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Proteomic Comparison of Human and Great Ape Blood Plasma Reveals Conserved Glycosylation and Differences in Thyroid Hormone Metabolism

Pascal Gagneux,[1] Bob Amess,[2] Sandra Diaz,[1] Stephen Moore,[2] Thakor Patel,[2] Wolfgang Dillmann,[1] Raj Parekh,[2] and Ajit Varki[1]*

[1]*Department of Medicine and Glycobiology Research and Training Center, University of California at San Diego, La Jolla, California 92093-0687*
[2]*Oxford Glycosciences, Abingdon, UK*

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ABSTRACT Most blood plasma proteins are glycosylated. These glycoproteins typically carry sialic acid-bearing sugar chains, which can modify the observed molecular weights and isoelectric points of those proteins during electrophoretic analyses. To explore changes in protein expression and glycosylation that occurred during great ape and human evolution, we subjected multiple blood plasma samples from all these species to high-resolution proteomic analysis. We found very few species-specific differences, indicating a remarkable degree of conservation of plasma protein expression and glycosylation during ~12 million years of evolution. A few lineage-specific differences in protein migration were noted among the great apes. The only obvious differences between humans and all great apes were an apparent decrease in transthyretin (prealbumin) and a change in haptoglobin isoforms (the latter was predictable from prior genetic studies). Quantitative studies of transthyretin in samples of blood plasma (synthesized primarily by the liver) and of cerebrospinal fluid (synthesized locally by the choroid plexus of the brain) confirmed ~2-fold higher levels in chimpanzees compared to humans. Since transthyretin binds thyroid hormones, we next compared plasma thyroid hormone parameters between humans and chimpanzees. The results indicate significant differences in the status of thyroid hormone metabolism, which represent the first known endocrine difference between these species. Notably, thyroid hormones are known to play major roles in the development, differentiation, and metabolism of many organs and tissues, including the brain and the cranium. Also, transthyretin is known to be the major carrier of thyroid hormone in the cerebrospinal fluid, likely regulating delivery of this hormone to the brain. A potential secondary difference in retinoid (vitamin A) metabolism is also noted. The implications of these findings for explaining unique features of human evolution are discussed. Am J Phys Anthropol 115:99–109, 2001.

The closest living relatives of humans are *Pan troglodytes* (chimpanzee) and *Pan paniscus* (bonobo) (~98.5% genomic sequence identity), which in turn are related to *Gorilla gorilla* (gorilla) and *Pongo pygmaeus* (orangutan) (~98% and ~97% genomic sequence identity, respectively, with humans) (Sarich and Wilson, 1967; King and Wilson, 1975; Ruvolo, 1997; Takahata and Satta, 1997; Kumar and Hedges, 1998). The divergence of the orangutan lineage from the others is estimated at 12–14 million years ago (Goodman et al., 1998). This remarkable degree of sequence identity led to the suggestion that humans and chimpanzees are sister species, and the proposal that regulatory mutations would account for the major biological differences (King and Wilson, 1975).

Detailed genomic data for the great apes remain rather limited, but so far relatively few specific differences have been found between humans and the great apes (reviewed in Gagneux and Varki, 2001). At the level of chromosomes, there are differences in chromosomal packaging, centromeric inversions, redistribution of heterochromatin, and subterminal satellite DNA adjacent to telomeres (Yunis and Prakash, 1982; Nickerson and Nelson, 1998; Royle et al., 1994). At the level of genes there are multiple differences in the number of duplicated genes and pseudogenes (Westhoff and Wylie, 1996; Salvignol et al., 1995; Craig et al., 1991; Trask et al., 1998), as well as differences in copy number of retroviral or transposon sequences (Holmes et al., 1994; Zhu et al., 1994; Bonner et al., 1982; Jorgensen et al., 1992; Hamdi et al., 1999; Leeflang et al., 1993). However,

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*Correspondence to: Ajit Varki, Glycobiology Research and Training Center, University of California at San Diego-0687, La Jolla, CA 92093-0687. E-mail: avarki@ucsd.edu

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none of these differences have yet been linked to significant structural, biochemical, or functional changes.

There are numerous genetic polymorphisms present in humans but apparently absent in chimpanzees (Onda et al., 1993; Huang et al., 1995; Saitou and Yamamoto, 1997; Maeda et al., 1984; Maeda and Kim, 1990; Erickson et al., 1992; Erickson and Maeda, 1994; Voevodin et al., 1998; Dufour et al., 2000), as well as differences in polymorphisms which are present in each species (Jaeger et al., 1998; Bergstrom et al., 1998; Hanlon and Rubinsztein, 1995). Obviously, the apparent lack of many of these polymorphisms in chimpanzees could reflect a sampling bias in favor of humans. In any case, as polymorphic genes, these loci show extensive variability within species and therefore are unlikely candidates for explaining biological differences between the two species. At the level of gene expression, a difference in expression pattern is found with one of the two relaxin genes shared by humans and chimpanzees that appears to be expressed only in the corpus luteum of the latter (Evans et al., 1994). Differences in number of isoforms generated by alternative splicing of the tyrosine hydroxylase gene in the brain generate increased heterogeneity in humans (Ichinose et al., 1993). Differences in DNA sequences of genes coding for functional enzymes 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). Again, no biochemical, structural, or functional consequences of these differences have been reported, for either humans or great apes. We also remain completely ignorant of the underlying genetic basis for notable differences in disease susceptibility of humans and chimpanzees, including the apparent rarity in chimpanzees of diseases such as falciparum malaria (Ollomo et al., 1997), epithelial cancers (Schmidt, 1978; McClure, 1973), Alzheimer's disease (Gearing et al., 1994), and AIDS (Novembre et al., 1997).

There are three examples of inactivation of functional genes. The V10 variable gene of human T-cell receptor gamma locus is inactive in humans (Zhang et al., 1996), but it is one of several such V genes, and no specific consequences to immune function are known. A human type I hair keratin pseudogene was recently shown to have functional orthologs in the chimpanzee and gorilla (Winter et al., 2001). The only other genetic change giving a human-specific loss of function is a 92-bp exon deletion in human CMP-sialic acid hydroxylase (Irie et al., 1998; Chou et al., 1998; Muchmore et al., 1998). The loss of this enzyme activity leads to the absence of a particular kind of sialic acid (N-glycolyl-neuraminic acid) on all cell types in humans. 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 (Brinkman-Van der Linden et al., 2000).

