

Evolution of hominoids and the search for a genetic basis for creating humanness

N. Saitou

Division of Population Genetics, National Institute of Genetics, Mishima (Japan)

This work was supported by Grants-in-Aid for Scientific Research on priority areas from the Ministry of Education, Culture, Sport, Science, and Technology of Japan.

Received 28 October 2003; manuscript accepted 11 March 2004.

Request reprints from: Dr. Naruya Saitou, Division of Population Genetics, National Institute of Genetics, Mishima 411-8540 (Japan); telephone: +81-55-981-6790; fax: +81-55-981-6789; e-mail: nsaitou@genes.nig.ac.jp

Abstract. The phylogenetic relationship of human and apes are reviewed. The history of molecular phylogenetic studies in this field is then discussed, as is the role of natural selection at the molecular level. It is argued that approximately 10,000 genetic changes are responsible for creating human specific phenotypes. A genome-wide comparison is necessary to decipher those changes.

The phylogenetic relationship of human and apes

We now have a clear picture of the phylogenetic constellation of human (*Homo sapiens*) among the hominoids. Hominoids include human and apes, and correspond to the superfamily Hominoidea (Fig. 1). Chimpanzee (*Pan troglodytes*) and bonobo (*Pan paniscus*) are equally close

to human, followed by gorilla (*Gorilla gorilla*) (e.g, Sibley and Ahlquist, 1984; Saitou, 1991; Horai et al., 1995). The close phylogenetic relationship with these African great apes suggests that the human lineage originated somewhere in Africa, as first proposed by Darwin (1871). In fact, the oldest fossils considered to be part of the human lineage immediately after the divergence from the chimpanzee/bonobo lineage have so far been found only in Africa.

Chimpanzee has traditionally been classified into three subspecies (*P. t. troglodytes*, *P. t. verus*, and *P. t. schweinfurthii*) mainly according to their geographical distribution (Central Africa, West Africa, and East Africa, respectively) (Groves, 1997). Mitochondrial DNA sequence comparisons have confirmed the existence of these three lineages (Morin et al., 1992; Gagneux et al., 1999). Recently, however, one group of West African chimpanzees (*P. t. verus*) in Nigeria was shown to have a rather distinct mitochondrial DNA lineage, and a new subspecies status (*P. t. vellerosus*) was proposed (Gonder et al., 1997). The bonobo, or birya (the name given by the local people of Congo), are distributed to the south of the Congo (Zaire) river, and is congeneric with chimpanzee. However, a hybrid offspring of chimpanzee and bonobo has been reported (Vervaecke and van Elsacker, 1992). This suggests that the reproductive barrier between these two species is relatively low. It should also be added that Goodman and his colleagues have recently proposed that chimpanzee and bonobo should be included in the genus *Homo* (Wildman et al., 2003).

Gorilla has been traditionally classified into three subspecies, *G. g. gorilla*, *G. g. graueri*, and *G. g. beringei*, corresponding to western lowland gorilla, eastern lowland gorilla and mountain gorilla, respectively (Nowark, 1991). However, recent molecular studies only indicated a clear genetic differentiation of the west and east lowland gorilla, while mountain gorilla is genetically close to the east lowland gorilla (Garner and Ryder, 1996).

Orangutan (*Pongo pygmaeus*), distributed in Southeast Asia, is much more remotely related to human than are the African apes (see Fig. 1). The geographical distribution of the extant orangutan is restricted to the islands of Borneo and Sumatra, and the two populations of which in turn correspond to two subspecies (*P. p. pygmaeus* and *P. p. abelii*, respectively). Although these two subspecies are genetically quite divergent (Xu and Arnason, 1996), hybrids are fertile.

The phylogenetic relationship of the gibbons is not yet well known, and even the number of species varies depending on different reports. For example, Groves (1997) classified gibbons (Hylobatidae) into one genus (Hylobates), four subgenera, and 11 species, while Napier and Napier (1985) identified only six species. A comparison of the various classification schemes of hominoid species can be seen at the "All About Apes" section of the Silver Project webpage (<http://sayer.lab.nig.ac.jp/~silver/taxonomy.html>). Common gibbon and siamang are estimated to have diverged more than 7 million years ago, but one example of hybridization was reported (Myers and Shafer, 1979).

