

# 1 Effects of ancestry, agriculture, and lactase persistence 2 on the stature of prehistoric Europeans

3 Samantha L Cox<sup>1\*</sup>, Kaeli Kaymak-Loveless<sup>1</sup>, Carson Shin<sup>1</sup>, Timka Alihodžić<sup>2</sup>, Kurt W.  
4 Alt<sup>3</sup>, Nadezhda Atanassova<sup>4</sup>, Dider Binder<sup>5</sup>, Morana Čaušević-Bully<sup>6</sup>, Alexander  
5 Chohadzhiev<sup>7</sup>, Stefan Chohadzhiev<sup>8</sup>, Henri Duday<sup>9</sup>, Bisserka Gaydarska<sup>10</sup>, Anahit  
6 Khudaverdyan<sup>11</sup>, Rafael Micó Perez<sup>12</sup>, Nicole Nicklisch<sup>3</sup>, Mario Novak<sup>13,14</sup>, Camila Oliart  
7 Caravatti<sup>12</sup>, Hélène Réveillas<sup>9,15</sup>, Maïté Rivollat<sup>9,16</sup>, Stephane Rottier<sup>9</sup>, Domagoj  
8 Tončinić<sup>17</sup>, Steve Zaüner<sup>18</sup>, and Iain Mathieson<sup>1\*</sup>

9 <sup>1</sup>Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA  
10 <sup>2</sup>Archaeological Museum Zadar, Zadar, Croatia

11 <sup>3</sup>Center of Natural and Cultural Human History, Danube Private University, Krems-Stein, Austria

12 <sup>4</sup>Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of  
13 Sciences, Sofia, Bulgaria

14 <sup>5</sup>Université Côte d'Azur, CNRS, CEPAM-UMR, 7264 Nice, France.

15 <sup>6</sup>Université de Franche Comté / UMR Chrono-Environnement, Besançon, France

16 <sup>7</sup>Regional History Museum, Veliko Tarnovo, Bulgaria

17 <sup>8</sup>Department of Archaeology, University of Veliko Tarnovo “St. Cyril and St. Methodius” 5003 Veliko  
18 Tarnovo, Bulgaria.

19 <sup>9</sup>PACEA – UMR 5199, Université de Bordeaux, Pessac, France

20 <sup>10</sup>Department of Archaeology, Durham University, Durham DH1 3LE, UK.

21 <sup>11</sup>Institute of Archaeology and Ethnography, NAS RA, 0025 Yerevan, Armenia.

22 <sup>12</sup>Department of Prehistory, Universitat Autònoma de Barcelona, Barcelona, Spain

23 <sup>13</sup>Centre for Applied Bioanthropology, Institute for Anthropological Research, 10000 Zagreb, Croatia.

24 <sup>14</sup>Department of Archaeology and Heritage, Faculty of Humanities, University of Primorska, Koper,  
25 Slovenia

26 <sup>15</sup>Service Archéologie de Bordeaux Métropole

27 <sup>16</sup>Department of Archaeogenetics, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

28 <sup>17</sup>Faculty of Humanities and Social Sciences, University of Zagreb, Zagreb, Croatia

29 <sup>18</sup>anthropol, 95349 Thurnau, Germany

30 \*Correspondence to SLC (coxsl@sas.upenn.edu) or IM (mathi@pennmedicine.upenn.edu)

