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Evolution of man in the light of molecular genetics: a review. Part II. Regulation of gene function, evolution of speech and of brains

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In the first part of this review the evolutionary history and genomics of the human species were considered in the light of molecular genetic evidence. In this second part the emphasis will be put on the regulation of the function of the genes and evolution of the human-specific traits such as enormously large brains and the capacity to communicate with a spoken language. The age-old question of what specifically makes us humans is also dealt with in its new lightning of molecular genetics of the genome era. It is concluded that, in addition to the structural differences of the genomes, it is most likely that it is different pattern of the regulation of the function of the genes, which evolved for most part through positive natural and sexual selection where the growth and the structure of the human population played a significant role, that differentiates us from our closest living relatives. In this process of the evolution of the most human-specific characteristics, like the size of brains, specifically that or the neocortex, and ability to speak, interbreeding with other forms of the *Homo*-genus may have played a role. In addition to the role of positive selection in general in the evolution of different human-specific traits, it is evident that this progressive selection has been quite effective, thus leading to accelerated evolution of these traits. Finally it can also be concluded that genetic and cultural evolution have gone hand in hand during the recent, and still continuing, evolution of the mankind interacting with each others in a bidirectional fashion.

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The last 50 years of molecular genetics have produced an abundance of new discoveries and data that make it useful to revisit some basic concepts and assumptions in our thinking about genomes and evolution. Chief among these observations is e.g. the complex modularity of genome organization. Genomes are hierarchically organized as systems assembled from DNA modules (Shapiro 2002, 2005). In particular the analysis of genome-wide messenger RNA expression profiles in man revealed a clustering of highly expressed genes to specific chromosomal regions (Caron et al. 2001).

The first statistical rigorous analyses of complete genomes, together with the availability of abundant gene-expression data, have shown that, contrary to the earlier assumptions, gene order in eukaryotic organisms is not random. It seems that genes that have similar and/or coordinated expression are often clustered (Hurst et al. 2004).

Genetic variation, through its effects on gene expression, plays a crucial role in phenotypic variation. Recent studies of many groups have integrated a number of resources and technologies to assess several aspects of genome variation affecting gene expression, and some of these large-scale mapping studies involving the expression of quantitative traits in model organism and man have recently been reviewed (de Koning and Haley 2005; Gibson and Weir 2005; Pastinen et al. 2006).

The most extensive review of transcriptional regulatory elements in the human genome is, however, that of Maston et al. (2006). They stated that the expression of eukaryotic protein-coding genes can be regulated at several steps, including transcription initiation and elongation, and mRNA processing, transport, translation and stability. Most regulation, however, is believed to occur at the level of transcription initiation. Further, Maston et al. (2006) concluded that the emerging picture about transcriptional regulation in man suggests that this regulation is a much more dynamic process than was once perceived. Interplay between the entire suite of core promoters, proximal regulatory elements, and distal regulatory elements, as well as their binding factors and cofactors, contribute to the precise nature of transcriptional output of a given promoter.

As first suggested by King and Wilson (1975), and subsequently nowadays also confirmed by many studies, the morphological and anatomical differences between humans and chimpanzees are mainly caused by differences in the regulation of the function of the genes. Similarly, inter-individual variation in gene expression also in man has proven to be in part governed by regulatory genetic determinants, which may be *trans*- or *cis*-acting, and which may harbour

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common haplotypes that affect total expression of a gene (Pastinen et al. 2005). In fact, even the variation in gene expression between different ethnic groups is accounted for by common genetic variants of *cis* determinants of the gene function (Spielman et al. 2007). It has been estimated that even though heritable expression differences resulting from *trans*-acting mechanisms appears to be quantitatively more important, *cis*-acting variation may explain up to 25 to 35% of inter-individual differences in gene expression (Pastinen and Hudson 2004).

In addition to the single-nucleotide polymorphism (SNP), copy number variation (CNV) has also been proven to affect the gene expression phenotypes in man among and across populations (Stranger et al. 2007). SNP accounted for ca 84% and CNV ca 18% of the variation in gene expression found in this study between individuals, but the two types had little overlap.

Most recent research results shows that at least 58% of the human transcriptome is expressed in the cortex of the brain, and in addition suggest that genetic variability in the form of single-nucleotide polymorphism (SNP) can contribute to the variability of cortical transcript expression (Myers et al. 2007).

The advent of whole-genome sequencing and increasingly complete surveys of genetic variation in man represents a turning point in the study of positive natural selection in humans. It is now possible to identify new candidates for selection and to re-evaluate previous claims by comparison with empirical distributions of DNA sequence variation across the human genome and among populations (Sabeti et al. 2006, 2007). Most recent applications of this type of surveys have already revealed many candidate regions for positive natural selection in the human genome (Sabeti et al. 2007). Such endeavours will be enhanced in the near future to define coding and regulatory regions of the genome, which have been or presently are involved in positive natural selection. True understanding of the role of adaptive evolution in man will require collaboration across multiple disciplines, including molecular and structural biology, medical and population genetics, and history and anthropology (Sabeti et al. 2007).

