Response to Wyckelsma et al.: Loss of α-actinin-3 during human evolution provides superior cold resilience and muscle heat generation

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Response to Wyckelsma et al.: Loss of α-actinin-3 during human evolution provides superior cold resilience and muscle heat generation

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Summary

The common loss-of-function mutation R577X in the structural muscle protein ACTN3 emerged as a potential target of positive selection from early studies and has been the focus of insightful physiological work suggesting a significant impact on muscle metabolism. Adaptation to cold climates has been proposed as a key adaptive mechanism explaining its global allele frequency patterns. Here, we re-examine this hypothesis analyzing modern (n = 3,626) and ancient (n = 1,651) genomic data by using allele-frequency as well as haplotype homozygosity-based methods. The presented results are more consistent with genetic drift rather than selection in cold climates as the main driver of the ACTN3 R577X frequency distribution in human populations across the world. This Matters Arising paper is in response to Wyckelsma et al. (2021),[1] published in *The American Journal of Human Genetics*. See also the response by Wyckelsma et al. (2022),[2] published in this issue.

The loss-of-function (LoF) mutation rs1815739C>T in *ACTN3* (MIM: 102574), also known as the *ACTN3* R577X allele, impacts muscle performance in elite athletes[3] and muscle mass, bone mineral density, and other health parameters in life and during the aging process.[4] The derived LoF allele is frequent (>0.25) among non-African populations, while in West and South African populations, its frequency is low (<0.15). Because of its latitudinal correlations in a small number of populations representing intercontinental variation and longer haplotype homozygosity in non-Africans, recent positive selection either for increased efficiency in skeletal muscle metabolism and endurance,[5,6] a partial protection from muscle wasting[7] or adaptation to cold environments[8] have been suggested as the adaptive mechanisms behind its allele frequency patterns across the world. However, it has remained unresolved when and where the R577X frequency shift occurred, whether populations exposed to extreme cold environments, such as those living in Siberia, follow the expected patterns of increased latitudinal R577X frequency, as predicted by the cold adaptation model, and what exactly the physiological mechanism of adaptation has been.

Recently, Wyckelsma and colleagues[1] have shown a significant effect of the *ACTN3* LoF allele on skeletal muscle thermogenesis and core body temperature maintenance through cold water immersion experiments on healthy volunteers and suggest these physiological effects to be the mechanism for cold adaptation responsible for the increase of allele frequencies, potentially by increasing infant survival in cold environments. While the physiological evidence presented by Wyckelsma et al. is undeniably insightful, it leaves open the question of where and when adaptation occurred and the role of other physiological aspects of muscle performance related to *ACTN3* inactivation. The arguments for the mechanism of latitudinal adaptation to cold environments—either through differential survival of infants or some other mechanism—are only relevant if the premises for the adaptation linked to survival in cold environments hold. Here, we re-examine currently available data from 89 locations (Figure 1) of R577X frequency in the world from modern genomes.

We analyze them together with ancient genomes to test for global versus local correlations between the presence of R577X and latitude and to determine where the LoF allele became common.

Consistent with previous studies,[8] we find a modest but significant correlation between latitude and R577X frequency ($r = 0.251$, $p = 0.018$) in a global panel[9–13] of individuals from 89 regional groups (n = 3,616) (Figure 2A, Table 1, Table S1). However, this positive correlation between latitude and the derived allele frequency (DAF) of R577X appears to be entirely driven by inter-continental differences rather than latitude, as we find no significant intra-continental relationships (Figures 2C–2F). There is also no significant correlation when considering only East African and non-African variation (Figure 2B). Four populations appear to be outliers with the highest R577X frequencies. These are the Kalash from Pakistan (DAF of 0.89) and three Native American populations (0.88), each

