sialic acid (*N*-glycolyl-neuraminic acid, Neu5Gc) on the surface of all cell types in humans and a secondary increase in the precursor molecule *N*-acetyl-neuraminic acid (Neu5Ac). This remains the only major biochemical difference known to date between chimpanzees and humans. Since this change affects the cell surface of almost all cell types in the body and could potentially alter cell–cell interactions (Varki, 1997), its biological implications are currently being explored. In this regard, a sialic acid-binding human macrophage receptor called sialoadhesin (siglec-1) strongly prefers Neu5Ac over Neu5Gc. Thus, human cells have a higher density of sialoadhesin ligands than great ape cells (Brinkman-Van der Linden *et al.*, 2000). Interestingly, sialoadhesin-positive macrophages in humans are found primarily in the perifollicular zone of the spleen, whereas in chimpanzees they also occur in the marginal zone and surrounding the periarteriolar lymphocyte sheaths. Also, whereas only a subset of chimpanzee macrophages express sialoadhesin, most human macrophages do (Brinkman-Van der Linden *et al.*, 2000). The biological consequences of these changes for macrophage biology remain uncertain. The absence of Neu5Gc in humans could also explain some differences in susceptibility to certain bacterial and viral organisms that use sialic acids as cognate ligands to attach to and gain entry into cells (Varki, 1997; Karlsson, 1998). For example, humans (unlike piglets and calves) do not appear to suffer from diarrhea caused by *Escherichia coli* K99, which requires Neu5Gc for recognizing gut epithelial cells (Kyogashima *et al.*, 1989), and the absence of Neu5Gc could potentially affect interactions with some strains of Influenza A and B viruses (Higa *et al.*, 1985; Ito *et al.*, 1997; Suzuki *et al.*, 1997). There are many other pathogens that utilize sialic acids as specific binding sites on mammalian cells (Karlsson, 1995, 1998; Varki, 1997; Ollomo *et al.*, 1997; Escalante *et al.*, 1995). In most such instances, the consequences of having Neu5Gc versus excess Neu5Ac have yet to be investigated.

Perhaps the most intriguing fact is that in a variety of mammals studied, including the chimpanzee, the amounts of NeuGc found in the brain are always very low, irrespective of the level of Neu5Gc present in other organs of the body (Tettamanti *et al.*, 1965; Ecsedy *et al.*, 1997; Mikami *et al.*, 1998; Muchmore *et al.*, 1998). The near absence of Neu5Gc from brains of other mammals correlates with very low levels of hydroxylase message expression in the brain (Kawano *et al.*, 1995). Thus, Neu5Gc is apparently not desirable in the mammalian brain and expression of the hydroxylase has been selectively suppressed in the brain for tens of millions of years of mammalian evolution. In humans, the last traces of Neu5Gc in the brain are eliminated, because of the genomic mutation in the hydroxylase gene. It remains to be seen whether this had any significant impact on human brain evolution. Finally, since humans show an immune reactivity to Neu5Gc, there are potential implications for the consumption of animal foods by humans and the use of animals as sources for recombinant therapeutics or organ transplantation.

Gene Sequence Differences

Known differences in DNA sequences of genes coding for functional proteins include a 12-bp deletion in the dopamine D4 receptor gene of chimpanzees and gorillas (Livak *et al.*, 1995) and a minimum of eight amino acid changes in the human melanocortin 1 receptor locus (Rana *et al.*, 1999). The dopamine D4 receptor is one of five receptors that are involved in mammalian dopaminergic pathways. The DNA sequence of this gene is known to be highly polymorphic in humans, with at least 25 alleles reported, which represent differences in the number and DNA sequence of a 48-bp (16-amino acid) repeat unit. However, chimpanzees and gorillas share a unique 12-bp deletion in the coding region of this gene that is outside the repeat-unit segment. Functional differences due to these differences remain to be studied.

Humans are also missing two ancestral exons in the tropoelastin gene (Szabo *et al.*, 1999). Unlike the case with the CMP-sialic hydroxylase gene mentioned above, this mutation is at a position coding for the carboxyl end of the protein and causes only minor changes in the protein product. The two missing exons (34 and 35) at the 3′ end of the gene are found in the elastin gene of other vertebrate species examined. Fur-

ther studies have shown that while the loss of exon 35 occurred before the divergence of Old World monkeys, the loss of exon 34 occurred only after the last common ancestor with chimpanzees. Each of the exon losses appears to have been facilitated by *Alu*-mediated recombination events. It is not known whether this resulted in any changes in the connective tissue or muscles of humans. The differing demands on biomechanics due to bipedalism come to mind in this context.