Molecular phylogenetic studies on human and apes

The molecular evolutionary study of human probably goes back to the work of G.H.F. Nuttall, who originally studied the immunological differences found in the blood of various animals including human (Gribbin and Chérfas, 1982). However, a clear phylogenetic relationship of the hominoid species was first obtained by Goodman (1962). Goodman indicated a closer relationship of the African apes (chimpanzee and gorilla) with human than the Asian apes

(orangutan and gibbon), using the immunodiffusion method. However, his results remain semi-quantitative.

The later application of the microcomplement fixation method of Sarich and Wilson (1967) greatly enhanced the sensitivity of the immunological reaction, and allowed the estimation of the divergence time of human, chimpanzee, and gorilla to be about 5 million years ago, when the time of divergence of hominoids and the Old World monkeys was assumed to be 30 million years. That classic paper set a standard time frame for the human-ape divergence. Many molecular studies have rapidly accumulated since then in the field of human evolutionary studies, and we now have ample findings which strongly support the phylogenetically closer relationship of human to African apes, rather than with Asian apes, as first showed by Goodman (1962). Findings supporting this clustering pattern include amino acid sequences (Goodman et al., 1971), chromosome banding patterns (Yunis and Prakash, 1982), DNA-DNA hybridization (Sibley and Ahlquist, 1984, 1987), and nucleotide sequences (Brown et al., 1982; Koop et al., 1986; Hixson and Brown, 1986; Miyamoto et al., 1987; Maeda et al., 1988; Ueda et al., 1989). However, despite the accumulation of a large amount of data, there was still at that time no consensus on the problem of the branching order of human, chimpanzee, and gorilla (Nei et al., 1985; Saitou, 1991). Gonzalez et al. (1990) studied ribosomal RNA genes and supported the human-chimpanzee cluster statistically significantly for the first time. Horai et al. (1995) also found the statistically significant support for the human-chimpanzee cluster by determining the complete mitochondrial DNA sequences of apes.

There is, however, a further very important aspect to take into account when reconstructing the evolutionary branching pattern of these three hominoid species. In the presence of genetic polymorphisms, a phylogenetic tree reconstructed from a single gene from each species (gene tree) may be different from the true species phylogeny (species tree), even if a large number of nucleotides are used (Nei, 1985; Takahata and Nei, 1985; Saitou and Nei, 1986; Nei, 1987). This is especially true when the two speciation events occurred during a short period, and it has been shown that several gene trees may be necessary for obtaining the correct species tree in the case of human, chimpanzee, and gorilla (Saitou and Nei, 1986).

Recently, several studies were conducted with the aim of determining the phylogenetic relationship among human, chimpanzee, and gorilla. Satta, Klein, and Takahata (2000), Chen and Li (2001), O'hUigin et al. (2002), and Kitano et al. (2004) compared 34, 53, 51, and 103 genes or DNA segments, respectively, and in all of these studies it was found that about 40 % of the gene trees were different from the species tree. Although all three possible gene trees were observed, the tree showing the human-chimpanzee cluster was always the most common in each of the four studies. Taken together, these studies would seem to finally establish that chimpanzee and bonobo are the closest organisms to human.

The next question is therefore the divergence time between human and chimpanzee. Sarich and Wilson (1967) estimated that human, chimpanzee, and gorilla diverged around 5 million years ago (MYA) based on an assumed divergence time of hominoids and Old World monkeys of 30 MYA. Numerous divergence time estimates have since then been proposed based on molecular data (Table 1). Because molecular data alone can give only relative divergence times, ratios of divergence times between $D[\text{human-chimpanzee}]$ and $D[\text{human-orangutan}]$ are also shown in Table 1, where $D[a-b]$ is the molecular distance between species a and b.