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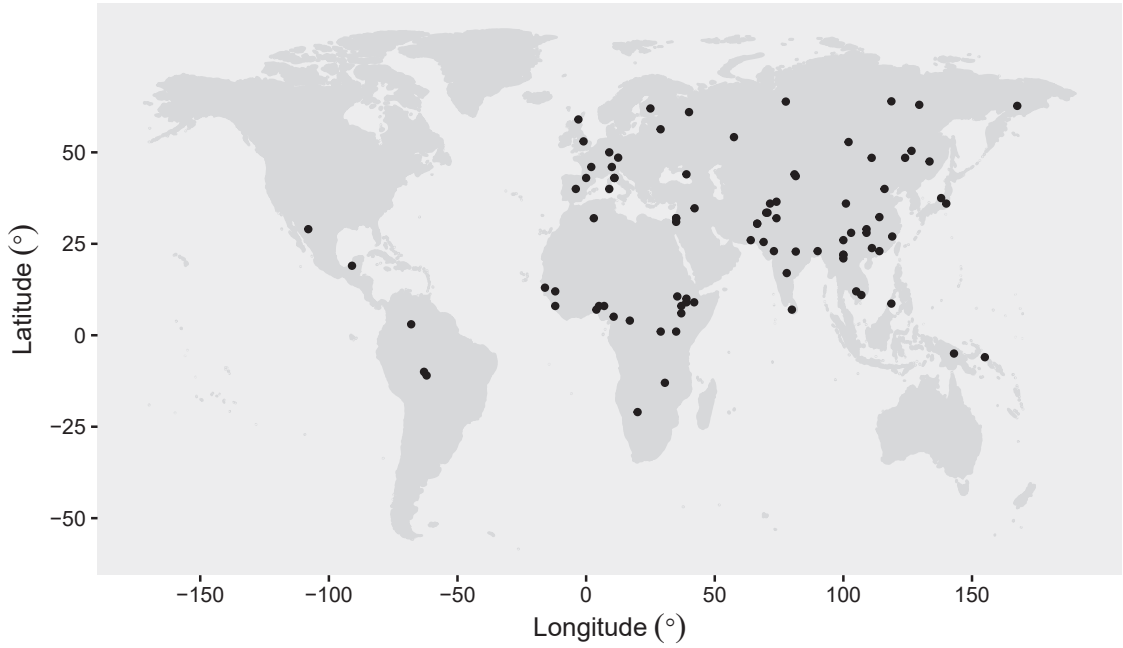

Figure 1. Locations of origin for modern genetic data
World map showing as circles the approximate ancestral coordinates for modern data. Please note that for the 12 macro-groups from the EGDP panel these represent the average of individual locations.

characterized by small effective population size, making genetic drift the most likely explanation of their outlier status. The lack of significant correlations at an intra-continental level is informative for distinguishing the effects of latitude and genetic ancestry on R577X allele frequency.

Notably, the Ethiopian R577X frequency (0.325, n = 318) is comparable to most non-Africans, being substantially higher than the frequency in Bantu or Khoisan speakers (Figure 2C). To address whether this increase in frequency reflects possible selection or neutral demographic history, we re-analyzed data from Pagani et al. (2015).[13] First, we find that the R577X frequency in the Ethiopian Gumuz people, who putatively are not affected by non-African admixture, is 0.13 (Table S1, Table S2). Second, we make use of the local ancestry deconvolution results reported in Pagani et al. (2015).[13] Thereby, we observe that the unadjusted mean R577X DAF in the four admixed Ethiopian populations (0.32) is between the DAF measured on haplotypes in these populations that could be confidently assigned to African (0.19) or non-African (0.53) ancestries (Table S2). In conclusion, the Gumuz and the African deconvoluted frequencies broadly match the ones found for other Sub-Saharan African populations, while the non-African deconvoluted frequency matches the one for other West Eurasian populations. These findings suggests that the R577X allele frequency in the population ancestral to West and East Africans was most likely <0.2 and that the currently observed higher DAF in East Africa is likely to be the result of the previously documented West Asian admixture ~3,000 years ago.'[14]

The absence of latitudinal correlation is most apparent in East Eurasia where populations from the extremely cold environments of Northeast Siberia have a mean frequency of R577X (0.28) substantially lower than South Asian (0.60) or Melanesian (0.73) populations living in tropical environments (Table 1, Figure 2E; Table S1).

