**ACTN3 Allele Frequency in Humans Covaries with Global Latitudinal Gradient**

Scott M. Friedlander¹, Amanda L. Herrmann¹, Daniel P. Lowry¹,², Emily R. Mepham¹, Emily R. Mepham¹, Monkol Lek³,⁴, Kathryn N. North³,⁴, Chris L. Organ⁵,⁶

¹Department of Ecology and Evolutionary Biology, Brown University, Providence, Rhode Island, United States of America, ²Department of Earth and Environmental Sciences, University of Michigan, Ann Arbor, Michigan, United States of America, ³Institute for Neuroscience and Muscle Research, The Children’s Hospital at Westmead, Sydney, New South Wales, Australia, ⁴Discipline of Paediatrics and Child Health, University of Sydney, Sydney, New South Wales, Australia, ⁵Department of Anthropology, University of Utah, Salt Lake City, Utah, United States of America, ⁶Department of Paleontology, Natural History Museum of Utah, University of Utah, Salt Lake City, Utah, United States of America

**Abstract**

A premature stop codon in ACTN3 resulting in α-actinin-3 deficiency (the ACTN3 577XX genotype) is common in humans and reduces strength, muscle mass, and fast-twitch fiber diameter, but increases the metabolic efficiency of skeletal muscle. Linkage disequilibrium data suggest that the ACTN3 R577X allele has undergone positive selection during human evolution. The allele has been hypothesized to be adaptive in environments with scarce resources where efficient muscle metabolism would be selected. Here we test this hypothesis by using recently developed comparative methods that account for evolutionary relatedness and gene flow among populations. We find evidence that the ACTN3 577XX genotype evolved in association with the global latitudinal gradient. Our results suggest that environmental variables related to latitudinal variation, such as species richness and mean annual temperature, may have influenced the adaptive evolution of ACTN3 577XX during recent human history.

**Introduction**

Genes important for metabolism include the α-actinin family that code for contractile proteins in muscle [1]. In humans, two different genes code for skeletal muscle α-actins: ACTN2 is expressed in all skeletal muscle fibers, while ACTN3 is expressed in fast-twitch muscle fibers. Mammalian ACTN2 and ACTN3 genes appear to have arisen from a gene duplication that occurred over 310 million years ago, before the divergence of birds and mammals [2]. Following the duplication of the ancestral ACTN 2/3 gene, ACTN3 evolved specific expression patterns in fast twitch muscle fibers, which are responsible for generating force at high velocity. Yet, approximately 18% of the human population is α-actinin-3 deficient (the ACTN3 577XX genotype) resulting in a premature stop codon in ACTN3 [3,4]. In the homozygous condition (577XX genotype) this leads to a complete deficiency of the α-actinin-3 protein, although α-actinin-2 partially compensates for the deficiency [5]. The absence of α-actinin-3 in humans has been shown to reduce strength, muscle mass, and fast-twitch fiber diameter, but increase skeletal muscle metabolic efficiency and resistance to fatigue [6]. Consequently, the ACTN3 577XX genotype has been hypothesized to be adaptive where efficient muscle metabolism is concerned, such as in environments with scarce resources or where endurance running has an impact on survival [7]. Together, these Darwinian hypotheses predict a significant evolutionary relationship of ACTN3 577XX genotype frequency and global latitudinal patterns of biodiversity. This relationship is predicted because factors related to food acquisition and hunting, such as species richness or mean annual temperature, show well-established global latitudinal patterns [8].

The ACTN3 577X substitution likely preceded the arrival of anatomically modern humans in Europe and Asia 40,000–60,000 years ago [9]. Since the evolution of modern humans, approximately 100,000–200,000 years ago, populations have occupied a wide range of habitats and adapted to use a variety of resources. Differences in environmental resources can drive even small genetic changes, such as single nucleotide polymorphisms, to high frequency in human populations. For example, using demographic HapMap data, allele frequencies related to subsistence have been found to change across different ecoregions [10]. It is therefore plausible that differences in the frequency of ACTN3 577XX may have arisen during the recent past, specifically in relation to the global latitudinal gradient. Here we test the ACTN3 577XX adaptation hypothesis using global genotype and biodiversity data, with Bayesian comparative methods that account for phylogeny and migration.