It is interesting to note that except for two rather small and large divergence estimates (Hasegawa et al., 1985, 2003), most of the estimates are in the small range of 4.5–6 million years. The relative ratio ($D[\text{human-chimpanzee}]/D[\text{human-orangutan}]$) is also more or less the same

within the range of 0.38–0.48, except for the two large and small estimates based on earlier and limited molecular data (Sarich and Wilson, 1967; Hasegawa et al., 1985) and an estimate by Kumar and Hedges (1998). It should be noted that the calibration method described by Kumar and Hedges (1998) was recently criticized by Graur and Martin (2004). If we therefore assume that the divergence time of human and chimpanzee is about 6 million years ago, and the $D[\text{human-chimpanzee}]/D[\text{human-orangutan}]$ is 0.40, the divergence time of human and orangutan becomes 15 million years ago. Figure 1 displays the phylogenetic relationship of the extant hominoid species based on these strands of molecular evidence.

Positive selection for ape and human genes

Hughes and Nei (1988, 1989) showed that both MHC class I and class II genes experienced positive selection, probably of the overdominant type, through the comparison of synonymous and non-synonymous nucleotide substitutions. This D_n/D_s test is quite powerful when being used to detect positive selection based on the neutral theory (Kimura, 1983), and many studies were conducted to detect such selection. However, one drawback of this test is that it can detect only a limited type of genes under positive selection, and to obtain a statistically significant result, we need to have multiple substitutions. Therefore, a protein gene must have many variable amino acids, a pre-condition that may be satisfied in protein-coding genes that are involved in the immune system, such as MHC. If a single amino acid change were responsible in enhancing fitness, however, it would be difficult to detect it by the D_n/D_s test.

Table 2 lists the primate genes found to be under the influence of positive selection in relatively recent studies. Although the majority of protein-coding genes have lower non-synonymous substitutions than synonymous ones (Endo et al., 1996), a certain proportion are under positive selection. Although most of the genes listed in Table 2 were found to have experienced positive selection thorough the D_n/D_s test, the FOXP2 gene studied by Enard et al. (2002) and Zhang et al. (2002) did not show significantly higher D_n than D_s . They instead studied the within-human variation of FOXP2 across a number of populations, and found that the gene had little variation, suggesting a recent fixation of an advantageous mutation. This result is particularly intriguing as the FOXP2 gene was found by Lai et al. (2001) to be related to language ability and as such it may have been involved in the development of language in human history. However, one of the two amino acid substitutions occurred in the human lineage after the divergence of the human-chimpanzee common ancestor also occurred in the carnivores (Zhang et al. 2002). It is therefore not yet clear if these amino acid changes were truly responsible for the emergence of language.

Amount of genetic changes responsible for human specific phenotypes

The overall level of nucleotide substitutional divergence between human and chimpanzee is 1.23 % (Fujiyama et al., 2002). Many of those differences are due to mutations that occurred in junk DNA (Ohno, 1972), and most probably have no effect on the level of phenotypic difference between human and chimpanzee. However, some proportion of nucleotide changes, in particular, amino acid replacing non-synonymous ones, must be responsible for human-specific characters. There are 30,000–40,000 protein-coding genes in the human genome (International Human

Genome Sequencing Consortium 2001), and a certain proportion must code proteins that slightly differ in amino acid sequences between human and chimpanzee. Some of those amino acid differences may be responsible for human-specific characters. Amino acid differences are classified into replacement (substitution) and insertions/deletions (indels). Both types can lead to slight-to-considerable changes of the protein 3D structure, which is the source of human specific phenotypes.

DNA regions responsible for the modification of gene expression are also important, as King and Wilson (1975) pointed out. The discovery of such regions is difficult and we need to obtain multiple species comparisons of contiguous genomic sequences including both coding and non-coding DNA.