Most extracellular and cell surface proteins and lipids are glycosylated with a complex array of oligosaccharide chains (glycans) (Rademacher et al., 1988). These glycans can mediate structural and physical roles, and function as ligands for carbohydrate-binding proteins (lectins) of endogenous or exogenous origin (Rademacher et al., 1988; Varki, 1993; Gagneux and Varki, 1999). While some intra- and interspecies differences in glycosylation have been reported, very little is known about the rates and magnitude of changes in glycosylation during evolution and speciation (Gagneux and Varki, 1999). For example, it remains unknown whether the overall glycosylation of human and chimpanzee proteins reflects the almost 99% identity found in genomic DNA sequences, or if it is markedly divergent. Considering the prominent role of glycans in mediating host-pathogen interactions, one could assume high rates of evolution for glycan structures of target tissues. Glycans are also involved in many important mammalian polymorphisms such as the ABO, M, and Lewis blood groups (Hakomori, 1999). To explore this issue, we sought to exploit the fact that the glycan chains attached to glycoproteins can significantly affect the apparent molecular weight and isoelectric points of glycoproteins in electrophoretic analyses. Thus, gains or losses of glycosylation sites, changes in the extent of branching, and/or changes in the number of sialic acid residues per glycosylation site can all result in major changes of migration of proteins in two-dimensional (2-D) gel electrophoresis. Since most proteins in blood plasma have attached N-glycans, we subjected multiple samples of blood plasma from humans and great apes to proteomic analysis, using a new high-resolution 2-D gel electrophoretic approach that incorporates fluorescent dye staining of proteins for improved detection, computer-aided comparison of electrophoretic patterns, robotic excision of specific dots (protein isoforms), and subsequent characterisation of proteins (Page et al., 1999). In carrying out this comparative analysis, we also intended to detect any other specific differences in protein expression that could potentially explain features unique to human evolution.

METHODS

Proteomic analysis of blood and cerebrospinal fluid samples

Human plasma samples for proteomic analysis were from normal members of the laboratory. The great ape samples were provided by the Yerkes Primate Center (Emory University, Atlanta, GA) and were obtained from normal adults undergoing annual veterinary inspection. EDTA-anticoagulated blood samples from normal adult humans and great apes were centrifuged to obtain plasma, which was

frozen at −70°C until analysis. Chimpanzee cerebrospinal fluid samples were obtained from normal adults undergoing annual health checks at the Yerkes Primate Center under complete IACUC approval for such collection. Normal adult human cerebrospinal fluid samples were obtained from the San Diego Veterans Administration Hospital, courtesy of Dr. E. Muchmore. We have IRB approval to use clinical samples from humans that are otherwise discarded when found to be normal. All samples were stored at −70°C after collection. After optimizing conditions, aliquots containing 120 μg of protein from 4 individuals of each species were compared for final analyses. Immobilized pH gradient (IPG) gels (Immobiline DryStrip 3-10 NL, Amersham Pharmacia Biotech) were rehydrated according to the manufacturer's instructions using an IPG rehydration cassette and a solution containing 8 M urea, 2% w/v CHAPS, 0.8% w/v Resolyte 3.5–10 (Bio-Rad), 10 mM DTT, and a trace of bromophenol blue in Milli-Q water. Sample protein concentrations were determined using the Pierce Coomassie Plus protein assay kit, with Pierce Standard Albumin as reference. Samples were then solubilized in an equal volume of 10% w/v SDS with 2.3% w/v DTT, vortexed, and briefly centrifuged before heating at 95°C for 5 min, cooled on wet ice for 5 min, and briefly centrifuged again. After mixing with a solution containing 8 M urea, 4% w/v CHAPS, 65 mM DTT, 40 mM Tris base, and 0.2% w/v bromophenol blue to give a final protein concentration of 2.4 mg/ml, samples were vortexed thoroughly and centrifuged for 5 min at 13,000 rpm and 20°C for immediate use. Each gel was loaded with 50 μl of solubilized sample (120 μg protein) via sample cups placed at the basic end and focused overnight (70 kVh, 20°C). Immediately after focusing, IPG gels were equilibrated in 6 M urea, 2% w/v SDS, 2% w/v DTT, 50 mM Tris (pH 6.80), and 30% v/v glycerol for 15 min before running in the second dimension on 9–16% T, 2.7% C gels, cast with the gel bound to one of the glass plates in an electrophoresis tank similar to that previously described (Amess and Tolkovsky, 1995) at 30 mA per gel and 20°C. Immediately after electrophoresis, gels were fixed in 40% v/v ethanol:10% v/v acetic acid and stained with the OGS fluorescent dye EPDF IV, and the protein expression maps (PEMs) for each sample were obtained by scanning with a Molecular Dynamics Storm™ scanner. Analysis of gel images was done using a custom version of Melanie II (BioRad). Primary PEMs were processed with MelBatch to crop images, and detect and quantify (based on fluorescence signal) features. Using MelView, images were compared manually, and features appearing consistently in samples of one species relative to the others were noted. Since the PEMs from the various species cannot necessarily be expected to match, this was deemed to be the most reliable approach and was also manageable, given the relatively small number of gels. Protein features of interest were excised from the gel by a software-driven robotic cutter, delivered into separate wells of a 96-well plate, and processed by a proteolysis workstation to yield tryptic peptide pools. A mass list of possible peptides from each protein was obtained using matrix-assisted laser desorption mass spectrometry, using an Elite matrix-assisted laser desorption-time of flight (MALDI-TOF) mass spectrometer (PerSeptive Biosystems, Framingham, CT). Fragmentation spectra from 1-Da mass windows (obtained from the MALDI mass list) were recorded using a nanospray ionization source (Z-spray) on a Q-TOF instrument (Micromass, Manchester, UK). The continuum fragmentation spectra were converted to centered spectra and used to search the SWISS-PROT (version 36.0, October 1998) database with the Sequest computer program (Finnigan, San Jose, CA). Candidate sequences were confirmed when an ion series consistent with y-type fragmentation was observed for the complete peptide sequence.