**Methods**

ACTN3 XX frequency data were obtained from the literature (Table 1). These data were derived from studies that compared the frequencies of the ACTN3 polymorphism in elite athletes to the
differences in glaciation and aridification of North Africa) over the time in which should be noted that large climatic changes have occurred (e.g. back through the Mesozoic into the Paleozoic [14]. However, it the tropics) has been a striking large-scale pattern that extends (increasing numbers of species and higher taxa from the poles to

| Population     | Latitude (Mean) | Temp (Mean, °C) | Ln Net Primary Productivity (g of carbon) | Ln Species Richness XX frequency | XX Ref |
|----------------|-----------------|-----------------|-----------------------------------------|----------------------------------|--------|
| Australian     | 26.42           | 20.47           | 11.25                                   | 6.07                             | 10%    | [30]   |
| Bantu          | 27.96           | 18.44           | 11.27                                   | 6.29                             | 1%     | [30]   |
| China          | 32.00           | 11.52           | 11.23                                   | 6.12                             | 16.9%  | [31]   |
| Ethiopia       | 9.76            | 24.15           | 11.12                                   | 6.23                             | 12%    | [30]   |
| Greece         | 40.00           | 12.06           | 11.39                                   | 5.86                             | 18.3%  | [32]   |
| Israel         | 34.12           | 17.97           | 10.94                                   | 5.98                             | 18%    | [33]   |
| Italy          | 42.61           | 11.13           | 11.50                                   | 5.78                             | 21.57% | [34]   |
| Japan          | 41.50           | 6.71            | 11.42                                   | 5.73                             | 24%    | [30]   |
| Kenya          | 0.61            | 21.47           | 11.57                                   | 6.62                             | 1%     | [30]   |
| Lithuania      | 47.66           | 6.35            | 11.49                                   | 5.82                             | 10.4%  | [35]   |
| Nigeria (Yoruba)| 7.96           | 25.34           | 11.49                                   | 6.41                             | 0%     | [30]   |
| Papua New Guinea| 6.61           | 24.99           | 11.68                                   | 5.74                             | 15%    | [30]   |
| Russia         | 48.95           | 6.06            | 11.08                                   | 5.81                             | 14.2%  | [36]   |
| Spain          | 40.64           | 12.62           | 11.36                                   | 5.8                              | 19.9%  | [30]   |
| Sweden         | 61.33           | 3.63            | 11.04                                   | 5.65                             | 25%    | [37]   |

References for XX frequency are included in brackets after ACTN3 data.
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Table 1. Latitude, mean annual temperature, net primary productivity, and species richness data were obtained from the World Wildlife Fund Terrestrial Ecoregion Database [12].

frequencies in control groups of the general population. We only used frequency data from the control groups (n = 3,351). ACTN3 XX frequency data were Arcsin transformed before statistical analysis, a transform suitable for nominal and percentage data [11].

Using the World Wildlife Fund Ecoregion database [12], we determined the average latitude, mean annual temperature (°C), net primary productivity (grams of carbon), and total species richness (tetrapods) data for each country/region. Although some of these metrics have changed over the time period in which ACTN3 polymorphisms arose in human populations, they nonetheless show a consistent relationship with the global latitudinal gradient [13]. For example, the latitudinal biodiversity gradient (increasing numbers of species and higher taxa from the poles to the tropics) has been a striking large-scale pattern that extends back through the Mesozoic into the Paleozoic [14]. However, it should be noted that large climatic changes have occurred (e.g. glaciation and aridification of North Africa) over the time in which differences in ACTN3 allele frequencies arose among human populations. Even so, we expect differences in our gene frequency data to have arisen relatively recently in line with modern climatic variation (as opposed to a novel mutation that may have had specific adaptive value in a past environment).

We curated our data by compiling a list of every ecoregion within each country. The list was constructed first using maps to form overestimated rectangles of minimum and maximum latitude and longitude around each country to account for irregular borders. Every ecoregion within these rectangles was gathered and narrowed down further using Google Earth (we limited Russia to west of Mongolia). By using the average latitudinal and longitudinal coordinates of each ecoregion, we were able to see if the ecoregion fell within the borders of the country in question. Ecoregions within the country were retained, while ecoregions outside of the country were removed from the list. One exception was Israel, which had no ecoregions within its borders, so the two closest ecoregions were used.

We produced a Bayesian posterior distribution of phylogenetic trees from whole mitochondrial sequences for the following human lineages: Aboriginal Australian (NCBI 134303155), Bantu (NCBI 160426755), Israeli (NCBI 145968101), Japanese (NCBI 61287226), Chinese (NCBI 292597164), Kenyan (NCBI 13272962), Papua New Guinean (NCBI 256946671), Lithuanian (NCBI 301017707) Russian (NCBI 2905535741), Swedish (NCBI 213579486), Nigerian (EMBL AF346983), Italian (EMBL AF346983), Greek (EMBL GQ29165), Spanish (NCBI 74475826), and Ethiopian (NCBI 156459510). The whole mitochondrial genome of Homo Neanderthalensis (NCBI 196123578) was included as an outgroup.