The first part of this review (Portin 2007) dealt with the genomics and evolutionary history of man. Here I review recent findings and hypotheses concerning molecular evolution of man with special emphasis on the variation in the regulation of gene function, and focus on the evolution of brain and speech, characters that genetically make us specifically human.

THE IMPORTANCE OF REGULATORY ELEMENTS OF OUR DNA

The faithful execution of biological processes such as development, proliferation, apoptosis, aging and differentiation requires a precise and carefully orchestrated set of steps that depend on the proper spatial and temporal expression of genes. To understand the molecular mechanisms that govern specific expression patterns on a global scale, it is important to identify the regulatory elements associated with each gene (Maston et al. 2006).

Natural variation in gene expression is extensive in humans and other organisms, and the variation in the baseline expression level of many genes has a heritable component (reviewed by Morley et al. 2004). To localize the genetic determinants of these quantitative traits or expression phenotypes, Morley et al. (2004) used microarray to measure gene expression levels and performed genome-wide linkage analysis for expression levels of 3554 genes in 14 large families. For approximately 1000 expression phenotypes there was significant evidence of linkage to specific chromosome regions. Both *cis*- and *trans*-acting loci were found to regulate variation in the levels of genes, although most of these act in *trans*. In addition to genomic regions with regulatory loci that affect single phenotypes in *cis* or *trans*, genomic regions containing transcriptional regulators that influence multiple expression phenotypes, were also found.

In a further study the same research group (Cheung et al. 2005) carried out association analysis with dense sets of single-nucleotide polymorphism (SNP) markers from the International HapMap Project (The International HapMap Consortium 2003). Firstly, for a set of 374 phenotypes with evidence of *cis*-linked determinants, association analysis was performed. Secondly, attention was restricted to 27 phenotypes with the strongest linkage evidence for *cis*-acting determinants and over 770 000 SNPs in the human genome were tested for association with these.

Of the 374 phenotypes studied, 65 (17%) showed also highly significant ($P < 0.001$) evidence for association with at least one SNP marker, and 133 (36%) showed less, but in any case significant ($P < 0.01$) evidence for association. Moreover, the strength of linkage observed in the family studies predicted the results of the association studies. For example, among the 27 phenotypes with highly significant *cis* linkage, 70% also showed evidence of *cis* association at $P < 0.001$ level. These findings suggest that, beside linkage studies, genome-wide association studies with dense SNP maps are also able to identify *cis*-acting regulatory elements in genomes.

Using SNPs as markers Pastinen et al. (2004) carried out a survey of genetic and epigenetic variation affecting human gene expression in a lymphoblastoid cell line of the HapMap panel. This survey identified 23 genes out o 129 with common allele-specific transcripts whose expression deviated from the expected equimolar ratio. Thus, the survey demonstrated allelic differences in gene expression, reflecting the presence of putative allele-specific *cis*-acting factors of either genetic or epigenetic nature.

Similar results suggesting that regulatory polymorphism is widespread in the human genome were obtained by Stranger et al. (2005) by performing genome-wide quantitative trait analysis for the level of gene expression of 630 genes in 60 unrelated individuals and also using the publicly available data of the International HapMap Project. For the 374 expressed genes, they found many regions with statistically significant association of single nucleotide polymorphisms (SNPs) with expression variation in lymphoblastoid cell lines.

Using association methods Pastinen et al. (2005) mapped *cis*-acting variants in HapMap samples. They identified 16 loci out of 64, each of which harbours common haplotypes that affect total expression of genes, and a further 17 loci with evidence of haplotypes affecting relative allelic expression.

In the most extensive study conducted so far, Stranger et al. (2007) observed in 14 072 genes tested for their expression levels significant association with at least one SNP in 888 cases. In 99 cases association to copy number variation (CNV) in at least two of the four HapMap populations studied was observed. Without going into details, it can be said that the research group detected a large number of regions that appear to carry genetic variation affecting gene expression, and a considerable amount of variation concerning the presence of regulatory elements between populations was also found.

Lettice et al. (2002) were the first to postulate that chromosomal regions in man that are gene-poor, and therefore often called gene deserts, harbour gene regulatory elements that have the ability to modulate gene expression over very long distances. This hypothesis was supported by the observation of Nobrega et al. (2003) indicating that several of the enhancers, which they characterized in their study of the genomic regions surrounding the DACH locus of man, reside in gene deserts.

Subsequent studies reviewed by Kleinjan and van Heyningen (2005) and extended by Pennacchio et al. (2006), Xie et al. (2007), Margulies et al. (2007) and King et al. (2007), have shown that enhancers and repressors of gene activity are often located in evolutionary conserved non-coding regions of the genome. Some of these regulatory regions, as well as binding sites of microRNAs, presently known to be involved in the control of gene function (Bartel 2004), also show evidence for recent selection (King et al. 2007; Chen and Rajewsky 2006).

So called ultraconserved elements, first found by comparing the human, chimpanzee, mouse and rat genomes (Bejerano et al. 2004), most of which are non-protein coding regions, are unique to vertebrates and have undergone little or no change since mammal and bird ancestors diverged about 300 million years ago. The reason for their extreme conservation has been supposed to be that they are mutation cold spots, but more likely they are unusually large patches (over 200 base pairs long) of sites under negative selection (Keightley et al. 2005; Kryukov et al. 2005; Drake et al. 2005).