Transthyretin and thyroid hormone measurements

Western blotting for quantitiation of transthyretin was done with a polyclonal goat anti- Human transthyretin Ab (IgG fractionated, Incstar, Stillwater, MN), after running ~5 μg of protein from each plasma sample on 15% SDS-PAGE gels. For horseradish peroxidase detection, the primary Ab was used at a dilution 1:1,000, and secondary labeled chicken anti-goat antibody at 1:20 000. Final detection was with SuperSignal® West pico substrate (Pierce Corp., Rockford, IL). Signals were quantified on a Biorad GS 525 chemifluorescence reader, using Molecular Analyst software. Because the polyclonal antibody used was originally generated against human transthyretin, cross-reactivity with chimpanzee transthyretin is likely, if anything, to underestimate the latter. Radio-immunoassays for thyroid hormone parameters were performed, following the manufacturer's instructions using commercially available diagnostic kits (Diagnostic Products Corporation, Los Angeles, CA). The ^{125}Iodine readouts from Coat-a-Count tubes were made on a Cobra II Gamma counter from Packard Instruments.

RESULTS

Proteomic comparisons of great ape and human blood plasma show very few differences

Examples of typical 2-D gels from each species are shown in Figure 1. There was virtual identity in profiles within members of each species (example in Fig. 1, for humans). Considering the large amount of intraspecific variation in surface glycans in humans (blood groups, glycoforms), one could have expected to find more differences between species. To the contrary, there was a high degree of similarity among all five species, indicating that very few major changes in overall expression levels, glycosyla-

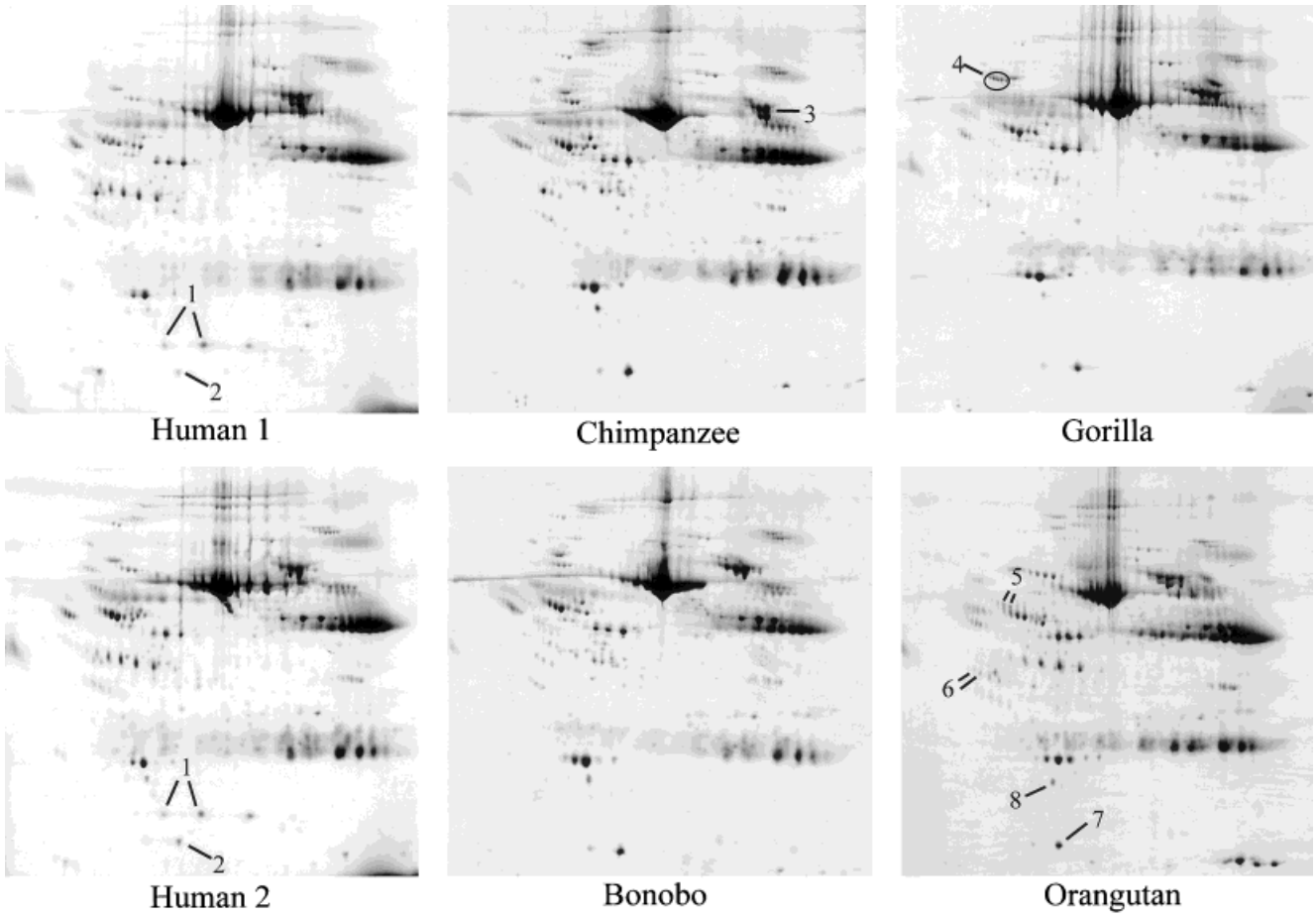

Fig. 1. Typical 2-D gel profiles of plasma samples from humans and the four great apes. The two human samples demonstrate the reproducibility of gel profiles. One profile from each of the great apes is shown. In each case, the major species-specific features are marked with an identification number (see Table 1 for summary and identifications). Since the bonobo profile was otherwise identical to that of the chimpanzee, there are no features noted on this gel. Since the primary goal was to identify human-specific differences, an exhaustive attempt was not made to define all other minor species-specific changes. It should also be noted that less abundant features that happen to coelectrophorese with the major plasma proteins may not be identified by this method. Each feature spot was identified by robotic excision, digestion with trypsin, and analysis of resulting peptides by mass spectrometric analysis.

