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Authors

Wallace, IJ
Botigué, LR
Lin, M
et al.

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SHORT COMMUNICATION

Worldwide variation in hip fracture incidence weakly aligns with genetic divergence between populations

I. J. Wallace[1,2] · L. R. Botigué[3] · M. Lin[4] · J. B. Smaers[1] · B. M. Henn[3,4] · F. E. Grine[1,5]

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Abstract
Summary This study investigates the influence of genetic differentiation in determining worldwide heterogeneity in osteoporosis-related hip fracture rates. The results indicate that global variation in fracture incidence exceeds that expected on the basis of random genetic variance.
Introduction Worldwide, the incidence of osteoporotic hip fractures varies considerably. This variability is believed to relate mainly to non-genetic factors. It is conceivable, however, that genetic susceptibility indeed differs across populations. Here, we present the first quantitative assessment of the effects of genetic differentiation on global variability in hip fracture rates.
Methods We investigate the observed variance in publically reported age-standardized rates of hip fracture among 28 populations from around the world relative to the expected variance given the phylogenetic relatedness of these populations. The extent to which these variances are similar constitutes a "phylogenetic signal," which was measured using the *K* statistic. Population genetic divergence was calculated using a robust array of genome-wide single nucleotide polymorphisms.
Results While phylogenetic signal is maximized when $K > 1$, a *K* value of only 0.103 was detected in the combined-sex fracture rate pattern across the 28 populations, indicating that fracture rates vary more than expected based on phylogenetic relationships. When fracture rates for the sexes were analyzed separately, the degree of phylogenetic signal was also found to be small (females: $K = 0.102$; males: $K = 0.081$).
Conclusions The lack of a strong phylogenetic signal underscores the importance of factors other than stochastic genetic diversity in shaping worldwide heterogeneity in hip fracture incidence.

Keywords Bone fragility · Ethnicity · Genetics · Osteoporosis · Phylogenetic comparative methods · Phylogenetic signal

Electronic supplementary material The online version of this article (doi:10.1007/s00198-016-3591-x) contains supplementary material, which is available to authorized users.

✉ I. J. Wallace
iwallace@fas.harvard.edu

[1] Department of Anthropology, Stony Brook University, Stony Brook, NY 11794, USA

[2] Present address: Department of Human Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA

[3] Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY 11794, USA

[4] Graduate Program in Genetics, Stony Brook University, Stony Brook, NY 11794, USA

[5] Department of Anatomical Sciences, Stony Brook Medicine, Stony Brook, NY 11794, USA

Introduction

The incidence of osteoporosis-related fractures varies substantially around the world. For example, age-standardized rates of hip fracture differ by at least 10-fold between countries exhibiting the highest and lowest incidences [1, 2]. Hip fracture rates are generally highest in northern Europe, Central Europe, and the Caucasus; moderate in North America, Japan, and Oceania; and lowest in South Asia and Africa [1]. Similar patterns of international disparity also exist for rates of other types of osteoporotic fracture [2]. Currently, the underlying causes of this heterogeneity in fracture incidence are not known, although genetic factors have been

hypothesized to have a limited influence relative to environmental conditions [1].

To date, no study has investigated the potential contribution of genomic variation to global variability in fracture incidence, but it is conceivable that genetic susceptibility does indeed differ between populations [2]. Fracture risk has been shown to be heritable and defined by many loci across the genome [3, 4]. Moreover, the frequency of alleles affecting bone mineral density—the primary clinical risk factor for fracture—has been demonstrated to differ markedly between ethnically distinct populations [5]. Therefore, one way in which allelic variation might affect fracture rates is by influencing the average bone mass of populations [2]. It is also possible that other phenotypic traits related to fracture risk are similarly biased by genetic differences between populations, including traits such as bone geometry, body size, body mass index, muscle strength, and circulating hormone levels [6].

