Positive selection drives population differentiation in the skeletal genes in modern humans

Dong-Dong Wu¹,³ and Ya-Ping Zhang¹,²,*

¹State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China, ²Laboratory for Conservation and Utilization of Bio-Resources, Yunnan University, Kunming 650091, China and ³Graduate School of the Chinese Academy of Sciences, Beijing, China

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During the course of evolution, the human skeletal system has evolved rapidly leading to an incredible array of phenotypic diversity, including variations in height and bone mineral density. However, the genetic basis of this phenotypic diversity and the relatively rapid tempo of evolution have remained largely undocumented. Here, we discover that skeletal genes exhibit a significantly greater level of population differentiation among humans compared with other genes in the genome. The pattern is exceptionally evident at amino acid-altering sites within these genes. Divergence is greater between Africans and both Europeans and East Asians. In contrast, relatively weak differentiation is observed between Europeans and East Asians. SNPs with higher levels of differentiation have correspondingly higher derived allele frequencies in Europeans and East Asians. Thus, it appears that positive selection has operated on skeletal genes in the non-African populations and this may have been initiated with the human colonization of Eurasia. In conclusion, we provide genetic evidence supporting the rapid evolution of the human skeletal system and the associated diversity of phenotypes.

INTRODUCTION

Modern humans are characterized by dramatic differences from other primates in skeletal anatomy, especially compared with our closest relatives, the chimpanzees (1,2). A primary example is bipedalism, the ability to walk and run upright on two feet. Although bipedalism is evident in the earliest hominins, in humans, with our specialized adaptations of the skeleton and muscles, it is more energy economical than in other apes (1). Endurance running, a derived capability of Homo, also needs musculoskeletal specializations (2). In addition, modern human populations exhibit substantial phenotypic variation as evidenced by body mass, height and craniofacial differences shaped by the skeletal system. The human skeletal system has evolved rapidly with the advent of agriculture (3) and it is possible that these changes would have likely been accompanied by changes in selective pressures on the underlying genes, where adaptive evolution probably occurred.

Positive selection is the main force driving rapid evolution and adaptation. However, unlike other traits, e.g. brain development and skin pigmentation (reviewed in 4), fewer genes have been documented to have evolved under positive selection accounting for the rapid evolution of the skeletal system. To address this possibility, we investigate the population differentiation pattern of skeletal genes in modern humans to evaluate specifically the potential positive natural selection based on HapMap phase II data (5). In this study, we found a significantly higher population differentiation at single nucleotide polymorphism (SNPs) in skeletal genes, exceptionally at amino acid-altering sites. SNPs with higher levels of differentiation have correspondingly higher derived allele frequencies (DAFs) in Europeans and East Asians. Since we could exclude the possibility of relaxed purifying selection, as skeletal genes play crucial roles in development and have very low average dN/dS values between humans and chimpanzees, and demographic history should affects all genomic loci similarly, we conclude that positive selection has operated on the skeletal genes in the non-African populations and this may have been initiated with the human colonization of Eurasia.

RESULTS

As no systematical definition of skeletal genes is presently available by gene classification methods, we compiled a list

*To whom correspondence should be addressed. Tel: +86 8715190761; Fax: +86 8715195430; Email: zhangyp@mail.kiz.ac.cn or zhangyp1@263.net.cn

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of 132 genes, all of which are located on autosomal chromosomes that are involved in the skeletal system by searching the literatures (Supplementary Material, Table S1). We evaluated $F_{ST}$, population differentiation among the three populations: African (YRI), European (CEU) and East Asian (EA), according to previously described methods (6,7). Since genic regions have higher levels of population differentiation than non-genic regions (8), we used genome-wide SNPs at gene regions rather than the entire genome, thus this is a more conservative approach. Skeletal genes exhibit an excess of SNPs with higher $F_{ST}$-values ($\geq 0.6$) relative to whole genome-wide genes ($\chi^2 = 139.39, P = 3.61 \times 10^{-32}$, Fig. 1A). Four bins with $F_{ST} \geq 0.6$ had $P$-values of $7.33 \times 10^{-16}, 4.35 \times 10^{-36}$ and $1.30 \times 10^{-3}$, respectively, all with statistical significance. We calculated the proportion of SNPs with $F_{ST}$-value $\geq 0.6$ for 132 randomly chosen genes, with 100 replications, yet the proportion of higher $F_{ST}$-value SNPs in skeletal genes, 1.249%, was significantly higher than for the random gene panel (98.4% percentile rank). However, a major problem with HapMap SNPs is its ascertainment bias, a problem that could complicate the detection of natural selection, and is very difficult to exclude (9). Accordingly, we performed a further study using the ‘class A’ SNPs from the Perlegen data (10), which were discovered in a genome-wide homogeneous resequencing scheme, and thus should have no ascertainment biases. The Perlegen data are much more limited (only $\approx 5400$ SNPs at skeletal genes) compared with the HapMap data ($\approx 11000$ SNPs at skeletal genes) thus has less power to demonstrate a difference between the African and European populations. These results suggest that the observation of higher population differentiation of SNPs at the skeletal genes cannot solely be attributable to ascertainment biases, although we cannot absolutely exclude this possibility.