These ultraconserved elements of the human genome are important regulatory elements of gene function. They are most often located in either overlapping exons in genes involved in RNA processing or in introns or nearby genes involved in the regulation of genetic transcription and development (Bejerano et al. 2004). Most interestingly, mutations in these non-protein coding regulatory elements of the human genome are negatively very strongly selected – even stronger than in protein coding region (Katzman et al. 2007). Actually, the data argue that the ultraconserved elements in the human genome are currently, as well as historically, strongly constrained functional elements.

The hypothesis that the regulation of the function of the genes has been important in the evolution of man can also be supported by the study of Kitano and Saitou (2005) involving the rate of nucleotide substitutions in the 5′ upstream sequences of genes. They compared nucleotide sequences of nine 5′ upstream gene regions for human, chimpanzee, gorilla and orang-utan, and observed that the rate of nucleotide substitutions in the 5′ regions was lower than that of synonymous sites in the coding regions, suggesting that 5′ upstream regions have evolved under some functional constraints.

Together the evidence provided by these large-scale surveys of regulatory genetic elements in man demonstrates the importance of the regulatory elements of gene expression in the phenotypic variation of mankind both within and between populations and ethnic groups. Gene expression is the basis for many crucial functions in the cell, so the relative contribution of the genetic variants affecting regulation of gene expression is an indication of the nature of the mutational and

natural selection processes that contribute to the phenotypic diversity and divergence.

Following Maston et al. (2006), it can be stated that regulatory systems of gene function in man are robust and redundant, and yet highly sensitive as well. Even single-nucleotide differences in the regulatory sequence can have significant effects on gene expression. Therefore, it can be suggested that transcriptional regulation can cover a broad, continuous spectrum of regulatory control, such that it is likely that discrete models of regulatory action may apply to only a limited sets of promoters.

It is most likely that the evolution of the regulation of the function of genes has played a significant role in the emergence of man being probably the most important of the biological factors that distinguish the humans from other species.

THE PUTATIVE ONGOING EVOLUTION OF OUR BRAINS

A general view of the evolution of human brains

One of the most distinctive characteristics of humans among primates is the size, organization and function of the brain. We humans are proud of our big brains, and rightly so. The human brain is proportionally larger than that of any other animal. Its highly advanced cognitive powers have spurred us to create art and science, build cities and cultivate earth. But just why and how natural selection blessed us with these talents has been poorly understood. The fossil record and modern genetic studies clearly show that the evolution of higher cognition began sometime after the chimp and human lines split, some 5 to 6 million years ago, and continued at least until the rise of modern humans, roughly 200 000 years ago. Rapidly advancing knowledge of genome structure and sequence enables new means for the analysis of specific DNA changes associated with differences between the human brain and that of other mammals and consequently the evolution of our brains. Recent studies have implicated evolutionary changes in messenger RNA and protein expression levels, as well as DNA changes that alter amino acid sequences (Hill and Walsh 2005). Likewise, the most recent studies of the expression of non-coding RNAs in the human brains have already significantly increased our knowledge and understanding of the evolution of the human brain.

Some recent studies seem to suggest the possibility that the evolution of brains might still be going on. On the other hand, the molecular basis of the evolution of brains is still an open question, but at the same time we start to understand the conditions, which constituted the selection pressures that lead to the evolution of our big brains.

A whole-genome genotyping and expression analysis on a series of 193 neuropathologically normal postmortem human brain samples showed that 58% of the transcriptome is cortically expressed in at least 5% of the samples, and that of these cortically expressed transcripts, 21% have expression profiles that correlate with their genotypes (Myers et al. 2007). More precisely, associations between single SNPs and expression levels were found, suggesting that genetic variability can contribute to the variability of transcript expression. Results suggesting the same conclusion, were derived also by Bray et al. (2004) even though they had a much smaller sample of 19 subjects. They namely found that the presence of common *cis*-acting variation influenced the expression level of seven of the 15 assayed genes.

Recent evidence, reviewed by Dunbar and Schultz (2007), suggests that it was population structure, rather than pure ecological conditions, which constituted the selection pressure leading to the evolution of our big brains. The evidence clearly favours the view that it was the computational demands of living in large, complex societies that selected for large brains. Moreover, recent analyses also suggest that it may have been the particular demands of the more intense forms of pair-bonding that was the critical factor that triggered this evolutionary development.

It seems to me that of the different theories presented on the evolution of our brains, the one which suggests co-evolution of sociality, size and function of brains, and spoken language, is the most plausible. The increase in the size of the social group conditioned an increase of the size of the brains and communication by spoken language, and vice versa.

Protein coding genes affecting the size of the brains

Autosomal recessive primary microcephaly (MCPH) is a neuro-developmental disorder that causes a great reduction in brain growth *in utero*. MCPH is hypothesized to be a primary disorder of neurogenic mitosis, leading to reduced neuron number. Hence, MCPH proteins are likely to be important components of cellular pathways regulating brain size (Cox et al. 2006). Defects of at least six genes can cause this disorder and four of these have recently been identified and analysed. The analyses have been reviewed by Bradbury (2005), Cox et al. (2006) and Tang (2006). These four genes are: autosomal primary microcephaly 1 (*microcephalin* or *MCPH1*), abnormal spindle-like, microcephaly associated (*ASPM*), cyclin-dependent kinase 5 regulatory subunit-associated

protein 2 (*CDK5RAP2*) and centromere protein J (*CENPJ*).

