

Functional genomics in the chimpanzee

Wolfgang Enard, Ingo Ebersberger, Anne Fischer, Florian Heissig, Ines Hellmann, Barbara Höffner, Philipp Khaitovich, Takashi Kitano, Katrin Köhler, Dirk Metzler, Birgit Nickel, Molly Przeworski, Carsten Schwarz, Katja Nowick, Victor Wiebe, Michaela Winkler, Sebastian Zöllner and Svante Pääbo

Max-Planck-Institute for Evolutionary Anthropology, Leipzig

In 1607, an Englishman by the name of Andrew Battell returned to Europe from Africa and described chimpanzees and gorillas for the first time to the western World. Their similarity to humans was soon realized and in 1758 Linnaeus joined the great apes and humans in the taxon "Primates". The relationship of apes and humans has ever since that time caused a lot of interest and stirred much debate. Where do we stand today in these debates? The question of the relationship among the great apes and humans can be regarded as solved, mainly due to the accumulation of DNA sequences in recent years. The two extant chimpanzee species, the common chimp and the bonobo, are the closest living relatives of humans, followed closely by the gorilla and somewhat further away by the orangutan (figure 1).

We also know that humans and chimpanzees share a large amount of their genetic material. If one compares a piece of DNA from a chimpanzee with the correspon-

ding piece from a human, one finds roughly 1 out of 100 nucleotides to be different. This is only a little more than e.g. between African and Indian elephants or about as much as two individuals from one and the same fruitfly species differ. The difference of 1% can be used to roughly measure the time since humans and chimpanzees last shared a common ancestor. From an evolutionary perspective this turns out to be quite recently – probably about 5 to 6 million years ago. Yet, most humans feel that a lot of obvious differences exist when they look at a chimpanzee. In other words: the genotypes are very similar, but the phenotypes differ considerably. Unfortunately, next to nothing is known about which genotypic differences are responsible for the phenotypic differences we observe between humans and chimpanzees. In fact, this is not unknown only for humans and chimpanzees, but for any other species as well. Therefore, a better understanding of the genetic background of the phenotypic differences between humans and chimpanzees would be helpful for the general understanding of the evolution of species. We have tried to tackle these questions using three different molecular approaches.

In order to get a more detailed picture on how humans and chimpanzees differ in their genome, we sequenced 10,000 random short pieces of the chimpanzee genome. This adds up to about 0.1 % of the chimpanzee genome. We then compared these pieces to the corresponding parts of the human genome. Whereas the average difference was 1.24 %, we also found that the differences are not equally distributed among the autosomes (figure 2). This suggests that large regions of the genome accumulate differences at different rates. A major challenge for the future is to try to understand the underlying reason for this.

We also explored the evolutionary patterns in genes, since genes encode proteins and therefore can be suspected of more directly influencing the phenotype. To this end, we constructed cDNA libraries from chimpanzee testis and brain, generated a total of 5,000 DNA sequences (ESTs) from these libraries, and compared them to the corresponding human genes. This resulted in 2,844 gene fragments that could be compared between the two species. We divided the observed differences into changes that occurred in the part before the coding region starts (5' UTR), changes that result in a change of the encoded amino acid (non-synonymous substitutions), changes that do not change the encoded amino acid (synonymous substitutions) and changes that occurred in the part downstream of the coding region (3'UTR). By comparing these patterns with the data from the genomic comparison and with data available for human polymorphisms, one can make inferences about the evolutionary forces that shape the different regions. Further, single genes can be identified

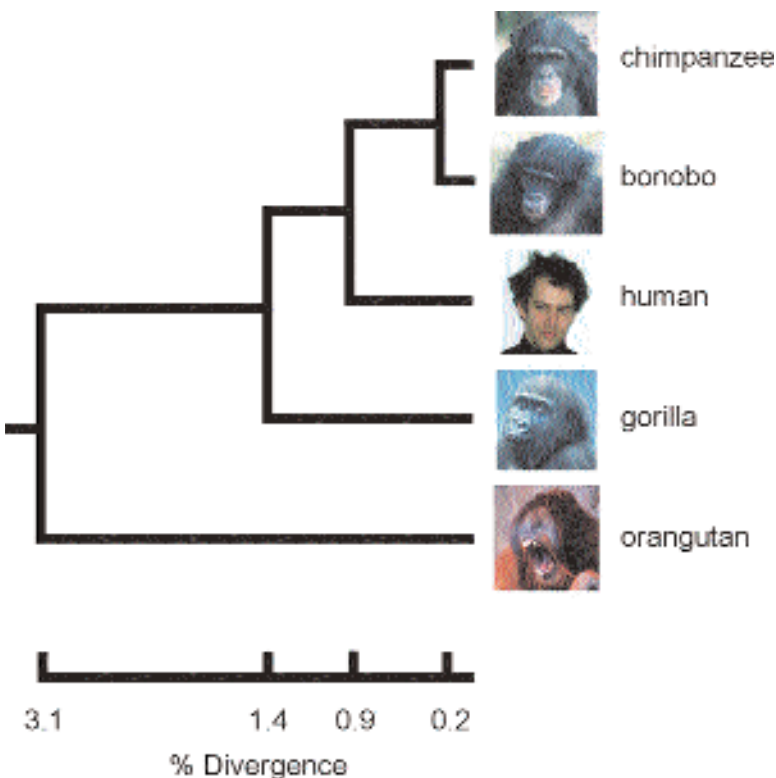


Fig 1: Phylogenetic tree of the extant great apes, represented by a locus on the X chromosome (Xq13.3). The observed differences between two species at that locus are given as percent divergence for the respective nodes. Pictures of non-human primates by M.Seres, MPI-EVAN.

