

Published in final edited form as:

Nat Rev Genet. 2008 October ; 9(10): 749–763. doi:10.1038/nrg2428.

## Explaining human uniqueness: genome interactions with environment, behaviour and culture

Ajit Varki<sup>\*,‡</sup>, Daniel H. Geschwind<sup>\*,§</sup>, and Evan E. Eichler<sup>\*,||</sup>

<sup>\*</sup>Center for Academic Research and Training in Anthropogeny (CARTA)

<sup>‡</sup>Departments of Medicine and Cellular and Molecular Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093, USA

<sup>§</sup>Program in Neurogenetics, Department of Neurology, Semel Institute for Neuroscience and Human Behaviour, David Geffen School of Medicine, University of California, Los Angeles, California, USA

<sup>||</sup>Department of Genome Sciences, and Howard Hughes Medical Institute University of Washington, Seattle, Washington, USA

### Abstract

What makes us human? Specialists in each discipline respond through the lens of their own expertise. In fact, ‘anthropogeny’ (explaining the origin of humans) requires a transdisciplinary approach that eschews such barriers. Here we take a genomic and genetic perspective towards molecular variation, explore systems analysis of gene expression and discuss an organ-systems approach. Rejecting any ‘genes versus environment’ dichotomy, we then consider genome interactions with environment, behaviour and culture, finally speculating that aspects of human uniqueness arose because of a primate evolutionary trend towards increasing and irreversible dependence on learned behaviours and culture — perhaps relaxing allowable thresholds for large-scale genomic diversity.

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After millennia of speculation, we can approach the question of what makes us human in a scientific manner, considering all the dimensions represented by many relevant disciplines. Studies of human genetic and genomic differences from our closest evolutionary relatives have much to offer. The evolutionary relatedness of humans and the African ‘great apes’ (now reclassified and grouped with humans and orangutans as hominids) was predicted by Huxley and Darwin<sup>1,2</sup>, and given molecular credence a century later by investigators such as Sarich, Wilson and Goodman<sup>3,4</sup>. Since the late 1800s, there has been an increasing interest in comparing humans with non-human hominids (NHHs), particularly chimpanzees, our closest living evolutionary relatives<sup>5</sup>. Initial studies involved anatomical and skeletal analyses of dead chimpanzees. Then came behavioural studies in captivity, particularly by Kohts, Köhler and Yerkes — and the field observations of Goodall, Imanishi, Nishida and others<sup>5</sup>. Much additional data concerning the behaviour, cognition, physiology and pathology of chimpanzees and other NHHs has since accumulated, showing how remarkably similar we are, and yet how different.

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Correspondence to A.V. [avarki@ucsd.edu](mailto:avarki@ucsd.edu).

#### FURTHER INFORMATION

The Varki laboratory: [http://cmm.ucsd.edu/Lab\\_Pages/varki/varkilab/index4.htm](http://cmm.ucsd.edu/Lab_Pages/varki/varkilab/index4.htm)

The Geschwind laboratory: <http://geschwindlab.neurology.ucla.edu>

The Eichler laboratory: <http://eichlerlab.gs.washington.edu>

miRBase, Sanger Institute: <http://microrna.sanger.ac.uk/sequences>

Early molecular comparisons by King and Wilson<sup>6</sup> showed that the problem was going to be difficult, as all of the protein sequences they studied were practically identical. Ironically, this classic paper might have diminished enthusiasm for further molecular comparisons, because of the fear that significant differences would be difficult to determine. This attitude changed in the 1990s with the discovery of specific genetic differences between humans and other hominids<sup>7-9</sup>, and there were calls for the sequencing of the NHH genomes<sup>10-12</sup>, including a biomedical rationale<sup>13</sup>. Subsequent sequencing of the chimpanzee genome<sup>14</sup> spawned many molecular comparisons between humans and other hominids, some aspects of which are cited throughout this Review. The possibility of obtaining genomic information from our closest extinct evolutionary cousins, the Neanderthals<sup>15,16</sup>, has further raised hope of elucidating the genetic components of what makes us human.

Of course, studies of genotypic variation need to be related to phenotypic differences<sup>12,17</sup> (BOX 1); however, the gap between phenomic and genomic studies remains large. It is time to set aside divisive and unproductive ‘genes versus environment’ arguments<sup>18</sup>, and explicate the human phenotype as the outcome of complex and ongoing interactions among genomes and the environment — and the effects of behaviour and cultural activities. Two approaches are taken here. The first is a genome-wide one, in which we consider the genomic and other molecular mechanisms that could be involved in uniquely human features, surveying roles of protein-coding changes, gene expression differences and genomic structural variation. The second approach considers potential contributions of genomic changes to organ-system differences. In both instances, we have selected examples largely from our own work as representative of the spread of topics and results in this field. Investigations in this area also have potential implications for understanding uniquely human aspects of disease processes<sup>13,19,20</sup>.

#### Box 1 Human uniqueness — what do we need to explain?

Ultimately, it is necessary to understand uniquely human aspects of phenotype in the context of genotypic differences from the non-human hominids (NHHs). Many lists of such differences have been published and it is becoming increasingly clear that several of these differences are relative rather than absolute. Some commonly discussed features are relative brain size, hairless sweaty skin, striding bipedal posture, long-distance running, ability to learn to swim, innate ability to learn languages in childhood, prolonged helplessness of the young, ability to imitate and learn, inter-generational transfer of complex cultures, awareness of self and of the past and future, theory of mind, increased longevity, provisioning by post-menopausal females, difficult childbirth, cerebral cortical asymmetry and so on (see ref. <sup>20</sup> for a more extended listing).

The type of approach proposed in this Review is necessary in order to eventually correlate the genotype with these and many other phenotypic differences. In this regard it is striking that although we know a lot about the human phenotype (that is, the human phenome), remarkably little detail is known about the phenome of the NHHs. Thus, a decade ago we proposed a ‘great ape phenome project’<sup>17</sup> that would attempt to identify all of these differences, with the goal of understanding which are indeed uniquely human characteristics.

Although this concept has gained interest, the opportunity to study the phenome of the NHHs is now greatly reduced, owing to recent decisions by the National Institutes of Health and other research agencies around the world that will markedly restrict or ban research on chimpanzees, and also stop all further breeding in captivity<sup>17,5</sup>. It is ironic that this ban is occurring just when the greatest opportunity exists in terms of emerging genomic information. Although there are clearly special ethical issues to be considered when exploring the phenotype of NHHs, it seems reasonable to suggest that studies that can be

ethically done in humans can also be done in other hominids, of course with appropriate mechanisms for protection of individual rights and dignity<sup>175</sup>. Without such an approach, we will be left with piecing together limited information on the NHHs, and arbitrarily trying to relate them to our extensive knowledge of the human phenome<sup>174</sup>.

As it is unlikely that we will fully answer questions about human uniqueness soon, we will be somewhat speculative and bold in the last part of this Review, closing by presenting a somewhat contrarian view of human genome dynamics. These final sections are meant to provoke discussion and debate. We will suggest that some of the common ‘rules’ of Darwinian genomic evolution through natural selection do not fully apply in humans, and that primate genomes (and the human genome in particular) have partially escaped such mechanisms because of buffering by culturally transmitted learned behaviours.

## Sources of molecular variation

### Going beyond the ‘1% difference’

Remarkable similarities of known human and chimpanzee protein sequences initially led to the suggestion that significant differences might be primarily in gene and protein expression, rather than protein structure<sup>6</sup>. Further analysis of alignable non-coding sequences affirmed this ~1% difference. However, the subsequent identification of non-alignable sequences that were due to small- and large-scale segmental deletions and duplications<sup>21-23</sup> showed that the overall difference between the two genomes is actually ~4%. Also, apart from coding regions (which make up ~2% of the entire genome), there is another ~2% of the two non-coding genomes that is highly conserved, but the function of which remains mostly unknown<sup>24</sup>. More recently it has emerged that many genes have undergone differential deletions or duplications in both humans and chimpanzees<sup>25-28</sup>. The discovery of various non-coding RNAs and the increasing appreciation of the role of post-translational modifications and epigenetic factors add even more complexity when translating genotype to phenotype. Together, these findings have dashed the hope that it would be simple to determine the key genetic differences between humans and our closest evolutionary relatives, that is, the genomic aspects of ‘what makes us human’.