One particular set of genes that seem worthy of special attention consists of those that show a high rate of evolution, i.e., a rate of nonsynonymous nucleotide substitution exceeding that of synonymous substitutions, which is an indicator of adaptive selection (Stewart, 2000). In this regard, there appears to be a rapid evolution of male reproductive genes in primates that is particularly notable in lineages leading to humans and chimpanzees (Wyckoff *et al.*, 2000; Stewart, 2000). The significance of these findings for human uniqueness remains uncertain. Several other sets of sequence data are currently available for comparison. For example a recent reanalysis of the human/chimpanzee/gorilla clade is based on 45 mostly autosomal sequences for all African hominoids (Satta *et al.*, 2000). Comparisons of sequences between humans and apes for over 70 genes can also be found on the Silver Ape Genome web page of Saitou *et al.* (http://sayer.lab.nig.ac.jp/~silver/index.html) (Saitou, 2000). There are of course many scattered single-amino acid differences between these various chimpanzee and human proteins, but none have yet been shown to be of functional significance.

Gene Duplication

Duplication and gene loss cause differential expansions or contractions of gene families. Differences in the number of duplicated genes and pseudogenes have been reported in humans and chimpanzees. For example, chimpanzees have three copies of the RhD blood group gene (Westhoff and Wylie, 1996; Salvignol *et al.*, 1995; Apoil and Blancher, 2000) and an additional triosephosphate isomerase pseudogene (Craig *et al.*, 1991) in comparison to humans. The hominid lineage also underwent a duplication of V_k immunoglobulin light-chain genes (Ermert *et al.*, 1995). However, some humans carry only a single copy due to a subsequent loss of the duplication. Members of the olfactory receptor gene family have been duplicated and chromosomally rearranged in humans (Trask *et al.*, 1998). Whereas humans carry 7 to 11 copies of the olfactory receptor gene-containing block on 3q, 15q, and 19p, its counterpart is found at a single site in the chimpanzee and gorilla, in a different location from any of the sites in the human genome. Keratinocyte growth factor (KGF) gene amplification and dispersion also occurred during hominoid evolution, with humans having eight sites on five chromosomes, chimpanzees having five sites on five chromosomes, and gorillas having only four sites on four chromosomes (Zimonjic *et al.*, 1997). Two sequential duplications have been documented for the genes of the human high-affinity receptor for immunoglobulin G (FCGR1), and these were followed by a pericentric inversion causing humans to have three FCGR1 genes, one on the short arm and two on the long arm of chromosome 1 (Maresco *et al.*, 1998). Overall, the extent of this form of paralogous evolution of certain gene families in hominoids appears quite striking. However, there have not yet been any links to functional outcomes that can explain biological differences between humans and great apes. Tissue-specific expression studies of these gene products at different developmental stages seem warranted.

Among the many multigene families, the immune system gene cluster known as the major histocompatibility complex (MHC) in animals and human leukocyte antigen (HLA) system in humans shows massive differences in gene number between species and polymorphisms among humans. The recently sequenced human HLA region is over three million bases long and among others contains many genes involved in antigen presentation and processing (The MHC Sequencing Consortium, 1999). Many individual MHC genes are strictly conserved in all hominoids but the region has been involved in substantial amounts of combinatorial diversification. The HLA region in humans is characterized by a very high level of recombination (McAdam *et al.*, 1994). The MHC region in the primate genome is characterized by intense evolutionary dynamics, such as multiple insertion of HERV and *Alu* sequences, expansion and contraction by gene duplication and subsequent loss, and gene conversion as well as transspecific evolution and positive selection for diversity (Bergstrom *et al.*, 1999; Watkins, 1995). Chimpanzees and bonobos share identical HLA alleles after 2 million years of divergence, and some human alleles are more similar to chimpanzee and gorilla alleles than to other human alleles (Cooper *et al.*, 1998), many of which have coalescent times of $>$30 million years (Klein *et al.*, 1993). Uniquely human MHC genes will almost certainly be identified, as sequence from more great ape MHC genes is obtained. However, these will likely represent part of the overall polymorphism in immune response of the human population, rather than genes that dictate human uniqueness.

New Genes

Apart from additional copies within the multigene families mentioned above, there is no evidence for novel functional genes unique to the human lineage. However, an unusual insertion of 2.2 kb of noncoding DNA in the human genome (called HS5) was discovered by subtraction of human and chimpanzee genomic DNA (Ueda *et al.*, 1990). Chimpanzees simply lack

these 2.2 kb but show remarkably similar homologous flanking sequences. The fragment contains part of a LINE 1 family related sequence and another part with no known homology. The mechanism of HS5 insertion in the human genome and its chromosomal localization remain unknown and there is no known effect on the expression of nearby genes.