TABLE 1. Unique features of 2-D gels of human and great ape plasma

Species	Identification[1]	Protein[2]	Nature of unique difference
Human	1	Haptoglobin-2	Presence
	2	Transthyretin	Decreased amount?
Chimpanzee	3	Transferrin	Basic pI shift
Gorilla	4	α_1 acid glycoprotein	Acidic pI shift
Orangutan	5	α_1 anti-trypsin	Higher molecular weight forms (possible variant)
	6	SP40 (sulfated glycoprotein)	Higher molecular weight forms present
	7	Transthyretin	Acidic pI shift
	8	Retinol-binding protein	Acidic pI shift

[1] See Figure 1.
[2] Identified by mass-spectrometric sequencing, except retinol-binding protein, which was identified by comparison to the Swiss Prot 2D human plasma map and peptide masses consistent with the human sequence.

tion sites, glycan branching, or sialylation of plasma proteins have occurred over the ~12 million years since the divergence of the orangutan lineage from that of the African great apes and humans. The fact that so few differences were found allowed us to focus on individual dots on the 2-D gels. Computer-aided analysis of the profiles allowed identification of a few major species-specific features, which are marked in Figure 1 and summarized in Table 1. Most of the differences in great apes represented altered electophoretic mobility of known proteins, e.g., transferrin in chimpanzees showed a basic pI shift, and alpha$_1$-acid glycoprotein from the gorilla showed an acidic pI shift, while four changes were detected in the orangutan. Here we focus only on the human-specific differences found.

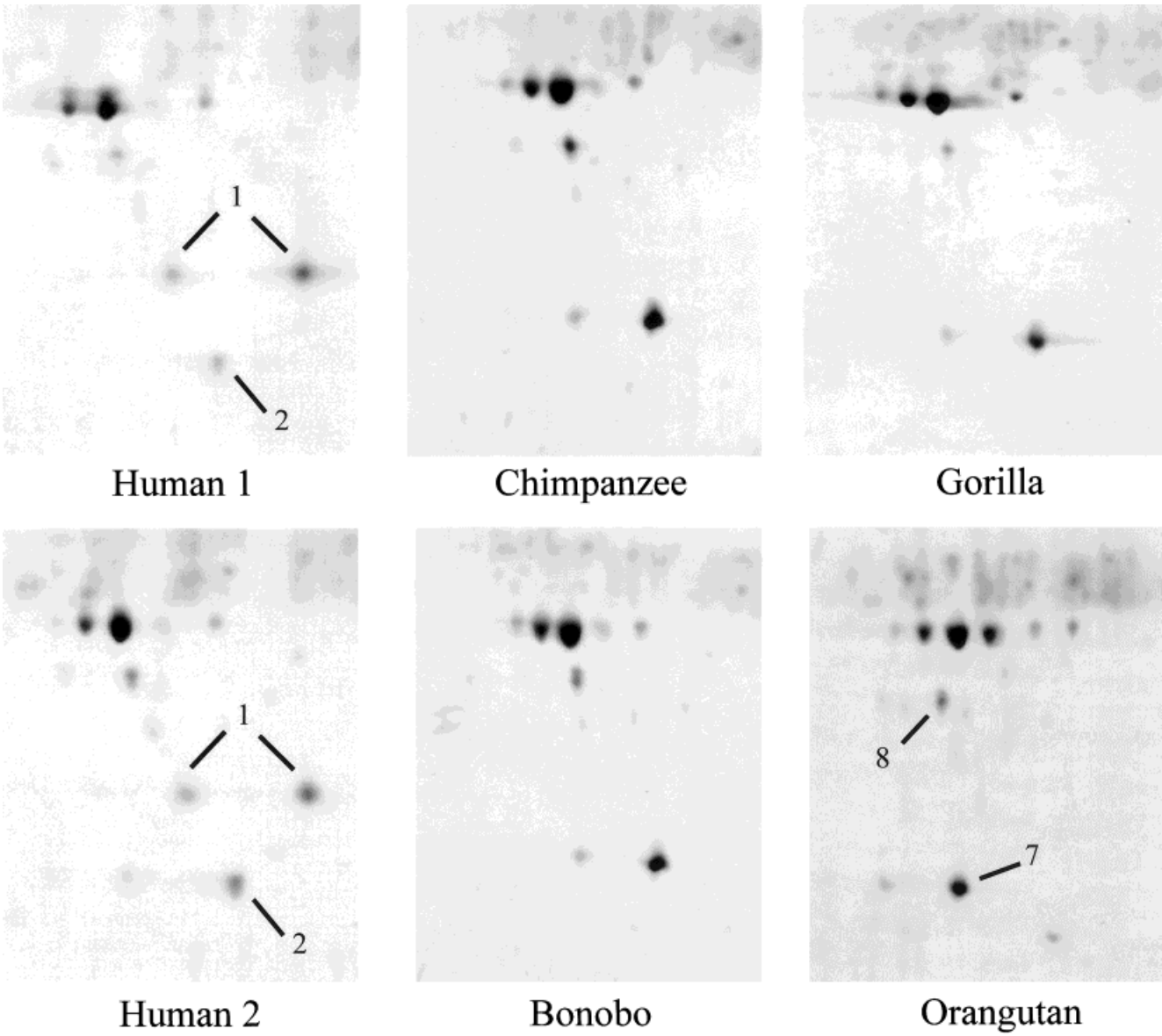

Fig. 2. Group-specific differences in 2-D gel profiles of plasma samples from humans compared to the four great apes. The regions of one gels from each species that include human-specific differences are shown (see Fig. 1 and Table 1 for identifications).

Only two protein features are unique to humans

As the higher-resolution views of a selected region of the gels show (Fig. 2), only two features are unique to humans: haptoglobin-2 (two new spots representing haptoglobin alpha 2 chain glycoforms) and transthyretin (apparent decreased intensity). Transthyretin is a ~55-kDa plasma and cerebrospinal fluid protein consisting of four identical subunits of 127 amino acids each. See discussion below of uniquely human haptoglobin isoforms, which actually represent allelic variants within the human population.