### Differences in protein-coding sequences

Classic analyses of adaptive evolution focused largely on regions of the genome that code for protein products, using well defined metrics based on the ratio of functional changes (amino-acid changes,  $K_a$ ) to neutral changes (typically based on the surrounding non-coding variation rate,  $K_i$ , or the rate of synonymous changes,  $K_s$ ). Genome-wide analysis of positive selection in the human versus chimpanzee by organ system revealed the anticipated result that more widely expressed or ‘housekeeping’ genes have a lower  $K_a/K_i$  ratio, as they are under more functional constraint than genes with more restricted or organ-specific expression patterns<sup>29, 30</sup>. There has been some controversy around whether brain genes are under more constraint than genes in non-neural tissues<sup>30-34</sup>, and a recent paper argues that there are large numbers of selected amino-acid differences between humans and chimps<sup>35</sup>. A combination of methodological issues, as well as differences in interpretation, probably account for these apparent discrepancies, highlighting the current state of confusion around the role of protein sequence divergence in the evolution of human brain function in particular. In some cases, the conclusions drawn are probably due to the specific comparisons that were made (for example, the choice of genes or outgroup)<sup>36</sup>. More comprehensive analyses, using several metrics of brain specificity and a more complete list of genes, support an increase in functional constraint on hominid brain genes relative to most other tissues<sup>30,32-34,37</sup>.

Although using single nucleotide changes in coding sequence to assess genome evolution is well documented in the literature, one must be cautious about its general applicability<sup>24,38</sup>. The classic approach treats all exons as functionally equal, which can be a faulty assumption. For example, rapid evolution within a single domain of a protein can be missed if its other domains are undergoing purifying selection<sup>39</sup>. Thus it is preferable to use more recent methods that can detect positive selection on a small number of sites<sup>40</sup>. These concerns do not invalidate studies that are based on classic Ka/Ks or Ka/Ki ratios, which deservedly continue to have an important role in molecular analyses of genome evolution — but we should be aware of their potential limitations.

Over half of mammalian genes are alternatively spliced, several in a lineage-specific manner<sup>41</sup>, and mutations that change splicing are significant causes of Mendelian-inherited disease in humans. However, the potential adaptive regulation of splicing<sup>42-44</sup> is not addressed in many studies of protein-sequence evolution. No detailed comparison of alternative splicing between humans and NHHs has been carried out. A recent candidate is neuropsin (also known as kallikrein-related peptidase 8, *KLK8*), which is a secreted serine protease preferentially expressed in the central nervous system and is involved in learning and memory. The longer spliced form of this mRNA is expressed only in humans, and not in non-human primates (NHPs), owing to a human-specific T-to-A mutation<sup>44</sup>.

### Functionally important sequences might not encode proteins

There is no *a priori* reason to believe that protein-coding regions are more relevant to hominid evolution than changes in enhancers, promoters, 3' UTRs<sup>45</sup>, non-coding RNAs<sup>46-48</sup> or even more cryptic regulatory regions<sup>49-51</sup>. Indeed, many deleterious mutations have arisen in gene control elements during the evolution of humans and chimpanzees<sup>45</sup>. The calculated rate of adaptive evolution (positive selection) depends on the neutral background rate. Although this has been a powerful approach, which sequence is truly non-functional and thus an appropriate measure for neutrality is currently unknown<sup>52</sup>. Thus, intronic regions, synonymous coding changes and surrounding non-coding regions that we now know to be functionally relevant<sup>53-54</sup> have been used to assess the rate of neutral evolution. Current estimates of adaptive protein evolution, therefore, might not be accurate in some cases, nor provide a complete picture of genome evolution. This is highlighted by the recent identification of 49 highly conserved non-coding sequences showing significant nucleotide divergence in humans (human accelerated regions; HARs), and one of which (HAR-1) encodes a novel non-coding RNA highly expressed during brain development<sup>47</sup>. Almost 1,000 human non-coding microRNAs (miRNAs) are listed in the [miRBase](#) at the Sanger Institute; many miRNAs are species specific, including those identified only in primate genomes, such as chimpanzee or human<sup>46</sup>. The extent to which non-coding RNAs regulate gene expression in mammals was unknown 10 years ago, and still almost nothing is known about primate- or human-specific miRNA selection or function. More surprises will require us to integrate new concepts of gene function and regulation<sup>49</sup> into the assessment of human evolution<sup>55</sup>.

### Differences in mRNA and protein expression

The idea that regions controlling mRNA expression are more important than coding sequences<sup>6</sup> gave theoretical support for studies comparing genome-wide mRNA expression across different tissues between humans and chimpanzees<sup>56-60</sup>, particularly in the brain. As well as relating gene expression to organ-specific phenotypes, such expression studies can also provide an important platform for novel phenotype discovery, for example, by identifying molecular differences in specific cell types<sup>60,61</sup>.

Although microarray-based studies of inter-species gene expression are affected by many technical or methodological issues (reviewed in REFS<sup>61-64</sup>), they revealed a few main themes.

First, there is an apparent acceleration in the evolution of brain-enriched genes in humans versus chimpanzees, relative to other tissues<sup>29,57,60,65</sup>. This finding has been interpreted to signify positive selection, but is also consistent with relaxation of constraint<sup>66</sup>. A second, related observation is that in the human lineage there are more increases in gene expression than decreases<sup>60,65</sup>. This could indicate a general upregulation of brain energy metabolism in humans, a feature consistent with the expansion of the neocortex in the human lineage<sup>59,60</sup>. Third, a neutral model has been proposed to account for much of the gene expression changes in the brain, because there is more variation in non-nervous-system enriched genes than in brain-enriched genes<sup>67</sup> (BOX 2).

### Box 2 High degree of purifying selection in brain-expressed genes

Why are coding-sequence changes in brain genes under a larger degree of purifying selection than in other tissues? The reason for this is not immediately clear as a wide range of brain function supports life to reproductive age in humans. Perhaps the complexity of brain function places limits on adaptive changes in sequence. Here, the supposition is that the rapid expansion of cortical surface area, and the subsequent interconnections between higher-order association areas from multiple brain regions that are necessary to support higher cognition, places a constraint on the sequence of involved genes.

But this notion, which is based on single nucleotide changes in protein-coding sequence, has to be reconciled with the CNV data, because CNVs in humans seem to be enriched among genes involved in neurodevelopmental processes. It is also possible that the actual selection of brain-enriched genes for analyses might be limited by tissue heterogeneity<sup>60</sup>; this might mean that the brain-enriched genes identified by microarrays<sup>29</sup> are those with moderately high levels of expression across many brain regions, and thus the analyses would be biased towards genes involved in fundamental neural cellular functions. Methods that are based on deep library sequencing or single-cell analysis will be needed to address this issue more conclusively. It would be ironic if, overall, increased purifying selection of protein-coding regions on human brain genes is a necessary consequence of the phenotypic adaptations that have led to our social-cultural flexibility, which might be buffering other genomic changes. Of course, any overall metric of selection on the functional genome should consider the role of structural variation and non-coding regions, which might significantly alter estimates of the direction of selection.

In fact, of all the organs studied, the strongest evidence for positive selection in gene expression and protein sequence is observed in the testes<sup>67</sup>, not in the brain. However, important methodological issues, such as tissue heterogeneity<sup>62</sup> and the manner in which differential gene expression is calculated (for example, the number of genes versus the sum of differential expression values), cloud the interpretation of these data<sup>64</sup>. Additionally, analyses by other investigators do not support a pervasive role for neutral evolution in hominid brain evolution<sup>68,69</sup>. Nevertheless, the neutral theory sets the context for the development of methods that distinguish between neutral and adaptive evolution on a gene-by-gene basis, as discussed later and in BOX 2.

### Epigenetic differences

Few studies have assessed epigenetic changes specific to the human lineage. A small-scale survey of the regulatory regions of 36 genes in 3 tissues showed that most of the 18 significant differences in promoter methylation were in the brain<sup>70</sup>. However, changes in methylation status could be due to dietary factors, including folate supplementation. A clearer understanding of the evolution of the molecular pathways involved in epigenetic regulation and chromatin structure, rather than just measurement of gene methylation, is necessary to interpret these data.