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## Abstract

32 Ancient DNA has revolutionized our understanding of human evolutionary history, but studies  
33 focusing solely on genetic variation tell an incomplete story by neglecting phenotypic out-  
34 comes. The relationships between genotype and phenotype can change over time, making it  
35 desirable to study them directly in ancient populations rather than present-day data. Here,  
36 we present a large-scale integration of ancient genomic and phenotypic data, analyzing femur  
37 length as a proxy for stature in 568 individuals with published whole-genome ancient DNA  
38 data across western Eurasia. Polygenic scores derived from modern European and East Asian  
39 genome-wide association studies retain predictive power in ancient populations, explaining up  
40 to 10% of phenotypic variance. Contrary to longstanding archaeological hypotheses, we find  
41 that Neolithic populations were only modestly shorter than preceding Mesolithic groups, with  
42 differences at least partly attributable to genetic rather than environmental factors, challenging  
43 narratives of systematic stature decline following the transition to agriculture. Finally, we find  
44 that the lactase persistence allele had a large positive effect on stature in ancient individuals  
45 (0.24 standard deviations), even though it shows no association with height in modern popula-  
46 tions. This gene-environment interaction highlights the limitation of using present-day genetic  
47 data to infer past phenotypic relationships. Our results underscore the value of integrating ge-  
48 netic and morphological data from ancient populations to reconstruct the dynamics of human  
49 adaptation.

## 50 1 Introduction

51 Adaptation and evolution reflect the cumulative outcomes of gene-environment interactions that  
52 shape phenotypic development within lifetimes and across generations. The genetics of these fun-  
53 damental processes can be difficult to study in human populations mainly because present-day  
54 populations only retain the scars of past events, giving little insight into the process itself. Reveal-  
55 ing the evolutionary history of complex traits necessitates the use of ancient DNA; however, there  
56 is less ancient data available and few quantifiable phenotypes with which genetic results can be  
57 verified.

58 Human stature represents an ideal model system for bridging this gap. As one of the most herita-  
59 ble complex traits, stature is both easily measured in skeletal remains and extensively characterized  
60 in modern genome-wide association studies. Its high polygenicity and environmental sensitivity  
61 make it particularly valuable for understanding how complex traits respond to changing selective  
62 pressures and how gene-environment interactions shift over time. Moreover, stature serves as a  
63 key indicator of population health and nutrition, making it central to debates about the major  
64 transitions of human prehistory.

65 One of the major environmental shifts in human prehistory was the global transition from  
66 hunting and gathering to farming, the causes and effects which have been a topic of intense study  
67 and debate for decades. One long standing hypothesis, first formalized by Armelagos and Cohen  
68 [1], posits that farming provided a stable environment that fueled population growth, but came  
69 at the cost of decreased individual health and a lower standard of living. Consequently, numerous  
70 researchers have investigated changes in European health between the Mesolithic and Neolithic,  
71 often using stature as a proxy, with conflicting results [e.g., 2, 3, 4, 5, 6, 7, 8]. Further, studies  
72 that do find stature decreases associated with the advent of farming have generally not accounted  
73 for the dramatic shifts in genetic ancestry that coincide with this major cultural and technological  
74 transition [9, 10] (with exceptions [11]). While anthropologists have always recognized that human  
75 biology is the result of complex interactions between genetics, culture, and environment, separating  
76 these effects has proved challenging.

77 Ancient DNA provides one potential approach. Several studies have combined GWAS data with  
78 ancient DNA to compute polygenic scores and track the evolution of stature in ancient individuals  
79 across time [12, 13], and to compare with changes in skeletal stature [14]. However, this approach  
80 has two major limitations. First, polygenic scores do not transfer well among present-day ancestry  
81 groups [15] and thus, presumably, not between present-day and ancient populations. Second, we  
82 do not know the relevant environmental effects and cannot account for their change through time.  
83 Though we assume a change in polygenic score reflects a change in phenotype, it can equally  
84 signal a shift in environment with no change in the resulting phenotype [16]. On the other hand,  
85 environmental perturbations can also lead to large changes in height without genetic change in both  
86 ancient [17] and present-day populations [18]. Finally, since questions about the magnitude and

87 prevalence of gene-environment interactions in present-day populations remain largely unanswered,  
88 it is still unknown whether the relationships between genes, environment and phenotype are the  
89 same in ancient populations.

