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Differences between Properties of Human and Chimpanzee Genomes

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Abstract

The genetic traits that distinguish us from chimpanzees and make us humans are still of great interest. Human and chimpanzee genomes have undergone several changes since ancestral divergence, including single nucleotide substitutions, deletions and duplications of DNA fragments of various sizes, insertions of transposable elements, and chromosomal rearrangements. Human-specific single nucleotide changes make up 1.23% of human DNA, whereas longer deletions and insertions make up ~3% of our genome. At the same time, a much larger proportion consists of differential chromosomal inversions and translocations, involving several megabases in length or even entire chromosomal sections.

However, despite our extensive knowledge of the structural genomic changes that accompany human evolution, we still cannot confidently identify the genes that drive human identity. Most of the structural changes affecting genes occurred at the level of expression regulation, which in turn caused major changes in the regulatory networks of interacting genes. In this review, we summarized the available information on genetic differences between humans and chimpanzees and their potential functional impact on differential molecular, anatomical, physiological, and cognitive traits of these species.

Key words: Chimpanzee, DNA, Genome, Human.

1. Introduction

The Chimpanzee Genome Project was an attempt to sequence the DNA of the chimpanzee genome. Sequencing began in 2005 and by 2013 24 chimpanzees had been sequenced. This project was included in the Great Ape Genome Project.

In 2013, high-resolution sequences from each of the four known chimpanzee subspecies were published: central chimpanzee, Pan troglodytes, 10 sequences; Western chimpanzee, Pan troglodytes versus, 6 sequences; Nigerian Cameroon chimpanzee, Pan troglodytes ellioti, 4 sequences; and eastern chimpanzee, Pan troglodytes schweinfurthii, 4 sequences. All of them were sequenced at an average of 25x coverage per capita.

This study showed significant diversity in the chimpanzee genome with many population characteristics. Central chimpanzees retain the most diversity in the chimpanzee lineage, while other subspecies show signs of population bottlenecks.

2. Background

Human and chimpanzee chromosomes are very similar. The main difference is that humans have one less pair of chromosomes than other apes. Humans have 23 pairs of chromosomes, while other apes have 24 pairs of chromosomes. In the human evolutionary lineage, two ancestral ape chromosomes merged at telomeres to form human chromosome 2. There are nine other major chromosomal differences between chimpanzees and humans. Human chromosomes 1, 4, 5, 9, 12, 15, 16, 17 and 18. After completion of the

Human Genome Project, the Common Chimpanzee Genome Project was initiated. In December 2003, a preliminary analysis of the 7,600 genes shared by the two genomes confirmed that some genes, such as the fork head-box P2 transcription factor involved in language development, are different from humans.

Several genes involved in hearing were also found to have changed during human evolution, suggesting selection involving human language-related behavior. Differences between individual humans and common chimpanzees are estimated to be about 10 times the typical difference between pairs of humans.

Another study showed that patterns of DNA methylation, which are a known regulation mechanism for gene expression, differ in the prefrontal cortex of humans versus chimpanzees, and implicated this difference in the evolutionary divergence of the two species.

Chimpanzee-human chromosome differences. A major structural difference is that human chromosome 2 (green color code) was derived from two smaller chromosomes that are found in other great apes (now called 2A and 2B).

Parts of the human chromosome 2 are scattered across parts of several feline and murine chromosomes of these species that are more distantly related to humans (an ancient common ancestor, about 85 million years from a common human/rodent ancestor).

3. Draft genome sequence of the common chimpanzee

An analysis of the chimpanzee genome sequence was published in Nature on September 1, 2005, in an article produced by the Chimpanzee Sequencing and Analysis Consortium, a group of scientists which is supported in part by the National Human Genome Research Institute, one of the National Institutes of Health. The article marked the completion of the draft genome sequence.

A database now exists containing the genetic differences between human and chimpanzee genes, with about thirty-five million single-nucleotide changes, five million insertion/deletion events, and various chromosomal rearrangements. Gene duplications account for most of the sequence differences between humans and chimps.