## Genomic mechanisms

### Genome structural changes

In addition to many single base-pair substitutions in the human genome, there is also a surprising abundance of larger structural changes, including insertions, deletions and duplications<sup>14,22,26</sup>. Chimpanzee–human structural changes are fewer in number but affect many more base pairs (~140 Mb) than do single base-pair substitutions (~35 Mb). These two forms of genetic variation are not readily comparable and should be considered separately. The gain and loss of genomic DNA segments has accelerated in humans and chimpanzees when compared with Old World monkeys (see FIG. 1a for an example) and other mammals<sup>23,25,28,71,72</sup>. Interestingly, this acceleration in genomic structural variation seems to coincide with a slow-down in single base-pair substitution in hominids<sup>73,74</sup>.

Structural variation in the hominid genome shows several interesting features. First, this variation, especially in events >15 kb, is distributed non-randomly throughout the genome, with segmental duplications coinciding with hot spots of evolutionary change<sup>75,76</sup>. Seven of the nine pericentric inversions that distinguish human from chimpanzee chromosomes, for example, map to regions of segmental duplication<sup>77</sup>. Second, genes associated with immunity, chemosensory activity and reproduction are enriched within regions of primate structural variation<sup>14,22,28,78</sup>. Third, structural changes have strong effects on distal and local gene expression patterns, both within and between species<sup>58,79</sup>. Finally, there have been recent episodic or punctuated events during hominid genome evolution<sup>80-82</sup>. Below we consider various forms of structural variation, based on comparisons within and between primate species. We should caution that none of the NHP genomes has been sequenced as thoroughly as the human genome, and so conclusions must be tempered by the quality of underlying genomic sequence, especially over such dynamic regions.

### Repeat elements and retroviruses

Approximately 50% of the human genome consists of various classes of repetitive DNA. A three-way comparison of chimpanzee, human and macaque genomes reveals few large-scale differences in repeat content since the divergence of humans and Old World monkeys over 25 million years (Myr) ago<sup>23</sup>. There are three notable exceptions. First, although NHH retrotransposition activity has generally decreased for both short and long interspersed nuclear elements (SINES and LINES), repeat activity of the *Alu* transposable element has been three times more active in humans than in chimpanzees. Thus, there are ~7,000 new *Alu* insertions within the human lineage compared with ~2,500 in the chimpanzee<sup>14</sup>. Second, the SVA element (a 2–3 kb repeat element composed of a SINE, a variable number of tandem repeat (VNTR) and an *Alu* sequence) emerged specifically within the hominid ancestor ~20 Myr ago. Human, chimpanzee and gorilla genomes show the greatest number (~2,500) of these elements, with specific subfamilies emerging within each lineage<sup>83</sup>. Younger elements are enriched within GC-rich genomic regions and show considerable (~25%) polymorphism<sup>14,83</sup>. This bias, and the fact that ~200 elements map within 5,000 bp of annotated genes, has led to speculation that these elements have altered the transcriptional landscape of some human genes<sup>14</sup>. Finally, both gorilla and chimpanzee genomes have undergone recent, episodic expansions of endogenous retrovirus repeat families near or shortly after the time of speciation (5–8 Myr ago)<sup>80</sup>. Thus, hundreds of full-length copies of the *Pan troglodytes* endogenous retrovirus 1 (PTERV1) exist at non-orthologous locations in the genomes of the chimpanzee and gorilla, but not in the human genome<sup>80</sup>. This burst of retroviral germline integrations near the time of African hominid speciation might thus have contributed to creating lineage-specific phenotypic differences over a short period<sup>80</sup>. Humans might have been spared this retroviral invasion owing to selective mutations of the tripartite motif 5 (TRIM5) immune-response protein<sup>84</sup>. It is also interesting that humans do not carry endemic infectious retroviruses (BOX 3).

### Box 3 Explaining the lack of endemic infectious retroviruses in humans

Other than the recent introductions of HIV and human T leukaemia virus (HTLV) into humans from other animals, humans seem to be devoid of species-wide endemic infectious retroviruses. By contrast, like most other mammals studied, other hominids and non-human primates (NHPs) do have such viruses. Indeed, given the remarkable corroboration between the phylogenetic trees of primates and their lineage-specific simian foamy viruses (SFVs)<sup>176</sup> our common ancestors with other hominids almost certainly had SFVs. The same is probably true of the lineage-specific simian infectious retroviruses (SIVs) found in most NHPs<sup>177</sup>. Assuming that the common ancestors of hominids carried multiple endemic infectious retroviruses, how did the human lineage eliminate them? Given that humans remain susceptible to re-infection with both SFVs<sup>178</sup> and SIVs<sup>177</sup> from other hominids, this seems unlikely to be explained solely on the basis of more efficient host restriction systems<sup>84</sup>. Rather, there seems to have been an episode in which the ancestral human lineage was somehow ‘purged’ of these endemic viruses. One testable hypothesis is that human-specific loss of the sialic acid N-glycolylneuraminic acid, which would normally be acquired by such enveloped viruses, could restrict viral transmission because of the simultaneous appearance of antibodies against this sialic acid in hominins<sup>179,180</sup>

### Gene deletions

Olson first suggested that deletions had a pivotal role in human evolution<sup>19,85</sup>. According to his ‘less-is-more’ hypothesis, the human was a “hastily made-over ape” in which irrevocable gene deletions were key factors in permitting human adaptations. Although a complete repertoire of such gene deletions does not exist, a total of 56 partially or predicted genes were confirmed as deleted in chimpanzees compared with humans and most were related to inflammatory response, parasite resistance or cell-surface antigens<sup>14</sup>. There is evidence for an almost twofold increase in gene loss in humans and chimpanzees when compared with macaques, and an almost fourfold increase in contrast to other mammals (for example, dogs, mice and rats)<sup>23,28,86</sup>. Interestingly, gene gain and gene loss might arise through the same mutational process (FIG. 1).

Most examples of human-specific gene losses have occurred within families of related genes with potentially redundant functions (for example, sialic acid binding Ig-like lectin 13, *SIGLEC13*)<sup>87</sup>, and/or are not universal to all humans (for example, caspase 12, *CASP12*)<sup>86</sup>. Thus, their significance for human uniqueness remains uncertain. There are few documented human-specific single-copy genes that have been lost from all living humans. The first example was the *Alu*-mediated inactivation of the cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase gene (*CMAH*)<sup>8</sup>, which probably occurred before the emergence of the *Homo* lineage<sup>88</sup> and resulted in large changes in the cell-surface distribution of sialic acid types, and apparent secondary changes in the biology of various sialic acid-recognizing proteins<sup>89</sup>. Such deletions might have occurred in conjunction with changes in diet, pathogens or immune changes of human ancestral species.

Another example of human-specific gene loss is the sarcomeric myosin gene (myosin, heavy chain 16 pseudogene, *MYH16*), which underwent a 2 bp frameshift mutation<sup>90</sup>. This gene is expressed primarily within muscles of the hominid mandible, and its loss is hypothesized to have caused an eightfold reduction in the size of type II muscle fibres in humans, with an evolutionary timing claimed to coincide with a shift towards a gracile masticatory apparatus within *Homo erectus* and *Homo ergaster*. It was also hypothesized that associated changes in muscle insertions at the apex of the skull were permissive for encephalization. However, the timing and the biological significance of this gene loss have been contested<sup>91,92</sup>.

## Gene and segmental duplications

The converse of the less-is-more hypothesis is that ‘more is better’, as argued by Ohno<sup>93</sup>. Comparisons of primate gene copy-number difference by array comparative genomic hybridization (ArrayCGH)<sup>25,27,94</sup>, and by experimental and computational analyses of segmental duplications<sup>22,23,72</sup>, in addition to comparisons of gene content between macaque, human and chimpanzee genomes<sup>28</sup>, support an overall increase in duplication activity in the common ancestor of chimpanzees and humans compared with other mammals (FIG. 1b). It is unclear, however, whether humans have an excess of such duplications compared with chimpanzees. After correcting for copy-number differences, the overall amount of lineage-specific duplications between humans and chimpanzees seems to be similar<sup>22</sup> — although human duplications are genetically more diverse when compared with chimpanzee duplications<sup>22</sup>. Data from other hominids should allow firmer conclusions to be drawn.