In this study, we investigate the influence of genomic differentiation in shaping worldwide patterns of hip fracture incidence using a methodological approach originally developed to address similar questions in evolutionary biology. The tendency for phenotypic traits, such as hip fracture rates, to vary according to genetic divergence between populations is referred to as "phylogenetic signal" [7]. A variety of statistical tools are available for measuring and testing for phylogenetic signal in comparative datasets, with the prerequisite that genomic variance among groups can be quantified and mapped into a phylogenetic framework [7]. Until recently, application of such tools to questions related to human phenotypic variation was limited by a lack of adequate data on the genetic relatedness of many of the world's populations. In the past decade, however, the rapid growth of single-nucleotide-polymorphism (SNP) array data has provided exceptionally detailed information on human genetic variation across the globe [8], including numerous populations for which high-quality data on hip fracture incidences are also available [1, 2].

Methods

The study sample consists of 28 populations from around the world for which comparable age-standardized hip fracture rates (per 100,000 individuals) have been published [1, 2] (Table 1), and genomic SNP array datasets are available in the public domain [9]. Criteria for including the studies reporting hip fracture rates, as well as the complete citations for those studies, are provided elsewhere [1, 2].

Genome-wide data were obtained from the Affymetrix AffyOrigins SNP array used by Haak and colleagues [9]. To maximize the comparability of SNP and hip fracture data, the population divisions of Haak et al. [9] were modified as follows: when hip fracture rates were derived from national studies, available genetic data were pooled for that country. An exception is that Sardinians were excluded from the Italian sample, as they are a known genetic isolate. When fracture rates came from regional studies within countries, genetic data from only the geographically most relevant populations were analyzed. For countries with pronounced ethnic diversity (e.g., UK, USA, and South Africa), genetic data were considered only for the specific populations from which fracture incidence data were obtained (e.g., British, African Americans, and Bantu-speaking, respectively), regardless of whether fracture data were from national or regional studies. In the case of Mexico, genetic data were available from only indigenous groups (including Mayan, Mixe, Mixtec, and Pima), whereas fracture data came from a national survey that presumably included indigenous people as well as Mestizos [10]. Despite this incongruity, we opted to include data from Mexico in our study because statistical analyses conducted with and without them yielded very similar estimates of phylogenetic signal.

Table 1 Age-standardized hip fracture rates (per 100,000 individuals) for the 28 populations analyzed in this study

Population	Women and men	Women	Men	Reference
African American	108	136	76	[1]
China	141	182	102	[2]
Croatia	157	177	135	[1]
Czech Republic	293	379	221	[2]
Finland	185	231	147	[2]
France	157	224	101	[2]
Greece	210	295	136	[2]
Hungary	282	371	207	[2]
Iceland	264	384	158	[2]
Iran	342	405	272	[2]
Italy	235	339	144	[2]
Japan	218	266	165	[1]
Jordan	158	198	114	[1]
Lebanon	196	315	114	[1]
Lithuania	216	270	156	[1]
Malta	221	319	138	[2]
Mexico	180	243	122	[2]
Nigeria	2	2	2	[1]
Norway	399	532	281	[2]
Russia	219	257	191	[2]
Saudi Arabia	107	135	77	[1]
South Africa	19	20	17	[1]
Spain	123	182	74	[2]
Taiwan	264	355	186	[2]
Thailand	146	205	91	[2]
Tunisia	50	58	41	[1]
Turkey	219	360	111	[2]
UK	201	276	140	[2]

We sampled $n = 10$ individuals per population for genetic analysis. For 9 groups, however, array data were available for only 6–9 individuals. The final genomic dataset consisted of ~500,000 autosomal SNPs from a total of 261 individuals. After pruning SNP data for linkage disequilibrium, genetic divergence among the 28 populations was quantified with Weir and Cockerham's F_{st} statistic [11]. Pairwise F_{st} values for each population were used to construct a population phylogenetic tree. A neighbor-joining algorithm [12] was used to infer branch internal lengths in units of genetic distance.

To obtain statistical support for the topology of the phylogenetic neighbor-joining tree, we utilized a bootstrap approach. From the 19 populations with SNP data available for >10 individuals, a random subset of 10 individuals was sampled for each bootstrap. F_{st} values for all pairwise comparisons in the complete dataset were then calculated for each bootstrap and used to construct a tree. We performed 1076 iterations, from which the most frequent tree was selected for analysis. Branch support was calculated as the proportion of trees generated that had the same branch splitting as the most frequent tree.