Single base pair substitutions account for about half of the genetic changes compared to gene duplication. The typical homologues of human and chimpanzee proteins differ on average by only two amino acids. About 30% of all human proteins have the same sequence as the corresponding chimpanzee proteins. As mentioned above, gene duplication is a major difference between human and chimpanzee genetic material, and currently about 2.7% of the genome differs due to gene duplication or deletion in the approximately 6 million years since humans and chimpanzees diverged from their common genetic material. The comparable variation within human populations is 0.5 percent.

About 600 genes were identified that may have been undergoing strong positive selection in the human and chimpanzee lineages; many of these genes are involved in immune system defense against microbial disease (example: granulysin is protective against Mycobacterium tuberculosis) or are targeted receptors of pathogenic microorganisms (example: Glycophorin C and Plasmodium falciparum). By comparing human and chimpanzee genes to the genes of other mammals, it has been found that genes coding for transcription factors, such as forkhead-box P2 (FOXP2), have often evolved faster in the human relative to chimpanzee; relatively small changes in these genes may account for the morphological differences between humans and chimpanzees. A set of 348 transcription factor genes code for proteins with an average of about 50 percent more amino acid changes in the human lineage than in the chimpanzee lineage.

Six human chromosomal regions were found that may have been under particularly strong and coordinated selection during the past 250,000 years. These regions contain at least one marker allele that seems unique to the human lineage while the entire chromosomal region shows lower than normal genetic variation. This pattern suggests that one or a few strongly selected genes in the chromosome region may have been preventing the random accumulation of neutral changes in other nearby genes. One such region on chromosome 7 contains the FOXP2 gene (mentioned above) and this region also includes the Cystic fibrosis transmembrane conductance regulator (CFTR) gene, which is important for ion transport in tissues such as the salt-secreting epithelium of sweat glands. Human mutations in the CFTR gene might be selected for as a way to survive cholera.

Another such region on chromosome 4 may contain elements regulating the expression of a nearby protocadherin gene that may be important for brain development and function. Although changes in expression of genes that are expressed in the brain tend to be less than for other organs (such as liver) on average, gene expression changes in the brain have been more dramatic in the human lineage than in the chimpanzee lineage. This is consistent with the dramatic divergence of the unique pattern of human brain development seen in the human lineage compared to the ancestral great ape pattern. The protocadherin-beta gene cluster on chromosome 5 also shows evidence of possible positive selection.

Results from the human and chimpanzee genome analyses should help in understanding some human diseases. Humans appear to have lost a functional gene for caspase 12, which encodes an enzyme that protects against Alzheimer's disease in other primates.

4. Human Genome

The human genome is the set of complete human nucleic acid sequences encoded in DNA on small DNA molecules found in the 23 pairs of chromosomes and individual mitochondria in the cell nucleus. They are usually considered as nuclear genome and mitochondrial genome separately. The human genome contains both DNA sequences that encode proteins and various types of DNA that do not encode proteins.

The latter is a diverse category that includes DNA coding for non-translated RNA, such as that for ribosomal RNA, transfer RNA, ribozymes, small nuclear RNAs, and several types of regulatory RNAs. It also includes promoters and their associated gene-regulatory elements, DNA playing structural and replicatory roles, such as scaffolding regions, telomeres, centromeres, and origins of replication, plus large numbers of transposable elements, inserted viral DNA, non-functional pseudogenes and simple, highly-repetitive sequences. Introns make up a large percentage of non-coding DNA. Some of this non-coding DNA is non-functional junk DNA, such as pseudogenes, but there is no firm consensus on the total amount of junk DNA.

Haploid human genomes, which are contained in germ cells (the egg and sperm gamete cells created in the meiosis phase of sexual reproduction before fertilization) consist of 3,054,815,472 DNA base pairs (if X chromosome is used), while female diploid genomes (found in somatic cells) have twice the DNA content.

Although there are significant differences between the human genomes (~0.1% due to single nucleotide variants and ~0.6% due to insertions), humans and their closest living relatives, bonobos and chimpanzees (~1.1% fixed single nucleotide variants and 4% with indels included).). Base pairs can also vary in size. Telomere length decreases after each cycle of DNA replication.