Hominid duplications are remarkable in two respects: they are more interspersed and show a higher degree of sequence identity, especially among intrachromosomal duplications, than those in other sequenced mammalian genomes<sup>71,95</sup>. This complex duplication architecture has led to the formation of hominid-specific gene families in humans and chimpanzees (for example, the morpheus gene family; FIG. 1) that lack clear orthologues in the mouse genome<sup>95-97</sup>. Although the specific function of these gene families is unknown, several of them show strong signatures of positive selection and dramatic changes in their expression pattern. For example, neuroblastoma breakpoint protein family (NBPF) genes are highly expressed in brain regions that are associated with higher cognition, and they show neuron-specific expression<sup>98</sup>. The segmental duplications associated with these genes served as the focal point for the evolution of the complex organization of interspersed duplications within the human genome<sup>81,99,100</sup>. Moreover, the expansion of hominid segmental duplications seems to be responsible in large part for the acceleration of gene duplication in humans and chimpanzees<sup>28,81</sup> and there is evidence that these ‘core duplicons’ have duplicated independently in different hominid lineages<sup>100</sup> (FIG. 1b).

## Copy-number variants

Copy-number variants (CNV) are abundant in humans and preferentially associate with duplicated genes<sup>101-103</sup>. Larger events (>500 kb) that affect genes occur more rarely but are enriched in individuals with cognitive disabilities, such as mental retardation<sup>104</sup>, autism<sup>105, 106</sup> and schizophrenia<sup>107</sup>. More limited surveys in the chimpanzee<sup>108</sup>, macaque<sup>109</sup> and mouse<sup>110,111</sup> reveal that copy-number variation is a common source of genetic variation for such mammals, although it is premature to determine whether humans have more CNVs than these other species.

There are, however, important differences in CNV distributions. Whereas human CNVs are enriched in genes that show evidence of positive selection<sup>112</sup>, mouse CNVs are more gene-poor and show potentially less adaptive evolution. Similar differences in gene density between humans and mice occur for segmental duplications<sup>72</sup> — which might be regarded as a more ancient form of copy-number variation. Although CNV gene density differs between mice and humans, CNVs in both species are enriched for genes that are important in environmental responses, for example, drug detoxification, olfaction and immune response. High-resolution surveys of human CNVs, however, reveal additional categories: transcription factors (especially zinc-finger genes) and genes that are important in the development of the central nervous system and in synaptic transmission<sup>113</sup>. Consistent with this finding is the association of larger *de novo* CNVs with two common neurodevelopmental disorders, autism and schizophrenia<sup>105-107</sup>, as described above. Also striking is human lineage-specific amplification of multiple copies of a protein domain of unknown function (DUF1220) and of the morpheus gene family on chromosome 16 (FIG. 1). These are hominid gene-family expansions that show



signs of positive selection and neuron-specific expression<sup>98</sup>, and the underlying duplications mediate rearrangements associated with neurocognitive disease.

Owing to its effect on gene dosage, the selective effect of large CNVs might be greater when compared with most single-nucleotide substitution events<sup>112</sup>. In populations with large effective population sizes, such as the laboratory mouse ( $n = 500,000$ ), selection would operate more efficiently, either fixing advantageous alleles or eliminating weakly deleterious alleles. By contrast, populations with smaller effective population sizes, such as humans ( $n = 10,000$ ), are more prone to the whims of genetic drift than they are to selection. The small effective population size in hominid ancestral populations would thus allow a greater fraction of weakly deleterious alleles to reach appreciable allele frequencies. As some copy-number changes are themselves mutagenic (that is, duplicated sequences can predispose to new mutation events, such as inversions, deletions and duplications through non-allelic homologous recombination), there is a cascading effect of increasing potential structural variation. This dynamic nature would lead to increased diversity and, we suggest, a broader range of adaptive responses owing to greater standing diversity and to the potential for new mutation.

### Gene-conversion events

Large-scale gene conversion probably accounts for <10% of highly identical segmental duplications<sup>22</sup>, whereas small-scale gene conversion events are thought to account for the increased sequence diversity observed within 10 Mb of primate telomeres<sup>14</sup>. Most gene conversions tend to disrupt gene function. However, a human-specific gene conversion that maintained an ORF occurred in the gene for Siglec-11; this is the first known ‘human-specific’ protein, and it is expressed in human but not NHH brains<sup>114</sup>. The significance of this human-specific event is unknown, and such small-scale gene conversion events have not been carefully searched for throughout the human genome.

### Are large-scale genomic changes accumulating more rapidly in humans?

As mentioned above, interspersed segmental duplications and deletions are prominent in hominid genomes<sup>111</sup>. Available evidence suggests a trend in the number of duplication and deletions, with human = chimpanzee > macaque > rodent > chicken > *Drosophila* = *Caenorhabditis elegans*. It is possible that humans are accumulating these large-scale genomic variations at a faster rate than other hominids, but the data are too limited to ascertain this. It would be useful to obtain comparative data on widely disparate human population groups, for example, African San versus Native Americans, as well as from various ‘subspecies’ of chimpanzee.

Similar questions arise about CNVs. Again, data from multiple species are lacking. CNV diversity might be higher in humans, despite the small effective population size. There could also be fitness benefits associated with the propensity to generate and tolerate more CNVs, for example, the expansion of amylase gene copies in humans<sup>115</sup>. It is interesting that in this case copy numbers progressively drop as we move from agricultural humans to non-agricultural humans to chimpanzee and bonobo. It is also interesting that CNVs are now being recognized as significant causes of neuropsychiatric conditions, and so the question is whether they are more common in more subtle forms of human-specific diseases related to brain function and social interaction.

We propose below that the frequencies of large interspersed segmental deletions and duplications are greatest in hominids, followed by monkeys and then rodents, because they are better tolerated by hominids owing to buffering by the increasing dependence of important functions on learned rather than hard-wired behaviour. In this scenario, individuals bearing variant genomes might survive and even be beneficial to a human population, by contributing

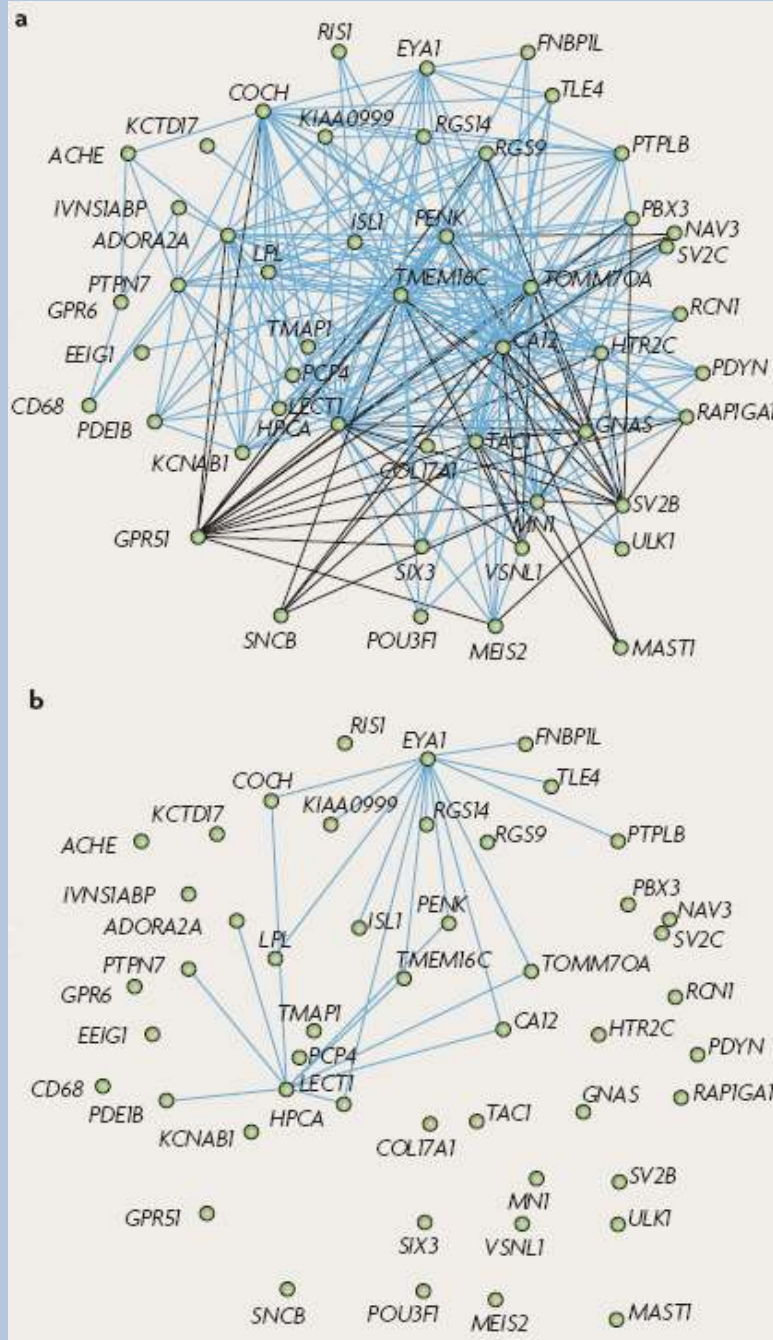
to genomic and phenotypic plasticity that is adaptive for the community at large, either in the short or long term.