How many significant changes are really responsible for creating humanness or "hominization"? The level of genomic nucleotide difference between human and chimpanzee, i.e., 1.23 % (Fujiyama et al., 2002), has been accumulated both in the human and chimpanzee lineage. Therefore, about half (ca. 0.6 %) of this difference can be assumed to have accumulated in the human lineage after divergence from the chimpanzee lineage. All the genetic changes responsible for "humanness" must reside in these differences. The human genome consists of about 3 billion nucleotides, and thus the 0.6 % difference is tantamount to about 18 million nucleotide changes. Although at least 95 % of the human genome may be attributed to be non-coding DNA, we still have some 900,000 nucleotide changes located in the coding DNA. Of these, how many changes are really responsible for creating humanness? First, we can safely omit synonymous changes in the coding region. Even if amino acid changes occur, the function of one protein may not drastically change. If we eliminate those non-significant nucleotide changes, the really interesting changes may only be ~1 %, or ca. 10,000 nucleotide changes. I would like to add that the genetic changes responsible for the humanness could also be accomplished through the fixation of neutral mutations, as well as that of advantageous mutations.

Ape genome sequencing efforts

We are now in the era of genomics. Therefore, apes, the closest organism group to human, have also become the target of genome sequencing. Our group has been studying the evolution of the ABO (Saitou and Yamamoto, 1997; Sumiyama et al., 2000; Kitano et al., 2000) and Rh (Kitano et al., 1998; Kitano and Saitou, 1999) blood group genes in hominoids. Figure 2 is a phylogenetic network of the ABO blood group gene intron 6 and exon 7 for human, chimpanzee, and bonobo. We expanded our interest to a much larger number of ape genes, and started a small-scale ape genome project in 1999. Because Ag, the acronym of "ape genome," is the atomic symbol for silver, we nicknamed this project "Silver" (<http://sayer.lab.nig.ac.jp/~silver/>). Currently, human and ape nucleotide sequences for 34 kinds of genes or DNA regions are available through this website, and all sequence data have been submitted to the DDBJ/EMBL/GenBank International Nucleotide Sequence database. Because of the open availability, two sequence datasets for chimpanzee have already been used for two human polymorphism studies (Sanders et al., 2001; Duan et al., 2003). We are also collaborating with various groups on the ape genome study: on fetuin (Osawa et al., 2001), cystatin B (Osawa et al., 2003), Spinocerebellar Ataxia 8 (Andres et al., 2003, 2004), and polyubiquitin (Tachikui et al., 2003). Because a genomic clone library is necessary for genome-wide study, we recently constructed a gorilla fosmid library and its PCR-based screening system (Kim et al., 2003).

The activity of other research groups as of February 2004 is as follows. Anzai et al. (2003) completed sequencing the chimpanzee MHC class I region, spanning 1.75 Mb. This is the longest chimpanzee genome segment sequence so far. Another project is the determination of the chimpanzee chromosome 22 long arm that is orthologous to the human chromosome 21 long arm. This is an international collaboration involving Japan, Germany, China, Korea, and Taiwan, headed by RIKEN GSC. A total of ca. 32.8 Mb nucleotide sequence data are now available through the DDBJ/EMBL/GenBank International Nucleotide Sequence database under accession numbers BS000001-BS000245 and AL954200-AL954259. A genomic comparison between human chromosome 21 and chimpanzee chromosome 22 has also been conducted (this paper was just published; International Chimpanzee Chromosome 22 Consortium, 2004). Recently, The Washington University Genome Sequencing Center and Whitehead Institute for Biomedical Research have both received funding from NHGRI to conduct a “4x” coverage of the chimpanzee genome. Sequence data from this project are now available at Ensembl database (http://pre.ensembl.org/Pan_troglodytes/). Given the amount of data already collected, along with the number and diversity of ongoing projects, we can confidently state that we are now really immersed in the human-ape comparative genomics era.

Acknowledgments

I thank Drs. Takashi Kitano and Alan Lovell for comments.