To discern which population(s) contribute more to the pattern, we generated three pairwise sets of $F_{ST}$-values based on the HapMap data: $F_{ST}$ (CEU–YRI), $F_{ST}$ (EA–YRI) and $F_{ST}$ (CEU–EA). $F_{ST}$ (CEU–YRI) population differentiation between the Europeans and Africans, and $F_{ST}$ (EA–YRI) that between the East Asians and Africans, consistently showed an excess of SNPs with higher $F_{ST}$-values ($\chi^2 = 31.09, P = 2.47 \times 10^{-8}$, Supplementary Material, Fig. S1). The level of population differentiation between the European American and Han Chinese populations did not show a statistically significant difference between the skeletal and genome-wide genes (Supplementary Material, Fig. S1). The Perlegen data are much more limited (only $\approx 5400$ SNPs at skeletal genes) compared with the HapMap data ($\approx 11000$ SNPs at skeletal genes) thus has less power to demonstrate a difference between the African and European populations. These results suggest that the observation of higher population differentiation of SNPs at the skeletal genes cannot solely be attributable to ascertainment biases, although we cannot absolutely exclude this possibility.

![Figure 1](https://academic.oup.com/hmg/article-abstract/19/12/2341/2527069/download?downloadUrl=https%3A%2F%2Facademic.oup.com%2Fhmg%2Farticle-abstract%2F19%2F12%2F2341%2F2527069)
The proportions of SNPs in the skeletal genes with \( F_{ST} \)-values \( \geq 0.6 \) were 1.996 and 2.693\% for \( F_{ST} \) (CEU – YRI) and \( F_{ST} \) (EA – YRI), respectively. These values were significantly higher than for randomly chosen genes (100 and 98.6\% percentile ranks, respectively). However, \( F_{ST} \) (CEU – EA), differentiation between the European and East Asian populations, did not have a higher proportion of higher \( F_{ST} \) SNPs (\( \chi^2 = 1.78, P = 0.18, \) Fig. 1B). Correspondingly higher \( F_{ST} \) patterns at skeletal genes were also observed when the analyses were constrained to SNPs having similar globally distributed minor allele frequencies (Supplementary Material, Fig. S2).

Genetic hitchhiking could potentially generate an enrichment of SNPs with higher \( F_{ST} \)-values, and result in a higher gene density surrounding higher \( F_{ST} \)-values (8). However, skeletal genes are broadly distributed among the chromosomes with few being neighbors, and the gene density and \( F_{ST} \)-values do not appear to be correlated (8). To further exclude the possibility of hitchhiking, we analyzed genes neighboring the skeletal genes. Increased proportions of higher \( F_{ST} \) (EA – YRI) and \( F_{ST} \) (CEU – EA) values were not observed (Supplementary Material, Fig. S3B and C). However, in the \( F_{ST} \) (all) and \( F_{ST} \) (CEU – YRI) panel, genes neighboring the skeletal genes showed an excess of higher \( F_{ST} \) (Supplementary Material, Fig. S3A and D, \( \chi^2 = 12.55, P = 0.0004; \chi^2 = 58.42, P = 2.12 \times 10^{-14} \)). This result is likely due to hitchhiking to the skeletal genes as the enrichment of higher \( F_{ST} \)-values in neighboring genes is lower than in the skeletal genes.