## Systems or network approaches

The inherent difficulty in determining whether a change in gene expression is adaptive or neutral argues strongly for the development of methods that place gene expression changes in a functional context. If gene expression changes were neutral they would not tend to accumulate in specific ontological or functional categories but, rather, would be randomly distributed. In this regard, it is notable that genes that are differentially expressed between human and chimpanzee brains are enriched for energy metabolism and protein-folding categories, consistent with their accelerated evolution<sup>62</sup>. One weakness of such an approach is that it does not assign any level of confidence to individual genes. Another is that genes can have multiple functions, and the Gene Ontology classifications do not take into account all biological processes (see below).

By contrast, network biology approaches allow individual genes to be placed within a functional context<sup>116,117</sup> (BOX 4). Complex systems, biological or otherwise, exhibit properties of scale-free networks<sup>118</sup>. Furthermore, clusters of highly co-expressed genes, or modules, define groups of genes that are functionally related<sup>119-122</sup>. There is a small but significant correlation between being on the periphery of protein networks and the tendency to have undergone adaptive selection<sup>123</sup>, consistent with the functional relevance of network position. Oldham *et al.*<sup>120</sup> reasoned that a comparative network approach would provide a general framework for identifying functional, or adaptive, changes in gene expression between chimpanzees and humans. Any changes in the network structure, for example, the network position of a specific gene, between two species provides a unifying manner in which to identify non-neutral (that is, functional) changes in gene expression relationship<sup>120</sup> (BOX 4). This approach was used to concentrate on the specific genes or regions of the genome driving these adaptive changes. Overall, genes in modules corresponding to cerebral cortical modules, including those involving neuronal plasticity, showed the greatest divergence relative to sub-cortical brain regions, consistent with the primary role of the cerebral cortex in the evolution of human cognition and behaviour<sup>120</sup>. These studies provide a proof of principle, supporting the use of systems-biology approaches to inform cross-species comparisons, and facilitating the connection of phenotypes within specific organ systems to changes at the level of the genome.

### Box 4 Use of scale-free networks to study gene expression differences



Complex systems, biological or otherwise, exhibit properties of scale-free networks<sup>118</sup>, which delineate an organization whereby a few nodes (that is, genes) are central, or ‘hubs’, serving as control points in the network, whereas most nodes are more peripheral and have few connections. This theoretical construct has been applied to gene expression data using a method called whole-genome network co-expression analysis (WGNCA)<sup>119-122</sup> to provide a functional context to view transcriptome organization. Rather than simply studying differential expression, WGNCA uses the information from the measurement of co-expression relationships to create gene expression networks. The position of each gene

in a given network is described by its connectivity ( $k$ ), which summarizes its degree of connection to all other genes in the network. Genes with high  $k$  (hub genes) are crucial to the integrity and proper functioning of the network, and changes in their position between the networks of two species aids in discerning between adaptive versus neutral changes. The degree of network relationship between two genes is summarized by topological overlap (TO), a measure of network neighbourhood sharing<sup>118,119</sup>. Plotting genes on the basis of their degree of TO permits visualization of network structure; genes that cluster together define modules of functionally related genes. Comparison of networks between species, or between normal and diseased tissue, allows one to identify key functional changes that have occurred.

The figure demonstrates that differential connectivity in gene co-expression networks distinguishes adaptive evolutionary changes. Analysis of gene co-expression relationships using WCGNA in the human brain identifies modules that correspond to specific brain regions. Here, a module corresponding to the caudate nucleus is shown (**a**). Comparison of modules between humans and chimps can identify species-specific co-expression relationships (**b**). In part **a** of the figure, 300 pairs of genes with the greatest topological overlap in humans are depicted in a module that represents the caudate nucleus. Genes with expression levels that are negatively correlated (using Pearson correlation) are connected by black lines. Connections from part **a** that are present in humans but absent in chimpanzees are depicted in part **b**. Note that overall the module is highly conserved between the species, and most connections are not human specific. However, nearly all connections that are human specific converge upon two genes, eyes absent homologue 1 (*EYAI*) and leukocyte cell-derived chemotaxin 1 (*LECT1*), demonstrating a large change in network position; this is consistent with non-neutral evolution of these two genes, which are differentially expressed between chimpanzees and humans.

Figure is modified, with permission, from REF.<sup>120</sup> © (2006) National Academy of Sciences, USA.

Despite their great potential, there are limitations in using genome-wide approaches to exploring human uniqueness. First, it can be complicated to translate the results into a specific biological process or organ system. Second, as mentioned earlier, Gene Ontology and Panther-type gene classification systems do not take into account all possible biological processes. For instance, the genes involved in one biological process (sialic-acid biosynthesis, addition and recognition) are randomly scattered throughout the Gene Ontology and Panther categories<sup>39</sup>. Thus, genome-wide approaches need to be complemented by candidate gene approaches, as described in the following section.

## An organ-systems view

Ultimately, it is necessary to connect specific genes and genomic processes to the phenotypes that are most relevant for human evolution. In particular, analyses could focus on genes and other genomic elements involved in those physiological and organ systems that show the most differences between humans and other hominids. Below, we consider relevant issues for some organ systems that feature aspects of human uniqueness.

### Brain and nervous system

In considering human uniqueness, encephalization is typically the first aspect to be given attention, partly because it is the easiest to measure, even in fossil hominids. However, modern human brain size was reached >100,000 years before archaeological evidence of modern human behaviours is seen<sup>124</sup>. Taken together with other evidence, such as poor correlation between brain size and cognitive abilities<sup>125</sup> and the remarkable abilities of individuals

surviving extensive brain injury or surgery in infancy<sup>126</sup>, it is reasonable to say that encephalization has been overrated as being the key to human cognitive abilities. It was probably an important and necessary step along the way, but insufficient to achieve modern human cognition. Even the notion that human brain frontal lobes are selectively enlarged has been questioned by modern studies<sup>127</sup>.

Conversely, some uniquely human cognitive phenotypes are well delineated and can be connected to regions of the cerebral cortex, including language (peri-sylvian cortex and hemispheric asymmetry)<sup>128-130</sup>, artistic, musical and mathematic abilities (multiple regions), and planning (frontal systems). Others, such as primary sensory regions, have received little attention, but when rigorously assessed show significant morphological and molecular differences between humans and chimpanzees<sup>61,131</sup>. Thus, phenotype discovery and genotype correlation must be an important component of studies of human brain evolution<sup>128,130,132,133</sup>.

Social awareness is a potentially central area of cognition in which humans might excel relative to their closest primate relatives<sup>134,135</sup>, and the cortical regions involved in these functions are implicated in disorders of human social behaviour, such as autism<sup>136-138</sup>. Parallel distributed circuits that are involved in language and that use specific regions that are more developed in humans than in non-human primates have also been identified<sup>130,139</sup>. Understanding how evolution of our genome maps onto changes in the size and connectivity of these specific cortical association areas — key regions that underlie the remarkable development and flexibility inherent in human cognition and behaviour — is of central importance<sup>128</sup>. One exciting example might be the spindle cell (Von Economo) neurons, which are enriched in the human cingulate and insular cortex relative to NHHs, and are not found in non-hominid primates<sup>137,140</sup>. This cell type is also selectively vulnerable in frontotemporal dementia<sup>141</sup>. This highlights the potential of human diseases to help delineate the underlying functional relevance of specific brain phenotypes or genes.