To further explore the relationship between climate variables and the DAF at R577X, we ran the same regressions as above by using mean annual temperature retrieved from the WorldClim2 database[15] as predictor. The observed patterns confirm the latitude analyses. There is no significant relationship between temperature and R577X DAF on a global scale (Figure S1A) or for most continental subsets (Figures S1D–S1F). The only exception is a moderate negative correlation among Sub-Saharan Africans ($r = -0.481$, $p = 0.043$). This is at a first glance consistent with cold adaptation. Visual inspection of the evidence shows that this signal is driven by populations from Ethiopia living at higher altitudes (Figure S1C). Here, it is crucial to note that as discussed above, these also carry substantial amounts of non-African ancestry and the latter is sufficient to explain the higher R577X frequencies in Ethiopians (see above).

As a further method to investigate neutral processes that affect allele frequencies as potential confounders of selection, we applied the phylogenetic generalized least-squares (PGLS) method,[16] adapting a framework developed by Key et al. (2018).[17] We focused on 12 regional groups from the Estonian Biocenter Genome Diversity Panel (EGDP) dataset where a pre-computed F_{ST} matrix was available and from which we generated a neighbor joining tree (Figure S2). When accounting for phylogenetic history, there is no significant relationship between latitude and the frequency of the T allele (beta = −0.0575, p = 0.169);