Demographic history, such as a reduction in effective population size, which strengthens genetic drift, could generate a similarly higher population differentiation (4). However, demographic history should affect all genomic loci similarly, thus, should play a minor role in shaping the structured patterns of polymorphism at the skeletal genes (4). In addition, it is difficult for demographic history to influence genes which are dispersely distributed in an irregularly fashion on chromosomes without a biological factor. Skeletal genes have a crucial function in development, as indicated by their very low average dN/dS value between humans and chimpanzees (0.14 versus 0.23 of the genome-wide genes) (11), therefore, relaxation of selective constraints on these genes should be also excluded as an explanation for our observations.

The pattern of higher \( F_{ST} \)-values in skeletal genes could be attributable to either (i) positive selection operating in African population driving its divergence from the other two populations or (ii) positive selection occurring both in Europeans and East Asians during and after the ‘out-of-Africa’ event. To distinguish between these two scenarios, we obtained the DAF of each SNP with \( F_{ST} \) all-values higher than 0.3716, which are values in the top 5\% of SNPs among all genome-wide genes. These SNPs demonstrated a significantly higher DAFs in European (Fig. 2A) and East Asian (Fig. 2B), but not African (Fig. 2C) populations. Furthermore, we compared the higher \( F_{ST} \) SNPs in skeletal genes with higher \( F_{ST} \) SNPs in genome-wide genes. Europeans demonstrated a significant excessive of higher DAF (\( \geq 0.9 \)) SNPs (Supplementary Material, Fig. S4A, \( \chi^2 = 26.37, P = 2.82 \times 10^{-7} \)), and East Asians had an even higher excess of DAF SNPs, although the difference was not statistical significant (Supplementary Material, Fig. S4B, \( \chi^2 = 2.14, P = 0.15 \)). In comparison, Africans have fewer in the skeletal genes (Supplementary Material, Fig. S4C). The higher DAFs of SNPs with higher \( F_{ST} \)-values cannot be attributed to demographic history or the relaxation of purifying selection for skeletal genes, as discussed above, therefore, positive selection appears to have operated on the genes in Europeans and East Asians, but not Africans, and this generated the observed pattern of population differentiation.
The 89 non-synonymous SNPs mapped to the skeletal gene regions also demonstrated a pattern concordant with the higher $F_{ST}$-values between African and both Europeans and East Asians (Fig. 3, $P = 0.13$ $F_{ST}$ (CEU–YRI), and $P = 0.001$ $F_{ST}$ (CEU–YRI) by the two-tailed Mann–Whitney $U$-test; and $P = 0.037$ $F_{ST}$ (EA–YRI), and $P = 3.56 \times 10^{-4}$ $F_{ST}$ (CEU–YRI) by $t$-test). When compared with the genome-wide total for non-synonymous SNPs, no significant differentiation was observed between the Europeans and East Asians ($P = 0.23$, by two-tailed Mann–Whitney $U$-test and $P = 0.21$ by $t$-test). $F_{ST}$-values of these 89 SNPs among the three populations also showed a statistically significant elevation ($P = 0.015$ by two-tailed Mann–Whitney $U$-test and $P = 0.005$ by $t$-test). Conversely, synonymous SNPs did not differ between skeletal (containing 112 SNPs) and genome wide genes ($P = 0.633, 0.954, 0.832$ and $0.866$ based on the four kinds of $F_{ST}$-values by the two-tailed Mann–Whitney $U$-test). The differences between non-synonymous and synonymous mutations also suggest that demographic history played a weaker role than positive selection.

Table 1 summarizes the top 8 non-synonymous SNPs in seven skeletal genes with high $F_{ST}$-values among the three populations. The RTTN (rotatin) gene is required for the early specification of left–right (L–R), and axial rotation and may play a role in notochord development (12). MSX2 regulates the expression of osteocalcin in mouse embryos and is implicated in the control of bone formation (13); mutations are associated with many types of human bone disorder, e.g. parietal foramina (14,15) and autosomal dominant mutations are associated with many types of human bone disease (16,17). A non-synonymous SNP (rs4242182) in the MSX2 gene shows high population differentiation. Global frequency distribution indicates that this SNP has a highly DAF in non-Africans (Supplementary Material, Fig. S5). The non-synonymous SNP (rs2306033) in the LRP4 gene is associated with bone mineral density (BMD) (18) and shows high population differentiation. ADAMTSL8 is associated with BMD (19). BMD is widely used for the diagnosis of osteoporosis and related fractures and BMD varies in modern humans with ethnicity (20,21). For example, Asians have lower BMD than Europeans, who are lower than Africans (20,21). Presumably, BMD has been a target of positive selection during the evolution of humans. The ACAN and CEP63 loci are associated with adult human height (22). The high genetic differentiation of these loci is consistent with the diversity of height in different human populations. Many of these SNPs occur in higher, DAFs in non-African populations (Supplementary Material, Fig. S5).