Thus, in the context of clear adaptive evolution of certain brain phenotypes in humans, the underlying specific genetic changes should be identifiable. Several nervous-system genes undergoing adaptive evolution at the protein level have been identified in humans, including genes involved in neurogenesis, hearing and developmental patterning<sup>30</sup>. Apparent adaptive evolution of forkhead box P2 (*FOXP2*), a gene involved in human speech production, has been reported in the human lineage<sup>142,143</sup>, with the derived variant being shared with Neanderthals<sup>144</sup>. Remarkably, recent studies show that *FOXP2* is also related to vocal learning in birds, in a circuit with functional homology to humans<sup>145-148</sup>, and is rapidly evolving in bats, which one can speculate might be related to their echolocation capacity<sup>149</sup>. This suggests that the phenotype involved is not language *per se*, but rather the development and function of circuits involved in sensory-motor integration that contribute to vocal motor learning in multiple species<sup>150</sup>. New evidence for adaptive selection on a subset of *FOXP2* transcriptional targets in humans raises the possibility of potential co-evolution of a transcriptional programme downstream of *FOXP2* (REF. <sup>151</sup>). Thus, one can speculate that it might be the adaptive evolution of such a pathway, rather than of *FOXP2* alone, that is connected to language and speech function in humans.

However, connecting such genes involved in disorders of human cognition to the specific phenotypes undergoing selection poses significant challenges. A salient example involves two genes, abnormal spindle homologue microcephaly associated (*ASPM*) and microcephalin (*MCPHI*), the adaptive evolution of these genes in humans was claimed to be related to normal variation in brain size, on the basis of the fact that Mendelian mutations in each results in microcephaly in humans<sup>152,153</sup>. However, not all investigators have found evidence for the adaptive evolution of *ASPM* or *MCPHI* (REF. <sup>154</sup>). Also, neither gene is likely to contribute

significantly to normal variation in human brain size<sup>155</sup>. This case illustrates the challenges of interpreting genetic data in the face of complex phenotypes, especially those that are poorly understood.

### Sensory systems

With the possible exception of our tricolour stereoscopic vision and finger-tip tactile sensation, most human sensory systems have, if anything, lost acuity during our evolution. An interesting case documented at the genomic level seems to be the sense of smell, with which humans seem to have lost, by pseudogenization, many of the hundreds of olfactory-receptor genes that are found in rodents and in other primates<sup>156</sup>. Interestingly, the situation was found to be intermediate for the chimpanzee<sup>156</sup> — although others have recently claimed that this is not the case<sup>157</sup>. As discussed later, buffering by cultural factors might have allowed such gene loss without negatively affecting the survival of the species. In this regard, it is of note that human olfactory perception differs widely between individuals, and that this might be related to genetic variation in human odorant-receptor genes<sup>157-159</sup>. Another interesting difference relates to the bitter-taste receptor genes in hominids, with humans having a higher proportion of pseudogenes than apes<sup>160-162</sup>.

### Skin and appendages

Of all the organ systems, it is the skin and its appendages (such as sweat glands, hair and breasts) that show the most striking differences between humans and other hominids. Furthermore, this organ system is most accessible to safe and ethical sampling. Despite this, we are unaware of any systematic comparisons of the biology of the skin between humans and other hominids. One frequently noted difference is the poor wound-healing abilities of humans, a phenotype that is partially recapitulated in a mouse line that has a human-like genetic defect in the *Cmah* gene<sup>163</sup>.

### Musculoskeletal system

Humans are generally more gracile than other hominids, with weaker muscles and less prominent muscle insertion points on our bones<sup>124</sup>. Apart from the *MYH16* mutation mentioned above, little has been done to study the molecular basis of these differences. Other striking differences include our upright state after infancy and our capacity for striding, bipedal walking and running<sup>164</sup>. Of course, it is not even clear if bipedalism is fully programmed genetically or if it is at least partially acquired by observation, learning and teaching. In this regard, the high frequency of human problems associated with bipedalism (for example, back and spine disorders) bears testament to the incomplete human adaptation to this locomotor state.

### Reproductive system

There are also several apparent differences between humans and NHHs in reproductive biology and disease, particularly in females. Examples include difficult childbirth, the full development of breasts before pregnancy, the process of menopause, and the high degree of blood loss during menstruation, often leading to chronic iron deficiency. Again, there are as yet few molecular correlates of these differences. One clue is the human-specific expression of *SIGLEC6* in the placental trophoblast, which seems to increase in expression during labour<sup>165</sup>.

### Immune system

The striking difference in the reaction of the human and chimpanzee immune systems to the HIV or chimpanzee immunodeficiency virus, respectively, with the latter being resistant to progression to AIDS, as well as the apparent rarity of other T-cell-mediated disorders in chimpanzees<sup>19,20</sup> indicates some fundamental differences in the responsiveness of the immune

system. One candidate for explaining this difference is suppression of expression of inhibitory *SIGLEC* genes in human T cells<sup>166</sup>.

### Practical implications

Overall, the organ-systems approach has much to offer, as it can complement genome-wide studies by focusing the efforts on specific phenotypes or features that seem to be uniquely human. Ultimately, the practical implications of understanding the genetic and genomic basis of uniquely human features range from understanding human cognition, with its frequent variations and abnormalities, to explaining diseases to which humans are particularly susceptible.

## Uniquely human evolutionary processes?

### Effects of behaviour and culture on the phenotype

Current understanding of genetic and phenotypic features of human evolution indicates that traditional evolutionary biology approaches have yet to explain most of the unique features of humans. We can therefore enter into more speculative discussions concerning how genome–environment interactions are modified by behaviour in warm-blooded animals in general and in primates in particular, and how these interactions are further modified by learning, teaching and culture in humans. Although we realize that much of what we suggest below is necessarily speculative there is general support from currently available knowledge, and we hope that this can form the basis for a rigorous and productive debate and research programme.

The phenotype of a fly or worm can be affected by its external and internal environment, but behavioural responses tend to be relatively hard-wired and stereotyped. With warm-blooded animals one sees a greater impact of postnatal care and of influence of learning from the prior generation — with humans being at one extreme end of this trend. Indeed, there is little doubt that, at least in mammals, behaviour can have profound effects on the genome and the phenotype by affecting the functional output of the genome either directly or indirectly. One example can be found in the two distinct developmental pathways of male orangutans, in which juveniles exposed to an aggressive mature conspecific male undergo permanent arrest of secondary sexual development<sup>167</sup>, allowing an alternative reproductive strategy.

In the case of hominids in general, and humans in particular, the further confounding issue is the effect of culture. For example, specific behaviours and their accompanying artefacts that are not hard-wired but are instead handed down from generation to generation by observation and, in the case of humans, by teaching, learning, conscious choice, and even by imposition through cultural practices or institutions. Thus, for example, genetically identical twins who happen to choose different careers (for instance, one an ascetic Buddhist monk the other a sumo wrestler) could end up with such markedly different physical, behavioural and cognitive phenotypes that an alien anthropologist might initially think they were different sub-species of humans.

In this regard, it is notable that even stereotyped mammalian behaviours that are considered crucial for species survival, such as effective mothering, seem to require observational learning in primates<sup>168-170</sup>. For example, one of the fears arising from the current National Institutes of Health ban on chimpanzee breeding<sup>171</sup> is the narrow time window before there will be no more fertile chimp females who have observed maternal care by an older female, something that is required for successful rearing of a new chimpanzee infant<sup>169,170</sup>. The situation is quite different in a dog or mouse mother who has not previously observed maternal behaviour, and is yet able to carry out these vital functions instinctively. Another example is the great difficulty in reintroducing primates in general and apes in particular back into the wild<sup>172</sup>, which is at least partly due to the fact that they lack so many of the hard-wired behaviours required for

survival<sup>173</sup>. Thus, we must consider the possibility that hominids in general and humans in particular have partially escaped from classic Darwinian selective control of some aspects of the genome (BOX 5; FIG. 2), and that humans have even escaped the final stage of Baldwinian genetic hard-wiring of long-standing species-specific learned behaviours (BOX 5). This might in turn help to explain the unusual degree of exaptation displayed by the human brain, presented as ‘Wallace’s Conundrum’ in BOX 6. The advantages of such novel changes are flexibility, plasticity, more rapidly developing population diversity and greater opportunities — but the disadvantages are that genomes cannot recover what has been irrevocably lost, and cultural advantages can be sensitive to the whims of history and fate.