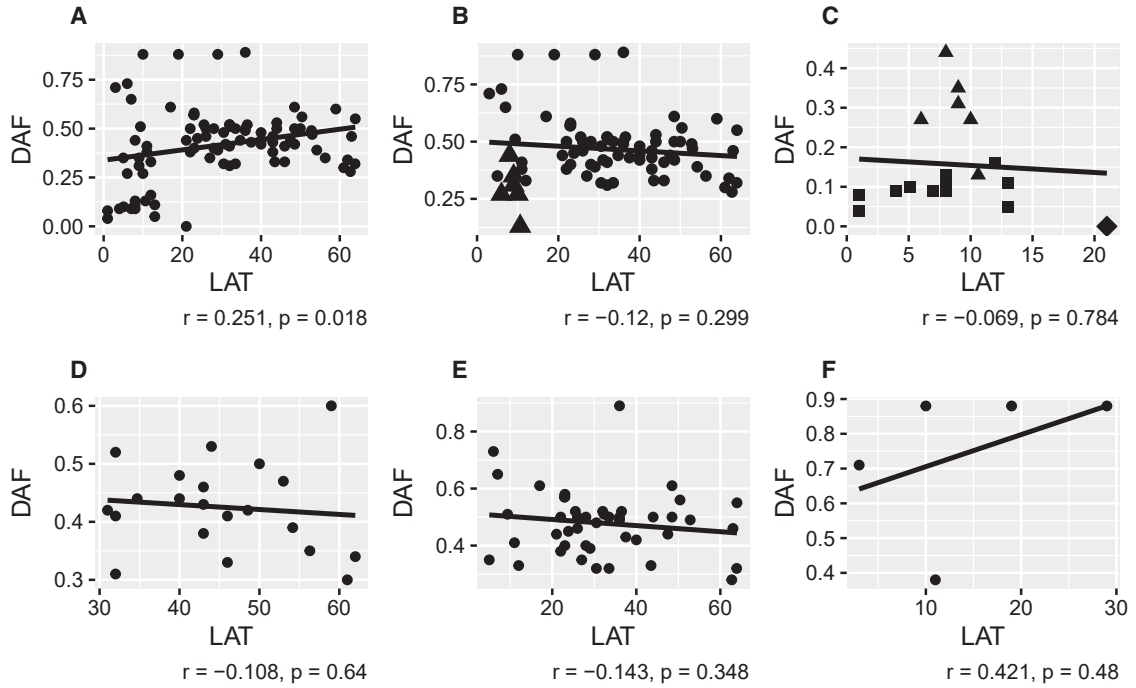

Figure 2. Relationship between *ACTN3* R577X allele frequency and latitude

(A–F) Dataset obtained from three global panels of human genomes,[9–11] a dataset of Northeast African genomes,[13] and a survey focusing on the *ACTN3* R577X allele.[12] The panels show simple linear regressions of *ACTN3* LoF allele frequency and latitude globally (A), in Ethiopians and non-Africans (B), in Sub-Saharan Africans (C), in West Eurasians (D), in East Eurasians (E), and in Native Americans (F). The respective correlation coefficients and p values are displayed in the bottom right corner of each panel. In (B) and (C), Ethiopians are represented by a triangle. In (C), Bantu speakers are marked by squares and Khoisan speakers by a diamond. Abbreviations: DAF, derived allele frequency of the *ACTN3* R577X allele; LAT, absolute value of latitude in degrees.

the same holds when temperature is used instead of latitude (beta = 0.044, p = 0.235) (Figures S3 and S4). This is further supported by the comparison of a null PGLS model, which is only based on shared ancestry, against the full PGLS model, which includes latitude as an additional predictor. We do not find evidence that the full model is superior to the null model in explaining the allele frequency patterns at R577X ($X^2 = 2.49$, df = 1, p = 0.138). This is also the case when temperature is added to the null model ($X^2 = 1.69$, df = 1, p = 0.206).

As climate represents a selection factor that acts over long time periods, it is important to reduce potential confounding due to populations that recently migrated to different latitudes/climates. First, we excluded the African American, African Caribbean, and Native American populations from the 1000 Genomes Project panel from all analyses in this paper, as their ancestry is substantially derived from European sources.[11] Second, we exploited available aDNA data to test whether the lower than expected DAFs at R577X in Europe and Siberia could potentially reflect recent admixture and/or replacement by populations from lower latitudes. Siberia was reached by modern humans by 45 kya[18] and has had at least three subsequent population expansions and large-scale complex admixture events.[19] Similarly, Europe has experienced major population turnover events since the Ice Age.[20] To examine temporal changes of R577X frequencies in Siberia and in Europe, we used pseudo-haploid genotype data of ancient individuals from Europe and from archaeological sites located in Russia east of 60 degrees east longitude from the comprehensive Allen Ancient DNA Resource (n = 1,651). We observe no consistent frequency increase of the LoF allele in the time series of the past 50 kya in European or Siberian subsets of the aDNA data as would be predicted by the cold adaptation model (Table 2). The unusually low frequencies observed in the period following the Last Glacial Maximum are unexpected but need to be interpreted with caution because of small sample sizes. These allele frequency patterns are inconsistent with trajectories previously identified for targets of recent strong positive selection in Europe and Siberia.[21–24]

To assess the linkage disequilibrium (LD)-based evidence for recent positive selection on the R577X allele globally in populations from different latitudes, we examined the haplotype homozygosity-based nS_L[26] statistic for R577X in 12 regional population groups from the EGDP data.[10] The nS_L is complementary to the allele frequency-based approaches discussed above and more robust to variation in recombination rates than comparable tests, such as the iHS.[27] We find that the empirical p values of the nS_L scores at R577X are not significant across 12 regional groups considered, including populations from extremely cold climates such as Northeast Siberians who, in fact, show a lower rather than a higher frequency of R577X. The nS_L

Table 1. Global distribution and evidence for selection for the *ACTN3* R577X allele

Population	n	Latitude	DAF	nS_L p value
AFR	26	5	0.10	0.61
MIE	26	35	0.44	0.52
WEU	32	49	0.42	0.33
EEU	53	56	0.35	0.48
VOL	23	54	0.39	0.34
SOA	28	23	0.57	0.04
WSI	17	64	0.32	0.20
SSI	34	53	0.49	0.49
CSI	31	64	0.55	0.61
NSI	25	63	0.28	0.97
SEM	29	24	0.45	0.14
SEI	45	9	0.51	0.29