In order to test whether unequal rates of evolution and gene flow among populations affect the phylogenetic signal detected in fracture rates, we used TreeMix software [13] to build an alternative topology of the phylogenetic tree that allows for differential genetic drift and gene flow among groups. After inferring the initial tree structure, estimated weights of directional gene flow among populations were added to best fit the data. We sequentially set the number of possible gene flow events from 0 (no admixture) to 10 to test for the best fitting model applied to the genetic data. For all models, the root position of the tree was defined as the South African population branch, and every 500 SNPs were grouped in windowed blocks to account for linkage disequilibrium among loci.

The degree of phylogenetic signal in hip fracture rates was quantified using the approach of Blomberg and colleagues [7]. With this method, phylogenetic signal is measured using the K statistic, which is the observed level of phylogenetic covariance in the population data divided by the expected level of covariance according to a Brownian motion model of evolution. A Brownian motion model describes evolution as a random process, with trait variance proportional to phylogenetic tree branch length (i.e., genetic distance). Thus, the degree of phylogenetic signal represents the extent to which genetic distance aligns with the variation present in the given phenotypic trait (in this case, hip fracture incidence). Phylogenetic signal is maximized when $K > 1$; whereas a K value < 1 indicates that populations resemble each other less than would be expected on the basis of their phylogenetic relationships alone. A low K value reflects trait variation that departs from random evolution (i.e., deviation from Brownian motion) and/or measurement error. Phenotypic variation that deviates from a Brownian motion model of evolution can arise from both phenotypic divergence between closely related populations and trait convergence between distantly related groups. Likely causes of trait variation that departs from Brownian motion are population-specific behavioral and physiological responses to individuals' environmental and lifestyle conditions. Another potential cause is natural selection differentially affecting groups over generations.

To statistically test the null hypothesis that variation in the hip fracture incidence data exhibits no phylogenetic signal (i.e., $K = 0$), a randomization procedure was used that compares observed K values to values calculated from 1000 permutations of the phenotypic data without regard to phylogeny [7]. Statistical significance was assessed using a 95 % criterion ($p < 0.05$). For comparative datasets with ≥20 groups, this test has been shown to detect significant phylogenetic signal in the vast majority of phenotypic traits (>90 %), even when K values are relatively small [7]. Thus, while statistical significance reveals whether variation can be considered to be non-random, the magnitude of the phylogenetic signal (i.e., the K value) reveals to what extent variance among closely related species is more or less similar than predicted given their phylogenetic relatedness (assuming Brownian motion).

To graphically represent the evolutionary pattern of hip fracture incidence among the 28 populations, a Brownian motion model was used to estimate ancestral values along phylogenetic space [14].

Results

Figure 1 presents the most parsimonious neighbor-joining phylogenetic tree depicting the genetic relatedness of the 28 global populations. Most nodes have strong bootstrap support, except for internal branches that determine the precise topology of European populations. Colors at the tips of the tree branches indicate age-standardized hip fracture rates (per 100,000 individuals) among the 28 groups for females and males combined. Colors along the tree branches represent the inferred pattern of evolution in hip fracture rates among the groups. As Fig. 1 illustrates, hip fracture rates are generally highest among a clade formed by populations from northern Europe, Russia, and the UK and lowest among groups from Sub-Saharan Africa. Hip fracture rates tend to be intermediate in three clades formed primarily by East Asian, southern European, and Near Eastern groups.