#### **Box 5 Have humans escaped Baldwinian hard-wiring of behaviours?**

The Baldwin effect considers the costs and benefits of learning, in the context of evolution. Baldwin and others proposed that learning by individuals could potentially explain evolutionary phenomena that superficially seem to involve Lamarckian inheritance of acquired characteristics<sup>181-183</sup>. Given sufficient organismal plasticity, abilities that initially require learning could be replaced by rapid evolution of genetically determined systems that no longer require that learning. Behaviours that were initially learned would thus become instinctive ones in later generations, either because of new mutations or by ‘genetic assimilation’ of pre-existing genomic variability<sup>182</sup>. If a learned behaviour fails to become hard-wired genetically, it should then be susceptible to rapid disappearance, as there is a significant cost to the individuals who have to display the phenotypic plasticity to be able to learn.

There remains some controversy about the exact definition of the Baldwin effect and its importance to evolution in general<sup>184</sup>. However, some authors have suggested a role for Baldwinian processes in the evolution of uniquely human features, such as human language abilities<sup>185,186</sup>. For example, Deacon’s proposal<sup>187</sup> is that complexes of genes can be integrated into functional groups as a result of environmental changes that mask and unmask selection pressures, that is, a reverse Baldwin effect facilitated by niche construction<sup>188</sup>. In this regard, it is interesting that learned behaviours can be carried for many human generations without becoming hard-wired. For example, some long-isolated and small populations, such as Tasmanian Aboriginals, partially or completely lost many ancestral material practices, such as the making of fire and exploitation of certain marine food resources<sup>189</sup>. Apparently, such long-standing learned behaviours never become genetically hard-wired in humans and they remain dependent on intergenerational transfer by observation, learning and/or teaching. Perhaps humans have escaped the need for the last phase of the Baldwin effect that genetically hard-wires behaviours, and instead utilize extended developmental plasticity to invent, disseminate, improve and culturally transmit complex behaviours over many generations, without the need to hard-wire them. Of course, this advantage comes with great risk, as failure of cultural transmission can then result in permanent loss of a useful behaviour.

#### **Box 6 Wallace’s Conundrum**

The 2009 Darwin Centenary celebrations will further downplay Alfred Russel Wallace’s co-discovery of the theory of evolution by natural selection<sup>190</sup>. Wallace lost favour with the scientific community partly because he questioned whether natural selection alone could account for the evolution of human mind, writing: “I do not consider that all nature can be explained on the principles of which I am so ardent an advocate; and that I am now myself going to state objections, and to place limits, to the power of ‘natural selection’. How could ‘natural selection’, or survival of the fittest in the struggle for existence, at all favour the development of mental powers so entirely removed from the material necessities of savage



men, and which even now, with our comparatively high civilization, are, in their farthest developments, in advance of the age, and appear to have relation rather to the future of the race than to its actual status?"<sup>191</sup>.

Although Wallace was criticized for apparently invoking spiritual explanations<sup>192</sup>, one of his key points remains valid — that it is difficult to explain how conventional natural selection could have selected ahead of time for the remarkable capabilities of the human mind, which we are still continuing to explore today. An example is writing, which was invented long after the human mind evolved and continues to be modified and utilized in myriad ways. Explanations based on exaptation<sup>193</sup> seem inadequate, as most of what the human mind routinely does today did not even exist at the time it was originally evolving. Experts in human evolution or cognition have yet to provide a truly satisfactory explanation. Thus, 'Wallace's Conundrum' remains unresolved: "[...] that the same law which appears to have sufficed for the development of animals, has been alone the cause of man's superior mental nature, [...] will, I have no doubt, be overruled and explained away. But I venture to think they will nevertheless maintain their ground, and that they can only be met by the discovery of new facts or new laws, of a nature very different from any yet known to us."<sup>191</sup>.

Perhaps this proposed novel 'Wallacean' evolutionary mechanism relates partly to our suggestion that aspects of human uniqueness arose when there was relaxation of selection for maintenance of genome integrity, thus allowing us to partially escape from conventional Darwinian and Baldwinian selection processes, and to become much more dependent on inter-generational cultural transfer.

## Conclusions and perspectives

With the exception of the *FOXP2* gene, in which mutations cause a defined phenotype in humans, and some human-specific consequences of the *CMAH* gene mutation, much of the discussion about genes involved in human uniqueness has been somewhat speculative. Indeed, it is difficult to predict which answers might be accessible to us in the next years, and which approaches will be most fruitful. How then should we best proceed towards explaining human uniqueness? More rigorous attempts to connect changes at the genome level to specific phenotypes are necessary<sup>174</sup>. Phenotypes as complex as human cognition and intelligence are unlikely to be explained by any of the current studies, many of which have relied on small sample numbers, limited methods and many unproven assumptions. Such attempts will only yield true success if experts from multiple disciplines coalesce into transdisciplinary teams that probe multiple hypotheses concurrently, while avoiding preconceived notions based on the understanding of the evolution of other species. From this perspective, there is also a need to study many more closely related species that can provide more solid outgroup information, and to address the need for larger sample sizes among each primate group. All of these approaches will be better informed by a broader understanding of what areas of the genome are functional, and improved methods for analysing differences between genomes will probably be fuelled by the emerging field of systems biology. In this manner, approaches beginning with genotype and those starting with phenotype will meet and provide a level of convergent evidence that has so far not been mustered in this arena. Perhaps this will direct us on a rational path towards understanding human uniqueness, taking into account the interlinked roles of changes in Darwinian, Baldwinian, Wallacean and other as yet unknown mechanisms that gave rise to the unusual features of our species.

## Acknowledgments

The authors gratefully acknowledge comments from P. Gagneux, R. Bingham, D. Nelson, P. Churchland, F. Ayala, S. Hrdy, and two anonymous reviewers; M. Oldham for adapting the figure in BOX 4; and funding from the Howard Hughes Medical Institute (HHMI), the Mathers Foundation and the Gordon and Virginia MacDonald Foundation (to D.H.G.). The authors have National Institutes of Health grant funding: GM32373 to A.V., H60233 to D.H.G. and GM58815 to E.E.E.

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## Glossary

### Hominid

The term that is now often used to refer to the clade that includes both humans and great apes (that is, chimpanzees, bonobos, gorillas and orangutans). The term hominoid is also no longer routinely used for great apes. In recognition of these changes, we have introduced the term non-human hominids in place of great apes in most places. However, we recognize that the nomenclature is still in flux.

### Positive selection

A form of natural selection that increases the frequency of beneficial alleles in a population.

### Outgroup

A related but taxonomically distinct species that can be used to infer the ancestral state of a particular characteristic.

### Tissue heterogeneity

The presence of a large and variable number of cell types within a given tissue. Tissue heterogeneity in the brain might blunt the ability to detect the most variable low abundant genes in the brain relative to less complex tissue.

### Neutral theory

The word ‘neutral’ has two different meanings in population genetics literature. The strictly neutral model assumes that all mutations are neutral, whereas the

biologically neutral model assumes that all mutations are either neutral or deleterious.

**Encephalization**

An increase in brain size relative to body size.

**Array comparative genomic hybridization**

(ArrayCGH). A technique used to measure the relative copy number of a test and reference DNA sample based on differential hybridization to DNA molecules fixed on a microarray.

**Copy-number variant**

(CNV). A gain or loss of a >1 kb DNA region that contains genes. Most copy-number polymorphisms tend to be small (<10 kb) in size. *De novo* CNVs are variants that largely arise by new mutation, as opposed to hereditary transmission.

**Gene conversion**

A non-reciprocal recombination process that results in an alteration of the sequence of a gene to that of its homologue during meiosis.

**Ontology**

A hierarchical organization of concepts. The Gene Ontology framework provides one means for determining whether gene expression differences represent enrichment for specific functional categories.

**Scale-free network**

A network in which a few nodes (for example, genes) are central (that is, they act as 'hubs') and therefore serve as control points in the network, whereas most nodes are more peripheral and have few connections.

**Parallel distributed circuits**

Interconnected brain regions that work coordinately, to yield cognition and behaviour.