DAF, derived allele frequency of the *ACTN3* R577X allele in 12 regional populations of the EGDP panel;[10] AFR, West and Central Africa; MIE, Middle East; WEU, South and West Europe; EEU, East and North Europe; VOL, Volga-Ural Region; SOA, South Asia; WSI, West Siberia; SSI, South Siberia and Mongolia; CSI, Central Siberia; NSI, Northeast Siberia; SEM, Mainland East and Southeast Asia; SEI, Island Southeast Asia. Latitude in degrees refers to the average absolute value of individual sampling sites. nS_L p value: the nS_L scores were standardized for derived allele frequency bins in relevant populations and then the empirical p values were generated.

statistic thus does not support recent selection on the derived allele at R577X in populations from high latitudes (Table 1, Figures S5–S7). This suggests that the low levels of diversity and recombination in HapMap Europeans and Asians detected previously[6] should either not be considered unusual in context of genome-scale variation in a broader set of non-African samples or at least not be interpreted as a signal of strong positive selection in populations living in cold environments.[1] Other tests of selection applied on global genetic datasets, including those incorporated in the 1000 Genomes Project[28] and HGDP selection[29] browsers or those specifically designed to identify signals of temperature-related adaptation,[30,31] have also failed to identify *ACTN3* as a significant target of selection in high latitude populations. Conversely, examples of cold adaptation genes identified previously, such as *TRPM8*,[17] show a clearly different latitudinal cline and temporal allele frequency pattern than *ACTN3* R577X.

Lastly, we examined how unusual a SNP showing an allele frequency pattern in Africans versus non-Africans comparable to that of *ACTN3* R577X is. The African populations from the 1000 Genomes Project (n = 504, excluding African Americans and African Caribbeans) exhibit an R577X frequency of 0.1 and the 1000 Genomes Eurasians (n = 1,496) a frequency of 0.49. We tested how likely it is for a SNP showing a frequency of 0.1 in Africans to have experienced an increase up to 0.49 in Eurasians in the 1000 Genomes Panel. Of the 37,970 SNPs with a non-reference frequency of 0.1 in Africans, 4,741 have a non-reference frequency of 0.49 or higher in Eurasians. We find that *ACTN3* R577X ranks approximately on the 12th percentile, i.e., it is not an outlier relative to the genome-wide empirical distribution. Therefore, it is plausible that the ACTN3 R577X frequency difference between Africans and non-Africans solely reflects the demographic events that led to the colonization of Eurasia.

Table 2. Frequency of the *ACTN3* R577X allele in ancient and modern genomes from Siberia and Europe

	Siberia			Europe		
Time period	n	DAF	90% CI	n	DAF	90% CI
modern	168	0.46	0.41–0.50	727	0.43	0.41–0.45
≤2,500	25	0.56	0.4–0.71	434	0.43	0.39–0.47
2,501–5,000	95	0.44	0.36–0.53	589	0.49	0.45–0.52
5,001–10,000	32	0.59	0.45–0.72	447	0.37	0.34–0.41
10,001–20,000	2	0	0.00–0.60	16	0.06	0.02–0.25
20,001–50,000	3	0.67	0.25–0.9	8	0.50	0.25–0.75

Note, time is given in years before present. DAFs (derived allele frequencies) are based on pseudo-haploid calls from Allen Ancient DNA Resource, and 90% credible intervals (CIs) were calculated with the beta distribution as described by Burger et al., 2020.[25]