Molecular genetic evidence pointed to the direction that the evolution of all these four genes has been accelerated in the human lineage or in primates in general, and consequently it was suggested that the molecular evolution of these genes would explain, at least partly, the evolution of the size of human brains (Zhang 2003; Wang and Su 2004; Evans et al. 2004, 2006a; Kouprina et al. 2004; Ponting and Jackson 2005).

For the *microcephalin* and *ASPM* genes it was even shown that their adaptive evolution is most likely still going on in the present human population (Evans et al. 2005; Mekel-Bobrov et al. 2005). This notion is based on the observation that the origin of the haplogroups, containing the adaptive alleles, substantially postdates the emergence of the anatomically modern man ca 195 000 years ago, and yet rose to high but variable frequencies under positive selection in the different present human populations. Very interestingly, evidence has also been presented for the hypothesis that the adaptive allele of the *microcephalin* gene has been introgressed into *Homo sapiens* from an archaic *Homo* lineage (Evans et al. 2006b). This hypothesis is based on the observation that the haplogroup containing the *microcephalin* allele prevailing in the present human population rose from a single copy $\sim$1.1 million years ago and introgressed into humans $\sim$37 000 years ago.

The role of natural selection on the evolution of *ASPM* and *microcephalin* genes has, however, been disputed by Currat et al. (2006) who suggested that models of human history that include both population growth and spatial structure can explain the observed pattern of gene frequencies without selection. This critique was, however, refuted by Mekel-Bobrov et al. (2006) mainly by pointing out that the demographic models presented by Currat et al. (2006) were outdated.

Subsequently Yu et al. (2007) criticized the hypothesis of adaptive evolution of the *ASPM* gene by pointing to the fact that Mekel-Bobrov et al. (2005) have based the hypothesis on computer simulations of demographic histories, and presented empirical comparisons of the gene frequencies of *ASPM* alleles and a large number of other loci. They observed that the variation in the *ASPM* gene frequencies was not unusual, and none of their tests showed significant evidence for selection. Mekel-Bobrov and Lahn (2007) responded by writing that the critic presented by Yu et al. (2007) was based on HapMap project's ENCODE data set. According to Ke et al. (2005) rare alleles are underrepresented in these data sets, wherefore the test of Yu et al. (2007) may have lower power than the test on which the original was based, taking ground on full resequencing data.

Timpson et al. (2007), on their part, criticized the idea of the adaptive evolution of *MCPH1* and *ASPM* genes on the ground of their observation that they found no meaningful association between the brain size or various cognitive abilities and the alleles of *MCPH1* or *ASPM* in a sample of 9000 children. To this critic Mekel-Bobrov and Lahn (2007) responded by saying that studies of positive selection could not be based on phenotype frequencies but rather on genotype frequencies, which they themselves have applied. Further Mekel-bobrov and Lahn (2007) indicated that their own studies on the association between *ASPM* and *microcephalin* on the one hand and increased intelligence did not explain the adaptive evolution of these genes (Mekel-Bobrov et al. 2007). Also Woods et al. (2006) reported, after genotyping and measuring brain volumes in 120 normal subjects, that normal variants of *microcephalin* and *ASPM* do not account for brain size variability.

Thus, it seems to me, that the microcephaly genes have been, and presumably still are, under positive selection, but this adaptive evolution is most likely not due to the effect of these genes on brain size and function but rather on their other phenotypic effects, presumably on their general effect on mitotic cell division. By this means, however, the microcephaly genes could have indirectly affected the evolution of human brains.

Besides of microcephaly genes, other genes affecting brain size and structure have also been found and analysed. These include ephrin-signalling genes (Depaepe et al. 2005) and genes involved in formation of synapses and neurite growth (Cáceres et al. 2007). The latter ones have been shown to be upregulated during human brain evolution (Cáceres et al. 2007), this being the first detailed characterization of gene-expression changes in human evolution that involves specific brain regions, including portions of cerebral cortex.

Did brain-specific genes have accelerated evolution in man?

The genetic changes that have been responsible for the emergence of the human-unique brain features, like big size, structural asymmetry and capacity to speech and language and other high-order cognitive functions, are a topic of enduring interest. Generally speaking, the marked evolution of the human brain could be due to modifications of either a small or a large number of genes, where the modifications might be in gene expression or protein function.

Dorus et al. (2004), analysed a set of nervous system genes at the protein sequence level and found that these genes evolved significantly faster in primates compared to rodents, faster in hominids compared to Old World monkeys, and faster in humans compared to chimpanzee. They further suggested that the accelerated evolution was due to positive Darwinian selection for advantageous amino acid changes.