Single-Nucleotide Polymorphisms

The application of high-throughput DNA typing technology is rapidly producing a wealth of data on single-nucleotide polymorphisms (SNPs). A recent study identified hundreds of ancestral alleles by typing 10–20 individuals of each great ape species, as well as hundreds of humans (Hacia *et al.,* 1999). Polymorphisms shared between bonobos and humans all involved CpG dinucleotides, which are known for their instability. Some of the uniquely human SNPs may be relevant to our understanding of human evolution, especially if located in the enhancer regions of genes, where even single base pair changes could have caused major changes in gene expression.

Gene Expression Differences

Gene regulation is possibly the most important yet hardest type of difference to document. Apart from the technical difficulties of obtaining samples under similar biological circumstances and the instability of mRNA, the important differences could include spatial as well as temporal variation in gene transcription in addition to actual expression levels. One of the two relaxin genes shared by humans and chimpanzees appears to be expressed in the prostate and possibly the placenta of humans but only in the corpus luteum of chimpanzees (Evans *et al.,* 1994). Whereas monkeys seem to have only a single relaxin gene, humans and chimpanzees have two (H1 and H2, or gene 1 and gene 2). In humans, H2 relaxin is synthesized in the corpus luteum during pregnancy and is also found in the placenta and prostate, but the expression of H1 has been difficult to detect. Gene 1 in the chimpanzee codes for a type A chain similar to H1 but codes for a B chain of the H2 type, possibly due to a gene conversion event (the A and B chains arise from proteolytic processing of a single polypeptide precursor). Messenger RNA for this gene can be detected by polymerase chain reaction in the corpus luteum of the chimpanzee. Relaxin is a peptide hormone that has many known physiological effects on tissues of the reproductive tract, as well as other organs such as the heart and brain. It remains to be seen if subtle differences in relaxin gene expression are relevant to explaining human uniqueness. In this regard, it is of note that one of the actions of relaxin is to relax the female pelvic joint ligaments prior to the act of giving birth, a process that is much more traumatic in humans than in chimpanzees.

The lack of availability of high-quality cDNA libraries from great ape cells and tissues poses a serious obstacle to progress in this area (Ruvolo, 2000). The recent advent of micro-array and gene-chip technology promises to revolutionize the search for additional differentially expressed genes. However, apart from the problems with sample collection, steady-state mRNA expression levels do not always correlate with protein levels. Thus, this approach may need to be complemented by proteomics, which directly analyses the repertoire of proteins expressed at steady-state level. In this regard, we recently used proteomics to discover a down-regulation of plasma and cerebrospinal fluid expression of a protein that binds thyroid hormone and retinoids, in humans compared to chimpanzees and bonobos (P. Gagneux *et al.* unpublished). This finding has not yet been explained at the genetic level. However, given the many roles of thyroid and retinoid hormones in the development of the brain and other organs, it could be of potential importance.

Heterochrony, the process by which novel form and function is created via changes in the timing or duration of gene expression (and the resulting maturation schedule) may be of particular importance. This is indeed a well-known mechanism for evolutionary novelty and, in many respects, humans appear to be "neotenous apes," as judged from their extended period of dependency and slow physical and mental maturation (Dean and Wood, 1984). Thus, important differences may be totally missed because of time period chosen for gene expression studies (e.g., early development may not be included in the analyses). For example, maximum brain size is attained by about age 7 years in captive chimps and age 15 years in humans, a phenomenon that may partly explain why the final brain size of humans is so much larger (Herndon *et al.,* 1999). It may well be that the critical gene expression differences that result in such human uniqueness actually occur prior to birth.

Messenger RNA Splicing Differences

This is a mechanism for generating potentially different gene products using the same genome. The only example to date is a difference in number of isoforms generated by alternative splicing of the tyrosine hydroxylase gene in the brain, which generates increased heterogeneity in humans (Ichinose *et al.,* 1993). No functional consequences have been elucidated.

Methylation and Genomic Imprinting

There is currently no information available concerning any differences between humans and great apes in DNA methylation (Mochizuki *et al.,* 1996), a process that can affect gene expression, typically in the downward direction. Genomic imprinting is another way in which gene expression can be altered, in a manner specific to the inheritance from the maternal or paternal chromosomal complement. Mating systems may

have an impact on the generation of polymorphisms, as multiple paternity may favor the evolution of strong imprinting (Mochizuki *et al.*, 1996). It is not known whether the degree of genomic imprinting is comparable between humans and apes. Genomic imprinting could be an important cause for polymorphism within a species, but is less likely to explain interspecific differences.