In sum, the *ACTN3* R577X shows a wide frequency range (0.28–0.89) in non-African populations not correlated with latitude or temperature regardless of whether shared phylogenetic history is adjusted for. Neither allele frequency nor haplotype homozygosity-based tests reveal evidence for positive selection for this allele in populations living in cold environments. Its weak correlations with latitude in the global dataset can be explained by inter-continental differences between Bantu- and Khoisan-speaking Africans and the rest of the world. Selection in Khoisan ancestry has not been supported by previous selection analyses.[32] The apparent relative increase in frequency in Ethiopians versus other Africans can largely be explained by West Eurasian gene flow into the former and argues against a selection event in East Africans preceding the out of Africa dispersal. Although Wyckelsma et al. provide insightful new physiological evidence on the relationship between R577X and skeletal muscle thermogenesis and core body temperature maintenance, multiple lines of population genetic evidence are more consistent with drift rather than selection behind its global frequency distribution. That being said, traits related to the impact of muscle performance on endurance[6] or on general health and aging[33] cannot be ruled out yet as potential alternative mechanisms that contribute to the global allele frequency patterns at *ACTN3* R577X.

Data and code availability

Whole genomes from the 1000 Genomes, HGDP, and EGDP projects are publicly available (see links under web resources). The Ethiopian data presented by Yang et al., 2007[12] only cover allele frequencies at the ACTN3 R577X locus and can be found in Table 1 of their paper. Details on how to access the 120 Ethiopian genomes from Pagani et al., 2015[13] can be found in the respective paper. No new methods were developed to analyze the data presented here. In the cases where the data were not pre-computed, e.g., to generate the figures, the relevant code is available from the corresponding author upon request.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.ajhg.2022.03.014.

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Declaration of interests

The authors declare no competing interests.

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Web resources

1000 Genomes VCFs, http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/

Allen Ancient DNA Resource, version 44.3, https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data

Complete Genomics data from Pagani et al. 2016[10] in PLINK and VCF formats, https://evolbio.ut.ee/CGgenomes.html

HGDP Selection Browser, http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP/

HGDP VCFs, ftp://ngs.sanger.ac.uk/production/hgdp/

The 1000 Genomes Selection Browser 1.0, https://hsb.upf.edu/

WorldClim2 data, http://www.worldclim.com/version2

References

1. Wyckelsma, V.L., Venckunas, T., Houweling, P.J., Schlittler, M., Lauschke, V.M., Tiong, C.F., Wood, H.D., Ivarsson, N., Paulauskas, H., Eimantas, N., et al. (2021). Loss of α-actinin-3 during human evolution provides superior cold resilience and muscle heat generation. Am. J. Hum. Genet. *108*, 446–457.
2. Wyckelsma, V.L., Venckunas, T., Houweling, P.J., Schlittler, M., Lauschke, V.M., Tiong, C.F., Wood, H.D., Henrikas Paulauskas, H., Eimantas, N., Andersson, D.C., et al. (2022). Response to Mörseburg et al. Am. J.Hum. Genet. 109, *bxs.
3. Yang, N., MacArthur, D.G., Gulbin, J.P., Hahn, A.G., Beggs, A.H., Easteal, S., and North, K. (2003). ACTN3 genotype is associated with human elite athletic performance. Am. J. Hum. Genet. *73*, 627–631.
4. Pickering, C., and Kiely, J. (2018). *ACTN3*, Morbidity, and Healthy Aging. Front. Genet. *9*, 15.
5. Amorim, C.E.G., Acuña-Alonzo, V., Salzano, F.M., Bortolini, M.C., and Hünemeier, T. (2015). Differing evolutionary histories of the ACTN3*R577X polymorphism among the major human geographic groups. PLoS ONE *10*, e0115449.
6. MacArthur, D.G., Seto, J.T., Raftery, J.M., Quinlan, K.G., Huttley, G.A., Hook, J.W., Lemckert, F.A., Kee, A.J., Edwards, M.R., Berman, Y., et al. (2007). Loss of ACTN3 gene function alters mouse muscle metabolism and shows evidence of positive selection in humans. Nat. Genet. *39*, 1261–1265.
7. Seto, J.T., Roeszler, K.N., Meehan, L.R., Wood, H.D., Tiong, C., Bek, L., Lee, S.F., Shah, M., Quinlan, K.G.R., Gregorevic, P., et al. (2021). ACTN3 genotype influences skeletal muscle mass regulation and response to dexamethasone. Sci. Adv. *7*, eabg0088.
8. Friedlander, S.M., Herrmann, A.L., Lowry, D.P., Mepham, E.R., Lek, M., North, K.N., and Organ, C.L. (2013). ACTN3 allele frequency in humans covaries with global latitudinal gradient. PLoS ONE *8*, e52282.
9. Bergström, A., McCarthy, S.A., Hui, R., Almarri, M.A., Ayub, Q., Danecek, P., Chen, Y., Felkel, S., Hallast, P., Kamm, J., et al. (2020). Insights into human genetic variation and