References

- Andres AM, Soldevila M, Saitou N, Volpini V, Calafell F, Bertranpetit J: Understanding the dynamics of Spinocerebellar Ataxia 8 (SCA8) locus through a comparative genetic approach in humans and apes. *Neurosci Lett* 336: 143–146 (2003).
- Anzai T, Shiina T, Kimura N, Yanagiya K, Kohara S, Shigenari A, Yamagata T, Kulski JK, Naruse TK, Fujimori Y, Fukuzumi Y, Yamazaki M, Tashiro H, Iwamoto C, Umehara Y, Imanishi T, Meyer A, Ikeo K, Gojobori T, Bahram S, Inoko H: Comparative sequencing of human and chimpanzee MHC class I regions unveils insertions/deletions as the major path to genomic divergence. *Proc Natl Acad Sci USA* 100:7708–7713 (2003)
- Brown WM, Prager EM, Wang A, Wilson AC: Mitochondrial DNA sequences of primates: Tempo and mode of evolution. *J Mol Evol* 18:225–239 (1982).
- Chen FC, Li WH: Genomic Divergences between Humans and Other Hominoids and the Effective Population Size of the Common Ancestor of Humans and Chimpanzees. *Amer J Hum Genet* 68:444–456 (2001).
- Darwin C: *The Descent of Man and Selection in Relation to Sex*. (Appleton, New York 1871).
- Duan J, Wainwright MS, JComeron JM, Saitou N, Sanders AR, Gelernter J, Gejman PV: Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. *Hum Mol Genet* 12:205–216 (2003).
- Enard W, Przeworski M, Fisher SE, Lai CS, Wiebe V, Kitano T, Monaco AP, Pääbo S: Molecular evolution of FOXP2, a gene involved in speech and language. *Nature* 418:869–872 (2002).
- Endo T, Ikeo K, Gojobori T: Large-scale search for genes on which positive selection may operate. *Mol Biol Evol* 13:685–690 (1996).
- Fujiyama A, Watanabe H, Toyoda A, Taylor TD, Itoh T, Tsai SF, Park HS, Yaspo ML, Lehrach H, Chen Z, Fu G, Saitou N, Osoegawa K, de Jong PJ, Suto Y, Hattori M, Sakaki Y: Construction and analysis of a human-chimpanzee comparative clone map. *Science* 295:131–134 (2002).
- Gagneux P, Wills C, Gerloff U, Tautz D, Morin PA, Boesch C, Fruth B, Hohmann G, Ryder OA, Woodruff DS: Mitochondrial sequences show diverse evolutionary histories of African hominoids. *Proc Natl Acad Sci USA* 96:5077–5082 (1999).