Shi et al. (2006), on the other hand, found in their genome-wide analysis no evidence of the proposed accelerated evolution of brain specific genes in humans as compared to chimpanzees. The main reason why they could not repeat the results of faster evolution of humans than chimpanzees even when they used the same list of nervous system genes that Dorus et al. (2004) compiled seemed, according to the authors, to be the fact that Dorus et al. did not compare all of the 214 nervous system specific genes between human and chimpanzee. Instead, between human and chimpanzees they compared only 24 genes that were known to evolve faster in the human lineage than in the macaque lineage when the squirrel monkey was used as an out-group. In other words, according to Shi et al. (2006), Dorus et al. (2004) used a small and biased gene set in their human-chimpanzee comparison.

Shi et al. (2006), analysed almost 14 000 human, chimpanzee and macaque genes to test the hypothesis that human brain-specific genes have undergone widespread accelerated protein-sequence evolution since the human lineage separated from the chimpanzee lineage, and found no evidence that would have supported this hypothesis. Consequently, Shi et al. (2006) concluded, because their data include over 50% of all human genes, that it is appropriate to reject the hypothesis. According to the authors, this conclusion, however, does not preclude the possibility that substantial acceleration occurred in the evolution of a few nervous system genes, like the microcephaly genes the results for which are given above.

Further, according to Shi et al. (2006), it also remains possible that the origin of the human-unique brain features was due to expression changes (rather than coding sequence changes) of many genes, as had earlier been suggested from microarray data by Khaitovich et al. (2005) and Enard et al. (2002a). Because different RNA genes seem to be important in the regulation of the expression of protein coding genes (Satterlee et al. 2007), it is also noteworthy that Pollard et al. (2006) observed that a RNA gene, *HAR1F*, which is expressed in the neocortex is the most dramatic of the examples of the accelerated gene evolution in man (see next section).

Significance of RNA-genes in the human brain

As recently reviewed by Mehler and Mattlick (2007), the progressive maturation and functional plasticity of the human nervous system involve a dynamic interplay between the transcriptome and the environment. There is now growing awareness that the previously unexplored molecular and functional interface mediating these complex gene-environmental interactions, particularly in brain, may encompass a sophisticated RNA regulatory network involving the twin processes of RNA editing and multifaceted actions of numerous subclasses of non-coding RNAs (ncRNAs). The evolving nervous system involves numerous developmental transitions, such as neurulation, neural tube patterning, neural stem cell expansion and maintenance, lineage elaboration, differentiation, axonal path finding and synaptogenesis. RNA-based epigenetic mechanisms appear to be essential for orchestrating these precise and versatile biological phenomena. The concerted modulation of RNA editing and the selective expression of ncRNAs during seminal as well as continuous state transitions may comprise the plastic molecular code needed to couple the intrinsic malleability of neural network connections to evolving environmental influences to establish diverse forms of short- and long-term memory, context-specific behavioural responses and sophisticated cognitive capacities.

Specific ncRNAs have been shown to regulate dendritic spine development, neuronal fate specification and differentiation, and synaptic protein synthesis in animals (Satterlee et al. 2007). Among ncRNAs belong for example the microRNA molecules (miRNAs) which are ca 22 nucleotides long, and processed from larger hairpin-formed precursors that can regulate gene expression both in animals and plants (Bartel 2004), and the role of these miRNAs in diverse developmental processes is increasingly recognized (Alvarez-Garcia and Miska 2005).

In a massive parallel sequencing project Berezikov et al. (2006) compared the miRNA content of human and chimpanzee brains, and observed that many of miRNA genes are not conserved beyond primates, indicating their recent origin. Some of the miRNAs seemed to be species specific, whereas others are expanded in one species through duplication events. The data of Berezikov et al. (2006) also suggested that the evolution of miRNAs is an ongoing process, and that along with ancient, highly conserved miRNAs, there are a number of emerging miRNAs. Seventy-five percent of beforehand known human miRNAs cloned in the study were conserved in vertebrates and mammals, 14% were conserved in

invertebrates, 10% were primate specific and 1% were human specific.

In contrast, the 447 new miRNAs found in the human transcriptome have a different conservation distribution: more than half of the human miRNAs were conserved only in primates, about 30% in mammals and 9% in non-mammalian vertebrates or invertebrates; 8% were specific in humans only. A similar pattern of distribution was also observed for the chimpanzee miRNAs. Notably, the authors identified 14 chimpanzee miRNAs that had orthologous matches with multiple loci in the human genome, suggesting a human-specific gene family expansion. Likewise, a similar expansion of 15 miRNA genes was observed in the chimpanzee genome as well. The authors suggested that the different miRNA repertoire, as well as differences in expression levels of conserved miRNAs, may contribute to gene expression differences observed by Enard et al. (2002a) in human and chimpanzee brain.

The recent ability to compare our genome to that of our closest relative, the chimpanzee, provides new ways to link genetic and phenotypic changes in the evolution of human brain. Consequently, Pollard et al. (2006) devised a ranking of regions in the human genome that show significant evolutionary acceleration. They reported that the most dramatic of these so-called 'human accelerated regions', HAR1, is part of a RNA gene (*HAR1F*) that is expressed specifically in Cajal-Retzius neurons in the developing human neocortex from 7 to 19 gestational weeks, a crucial period for cortical neuron specification and migration. *HAR1F* is co-expressed with relin, a product of the Cajal-Retzius neurons that is of fundamental importance in specifying the six-layer structure of the human cortex.