population history from 929 diverse genomes. Science *367*, eaay5012.
10. Pagani, L., Lawson, D.J., Jagoda, E., Mörseburg, A., Eriksson, A., Mitt, M., Clemente, F., Hudjashov, G., DeGiorgio, M., Saag, L., et al. (2016). Genomic analyses inform on migration events during the peopling of Eurasia. Nature *538*, 238–242.
11. Auton, A., Brooks, L.D., Durbin, R.M., Garrison, E.P., Kang, H.M., Korbel, J.O., Marchini, J.L., McCarthy, S., McVean, G.A., Abecasis, G.R.; and The 1000 Genomes Project Consortium (2015). A global reference for human genetic variation. Nature *526*, 68–74.
12. Yang, N., MacArthur, D.G., Wolde, B., Onywera, V.O., Boit, M.K., Lau, S.Y.M.-A., Wilson, R.H., Scott, R.A., Pitsiladis, Y.P., and North, K. (2007). The ACTN3 R577X polymorphism in East and West African athletes. Med. Sci. Sports Exerc. *39*, 1985–1988.
13. Pagani, L., Schiffels, S., Gurdasani, D., Danecek, P., Scally, A., Chen, Y., et al. (2015). Tracing the route of modern humans out of Africa by using 225 human genome sequences from Ethiopians and Egyptians. Am. J. Hum. Genet. *96*, 986–991.
14. Molinaro, L., Montinaro, F., Yelmen, B., Marnetto, D., Behar, D.M., Kivisild, T., and Pagani, L. (2019). West Asian sources of the Eurasian component in Ethiopians: a reassessment. Sci. Rep. *9*, 18811.
15. Fick, S.E., and Hijmans, R.J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int. J. Climatol. *37*, 4302–4315.
16. Freckleton, R.P., Harvey, P.H., and Pagel, M. (2002). Phylogenetic analysis and comparative data: a test and review of evidence. Am. Nat. *160*, 712–726.
17. Key, F.M., Abdul-Aziz, M.A., Mundry, R., Peter, B.M., Sekar, A., D'Amato, M., Dennis, M.Y., Schmidt, J.M., and Andrés, A.M. (2018). Human local adaptation of the TRPM8 cold receptor along a latitudinal cline. PLoS Genet. *14*, e1007298.
18. Pitulko, V., Pavlova, E., and Nikolskiy, P. (2017). Revising the archaeological record of the Upper Pleistocene Arctic Siberia: Human dispersal and adaptations in MIS 3 and 2. Quat. Sci. Rev. *165*, 127–148.
19. Sikora, M., Pitulko, V.V., Sousa, V.C., Allentoft, M.E., Vinner, L., Rasmussen, S., Margaryan, A., de Barros Damgaard, P., de la Fuente, C., Renaud, G., et al. (2019). The population history of northeastern Siberia since the Pleistocene. Nature *570*, 182–188.
20. Lazaridis, I. (2018). The evolutionary history of human populations in Europe. Curr. Opin. Genet. Dev. *53*, 21–27.
21. Clemente, F.J., Cardona, A., Inchley, C.E., Peter, B.M., Jacobs, G., Pagani, L., Lawson, D.J., Antão, T., Vicente, M., Mitt, M., et al. (2014). A Selective Sweep on a Deleterious Mutation in CPT1A in Arctic Populations. Am. J. Hum. Genet. *95*, 584–589.
22. Fumagalli, M., Moltke, I., Grarup, N., Racimo, F., Bjerregaard, P., Jørgensen, M.E., Korneliussen, T.S., Gerbault, P., Skotte, L., Linneberg, A., et al. (2015). Greenlandic Inuit show genetic signatures of diet and climate adaptation. Science *349*, 1343–1347.