Although the sequence of the human genome has been completely determined by DNA sequencing, it is not yet fully understood. Most, but not all, genes have been identified by a combination of high throughput experimental and bioinformatics approaches, yet much work still needs to be done to further

elucidate the biological functions of their protein and RNA products (in particular, annotation of the complete CHM13v2.0 sequence is still ongoing). And yet, overlapping genes are quite common, in some cases allowing two protein coding genes from each strand to reuse base pairs twice (for example, genes DCDC2 and KAAG1). Recent results suggest that most of the vast quantities of noncoding DNA within the genome have associated biochemical activities, including regulation of gene expression, organization of chromosome architecture, and signals controlling epigenetic inheritance.

Human DNA contains a significant number of retroviruses, at least three of which have important functions (e.g., the HIV-like HERV-K, HERV-W and HERV-FRD play a role in placental formation by causing cell fusion).

In 2003, scientists reported sequencing 85% of the entire human genome, but in 2020 at least 8% is still missing.

In 2021, scientists will have a complete female genome (i.e. lack of Y chromosome). These sequences identified 19,969 protein-coding sequences representing approximately 1.5% of the genome and a total of 63,494 genes (mostly non-coding RNA genes). The genome consists of DNA regulatory sequences, LINEs, SINEs, introns, and sequences whose function has not yet been determined.

5. Conclusions

Directly comparable DNA sequences between the two genomes are nearly 99% identical. When DNA insertions and deletions are accounted for, humans and chimpanzees still share 96% of the same sequences.

At the protein level, 29 percent of genes code for the same amino sequences in chimps and humans. In fact, the typical human protein has accumulated just one unique change since chimps and humans diverged from a common ancestor about 6 million years ago.

To put this into perspective, the number of genetic differences between humans and chimps is approximately 60 times less than that seen between human and mouse and about 10 times less than between the mouse and rat. On the other hand, the number of genetic differences between a human and a chimp is about 10 times more than between any two humans.

The researchers discovered that a few classes of genes are changing unusually quickly in both humans and chimpanzees compared with other mammals.

These classes include genes involved in perception of sound, transmission of nerve signals, production of sperm and cellular transport of electrically charged molecules called ions. Researchers suspect the rapid evolution of these genes may have contributed to the special characteristics of primates, but further studies are needed to explore the possibilities.

The genomic analyses also showed that humans and chimps appear to have accumulated more potentially deleterious mutations in their genomes over the course of evolution than have mice, rats and other rodents. While such mutations can cause diseases that may erode a species' overall fitness, they may have also made primates more adaptable to rapid environmental changes and enabled them to achieve unique evolutionary adaptations, researchers said.

Despite the many similarities found between human and chimp genomes, the researchers emphasized that important differences exist between the two species.

About 35 million DNA base pairs differ between the shared portions of the two genomes, each of which, like most mammalian genomes, contains about 3 billion base pairs. In addition, there are another 5

million sites that differ because of an insertion or deletion in one of the lineages, along with a much smaller number of chromosomal rearrangements. Most of these differences lie in what is believed to be DNA of little or no function. However, as many as 3 million of the differences may lie in crucial protein-coding genes or other functional areas of the genome.

"As the sequences of other mammals and primates emerge in the next couple of years, we will be able to determine what DNA sequence changes are specific to the human lineage.

The genetic changes that distinguish humans from chimps will likely be a very small fraction of this set," said the study's lead author, Tarjei S. Mikkelsen of the Broad Institute of MIT and Harvard. Among the genetic changes that researchers will be looking for are those that may be related to the human-specific features of walking upright on two feet, a greatly enlarged brain and complex language skills.

Although the statistical signals are relatively weak, a few classes of genes appear to be evolving more rapidly in humans than in chimps. The single strongest outlier involves genes that code for transcription factors, which are molecules that regulate the activity of other genes and that play key roles in embryonic development.

A handful of other genes have undergone even more dramatic changes. More than 50 genes present in the human genome are missing or partially deleted in the chimpanzee genome. The number of corresponding gene deletions in the human genome is not yet known precisely. For genes with known function, it is already possible to identify potential consequences of these changes.

For example, researchers found that three key genes related to inflammation were deleted from the chimpanzee genome, which may explain some of the known differences between chimpanzees and humans in terms of immune and inflammatory responses.

In contrast, humans appear to have lost function of the caspase-12 gene, which produces an enzyme that may help protect other animals from Alzheimer's disease.