Chimpanzees and bonobos have more transthyretin than do humans

Chimpanzees and bonobos are our closest living relatives, sharing a common ancestor with humans about ~5–6 million years ago (Ruvolo, 1997; Takahata and Satta, 1997; Kumar and Hedges, 1998). To confirm the qualitative 2-D gel finding, we undertook quantitative comparisons of transthyretin levels in multiple plasma samples from normal adult humans, chimpanzees, and bonobos. Western blotting of total plasma proteins, followed by quantitation on a chemifluorescent reader, revealed a mean plasma transthyretin concentration in chimpanzees about twice that seen in humans (Fig. 3). Similar differences were observed between human and bonobo plasma samples (n = 5, data not shown). This indicates that a change in expression of transthyretin occurred after our last common ancestor with chimpanzees and bonobos, making this a candidate for explaining morphological and functional features unique to human evolution.

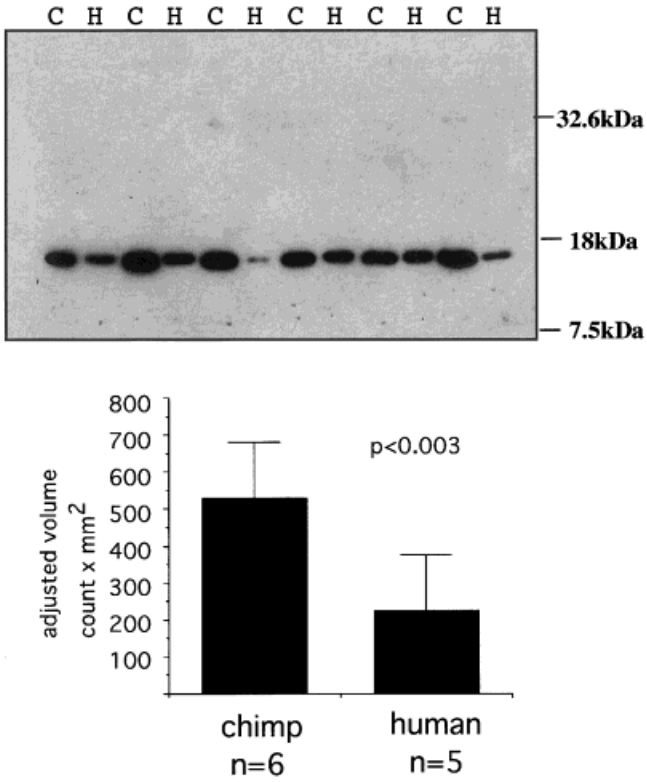

Fig. 3. Western blots of additional human and chimpanzee plasma, showing differences in level of transthyretin . Samples of 5 μl of 20-times diluted plasma were analyzed as described in Materials and Methods. Human (H) and chimpanzee (C) samples were run in alternating lanes.

Differences in thyroid hormone parameters between humans and chimpanzees

Although plasma transthyretin is only responsible for binding about 10–15% of the total plasma thyroid hormones, we decided to look for any differences between humans and chimpanzees using a radioimmunoassay to measure total plasma T4/T3 and free T3/T4, as well as T3 uptake, which measures the unoccupied hormone-binding sites on binding proteins. In spite of the higher levels of thyroid hormone-binding transthyretin, we observed higher levels of free thyroid hormone in chimpanzees (Fig. 4; similar results were obtained with five bonobo samples, data not shown). Taken together with the lower concentration of both free T3 and free T4, the higher concentration of total T4 in humans suggests that the affinities of transthyretin and/or thyroxine-binding globulin for thyroid hormone may be higher in humans. This seems to be confirmed by the higher T3 uptake values in chimpanzees.

Chimpanzees also have a higher concentration of transthyretin in the cerebrospinal fluid

Transthyretin is secreted not only by the liver into the blood plasma, but also by the choroid plexus into the cerebrospinal fluid, where it represents a substantial fraction of total protein, and is a major regulator of thyroid hormone availability. We therefore compared concentrations of transthyretin in the cerebrospinal fluid of normal adult chimpanzees and humans, using the same Western blotting/chemifluorescence quantitation as for plasma samples. As shown in Figure 5, a similar difference was found, with chimpanzees having about twice the level seen in humans.

A potential secondary difference in retinoid metabolism

Plasma retinol is bound to retinol-binding protein in a 1:1 complex, and this is the form delivered to peripheral tissues for conversion into bioactive retinoids that regulate gene transcription via the retinoid X receptors (Napoli, 1996; Xu et al., 1999). It is known that retinol-binding protein itself (molecular weight, 21 kDa) circulates predominantly bound to transthyretin, and one suggested effect of this complex formation might be the prevention of retinol-binding protein from being rapidly excreted by glomerular filtration (Wolf, 1995; Episkopou et al., 1993; Lohnes et al., 1992; Wei et al., 1995). Thus, transthyretin levels may also influence retinol-binding protein levels. Indeed, closer inspection of the 2-D gels shows that the spot corresponding to plasma retinol-binding protein in humans does in fact have a somewhat lower intensity on the 2-D gels compared with that for the other great apes (see Fig. 2, where retinol-binding protein was identified in the orangutan sample). However, when we directly measured retinol levels in the plasma of adult chimpanzees and humans, we found no significant differences (data not shown).

DISCUSSION

One previous comparative study examined 2-D gels of fibroblast proteins and used 383 spots to construct a molecular phylogeny of humans and great apes (Goldman et al., 1987). However, the functional significance of the noted differences was not pursued. Our proteomic comparisons revealed a high degree of similarity, even between humans and orangutans (who shared a common ancestor ~12–14 million years ago). This suggests that the general patterns of N-glycosylation, glycan branching, and sialylation of circulating plasma proteins have remained remarkably conserved for a long period of time. It remains to be investigated how characteristic this near-identity in glycosylation of (secreted) plasma proteins is of the situation in other tissue types, such as the large number of highly variable attached glycoconjugates present on the surface of virtually all cell types. While a few species-specific differences in protein spots were found in the present analysis, the most interesting human-specific one is the low level of transthyretin in humans. This in turn led us to find differences in thyroid hormone metabolism between humans and their closest living relatives, chimpanzees and bonobos.