Despite some similarity in hip fracture rates between groups within particular clades, the magnitude of phylogenetic signal across the entire sample, as measured using the K statistic, was found to be relatively small. Whereas phylogenetic signal is maximized when $K > 1$, a K value of only 0.103 was detected in the fracture rate pattern of females and males combined ($p = 0.011$). Similar results were obtained when fracture rates for the sexes were analyzed separately. That is, the degree of

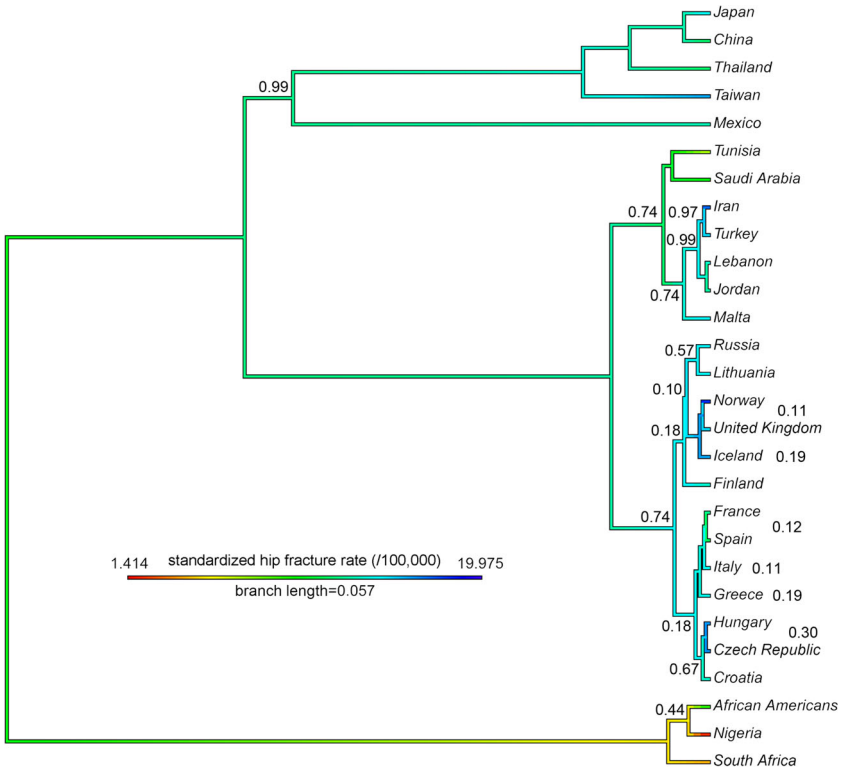

Fig. 1 Phylogenetic tree depicting the genetic relationships among 28 human populations. Tree branch lengths equal genetic distance. *Colors* at the tips of the tree branches indicate age-standardized hip fracture rates (per 100,000 individuals) for females and males combined. *Colors* along the tree branches represent the estimated evolutionary pattern of hip fracture incidence among the groups. *Numbers* separating populations indicate statistical support for the branching topology

phylogenetic signal was found to be minimal among both females ($K=0.102$, $p=0.008$) and males ($K=0.081$, $p=0.021$).

To assess the degree to which the branching pattern of European groups with low statistical support may have biased our results, K values were also calculated from other frequent topologies identified via our bootstrap analysis of trees (Supplementary Material Fig. 1). In agreement with the results from the most parsimonious neighbor-joining tree, K values were all found to be low (sexes combined, mean ± s.d.: $K=0.185\pm0.049$; females: $K=0.185\pm0.050$; males: $K=0.155\pm0.042$). When European branches with low support were excluded altogether from K calculations, the phylogenetic signal that was detected remained small (sexes combined: $K=0.118$, $p=0.11$; females: $K=0.099$, $p=0.19$; males: $K=0.110$, $p=0.15$).

Consistent with the results from the most parsimonious neighbor-joining tree, the degree of phylogenetic signal in hip fracture rates detected using the population tree that best accounted for differential genetic drift and gene flow (Supplementary Material Fig. 2) was also found to be low (sexes combined: $K=0.168$, $p=0.006$; females: $K=0.196$, $p=0.002$; males: $K=0.108$, $p=0.017$).

Discussion

This study represents the first quantitative assessment of the influence of genomic diversity on global variability in osteoporosis-related hip fracture rates. The degree of phylogenetic signal in fracture rates was measured within a broad sample of 28 populations from around the world. The variance in fracture incidence among these populations does not closely match the structure of their genetic relationships. Specifically, the degree of phylogenetic signal calculated using the K statistic was found to be markedly lower than if fracture rates were determined primarily by the genetic relatedness of populations. Our results are consistent with the notion that genetics play a minor role in affecting global patterns of fracture incidence, since the phylogenetic signal, albeit slight, was found to be statistically significant in most tests. Nevertheless, these results suggest that the heterogeneity in fracture rates worldwide is largely driven by factors other than random genetic differentiation [1].