- Garner KJ, Ryder O: Mitochondrial DNA diversity in gorillas. *Mol Phylogenet Evol* 6:39–48 (1996).
- Glazko GV, Nei M: Estimation of divergence times for major lineages of primate species. *Mol Biol Evol* 20:424–434 (2003).
- Gloves CP: Taxonomy and phylogeny of primates, in Blancher A, Klein J, Socha W (eds): *Molecular Biology and Evolution of Blood Group and MHC Antigens in Primates* (Springer Verlag Berlin 1997).
- Gonder MK, Oates JF, Disotell TR, Forstner MR: A new west African chimpanzee subspecies? *Nature* 388:337 (1997).
- Gonzalez IL, Sylvester JE, Smith TF, Stambolian D, Schmickel RD: Ribosomal RNA gene sequences and hominoid phylogeny. *Mol Biol Evol* 7:203–219 (1990).
- Goodman M: Evolution of the immunologic species specificity of human serum proteins. *Hum Biol* 34:104–150 (1962).
- Goodman M, Barnabas J, Matsuda G, Moore GW: Molecular evolution in the descent of man. *Nature* 233:604–613 (1971).
- Graur D, Martin W: Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends Genet* 20:80–86 (2004).
- Gribbin J, Charfas J: *The Monkey Puzzle* (Pantheon Books, New York 1982).
- Hasegawa M, Kishino H, Yano T: Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J Mol Evol* 22:160–174 (1985).
- Hasegawa M, Kishino H, Yano T: Man's place in Hominoidea as inferred from molecular clocks of DNA. *J Mol Evol* 26:132–147 (1987).
- Hasegawa M, Thorne JL, Kishino H.: Time scale of eutherian evolution estimated without assuming a constant rate of molecular evolution. *Genes Genet Syst* 78:267–283 (2003).
- Hixson JE, Brown WM: A comparison of the small ribosomal RNA genes from the mitochondrial DNA of the great apes and humans: Sequence, structure, evolution, and phylogenetic implications. *Mol Biol Evol* 3:1–18 (1986).
- Horai S, Hayasaka K, Kondo R, Tsugane K, Takahata N: Recent African origin of modern humans revealed by complete sequences of hominoid mitochondrial DNAs. *Proc Natl Acad Sci USA* 92:532–536 (1995).
- Hughes AL, Nei M: Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. *Nature* 335:167–170 (1988).
- Hughes AL, Nei M: Nucleotide substitution at major histocompatibility complex class II loci: evidence for overdominant selection. *Proc Natl Acad Sci USA* 86:958–962 (1989).
- International Chimpanzee Chromosome 22 Consortium: DNA sequence and comparative analysis of chimpanzee chromosome 22. *Nature* 429: 382–388 (2004).
- International Human Genome Sequencing Consortium: Initial sequencing and analysis of the human genome. *Nature* 409:860–921 (2001).
- Johnson ME, Viggiano L, Bailey JA, Abudul-Rauf M, Goodwin G, Rocchi M, Eichler EE: Positive selection of a gene family during the emergence of humans and African apes. *Nature* 413:514–519 (2001).
- Kim CG, Fujiyama A, Saitou N: Construction of a gorilla fosmid library and its PCR screening system. *Genomics* 100: 571–574 (2003).
- Kimura M: *The Neutral Theory of Molecular Evolution* (Cambridge University Press, Cambridge UK 1983).
- King MC, Wilson AC: Evolution at two levels in humans and chimpanzees. *Science* 188:107–116 (1975).
- Kitano T, Liu Y-H, Ueda S, Saitou N: Human specific amino acid changes found in 103 protein coding genes. *Mol Biol Evol* 21:936–944 (2004).
- Kitano T, Saitou N: Evolution of the Rh blood group genes has experienced gene conversions and positive selection. *J Mol Evol* 49:615–626 (1999).
- Kitano T, Sumiyama K, Noda R, Ferrell RE, Saitou N: Gene diversity of chimpanzee ABO blood group genes elucidated from intron 6 sequences. *J Hered* 91:211–214 (2000).
- Kitano T, Sumiyama K, Shiroishi T, Saitou N: Conserved evolution of the Rh50 gene compared to its homologous Rh blood group gene. *Biochem Biophys Res Commun* 249:78–85 (1998).
- Koop BF, Goodman M, Xu P, Chan K, Slightom JL: Primate η -globin DNA sequences and man's place among the great apes. *Nature* 319:234–238 (1986).
- Kumar S, Hedges SB: A molecular timescale for vertebrate evolution. *Nature* 392:917–920 (1998).
- Lai CSL, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP: A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature* 413:519–523 (2001).