"This is just the tip of the iceberg in understanding the genomic roots of biological differences. As we learn more about the different functional elements of the genome, we expect other important differences beyond the protein-coding genes to emerge."

Chimpanzee Sequences Armed with, the researchers also scanned the entire human genome for deviations from normal mutation patterns.

Such deviations may reveal regions of "selective sweeps," which occur when a mutation arises in a population and is so advantageous that it spreads throughout the population within a few hundred generations and eventually becomes "normal."

The researchers found six regions in the human genome that have strong signatures of selective sweeps over the past 250,000 years. One region contains more than 50 genes, while another contains no known genes and lies in an area that scientists refer to as a "gene desert." Intriguingly, this gene desert may contain elements regulating the expression of a nearby protocadherin gene, which has been implicated in patterning of the nervous system. A seventh region with moderately strong signals contains the FOXP2 and CFTR genes.

FOXP2 is involved in speech acquisition in humans. CFTR, which encodes a protein involved in ion transport and whose mutations can lead to lethal cystic fibrosis, is considered a target for positive selection in European populations.

References

1. Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., et al. 2000. Gene ontology: Tool for the unification of biology. The Gene Ontology Consortium. Nat. Genet. 25: 25-29.
2. Baum, J., Ward, R.H., and Conway, D.J. 2002. Natural selection on the erythrocyte surface. Mol. Biol. Evol. 19: 223-229.
3. Birtle, Z., Goodstadt, L., and Ponting, C.P. 2005. Duplication and positive selection among hominin-specific PRAME genes. BMC Genomics 6: 120.
4. Blanco-Arias, P., Sargent, C.A., and Affara, N.A. 2004. A comparative analysis of the pig, mouse, and human PCDHX genes. Mamm. Genome 15: 296-306.
5. Breuer, T., Ndoundou-Hockemba, M., and Fishlock, V. 2005. First observation of tool use in wild gorillas. PLoS Biol. 3: e380. Britten, R.J. 2002. Divergence between samples of chimpanzee and human DNA sequences is 5%, counting indels. Proc. Natl. Acad. Sci. 99: 13633-13635.
6. Burki, F. and Kaessmann, H. 2004. Birth and adaptive evolution of a hominoid gene that supports high neurotransmitter flux. Nat. Genet. 36: 1061-1063.
7. Caceres, M., Lachuer, J., Zapala, M.A., Redmond, J.C., Kudo, L., Geschwind, D.H., Lockhart, D.J., Preuss, T.M., and Barlow, C. 2003. Elevated gene expression levels distinguish human from non-human primate brains. Proc. Natl. Acad. Sci. 100: 13030-13035.
8. Canavez, F., Young, N.T., Guethlein, L.A., Rajalingam, R., Khakoo, S.I., Shum, B.P., and Parham, P. 2001. Comparison of chimpanzee and human leukocyte Ig-like receptor genes reveals framework and rapidly evolving genes. J. Immunol. 167: 5786-5794.
9. Carroll, S.B. 2003. Genetics and the making of Homo sapiens. Nature 422: 849-857.
10. Carroll, M.L., Roy-Engel, A.M., Nguyen, S.V., Salem, A.H., Vogel, E., Vincent, B., Myers, J., Ahmad, Z., Nguyen, L., Sammarco, M., et al. 2001. Large-scale analysis of the Alu Ya5 and Yb8 subfamilies and their contribution to human genomic diversity. J. Mol. Biol. 311: 17-40.
11. Chen, F.C. and Li, W.H. 2001. Genomic divergences between humans and other hominoids and the effective population size of the common ancestor of humans and chimpanzees. Am. J. Hum. Genet. 68: 444-456.
12. Cheng, Z., Ventura, M., She, X., Khaitovich, P., Graves, T., Osoegawa, K., Church, D., DeJong, P., Wilson, R.K., Pääbo, S., et al. 2005. A genome-wide comparison of recent chimpanzee and human segmental duplications. Nature 437: 88-93.
13. The Chimpanzee Sequencing and Analysis Consortium. 2005. Initial sequence of the chimpanzee genome and comparison with the human genome. Nature 437: 69-87.
14. Chou, H.H., Takematsu, H., Diaz, S., Iber, J., Nickerson, E., Wright, K.L., Muchmore, E.A., Nelson, D.L., Warren, S.T., and Varki, A. 1998. A mutation in human CMP-sialic acid hydroxylase occurred after the Homo-Pan divergence. Proc. Natl. Acad. Sci. 95: 11751-11756.
15. Chou, H.H., Hayakawa, T., Diaz, S., Krings, M., Indriati, E., Leakey, M., Pääbo, S., Satta, Y., Takahata, N., and Varki, A. 2002. Inactivation of CMP-N-acetylneuraminic acid hydroxylase occurred prior to brain expansion during human evolution. Proc. Natl. Acad. Sci. 99: 11736-11741.