In summary, as stated by Hill and Walsh (2005) we can anticipate having a systematic catalogue of DNA changes in the lineage leading to humans, but an ongoing challenge will be relating these changes to the anatomical and functional differences between our brain and that of our ancient and more recent ancestors and consequently to the evolution of the human brain.

EVOLUTION OF SPEECH

Language is a uniquely human trait likely to have been a prerequisite for the development of human culture. In accordance with Dunbar and Schultz (2007), it seems to me that of the different general theories presented on the evolution of our brains connected with the emergence of speech the one which suggests co-evolution of sociality, size and function of brains, and spoken language, is the most plausible. The increase in the size of the social group conditioned an increase of the size of the brains and communication by spoken language, and vice versa. However, it is very difficult to draw conclusions concerning the real conditions which led to the evolution of the capacity to speech, because we cannot restitute the ecological conditions that prevailed during the presumed time of the emergence of the spoken language in the evolving primitive human society about 200 000 – 100 000 years ago. On the other hand, molecular genetic research in recent years has given us some, even though small hints as to what kind of gene mutations might be connected to the specifically human capacity to speech.

The ability to develop articulate speech relies on capabilities, such as fine control of the larynx and mouth, that are absent in chimpanzees and other great apes. *FOXP2* (forkhead box 2) is the first gene found relevant to the human ability to develop language (Lai et al. 2001). A point mutation in *FOXP2* gene encoding a forkhead-domain containing transcription factor, co-segregates with a disorder in a family in which half of the members have severe articulation difficulties accompanied by linguistic and grammatical impairment (Fisher et al. 1998).

The human FOXP2 protein differs at only three amino-acid positions from its orthologue in the mouse. The chimpanzee, gorilla and rhesus macaque FOXP2 proteins are all identical to each other and carry only one difference from the mouse and two differences from the human protein, whereas the orang-utan carries two differences from the mouse and three from humans (Enard et al. 2002b). The pattern of nucleotide polymorphism in the *FOXP2* gene in the present human population also suggest that this gene has been the target of natural selection during recent human evolution (Enard et al. 2002b), and moreover comparative analysis of human, chimpanzee and mouse protein sequences suggest that it has experienced an enhanced evolutionary rate in the hominid lineage (Zhang et al. 2002).

The selective sweep, i.e. local loss of genetic variation in the vicinity of the critical exon of the *FOXP2* gene, caused by fixation of the advantageous *FOXP2* allele, occurred according to Enard et al. (2002b) in the last 100 000 years, and according to Zhang et al. (2002) in the last 200 000 years, the later dates being consistent with the emergence of modern humans and the putative development of human language (Hacia and Hey 2003). However, given the limited resolving power of statistical tests involving cases of low genetic variation, these estimates must be regarded with caution (Harris and Hey 2001).

Consequently, it is very interesting to note that the *FOXP2* variant found in modern humans was shared with Neanderthals, possibly suggesting that the genetic changes that led to the human variant and the selective sweep predate the common ancestor, which existed about 300 000 – 400 000 years ago, of modern human and Neanderthal populations (Krause et al. 2007). However, it is also possible that the Neanderthals received the human allele through interbreeding with the modern man.

WHAT GENETICALLY MAKES US SPECIFICALLY HUMAN?

We humans have many characteristics that are different from those of the great apes. These include enormously big brains, advanced cognitive abilities, complex vocal organs, bipedalism and opposable thumbs. These human-specific characters must have arisen through mutations accumulated in the genome of our direct ancestor after the divergence of the last common ancestor with chimpanzee. Recently Kehrer-Sawatzki and Cooper (2007) reviewed recent progress in comparing the human and chimpanzee genomes and discussed how the differences detected have improved our understanding of the evolution of the human genome. They concluded that to this end, interspecies comparisons have revealed numerous human-specific gains and losses of genes as well as changes in gene expression. The very considerable structural diversity polymorphism, evident in both the human and chimpanzee lineage has, however hampered the analysis of the structural divergence between the human and chimpanzee genomes. The concomitant evaluation of genetic divergence and the diversity at the nucleotide level has nevertheless served to identify many genes that have evolved under positive selection and may thus have been involved in the development of human lineage specific traits.

Genetic variation that distinguishes humans from the great apes

Kitano et al. (2004) conducted a systematic analysis of 103 protein-coding genes for human, chimpanzee, gorilla and orang-utan. The total number of amino acid changes in the human lineage was 147 for the 26 199 codons studies (0.56%). The total number of amino acid changes in the whole human genome was, thus, estimated to be about 80 000. According to the authors, these results suggest that only a small proportion of the protein-coding genes started to evolve differently in the human lineage after it diverged from the ape lineage.