**Frontotemporal dementia**

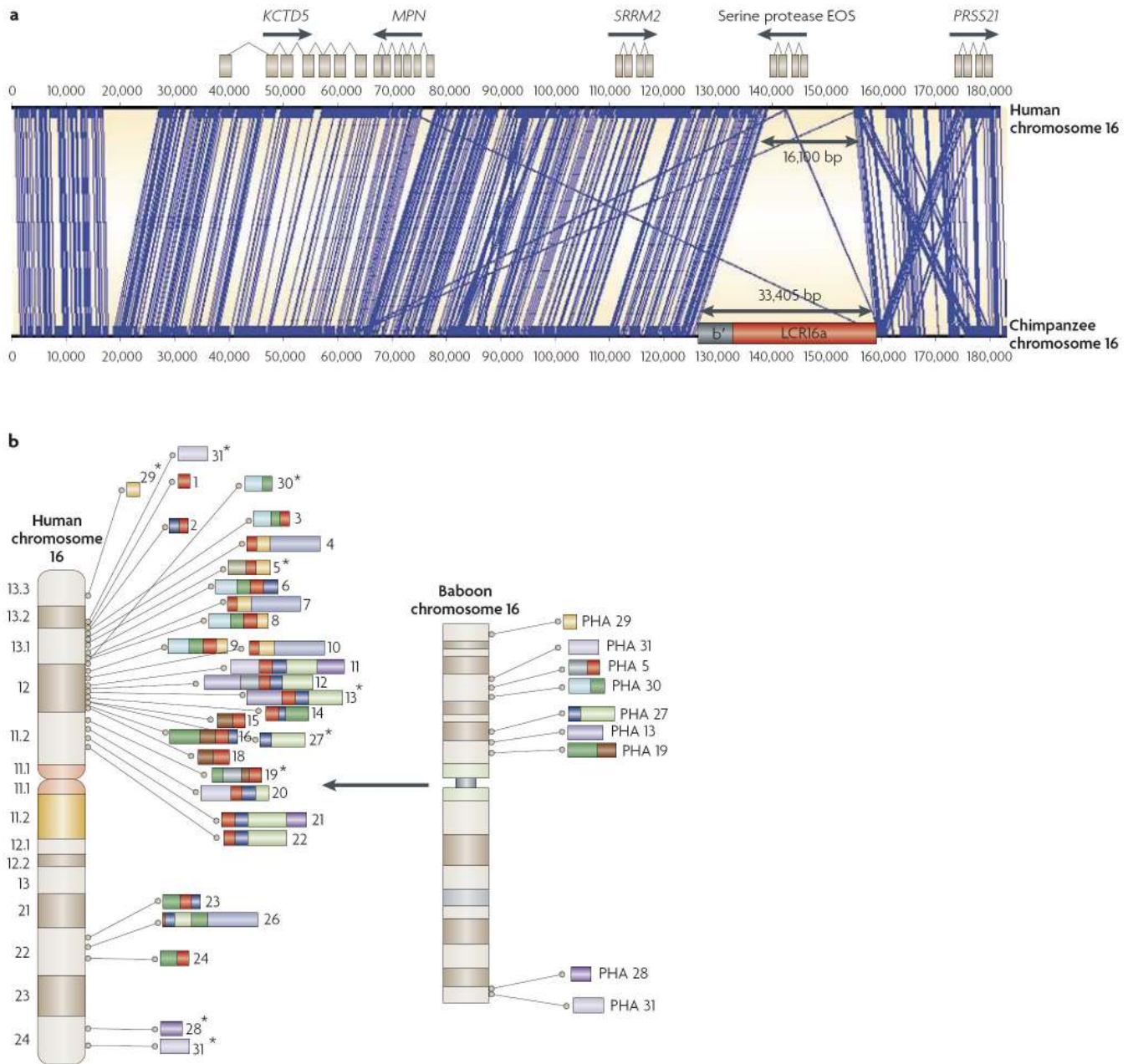
A degenerative disease that frequently involves dilapidation of social cognition.

**Pseudogenization**

Changes within the coding region of a gene that prevent transcription of a functional protein product.

**Exaptation**

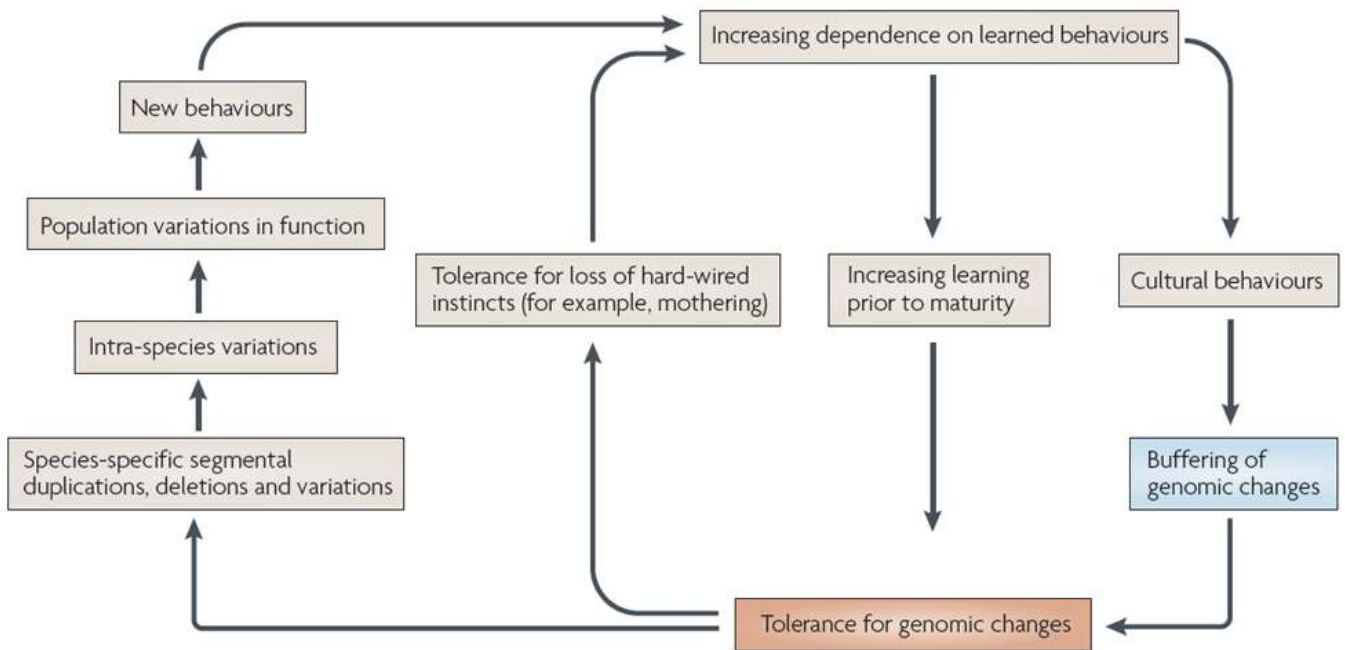
When a useful feature arises during evolution for a different reason, but is subsequently co-opted for its current function.



### Figure 1. Structural variations and segmental duplications

**a** Example of structural variation and segmental duplication difference between chimps and humans. An interspersed segmental duplication (33,405 bp) carries a copy of the rapidly evolving nuclear pore complex interacting protein gene (*NP1P*), a member of the morpheus gene family, into a new location on chromosome 16. The chimpanzee insertion results in a coordinated deletion (16,100 bp) of the serine protease EOS gene. As a result, chimpanzees carry an additional copy of the morpheus gene, carried within the duplicon LCR16a (low-copy repeat 16a), but have lost a serine protease gene that is present in humans. Blue lines identify regions of high sequence identity between chimpanzees and humans. **b** Human segmental duplication expansion on chromosome 16. Nine single-copy regions in Old World monkeys (as indicated on the baboon *Papio hamadryas* (PHA) chromosomal ideogram) became duplicated specifically within the human–great ape lineage of evolution. These loci were

distributed to 31 different locations on human chromosome 16; 24 of these carry a human–great ape specific gene family known as morpheus (as indicated by the red-coloured duplicons)<sup>99</sup>. Rearrangements between these interspersed duplications are associated with mental retardation and autism in humans. Coloured blocks show the distribution of the duplicons between the two species, with the position of the ancestral loci indicated by asterisks. Figure is modified, with permission, from REF.<sup>100</sup> © (2006) National Academy of Sciences, USA.



**Figure 2. Are human genomes escaping from Darwinian natural selection and Baldwinian fixation of learned behaviours?**

The figure shows potential mechanisms of behavioural and cultural buffering of genomic changes, potentially allowing hominid genomes to partially escape Darwinian selection and avoiding the need for the final phase of the Baldwin effect, in which learned behaviours eventually become hard-wired in the genome. The potential feedback loops shown could accelerate such processes, and even make some of them irreversible. See the main text and BOXES 5,6 for further discussion.