16. Clark, A.G., Glanowski, S., Nielsen, R., Thomas, P.D., Kejariwal, A., Todd, M.A., Tanenbaum, D.M., Civello, D., Lu, F., Murphy, B., et al. 2003. Inferring nonneutral evolution from human-chimp-mouse orthologous gene trios. Science 302: 1960-1963.

17. Currie, P. 2004. Human genetics: Muscling in on hominid evolution. Nature 428: 373-374.

18. Dennehey, B.K., Gutches, D.G., McConkey, E.H., and Krauter, K.S. 2004. Inversion, duplication, and changes in gene context are associated with human chromosome 18 evolution. Genomics 83: 493-501.

19. Dorus, S., Vallender, E.J., Evans, P.D., Anderson, J.R., Gilbert, S.L., Mahowald, M., Wyckoff, G.J., Malcom, C.M., and Lahn, B.T. 2004. Accelerated evolution of nervous system genes in the origin of Homo sapiens. Cell 119: 1027-1040.

20. Dykxhoorn, D.M., Novina, C.D., and Sharp, P.A. 2003. Killing the messenger: Short RNAs that silence gene expression. Nat. Rev. Mol. Cell. Biol. 4: 457-467.

21. Enard, W. and Pääbo, S. 2004. Comparative primate genomics. Annu. Rev. Genomics Hum. Genet. 5: 351-378.

22. Enard, W., Fassbender, A., Model, F., Adorjan, P., Pääbo, S., and Olek, A. 2004. Differences in DNA methylation patterns between humans and chimpanzees. Curr. Biol. 14: R148-R149.

23. Evans, B.A., Fu, P., and Tregear, G.W. 1994. Characterization of two relaxin genes in the chimpanzee. J. Endocrinol. 140: 385-392.

24. Evans, P.D., Gilbert, S.L., Mekel-Bobrov, N., Vallender, E.J., Anderson, J.R., Vaez-Azizi, L.M., Tishkoff, S.A., Hudson, R.R., and Lahn, B.T. 2005. Microcephalin, a gene regulating brain size, continues to evolve adaptively in humans. Science 309: 1717-1720.

25. Fan, Y., Linardopoulou, E., Friedman, C., Williams, E., and Trask, B.J. 2002a. Genomic structure and evolution of the ancestral chromosome fusion site in 2q13-2q14.1 and paralogous regions on other human chromosomes. Genome Res. 12: 1651-1662.

26. Fan, Y., Newman, T., Linardopoulou, E., and Trask, B.J. 2002b. Gene content and function of the ancestral chromosome fusion site in human chromosome 2q13-2q14.1 and paralogous regions. Genome Res. 12: 1663-1672.

27. Freimer, N. and Sabatti, C. 2003. The human phenome project. Nat. Genet. 34: 15-21.

28. Gagneux, P. 2004. A Pan-Oramic view: Insights into hominid evolution through the chimpanzee genome. Trends Ecol. Evol. 19: 571-576.

29. Gagneux, P., Moore, J.J., and Varki, A. 2005. The ethics of research on great apes. Nature 437: 27-29.

30. Grossman, L.I., Wildman, D.E., Schmidt, T.R., and Goodman, M. 2004. Accelerated evolution of the electron transport chain in anthropoid primates. Trends Genet. 20: 578-585.

31. Gu, J. and Gu, X. 2003. Induced gene expression in human brain after the split from chimpanzee. Trends Genet. 19: 63-65.