that have an excess of amino acid substitutions and are therefore good candidates for genes that changed their function during human or chimpanzee evolution. In addition to DNA sequence analyses, we also investigated differences in expression patterns of genes. For this, we used arrays (Affymetrix) that contain probes for approximately 12,000 human genes and compared mRNA levels in brain (prefrontal cortex) and liver samples among three adult male humans, three adult male chimpanzees and one adult male orang-utan. The results showed that whereas experimental variation between duplicate tissue samples from the same individual was small, the variation in gene expression between individuals within the species is substantial relative to the differences between humans and chimpanzee. However, the humans as well as the chimpanzees fall into two mutually exclusive groups when their gene expression patterns are related to that seen in the orang-utan (figure 3). This allows gene expression differences common to all humans and all chimpanzees to be identified for each tissue and revealed an apparent 3.8-fold acceleration of changes in gene expression differences in the brain on the human lineage. In contrast, changes in the liver accumulated about equally rapidly in the human lineage and the chimpanzee lineage. Thus, gene expression patterns may have changed more in the human brain than in the chimpanzee brain. The work also allowed us to identify 234 genes that seem to differ in their extent of expression between humans and chimpanzees in liver and 158

Ebersberger I *et al.*: **Genomewide Comparison of DNA Sequences between Humans and Chimpanzees.** *Am J Hum Genet* 2002, 70: 1490-1497

Enard W *et al.*: **Intra- and interspecific variation in primate gene expression patterns.** *Science* 2002, 296:340-343

Kaessmann H, Paabo S: **The genetical history of humans and the great apes.** *J Intern Med* 2002, 251: 1-18

Kaessmann H *et al.*: **Great ape DNA sequences reveal a reduced diversity and an expansion in humans.** *Nat Genet* 2001, 27: 155-156

Paabo S: **Genomics and society. The human genome and our view of ourselves.** *Science* 2001, 291: 1219-1220

such genes in the brain. Further work with these genes might allow us to identify the underlying genetic causes for the observed expression differences, like promoter changes, duplication of genes, deletions of genes, changes in regulatory proteins etc. These genes, or the pathways in which these genes are involved, may also explain some of the observed phenotypic differences between humans and chimpanzees.

In summary, we have taken a first step towards novel insights into evolutionary patterns of the genome and transcriptome of primates and humans. We have identified a first set of genes of potential importance for traits specific to chimpanzee or human. The integration of these and further approaches should make it possible to build more solid hypotheses that link genotypic differences between humans and chimpanzees to phenotypic differences.

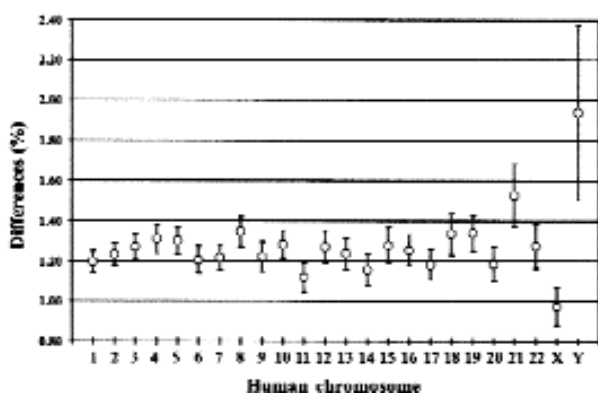


Fig 2: Mean DNA sequence differences between humans and chimpanzees by human chromosome. Bars indicate 95% confidence intervals.

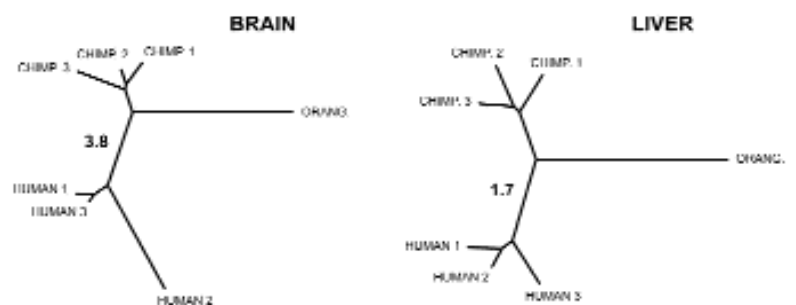


Fig 3: Distance trees representing the relative extent of expression changes among three primate species in brain and liver. Each individual was measured in duplicates. The pairwise distances were calculated by summing up the absolute values of the log₂ ratios for each gene that was assigned to be differently expressed in that pairwise comparison. The distance matrix that resulted from all pairwise comparisons was then used to draw the depicted neighbour joining trees. Numbers refer to the ratio between the changes common to humans and chimpanzees.