23. Hallmark, B., Karafet, T.M., Hsieh, P., Osipova, L.P., Watkins, J.C., and Hammer, M.F. (2019). Genomic Evidence of Local Adaptation to Climate and Diet in Indigenous Siberians. Mol. Biol. Evol. *36*, 315–327.
24. Mathieson, I., Lazaridis, I., Rohland, N., Mallick, S., Patterson, N., Roodenberg, S.A., Harney, E., Stewardson, K., Fernandes, D., Novak, M., et al. (2015). Genome-wide patterns of selection in 230 ancient Eurasians. Nature *528*, 499–503.
25. Burger, J., Link, V., Blöcher, J., Schulz, A., Sell, C., Pochon, Z., Diekmann, Y., Žegarac, A., Hofmanová, Z., Winkelbach, L., et al. (2020). Low Prevalence of Lactase Persistence in Bronze Age Europe Indicates Ongoing Strong Selection over the Last 3,000 Years. Curr. Biol. *30*, 4307–4315.e13.
26. Ferrer-Admetlla, A., Liang, M., Korneliussen, T., and Nielsen, R. (2014). On detecting incomplete soft or hard selective sweeps using haplotype structure. Mol. Biol. Evol. *31*, 1275–1291.
27. Enard, D. (2021). Types of Natural Selection and Tests of Selection. In Human Population Genomics, K.E. Lohmueller and R. Nielsen, eds. (Cham: Springer International Publishing), pp. 69–86.
28. Pybus, M., Dall'Olio, G.M., Luisi, P., Uzkudun, M., Carreño-Torres, A., Pavlidis, P., Laayouni, H., Bertranpetit, J., and Engelken, J. (2014). 1000 Genomes Selection Browser 1.0: a genome browser dedicated to signatures of natural selection in modern humans. Nucleic Acids Res. *42*, D903–D909.
29. Pickrell, J.K., Coop, G., Novembre, J., Kudaravalli, S., Li, J.Z., Absher, D., Srinivasan, B.S., Barsh, G.S., Myers, R.M., Feldman, M.W., and Pritchard, J.K. (2009). Signals of recent positive selection in a worldwide sample of human populations. Genome Res. *19*, 826–837.
30. Hancock, A.M., Witonsky, D.B., Alkorta-Aranburu, G., Beall, C.M., Gebremedhin, A., Sukernik, R., Utermann, G., Pritchard, J.K., Coop, G., and Di Rienzo, A. (2011). Adaptations to climate-mediated selective pressures in humans. PLoS Genet. *7*, e1001375.
31. Raj, S.M., Pagani, L., Gallego Romero, I., Kivisild, T., and Amos, W. (2013). A general linear model-based approach for inferring selection to climate. BMC Genet. *14*, 87.
32. Schlebusch, C.M., Skoglund, P., Sjödin, P., Gattepaille, L.M., Hernandez, D., Jay, F., Li, S., De Jongh, M., Singleton, A., Blum, M.G.B., et al. (2012). Genomic variation in seven Khoe-San groups reveals adaptation and complex African history. Science *338*, 374–379.
33. Houweling, P.J., Papadimitriou, I.D., Seto, J.T., Pérez, L.M., Coso, J.D., North, K.N., Lucia, A., and Eynon, N. (2018). Is evolutionary loss our gain? The role of *ACTN3* p.Arg577Ter (R577X) genotype in athletic performance, ageing, and disease. Hum. Mutat. *39*, 1774–1787.