Perspectives

Which of these reported differences can contribute to explaining the phenotypic differences between humans and great apes? Evaluation of the findings in all these categories indicates that the CMP-sialic hydroxylase mutation is the only one that is so far known to result in a global biochemical and structural difference between humans and great apes. Even in this case, any mechanistic relevance to human uniqueness has yet to be defined. Several of the other observations, such as the differences in the tropoelastin, keratinocyte growth factor, olfactory receptor, dopamine receptor, and relaxin genes, clearly deserve follow up. There is an acute need for more knowledge about great ape genomes before we can estimate how many genes are involved in the evolution of uniquely human characteristics. Ideally, one would want to have in hand not only the genomic sequences from multiple individuals of each species of great ape, but also those from some other well-studied primates, such as the baboon and the macaque. Meanwhile, the areas of focus for the future should include genes affecting development, mental maturation, reproductive biology, and other aspects of life history. Gene expression studies during ontogeny are also crucial and yet problematic because the relevant samples of great apes and human tissue samples are very difficult to obtain. In the United States, the current moratorium on the breeding of chimpanzees in NIH-funded facilities strongly limits the feasibility of developmental studies. Although several field studies with habituated animals are ongoing, the chances of obtaining tissue samples are small and involve numerous logistic difficulties. Increased interest by molecular biologists could, however, greatly facilitate the proper collection of fresh tissue samples from recently deceased animals at these study sites. Such studies may add a further (utilitarian) justification for the conservation of great apes, which are facing severe threats in all known habitats. Of course, great care is needed to avoid any potentially negative impacts of any such studies on the welfare of wild ape populations, which are dwindling rapidly due to other human activities. The large captive population of chimpanzees continues to provide sporadic tissue samples due to natural deaths of the animals. This population also provides certain "natural experiments," such as limited activity, changes in diets, and absence of reproduction. Coordinated efforts to make the appropriate samples available to different research groups could indeed provide much opportunity for research. Of course, since genetic manipulation of humans and great apes are ethically unacceptable, our final conclusions must be based only on indirect inferences and natural experiments.

It is also worth pointing out that we remain completely ignorant of the underlying genetic basis for notable differences in disease susceptibility between humans and chimpanzees, including the apparent rarity in chimpanzees of diseases such as falciparum malaria (Ollomo *et al.*, 1997), epithelial cancers (Schmidt, 1975; McClure, 1973), Alzheimer's disease (Gearing *et al.*, 1994), and AIDS (Novembre *et al.*, 1997). Exploring the MHC region of chimpanzees may well reveal the underlying causes of some of these differences. The olfactory receptors and the MHC genes also seem to be connected in interesting ways. For example, the mating system of each hominoid species is unique, and mate choice in humans seems to be impacted by olfaction of HLA diversity (Ober, 1999). Humans are also unusual in several aspects of reproductive biology, such as menopause, concealed ovulation, and the growth of breasts without lactation. Studies of the great ape genomes may thus teach us not only about human evolution and uniqueness, but also about the genetic basis of certain human diseases and about human reproductive biology (Varki, 2000).

Many "umbrella" hypotheses have been put forward to explain human uniqueness, mostly proposing novel aspects in the behavior and/or environment of hominid ancestors. These include bipedalism, rapid climate changes, spoken language, tool and weapon use, increased sociality, an aquatic phase, cooperative hunting, and the use of fire and cooking of food (Langdon, 1997; Wills, 1998). On the other hand, genetic processes such as chromosomal rearrangements and the amplification of transposable elements or endogenous retroviruses most likely have affected the genomes of our ancestors in profound ways that allowed subsequent novel adaptations. It is obvious that either approach, whether from the genome up to the adaptive potential of the organisms or from the novel adaptive regime down to the repercussions in the genome, is too simplistic. Organismic evolution is a simultaneous process in which genetic events can have immediate repercussions on the fitness of organisms and environmental selection pressures have immediate effects on which genes will be represented in future generations. By the same token, adaptation tends to lag, as it takes time for allele frequencies to change in populations. Any invoked selective regime must be shown to have been in place for a sufficient amount of time to allow the genetic changes to spread. The genetic changes on the other hand, must be shown to have occurred at the right time, i.e., prior to the novel adaptive landscape that favored them.

Any explanation of human uniqueness will also have

to take into account the sequential nature of such changes. As much as we desire a single explanation for the human phenomenon, it is clear from the fossil record that our evolution was due to multiple steps, some of which will possibly strike us as not very dramatic. Many of these must have left footprints in our genomes. Needless to say we would like to know the number of steps and their order. The temporal, spatial, environmental, and cultural contexts that determined their impact on human evolution cannot be ignored.

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