Haptoglobins belong to a multigene family characterized by duplication and complex insertion

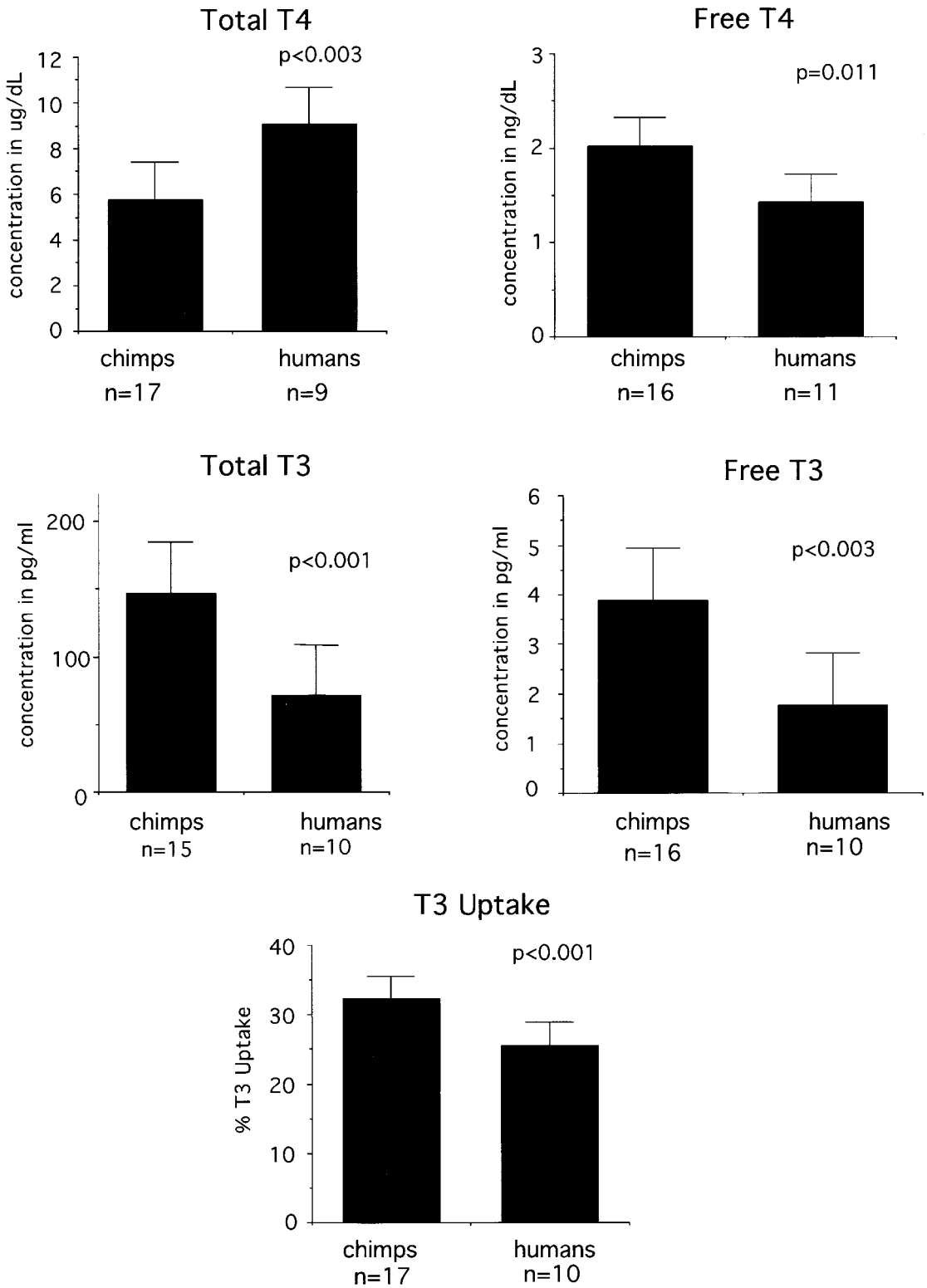

Fig. 4. Comparison of thyroid hormone parameters in plasma from humans and chimpanzees. Mean values are presented for total triiodothyronine (T3) total thyroxine (T4), free T3, and free T4, as well as for T3 uptake, which measures unoccupied binding sites on binding proteins. Bars indicate standard errors. *P*-values are for two-tailed *t*-tests, assuming unequal variances.

events in Old World primates. The two human haptoglobin genes (HP and HPR) are derived from an ancestral state still extant in apes, in which there are three genes. The human haptoglobin-related (HPR) gene resulted from an unequal crossing over facilitated by an Alu insert, from haptoglobin-related (Hpr) and haptoglobin primate (Hpp) genes (Maeda and Kim, 1990; Erickson et al., 1992; Erick-

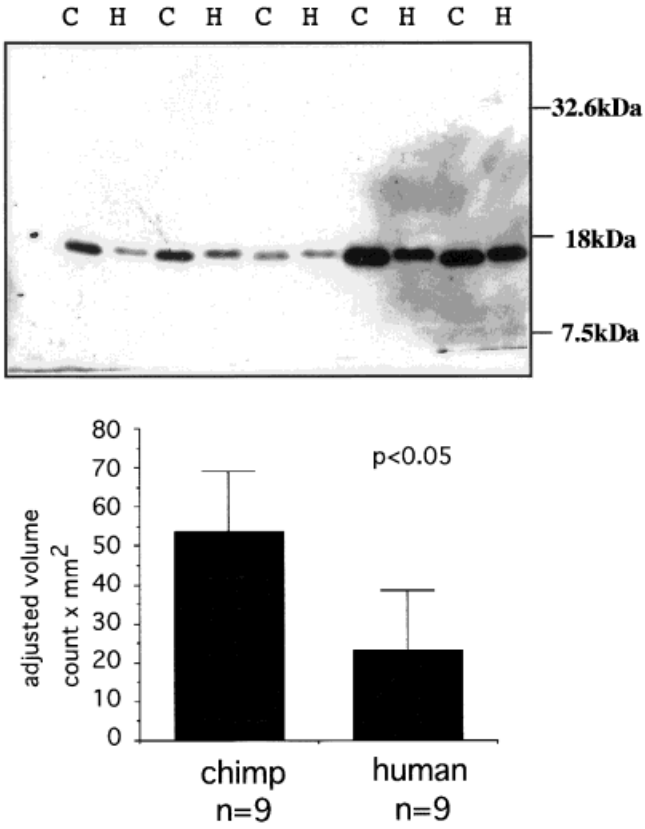

Fig. 5. Western blots of human and chimpanzee cerebrospinal fluid (CSF) samples, showing differences in levels of transthyretin. Samples of 5 μl 100-times diluted CSF were analyzed as in Figure 3.