90 Here, we investigate the evolutionary history of human height using maximum femur length  
91 as a proxy for attained stature. We collected a dataset consisting of maximum femur length and  
92 ancient DNA from 568 individuals from Eurasia, dated between 38,000 and 600 years BP. We then  
93 leveraged this combination of data to infer the contribution of environment to height across Neolithic  
94 agricultural communities and later societies, measure individual SNP effects on height in ancient  
95 individuals, and investigate the interaction between lactase persistence and environment. Our study  
96 represents the first systematic analysis of this scope in an ancient population, resulting in the first  
97 empirical evidence of gene-environment interaction in an ancient human sample.

## 98 2 Results

99 We collected femur lengths for 568 individuals with previously published ancient DNA (Supplemen-  
100 tary Table 1). The bulk of our data are concentrated in Western Eurasia and dated after 8000 BP  
101 ( $n=513/568$ ) (Fig. 1A). We either measured femur lengths directly or reverse engineered them from  
102 published stature estimations in order to remove error associated with combining stature estimates  
103 using different estimation methods. We previously showed that genotype imputation can improve  
104 PRS performance in low-coverage ancient samples [19]. Given the low coverage of our sample  
105 (median=0.515 $\times$ ), we imputed diploid genotypes using a previously published aDNA imputation  
106 pipeline [20, 21]. This causes many of our samples to shift consistently in PC space (Fig. 1B).

### 107 2.1 Identifying the optimal PRS for individual and population comparisons

108 We tested PRS constructed using clumping and thresholding on 1240k SNPs from several different  
109 source GWAS on both pseudohaploid and imputed genotype data: UK Biobank [23], East Asian  
110 [24], multi-ancestry [22] meta-analyses and between-sibling GWAS [25], as well as pre-trained PRS  
111 weights on HapMap3 SNPs [22]. To evaluate performance, we used linear regression of femur length  
112 on sex and four principal components, sample age and age<sup>2</sup>, calculating the incremental  $R^2$  when  
113 we add the PRS to the covariates. As our sample is largely from Europe, we were concerned that  
114 spurious associations between genetics and environment, known as population stratification, in the  
115 GWAS could confound results from PRS trained on present-day European ancestry populations  
116 [26, 27]. For this reason, we wanted to select two PRS for downstream analysis. The first, using  
117 European-derived summary statistics that maximized the incremental  $R^2$ , but could be confounded  
118 by stratification, and the second from an independent population that should be unconfounded by  
119 European population structure but has lower predictive power.

120 We found that the overall optimal PRS used European (EUR) summary statistics [22] and  
121 clumping and thresholding on imputed 1240k SNPs ( $R^2 = 0.099$ ; Fig. 1C). This is about four-