- Maeda N, Wu CI, Bliska J, Reneke J: Molecular evolution of intergenic DNA in higher primates: Pattern of DNA changes, molecular clock, and evolution of repetitive sequences. *Mol Biol Evol* 5:1–20 (1988).
- Messier W, Stewart CB: Episodic adaptive evolution of primate lysozymes. *Nature* 385:151–154 (1997).
- Miyamoto MM, Slightom JL, Goodman M: Phylogenetic relations of humans and African apes from DNA sequences in the $\phi\eta$ -globin region. *Science* 238:369–373 (1987).
- Morin PA, Moor JJ, Woodruff DS: Identification of chimpanzee subspecies with DNA from hair and allele-specific probes. *Proc R Soc Lond B* 249:293–297 (1992).
- Myers RH, Shafer DA: Hybrid ape offspring of a mating of gibbon and siamang. *Science* 205:308–310 (1979).
- Napier JR, Napier PH: *The natural history of the primates* (MIT Press, Cambridge MA 1985).
- Nei M: *Molecular evolutionary genetics* (Columbia University Press, New York 1987).
- Nei M: Human evolution at the molecular level, in Ohta T, Aoki K (eds): *Population Genetics and Molecular Evolution*, pp 41–64 (Japan Science Society Press, Tokyo 1985).
- Nei M, Stephens JC, Saitou N: Methods for computing the standard errors of branching points in an evolutionary tree and their application to molecular data from humans and apes. *Mol Biol Evol* 2:66–85 (1985).
- Nowark RM: *Walker's Mammals of the World Fifth edition, Volume I* (The Johns Hopkins University Press, Baltimore 1991).
- O'huigin C, Satta Y, Takahata N, Klein J: Contribution of homoplasmy and of ancestral polymorphism to the evolution of genes in anthropoid primates. *Mol Biol Evol* 19:1501–1513 (2002).
- Ohno S: So much "junk" DNA in our genome, in Smith HH (ed): *Evolution of Genetic Systems*, Brookhaven Symposium No. 26, pp 366–370 (Gordnand Breach Inc, New York 1972).
- Osawa M, Kaneko M, Horiuchi H, Kitano T, Kawamoto Y, Saitou N, Umetsu K: Evolution of the cystatin B gene: implications for the origin of its variable dodecamer tandem repeat in humans. *Genomics* 81:78–84 (2003).
- Osawa M, Yuasa I, Kitano T, Heinke J, Kaneko M, Saitou N, Umetsu K: Haplotype analysis of human alpha2-HS glycoprotein (fetuin). *Ann Hum Genet* 65:27–34 (2001).
- Saitou N: Reconstruction of molecular phylogeny of extant hominoids from DNA sequence data. *Amer J Phys Anthropol* 84:75–85 (1991).
- Saitou N, Nei M: The number of nucleotides required to determine the branching order of three species, with special reference to the human-chimpanzee-gorilla divergence. *J Mol Evol* 24:189–204 (1986).
- Saitou N, Yamamoto F: Evolution of primate ABO blood group genes and their homologous genes. *Mol Biol Evol* 14:399–411 (1997).
- Sanders AR, Cao Q, Taylor J, Levin TE, Badner JA, Cravchik A, Comeron JM, Saitou N, Del R, Salvi ADA, Walczyk KA, Goldin LR, Mowry BJ, Levinson DF, Crowe RR, Silverman JM, Gejman PV: Genetic diversity of the human serotonin receptor 1B (HTR1B) gene. *Genomics* 72:1–14 (2001).
- Sarich VM, Wilson AC: Immunological time scale for hominoid evolution. *Science* 158:1200–1204 (1967).
- Satta Y, Klein J, Takahata N: DNA archives and our nearest relative: the trichotomy problem revisited. *Mol Phyl Evol* 14:259–275 (2000).
- Sibley CG, Ahlquist JE: The phylogeny of the hominoid primates, as indicated by DNA-DNA hybridization. *J Mol Evol* 20:2–15 (1984).
- Sibley CG, Ahlquist JE: DNA hybridization evidence of hominoid phylogeny: Results from an expanded data set. *J Mol Evol* 26:99–121 (1987).
- Stauffer RL, Walker A, Ryder OA, Lyons-Weiler M, Hedges SB: Human and ape molecular clocks and constraints on paleontological hypotheses. *J Hered* 92:469–474 (2001).
- Sumiyama K, Kitano T, Noda R, Ueda S, Ferrell R, Saitou N: Sequence variation in the ABO blood group gene exon 7 of chimpanzee and bonobo. *Gene* 259:75–79 (2000).
- Sumiyama K, Saitou N, Ueda S: Adaptive evolution of the IgA hinge region in primates. *Mol Biol Evol* 19:1093–1099 (2002).
- Tachikui H, Saitou N, Nakajima T, Hayasaka I, Ishida T, Inoue I: Lineage specific homogenization of the polyubiquitin gene among human and great apes. *J Mol Evol* 57:737–744 (2003).
- Takahata N, Nei M: Gene genealogy and variance of interpopulational nucleotide differences. *Genetics* 110:325–344 (1985).
- Takahata N, Satta Y: Evolution of the primate lineage leading to modern humans: phylogenetic and demographic inferences from DNA sequences. *Proc Natl Acad Sci USA* 94:4811–4815 (1997).