32. Gygi, S.P., Rochon, Y., Franza, B.R., and Aebersold, R. 1999. Correlation between protein and mRNA abundance in yeast. Mol. Cell. Biol. 19: 1720-1730.

33. Hamann, J., Kwakkenbos, M.J., de Jong, E.C., Heus, H., Olsen, A.S., and van Lier, R.A. 2003. Inactivation of the EGF-TM7 receptor EMR4 after the Pan-Homo divergence. Eur. J. Immunol. 33: 1365-1371.

34. Hayakawa, T., Angata, T., Lewis, A.L., Mikkelsen, T.S., Varki, N.M., and Varki, A. 2005. A human-specific gene in microglia. Science 309: 1693.

35. Irie, A., Koyama, S., Kozutsumi, Y., Kawasaki, T., and Suzuki, A. 1998. The molecular basis for the absence of N-glycolylneuraminic acid in humans. J. Biol. Chem. 273: 15866-15871.

36. Johnson, M.E., Viggiano, L., Bailey, J.A., Abdul-Rauf, M., Goodwin, G., Rocchi, M., and Eichler, E.E. 2001. Positive selection of a gene family during the emergence of humans and African apes. Nature 413: 514-519.

37. Kim, V.N. 2005. MicroRNA biogenesis: Coordinated cropping and dicing. Nat. Rev. Mol. Cell. Biol. 6: 376-385. CrossRefMedlineGoogle Scholar

38. Lander, E.S., Linton, L.M., Birren, B., Nusbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., Fitzhugh, W., et al., 2001. Initial sequencing and analysis of the human genome. Nature 409: 860-921.

39. Li, W.H. and Saunders, M.A. 2005. News and views: The chimpanzee and us. Nature 437: 50-51.

40. Marshall, D., Hardman, M.J., Nield, K.M., and Byrne, C. 2001. Differentially expressed late constituents of the epidermal cornified envelope. Proc. Natl. Acad. Sci. 98: 13031-13036.

41. Mekel-Bobrov, N., Gilbert, S.L., Evans, P.D., Vallender, E.J., Anderson, J.R., Hudson, R.R., Tishkoff, S.A., and Lahn, B.T. 2005. Ongoing adaptive evolution of ASPM, a brain size determinant in Homo sapiens. Science 309: 1720-1722.

42. Nickerson, E. and Nelson, D.L. 1998. Molecular definition of pericentric inversion breakpoints occurring during the evolution of humans and chimpanzees. Genomics 50: 368-372.

43. Preuss, T.M., Caceres, M., Oldham, M.C., and Geschwind, D.H. 2004. Human brain evolution: Insights from microarrays. Nat. Rev. Genet. 5: 850-860.

44. Rearden, A., Phan, H., Kudo, S., and Fukuda, M. 1990. Evolution of the glycophorin gene family in the hominoid primates. Biochem. Genet. 28: 209-222.

45. Ross, N.L., Mavrogiannis, L.A., Sargent, C.A., Knight, S.J., Wadekar, R., DeLisi, L.E., and Crow, T.J. 2003. Quantitation of X-Y homologous genes in patients with schizophrenia by multiplex polymerase chain reaction. Psychiatr. Genet. 13: 115-119.

46. Satta, Y., Klein, J., and Takahata, N. 2000. DNA Archives and our nearest relative: The trichotomy problem revisited. Mol. Phylogenet. Evol. 14: 259-275.

47. Shillito, D.J., Gallup, G.G.J., and Beck, B.B. 1999. Factors affecting mirror behaviour in western lowland gorillas, Gorilla gorilla. Anim. Behav. 57: 999-1004.

48. Steinmetz, L.M. and Davis, R.W. 2004. Maximizing the potential of functional genomics. Nat. Rev. Genet. 5: 190-201.

49. Varki, A., Wills, C., Perlmutter, D., Woodruff, D., Gage, F., Moore, J., Semendeferi, K., Benirschke, K., Katzman, R., Doolittle, R., et al. 1998. Great Ape Phenome Project? Science 282: 239-240.

50. Wang, X., Thomas, S.D., and Zhang, J. 2004. Relaxation of selective constraint and loss of function in the evolution of human bitter taste receptor genes. Hum. Mol. Genet. 13: 2671-2678.