The program MUSCLE [15] was used to align the genome sequences and a posterior distribution of trees was inferred with the program BayesPhylogenies [16]. BayesPhylogenies implements a reversible jump mixture model that fits more than one model of sequence evolution to the data without partitioning. We used a GTR model with gamma-distributed rate variation across sites (4 rates) on one chain for 20,000,000 iterations, sampling every 2,000 iterations. We also implemented the reversible-jump mixture model for pattern heterogeneity. We checked for convergence by examining a time-series plot for the log-likelihoods in Tracer [17].

Statistical tests that accounted for phylogeny (regression and ancestral character state reconstruction) were performed in the program BayesTraits [18] (http://www.evolution.rdg.ac.uk). BayesTraits accounts for confounding phylogenetic relatedness in continuous data using a generalized least square (PGLS) approach. It can also account for phylogenetic uncertainty by integrating analyses over more than one tree (or a distribution of trees). Before we ran the regression analysis, we tested whether a random walk model of character evolution or a directional model of evolution best fit our data. We found no evidence for directional evolution (highest Bayes factor = 1.8, see below). Our analysis also estimated phylogenetic signal (λ), a parameter that assesses the degree to which covariation among trait values follows the phylogeny during the random walk and regression analysis [18–
hypotheses: species richness, mean annual temperature, and net primary productivity (natural log). We found no evidence for phylogenetic signal in the regression model relating ACTN3 577XX frequency and total vertebrate species richness (maximum likelihood estimate of \( \lambda = 0 \)), but high levels of phylogenetic signal (maximum likelihood estimate of \( \lambda = 1 \)) for both mean annual temperature and primary productivity. Accounting for phylogeny and migration, the model relating ACTN3 577XX frequency and total vertebrate species richness was supported, with a slope parameter of \(-0.23 \) (lower 95\% CI = \(-0.36 \), upper 95\% CI = \(-0.08 \), p-value = 0.016), as was the relationship between ACTN3 577XX frequency and mean annual temperature (slope of \(-0.006 \), lower 95\% CI = \(-0.0126 \), upper 95\% CI = 0.001, p-value = 0.09). However, we found no support for a model relating ACTN3 577XX frequency and net primary productivity (slope of \(-0.067 \), lower 95\% CI = \(-0.31 \), upper 95\% CI = 0.161, p-value = 0.56).

For present day latitude and associated data (such as species richness) to be meaningful in an evolutionary analysis, it must fairly represent latitudinal data of the past where genetic associations may have evolved. The relationship between latitudinal gradients and species richness, for example, is consistent through the recent past, with taxa preferentially originating in the
tropics and spreading toward the poles [13,14]. Moreover, the changes we analyze (differences in allele frequency) could have shifted relatively recently with respect to environmental change. However, it should be noted that we did not include marine species richness data in our analysis for coastal areas – and this could affect our species richness model. Moreover, although this study brings together disparate forms of data (which we view as a strength), the sample size for human populations was relatively small. Another limitation is the use of countries as demographic units, since countries vary in size, ecological diversity, as well as population size and heterogeneity (though our migration matrix should help account for this). For example, the 577X substitution is thought to have arisen in populations migrating out of Africa (Ethiopia) roughly 40,000-60,000 years ago [9]. Our analysis could therefore, be confounded by including African population data, but 40,000 years is potentially enough time to allow for gene flow to increase the 577X allele frequency within African populations. Given the mechanical and physiological roles of ACTN3 variation in the 577XX genotype, long-range linkage disequilibrium associated with ACTN3 577X (signature of positive selection) [6], and our results presented here, a neutral (demographically-driven) model of evolution is not supported. Available evidence instead suggests that changes in ACTN3 577XX frequency were driven by changing environmental conditions and resources associated with latitudinal migration.

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Author Contributions
Conceived and designed the experiments: SMF ALH DPL ERM. Performed the experiments: SMF ALH DPL ERM. Analyzed the data: SMF ALH DPL ERM CO. Contributed reagents/materials/analysis tools: SMF ALH DPL ERM CO. Wrote the paper: SMF ALH DPL ERM ML KNN CO.

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Author/s:
Friedlander, SM; Herrmann, AL; Lowry, DP; Mepham, ER; Lek, M; North, KN; Organ, CL

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