In contrast, Glazko et al. (2005) observed in their survey 127 human and chimpanzee orthologous proteins consisting of 44 000 amino acid residues that only 25 (20%) of these proteins showed the identical amino acid sequence between humans and chimpanzees. In other words, the proportion of different proteins was 80%. According to the authors, it is unclear at the present how these differences are related to the morphological differences between the two species. It is quite possible that a large proportion of morphological and anatomical differences are caused by a relatively small number of regulatory mutations as first suggested by King and Wilson (1975) or major effect genes as stated by Nei (1987). Consequently, Saitou (2005) in asking how many significant changes are really responsible for creating humanness or "hominization", proposed that only ~1% or ca 10 000 nucleotide changes of the total of some 900 000 (as she estimated), found in the coding regions of the human genome, would be really interesting regarding the phenotypic differences between man and the great apes, and suggested that genome-wide comparisons are necessary to decipher those changes.

As stated in the first part of this review (Portin 2007), a surprisingly large amount of both fine-scale and large-scale structural variations, like copy number polymorphism, insertions, deletions and inversions, has recently been found in the human genome (Sebat et al. 2004; Iafrate et al. 2004; Visser et al. 2005; Dhami et al. 2005; Sharp et al. 2005, 2006; Stefansson et al. 2005; Tuzun et al. 2005; Khaja et al. 2006). It has been estimated that there are for example some 100 copy-number variants per individual, each over 50 kb in size. In addition to these large copy-number variants, a significant number of intermediate-sized copy-number variants and inversions (8 to 40 kb) are being identified in the human genome, as are numerous smaller structural variants (1 to 8 kb) (Feuk et al. 2006). It is quite evident that, for a large part, it is the existence of this structural variation that makes the difference, at the basic genetic level, between us and the chimpanzees.

The earlier estimate, based on the amount of SNPs, showing 99.9% genome-sequence identity between humans, might thus be considered an overestimate. Moreover, recent analyses of the human and chimpanzee genomes indicate that different structural variations, notably segmental duplication events, have had a greater effect on altering the genome than single nucleotide pair changes. In fact Harris et al. (2007) using a new method, called genomic triangulation, compared the genomes of man, chimpanzee and rhesus macaque aiming at the analysis of

significance of the structural variation in the evolution of man. Applying this integrative method for reconstructing ancestral states and structural evolution of genomes, the authors identified 130 human-specific breakpoints in genome structure due to rearrangements at an intermediate scale (10 kilobases to 4 megabases), including 64 insertions affecting 58 genes. In addition, it was also found that many of these rearrangements are polymorphic in the human population.

The observations quoted above call for further studies on structural variation of the genome from the evolutionary perspective. In fact, it has been calculated that SNPs constitute a 1.2% genetic difference between man and chimpanzee, while large segmental duplications constitute a 2.7% difference (Cheng et al. 2005; Sharp et al. 2006). Varki and Altheide (2005) on their part estimated that the difference between human and chimpanzee genomes is about 4% comprising of ~35 million single nucleotide differences and ~90 Mb of insertions and deletions. Demuth et al. (2006) found an even bigger difference in percentages between the genomes of chimpanzee and man. They analysed mammalian gene families. Along the lineage leading to modern humans a gain of 689 genes and a loss of 86 genes inferred since the split from chimpanzees, including changes likely driven by adaptive natural selection. Their results implied that humans and chimpanzees differ by at least 6% (1418 of 22 000 genes) in their complement of genes.

These observations immediately suggest that large-scale variations play a significant and stronger than hitherto believed role in both micro- and macroevolution of man. However, it is in actual fact impossible to give an accurate percentile value for the basic genetic difference between man and chimpanzee because there is such a huge amount of individual variation in both species, and what concerns the phenotype, it is not only the number or quality of genes but interactions between genes, both regulatory and others, which play a significant role.

Positive selection has caused accelerated evolution of human-specific traits

As stated by Vallender and Lahn (2004) in their review concerning positive selection on the human genome, we humans as a species are inherently curious about our evolutionary origins. One powerful approach for studying human origins at the molecular and genetic levels is to identify genes that have been the targets of positive selection. With the rapid expansion of genome data and the availability of increasingly sophisticated analytical tools, positively selected genes are indeed being identified at an ever faster pace. According to these authors, judging from the currently available data, it appears that these genes largely belong to a limited number of functional domains. Of these they mentioned, and presented several examples, of the following seven: host-pathogen interaction, reproduction, dietary adaptation, physical appearance as well as sensory systems, behaviour and brain development.

For some of these domains, such as host-pathogen interactions and reproduction, the prevalence of positively selected genes is not surprising. For others, such as the regulation of brain development and behaviour, the identification of positively selected genes may offer valuable insight into the evolution of defining human-specific traits such as enlarged brain and highly advanced cognitive abilities.

A question of particular relevance to the understanding of human origins is whether the selective regimes driving human evolution are of exceptional quality or are more typical for biological evolution in general. One reason to suspect that selection on humans is exceptional is the remarkable rapidity with which some key traits were acquired. Allometrically scaled brain size, for example, grew by an order of magnitude since the lineage leading to humans diverged from old world monkeys some 20–25 million years ago, with a tripling in size occurring in just the last 2–3 million years of hominid evolution (Larson et al. 1998).

Such a dramatic change within a short period of time is extraordinary for any tissue system, but is particularly so for the brain, an exceedingly complex organ for which the growth in size is necessarily accompanied by the increase in organizational complexity (Vallender and Lahn 2004).