- Ueda S, Watanabe Y, Saitou N, Omoto K, Hayasida H, Miyata T, Hisajima H, Honjo T: Nucleotide sequences of immunoglobulin epsilon pseudogenes in hominoids and their phylogenetic relationships. *J Mol Biol* 205:85–90 (1989).
- Vervaecke H, van Elsacker L: hybrids between common chimpanzees (*Pan troglodytes*) and pygmy chimpanzees (*Pan paniscus*) in captivity. *Mammalia* 56:667–669 (1992).
- Wildman DE, Uddin M, Liu G, Li G, Goodman M: Implications of natural selection in shaping 99.4 % nonsynonymous DNA identity between humans and chimpanzees: enlarging genus *Homo*. *Proc Natl Acad Sci USA* 100:7181–7188 (2003).
- Wyckoff GJ, Wang W, Wu CI: Rapid evolution of male reproductive genes in the descent of man. *Nature* 403:304–309 (2000).
- Xu X, Arnason U: The mitochondrial DNA molecule of Sumatran orangutan and a molecular proposal for two (Bornean and Sumatran) species of orangutan. *J Mol Evol* 43:431–437 (1996).
- Yunis JJ, Prakash O: The origin of man: a chromosomal pictorial legacy. *Science* 215:1525–1530 (1982).
- Zhang J, Rosenberg HF, Nei M: Positive Darwinian selection after gene duplication in primate ribonuclease genes. *Proc Natl Acad Sci USA* 95:3708–3713 (1998).
- Zhang J, Webb DM, Podlaha O: Accelerated protein evolution and origins of human-specific features. *Foxp2* as an example. *Genetics* 162:1825–1835 (2002).

Fig. 1. The phylogenetic relationship of the extant hominoid species. It should be noted that the divergence time estimates are all quite rough.

Fig. 2. Phylogenetic network of the ABO blood group gene for human and great apes. The sequence data of Sumiyama et al. (2000) and Kitano et al. (2000) were combined. **(b)** and **(c)** denote bonobo and chimpanzee, respectively, and numbers following the dash designate haplotype IDs. See Sumiyama et al. (2000) and Kitano (2000) for more details.

Table 1. Estimates of divergence time between human and chimpanzee based on molecular data

Time* (MYA)	D[hc]/ D[ho]**	Type of molecular data	Reference
5	0.6	albumin	Sarich and Wilson (1967)
2.7	0.25	mtDNA	Hasegawa et al. (1985)
4.9	0.41	eta globin	Hasegawa et al. (1987)
5 - 6	0.44	mtDNA and nucDNA	Saitou (1991)
4.9	0.38	complete mtDNA	Horai et al. (1995)
4.5	-	nucDNA	Takahata and Satta (1997)
5.5	0.67	nucDNA	Kumar and Hedges (1998)
6.2-8.4	0.40	nucDNA non-coding	Chen and Li (2001)
5.4	0.48	nucDNA	Stauffer et al. (2001)
7.4	0.39	complete mtDNA	Hasegawa et al. (2003)
6	0.46	nucDNA	Glazko and Nei (2003)
4.3-5.9	0.44	nucDNA synonymous	Wildman et al. (2003)
-	0.40	nucDNA synonymous	Kitano et al. (2004)

*Divergence time estimate between human and chimpanzee.

**D[human-chimpanzee]/D[human-orangutan].

Table 2. Genes found to be under positive selection in primate evolution

Gene	Reference
ABO	Saitou and Yamamoto (1997)
BRCA1	Huttley et al. (2000)
FOXP2	Enard et al. (2002), Zhang et al. (2002)
IgA hinge region	Sumiyama et al. (2002)
LCR16a	Johnson et al. (2001)
lysozyme	Messier and Stewart (1997)
protamine 1 & 2	Wyckoff, Wang, and Wu (2000)
Rh50	Kitano et al. (1998)
Rh blood group	Kitano and Saitou (1999)
RNASE2 & 3	Zhang et al. (1998)
TNP2	Wyckoff, Wang, and Wu (2000)

Fig. 1 (Saitou)