son and Maeda, 1994). The uniquely human haptoglobin allele HP 2 polypeptide originated from an intragenic duplication of 1.7 kb by nonhomologous crossing over between two HP alleles (HP1F and HP1S). This explains the absence of the corresponding spots in the gels of great ape plasma. However, since its presence is an allelic variation in human populations (the humans studied here happened to be positive), it cannot explain primary differences between humans and great apes. On the other hand, the difference in transthyretin levels between humans and apes was noted in all samples that we studied.

Transthyretin is one of three known thyroid hormone carrying proteins in mammals (Schreiber and Richardson, 1997; Bartalena and Robbins, 1992, 1993). The others are albumin and thyroxine-binding globulin. These soluble carrier/distributor proteins are responsible for maintaining the vascular pool of thyroid hormones, and preventing them from partitioning into lipid membranes. Most thyroid hormone in plasma is present as protein-bound thyroxine (T4), a prohormone that gets converted into triiodothyronine (T3), the active hormone. A fraction of T3 and T4 is present in free form, which is functionally the more important fraction. Considering the higher transthyretin concentration in chimpanzee plasma, one might have expected to find a lower concentration of free T4 as well as free T3. Thus, while there is no simple correlation between transthyretin and thyroid hormone levels, the initial findings have led us to uncover a general difference in thyroid hormone status between chimpanzees and humans.

In addition to the regulated expression of plasma transthyretin by the liver, transthyretin is expressed constitutively in the choroid plexus and secreted directly into the cerebrospinal fluid. Unlike the situation in plasma, where transthyretin is a minor contributor to thyroid hormone binding, choroid plexus-derived transthyretin makes up about 25% of the cerebrospinal fluid protein fraction, and is considered the principal distributing protein for thyroid hormone on the brain side of the blood brain barrier, as both albumin and thyroxine-binding globulin are present in much lower concentrations in cerebrospinal fluid than in plasma (Schreiber and Richardson, 1997; Bartalena and Robbins, 1993). Thus, changes in cerebrospinal fluid transthyretin levels could have a major impact on the availability of thyroid hormones to the nervous system. Furthermore, we cannot rule out biologically significant differences that might occur during critical periods in development and/or physiological stress. Thyroid hormone and retinol levels in cerebrospinal fluid samples could not be directly measured due to the limited quantities of available samples. Because differences in the level of transthyretin in plasma are associated with differences in plasma thyroid hormone levels, we think it is reasonable to assume that changes in transthyretin concentrations in cerebrospinal fluid, where this protein is the major transporter of thyroid hormone, could affect the availability of thyroid hormone to the brain.

That the differences we found in plasma thyroid hormone levels were not exactly as predicted from the transthyretin levels is not too surprising. The thyroid hormone system is a very complex network of compartments and multiple hormone carrier proteins, where the absence of one element may be buffered by other components of the system (Schreiber and Richardson, 1997; Bartalena and Robbins, 1992, 1993). This is evident from the apparent functionally euthyroid state of transthyretin-deficient mice (Wolf, 1995; Episkopou et al., 1993; Wei et al., 1995), possibly because of a compensatory change in the biology of thyroxine-binding globulin. Serum albumin levels are similar between humans and chimpanzees, and further studies will be needed to examine thyroxine-binding globulin (glycosylation variants of this protein are known to cover a large area on the 2-D gel, making it difficult to discern any subtle difference in this analysis). We also cannot rule out differences in the affinity of human transthyretin and/or thyroxine-binding globulin for thyroid hormone. Furthermore, the thyroid hormone-mediated downregulation of thyroid-stimulating hormone could be at a different set point in the chimpanzee.

There are very few prior studies on thyroid hormones in great apes. We found a single report of acquired hypothyroidism in a captive chimpanzee (Miller et al., 1983). In contrast, the importance of thyroid hormone for embryogenesis in general and brain development in particular is well-studied in humans. Low thyroid hormone levels in utero affect development of the central nervous system, giving rise to "cretinism," which is characterized by serious mental retardation (Delange, 1994). Maternal thyroid deficiency during pregnancy can also impact the neuropsychological development of offspring in more subtle ways (Haddow et al., 1999). Adults with thyroid hormone deficiency also suffer from neuropsychiatric manifestations (Dugbartey, 1998; Denicoff et al., 1990). However, there is no model for human cretinism in the mouse, and the lack of transthyretin may have significant effects that are not evident from observing laboratory mouse behavior and survival.

Apart from its role in binding and distributing thyroid hormone, plasma transthyretin also carries retinol binding protein (vitamin A binding protein) (Wolf, 1995; Episkopou et al., 1993; Napoli, 1996; Wei et al., 1995). Indeed, transthyretin-deficient mice show reduced retinol (vitamin A) concentrations in plasma (Wolf, 1995; Episkopou et al., 1993; Wei et al., 1995). Further investigation will be needed to precisely measure any changes in plasma and cerebrospinal fluid retinol-binding protein levels secondary to transthyretin differences, and to ascertain if this is of biological significance at an earlier stage of ontogeny, when bioactive retinoids are known to be of particular importance (Napoli, 1996; Xu et al., 1999).