As stated above in the section concerning the evolution of brains, and also in the review of Mekel-Bobrov and Lahn (2006), accelerated evolution of brains was possibly due to a few nervous system genes only, or that the origin of the human-unique brain features was due to expression changes (rather than coding sequence changes) of many genes, i.e. in the regulation of the expression of protein coding genes.

One very interesting case of such brain-protein genes is the *MGC8902* gene, which is predicted to encode multiple copies of protein domain, DUF1220, of unknown function. Sequences encoding these domains are virtually primate-specific, show signs of positive selection, and are increasingly amplified generally as a function of a species′ evolutionary proximity to humans. Humans, namely, have on the average 212 copies of DUF1220, whereas chimpanzees, for example, have 37 copies, and monkeys only

30 copies (Popesco et al. 2006). Generally speaking, the number of DUF1220 copies is highly expanded in humans, reduced in African great apes, further reduced in orang-utan and Old World monkeys, single-copy in non-primate mammals, and absent in non-mammalian species. Of special interest is the observation that DUF1220 domains are highly expressed in brain regions associated with higher cognitive functions (Popesco et al. 2006).

Most recently Hawks et al. (2008) made a genetic survey of 3.9 millions HapMap single nucleotide polymorphism (SNP) dataset, and identified a large amount of recent positive selection in mankind. They found that selection has accelerated greatly during the past 40 000 years, and noted that human genetic variation appears consistent with a recent acceleration of positive selection. Moreover they suggested, quite rightly, that human demographic growth and changes in human culture and ecologies have contributed to the extraordinary rapid recent genetic evolution of our species.

Evolution of regulatory factors of gene expression is typical for the human lineage

Concerning evolution of human-specific regulation of the function of genes and interaction of genes in the brains, the recent investigations on an RNA gene expression of Pollard et al. (2006) and on gene networks by Oldham et al. (2006) are of special importance. As already mentioned, the former group searched for genome regions, the evolution of which is accelerated in the human lineage, and found that the most dramatic acceleration was observed for a novel RNA gene, *HART1*, that is expressed in the developing human neocortex. Oldham et al. (2006) on their part provided an integrated view of human brain evolution by examining the large-scale organization of gene co-expression networks in human and chimpanzee brains. They identified modules of co-expressed genes that correspond to discrete brain regions and quantified their conservation between the species. It was observed that module conservation in cerebral cortex is significantly weaker than module conservation in sub-cortical brain regions, revealing a striking gradient that parallels known evolutionary hierarchies. This finding clearly illustrates the significance of the genetic evolution of the cerebral cortex in the emergence of the modern man.

Taking the fossil record evidence for the rapid morphological and anatomical evolution of human-specific traits, specifically that of brains, for granted, and given the molecular genetic evidence presented in this review, it seems very likely that key genetic changes which have led to the emergence of man are changes in the regulation of the function of the genes. On the other hand, positive natural or sexual selection (Schillaci 2006) has undoubtedly played a critical role in the evolution of *Homo sapiens*. Of the many phenotypic traits mentioned above that define our species most, if not all, are likely the product of strong positive selection (cf. Vallender and Lahn 2004).

Human biological evolution may be still continuing

The human species is in an exceptional demographic and ecological transient. Rapid population growth has been coupled with vast changes in cultures and ecology during the Late Pleistocene and Holocene, creating new opportunities for adaptation. The past 10 000 years have seen rapid skeletal and dental evolution in human populations and the appearance of many new genetic responses to diets and disease (Armelagos and Harper 2005). Larger populations generate more new advantageous mutations that escape genetic drift and will rapidly increase in frequency. Human migrations from Africa into Eurasia created new selective pressures on features such as skin pigmentation, adaptation to cold and diet (Hawks et al. 2008). Over this time span, humans both inside and outside of Africa underwent rapid skeletal evolution (Hawks et al. 2008). Some of the most radical new selection pressures have most likely been associated with the transition of the way of living to agriculture (Armelagos and Harper 2005).

It is often claimed that the pace of human evolution should have slowed as cultural adaptation supplanted genetic evolution. However, the high empirical number of recent adaptive gene variants in the human population would seem to be sufficient to refute this claim (Wang et al. 2006; Voight et al. 2006). Large population size permitted large number of advantageous alleles in the human population. Ecological and cultural changes in the way of living created new selection pressures. Together these factors contributed to an extraordinary rapid recent genetic evolution of the human species. Cultural changes have reduced mortality rates, but variance in reproduction has continued to fuel genetic evolution (Crow 1966). Thus, contrary to the common belief, human biological evolution has not come to a halt, but rather it seems to continue – maybe at a fast rate (Voight et al. 2006). Specifically our evolution seems to continue for the traits that are specifically human, like the size and function of the brains. As suggested by Richerson and Boyd (2005) and perhaps most clearly indicated by the evolution lactase persistence and lactose intolerance (Hollox et al. 2001; Enattah et al. 2002; Swallow 2003; Tishkoff et al. 2006) and the rapid evolution of enzymes that help as to digest meat

(Sabeti et al. 2006), it seems apparent that biological and cultural evolution proceed hand in hand in a mutual relationship with each other.

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