Genes under recent positive selection also demonstrate long-range haplotype homozygosity (4). We obtained the selection data from Haplotter (http://haplotter.uchicago.edu/selection/) by iHS test (23) based on HapMap Phase II data (5). Two genes, i.e. BMP3 and C6orf106, were under positive selection in Europeans, and another two genes, i.e. GDF5 and HIST1H1D, in East Asians, but no evidence for positive selection in any skeletal genes in Africans were found, consistent with our conclusion of selection only in non-Africans.

**DISCUSSION**

Humans have encountered substantial environmental and climatic changes during their colonization of the world. This experience promotes the generation of phenotypic diversity, which is illuminated to a large extent by genetic diversity. For example, positive selection on genes results in higher population differentiation and morphological diversity as adaptations to local environments, including skin pigmentation (4,24), hair follicle development (25) and other traits (4). On the other hand, human genetic diversity has been profoundly influenced by demographic history, e.g. higher nucleotide diversity and more SNPs with high ancestral allele frequencies in Africans (26). However, demographic factors influence the whole genome equally, and should contribute weakly to elevated $F_{ST}$-values in skeletal genes. Our results show that skeletal genes, despite representing only a small fraction of the genome are outlier for $F_{ST}$-values and demonstrate different patterns between non-synonymous and synonymous SNPs. However, demographic history does also contribute to human phenotypic variation, e.g. geographic distance from Africa plays a determinant role in within-population human skull diversity (27,28). Our comparative study allowed us to identify a likely functional biologically factor (i.e. positive selection) that has been driving divergence of skeletal genes, and this should be informative to studies into the factors affecting human phenotypic variation.

We can conclude that positive selection operated on skeletal genes fairly recently, approximately <50 000–75 000 years ago (4), following the dispersal of humans from African. Although the human skeletal system has evolved rapidly during the past 10 000 years (3,29), supporting genetic evidence has been lacking. Presumably, both height and BMD have experienced positive selection. Environmental temperature has influenced body mass substantially (30,31). Increased body mass and height may have facilitated the adaptation of Caucasians to cold climatic areas (31). Decrease in BMD may have contributed to the smaller bone size in Asians (20). Regardless, genetic evidence at the genome level suggests that positive selection was the driving force in the rapid evolution of the human skeletal system.
To our knowledge, there has been no systematic studies on the evolutionary patterns of skeletal genes. In conclusion, the results of our study provide the evidence for positive selection driving higher population differentiation in modern human populations, which could explain the fact of the highly skeletal system related phenotypic variation among humans, and the rapid evolution of the human skeletal system. Positive selection should have been initiated with the human colonization of Eurasia, occurring after the out-of-Africa events. In addition, our study will provides insight into human evolution and the origin of human uniqueness.

MATERIALS AND METHODS

Allele frequency data of SNPs on autosomal chromosomes was retrieved from HapMap Phase II (release 21) (S) for three populations: African (YRI panel including 60 Yoruban individuals from Ibadan), European (CEU panel including 60 individuals of Utah residents with ancestry from northern and western Europe) and East Asian (EA panels including 45 Han Chinese (HCB) and 45 Japanese in Tokyo (JPT)). The ancestral alleles of SNPs were obtained from Haplotter (http://haplsetter.uchicago.edu/selection/) (23). Physical chromosome positions of the genes in the human genomes were downloaded from UCSC (http://genome.ucsc.edu/, hg17). Each gene was extended 500 bp upstream of 5′ terminus and downstream of 3′ terminus to identify its SNPs. ST-values of polymorphic SNPs with minor allele frequencies \( \geq 0.01 \) in at least one population were calculated as previously described (6,7) to evaluate the degree of population differentiation. Negative values have no biological explanation and were arbitrarily set to 0. The \( F_{ST} \) values were grouped equally into 10 bins. As described in (8), \( \chi^2 \) tests with one degree of freedom were used to test the significance of deviation of \( F_{ST} \)-value of skeletal genes from empirical data within each bin based on \( 2 \times 2 \) contingency tables constructed by the numbers of SNPs.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG online.

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