The few genetic differences between humans and apes known to date have not yet served to explain many of the obvious functional and morphological differences. To our knowledge, this is the first report of hormonal differences between humans and chimpanzees/bonobos. Given the numerous critical roles postulated for thyroid hormones and retinoids in cell differentiation and embryonic development (Napoli, 1996; Bavik et al., 1996; Jeannin et al., 1998; Xu et al., 1999), these differences are worthy of further exploration. A particularly intriguing question is whether differences in thyroid and/or retinoid metabolism during development could explain some of the differences between the chimpanzee and human central nervous system. Chimpanzee brains grow far less than human brains after birth and reach their maximal size at age 7, as opposed to age 19 in humans (Herndon et al., 1999; Dekaban, 1978). Dean and Wood (1984) pointed out that relatively simple modifications in the timing or pattern of cranial growth may account for differences between apes and humans. The differences reported here are of particular interest, considering the prominent role of cranial size and morphology for hominid taxonomy. In this regard, it is of interest that infants born to hyperthyroid mothers can suffer premature closure of the skull fontanelles and sutures (Krude et al., 1997; Cohen, 1991), a process which naturally occurs much earlier in the great apes (Schultz, 1969). Interestingly, a similar phenotype can also result from the teratogenic effects of therapeutic retinoids in pregnant human females (Cohen, 1991; Gardner et al., 1998). Further speculation must be curtailed until a more detailed analysis of thyroid and retinoid metabolism during the ontogeny of humans and great apes can performed.

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Thyroid Hormone Production in Chimpanzees and Humans: Implications for the Origins of Human Intelligence

Fred H. Previc*

Northrop Grumman Information Technology, San Antonio, Texas 78228

The recent findings by Gagneux et al. (2001) of a difference in thyroid output between chimpanzees and humans may provide important insights into the origins of human intelligence. In addition, their finding is consistent with other thyroid differences between chimpanzees and humans and with evidence of increased consumption of iodine-rich meat in our hominid ancestors.

The finding by Gagneux et al. (2001) that humans have lower plasma concentrations of transthyretin and free T3 (triiodothyronine) and T4 (thyroxine), but a higher total T4 concentration than chimpanzees, may indeed represent the first major endocrinological difference found between humans and apes. However, there have been previous suggestions of an evolutionary change in thyroid function in humans. In his anatomical observations, Crile (1934) observed a marked difference between the sizes of the thyroid and adrenal glands in humans and other primates: whereas nonhuman primates along with almost all other mammals have larger adrenal glands to thyroid glands, humans show the reverse ratio, favoring an enlarged thyroid gland. Crile (1934) interpreted this difference as reflecting the need for more sustained exertion in humans as opposed to more transient activation in nonhumans, which is consistent with theories that early humans may have engaged in extended locomotion and sustained exertion during such activities as scavenging and chase-hunting (Bortz, 1985; Coppens, 1996; Klein, 1989; Shipman, 1986). More recently, the anatomy of the chimpanzee skin and hand was viewed as analogous to those of humans with iodine deficiency (O'Donovan, 1996), which leads to hypothyroidism. A condition equivalent to iodine deficiency could explain the specific finding by Gagneux et al. (2001) of a lower T4 to T3 ratio in chimpanzees, as well as anatomical evidence of hypothyroidism (see Boyages and Halpern, 1993).

Gagneaux et al. (2001) noted the role of hypothyroidism in cretinism, an extremely debilitating disorder caused by iodine deficiency and associated with lowered T4 concentrations (Boyages and Halpern, 1993; DeLange, 2000). Besides affecting physical growth and stature, iodine deficiency causes numerous neurological impairments, including mental retardation; in fact, iodine deficiency is reputedly the leading worldwide cause of mental retardation, with over one billion individuals at risk (Boyages and Halpern, 1993; DeLange, 2000). One of the deleterious effects of hypothyroidism is to limit the conversion of tyrosine to dopa (the precursor to dopamine) in the central nervous system (CNS) (Diarra et al., 1989; Engstrom et al., 1974; Jacoby et al., 1975; Singhal et al., 1975). This mechanism is significant because dopaminergic activity is arguably the paramount contributor to the various intellectual skills that underlie abstract intelligence in humans and other species (Previc, 1999). Indeed, the expansion of dopaminergic systems in the prefrontal cortex, left hemisphere, and other areas of the CNS appears to explain the evolution of abstract intelligence in modern humans better than other factors such as increased brain size (Henneberg, 1999; Previc, 1999). That an evolutionary increase in thyroid T4 production could have, through a CNS dopaminergic action, resulted in increased intellectual capability in humans is supported by evidence that total T4 levels significantly predict overall cognitive status in older men, whereas overall levels of T3 do not (Prinz et al., 1999). The T4-cognitive link is consistent with evidence that cellular-bound T3 in the CNS is more likely to be synthesized from plasma T4 than from plasma T3 (Crantz et al., 1982).

It is tempting to speculate that the climatic changes occurring over the last several million years in sub-Saharan Africa contributed to both elevated thyroid production and increased dopamine. The emergence of vast, arid savannas is believed to have resulted in an expanded locomotory range in hominids and more midday activities such as scavenging of meat and chase-hunting (Bortz, 1985; Coppens, 1996; Klein, 1989; Shipman, 1986). The physical

*Correspondence to: Dr. Fred H. Previc, Northrop Grumman Information Technology, 4241 Woodcock Dr., Suite B-100, San Antonio, TX 78228. E-mail: fred.previc@brooks.af.mil

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endurance demands caused by greater locomotion and the associated thermal stress may have contributed to the increased dopamine (Previc, 1999), as could have the increased consumption of lean red meat (Draper et al., 1993) which is high in tyrosine as well as iodine. How much of the dopaminergic expansion in humans is derived from the increased T4 levels suggested by the results of Gagneux et al. (2001), and how much can be attributed to other factors, is presently unclear.

Whether or not the major changes that led to the evolution of human intelligence were primarily caused by genetic factors is also unknown. Although the genetic influence has generally been considered paramount in explaining the evolution of human intelligence, Gagneux et al. (2001, p. 100) correctly pointed out that genetic differences between chimpanzees and humans are extremely small and that "none of these differences have yet been linked to significant structural, biochemical, or functional changes." One alternative scenario is that prenatal influences on dopamine may have also contributed to the emergence of human intelligence (Previc, 1999), given that T4 levels in utero as well as subsequent brain development and intellectual capacity are all highly influenced by maternal iodine deficiency during pregnancy (Boyages and Halpern, 1999; DeLange, 2000).

I would encourage the provocative findings by Gagneux et al. (2001), however tentative, to be further replicated and expanded.

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