The lack of a strong phylogenetic signal within the worldwide pattern of hip fracture rates is perhaps somewhat surprising given that genetic factors account for a sizable portion of the variance in hip fracture risk among individuals within populations [3]. To be clear, however, the results of this study do not contradict the importance of a person's genetic complement in determining fracture risk. Instead, our findings suggest only that the bulk of alleles affecting fracture risk is not biased toward the fraction of stochastic genetic diversity that exists between populations, which is substantially less than the genetic diversity accounted for within populations [8]. Moreover, the relatively low K values estimated for hip fracture rates should not be interpreted as indicating that the

magnitudes of phylogenetic signal in traits underlying fracture risk are equally small. For example, femoral neck bone mineral density might be expected to exhibit a stronger phylogenetic signal than hip fracture rates, inasmuch as it is a less complex trait and potentially under greater genetic control than fracture risk [15], and there is evidence that differences in bone mass between particular ethnic groups are modulated by allele frequency divergence [5]; but this hypothesis remains to be tested.

The results of this study beg the question of which factors are most important in determining global heterogeneity in hip fracture incidence. At present, the single strongest correlate appears to be socioeconomic development, with people in highly developed countries being at greater risk than those in developing nations [2]. Exactly what this trend signifies is unclear, but it may reflect, at least in part, population-level differences in lifestyle features that are well known to affect individual fracture risk, such as habitual levels of physical activity and nutrient intake [16]. Indeed, to the extent that people in post-industrial societies tend to be more sedentary, have poorer-quality diets, and spend more time indoors shielded from sunlight than people in developing countries, they might be expected to be more susceptible to experiencing a fracture [17, 18]. It is also conceivable that some of the worldwide variation in fracture incidence is the outcome of natural selection differentially operating on group-specific phenotypes that affect fracture risk, for example, bone mineral density [5]. Future research is required to determine precisely how these and/or other factors relate to global fracture variability, which could provide insights for international strategies for fracture prevention.

This study has at least three important limitations, all of which relate to measurement error and its possible effects. First, because the SNP and fracture data did not originate from the same individuals, their comparability is potentially limited. Although care was taken to select populations for which the provenience of data matched reasonably well, it is probable that hip fracture rate calculations for some countries included individuals outside the genetic group analyzed [2]. Nevertheless, it is fair to assume that the measurement error in any one fracture rate estimate was relatively small compared to the large variation in rates that exists between populations [1]. Second, inaccuracy in the hip fracture data and/or the structure of the population phylogenetic tree might have impacted the observed low K values by making fracture rates of closely related populations appear less similar than expected under a Brownian motion model of evolution [7]. Some degree of error in the fracture data is inevitable given that they were derived from multiple studies employing different methodologies and from countries that vary in the completeness of their health databases and case ascertainment capabilities [1, 2]. The low support of some phylogenetic branches of European groups also suggests that the tree reported in Fig. 1 may not accurately reflect the genetic relationships of populations. Yet, any effect of topological variation among these branches on the phylogenetic signal detected in the global pattern was likely small because European branching patterns do not substantially change their relative branch lengths. Exploration of an alternative population tree method [13], allowing for differential genetic drift and gene flow, also did not dramatically alter K values. Thus, we believe that the overall influence of tree uncertainty on the phylogenetic signal detected in this study was likely minor. Third, the number of populations included in this study was limited by the availability of data in the public domain, with the result that our sampling was geographically unbalanced. Especially lacking were comparable genetic and fracture data from Africa and South America. With many closely related groups in the sample, our phylogenetic trees had many short branches near the tips, which can bias K values downwards [19]. Future studies like ours would benefit from a more comprehensive database of global genetic variation and fracture incidence rates, including additional data from the developing world in particular.

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Compliance with ethical standards

Conflicts of interest None.

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