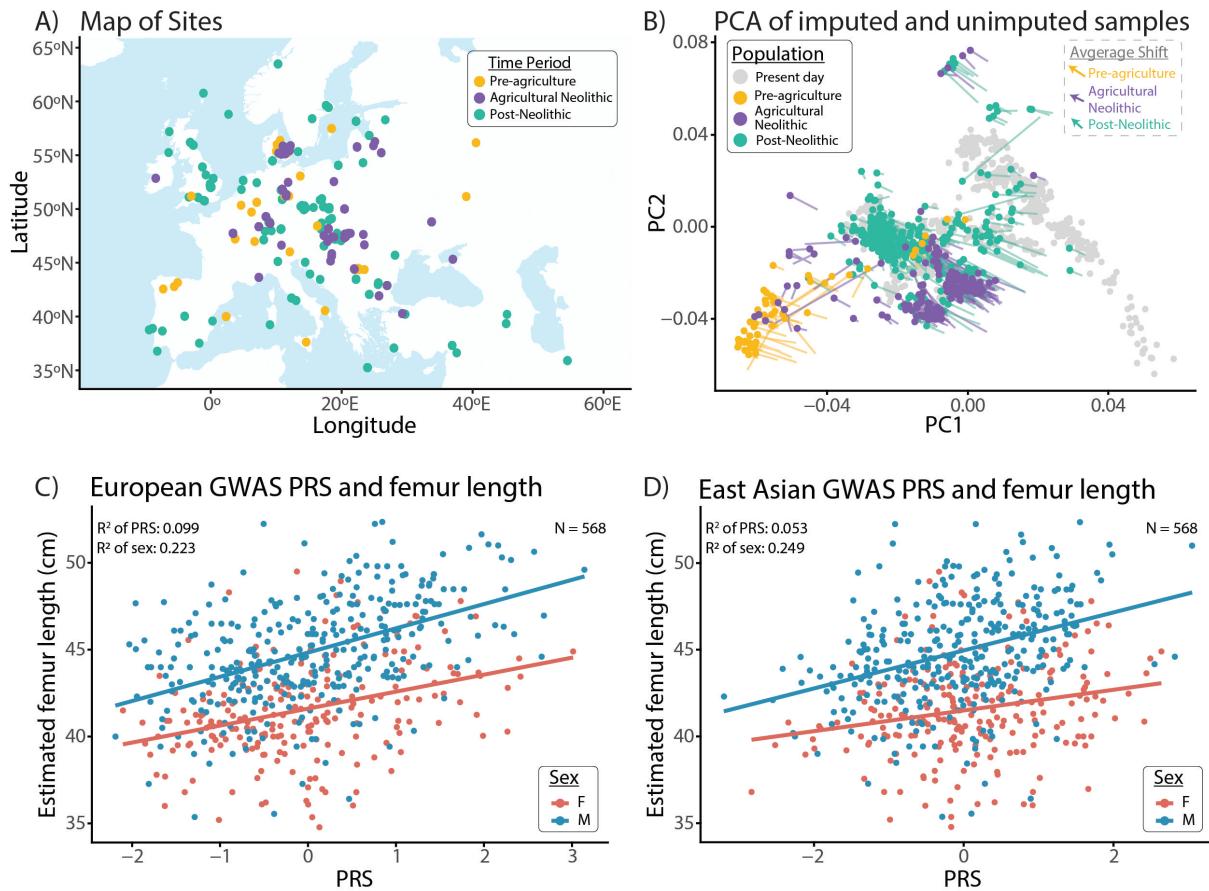


Figure 1: Sample characteristics *A)* Distribution of samples across Europe. Populations were defined based on the published archaeological evaluation of each site (see Methods); *B)* PCA showing the relationship between imputed (colored points) and unimputed samples projected into present-day European (Human Origins) PC space (gray points). Tails indicate the original location of the unimputed point for each individual. Dashed box in the top right shows the average shift distance and direction in PC space for samples in each population, arrows are shown to scale; *C)* relationship between PRS and femur length by sex for the best performing European PRS calculated using 1240k imputed genomes and [22] European GWAS summary statistics; *D)* relationship between PRS and femur length by sex for the best performing unconfounded PRS calculated using the HapMap3 imputed genomes and [22] East Asian meta-GWAS summary statistics.

122 fold lower than the  $R^2$  obtained in present-day European individuals [22], likely due to lower data  
 123 quality or increased distance to the training population from the ancient samples [28]. The optimal  
 124 unconfounded approach was to use pre-trained East Asian (EAS) PRS weights [22] with imputed  
 125 genotyped data on HapMap3 (HM3) SNPs ( $R^2 = 0.053$ ; Fig. 1D). For subsequent analyses we  
 126 therefore use the Yengo EUR 1240K PRS when we want to maximize individual prediction, and the  
 127 Yengo EAS HM3 PRS when we want to ensure our results are robust to population stratification,  
 128 for example to compare population means.

PRS	pseudohaploid $R^2$	imputed $R^2$
1240k SNPs C&T	UK Biobank (EUR)	0.065
	Chen et al., 2023 (EAS)*	0.030
	Tan et al., 2024 (Sibs)*	0.003
	Yengo et al., 2022 (ALL)	0.066
	Yengo et al., 2022 (EUR)	0.064
	Yengo et al., 2022 (EAS)*	0.015
HM3 SNPs Yengo PGS	Yengo et al., 2022 (ALL)	-
	Yengo et al., 2022 (EUR)	-
	Yengo et al., 2022 (EAS)*	-

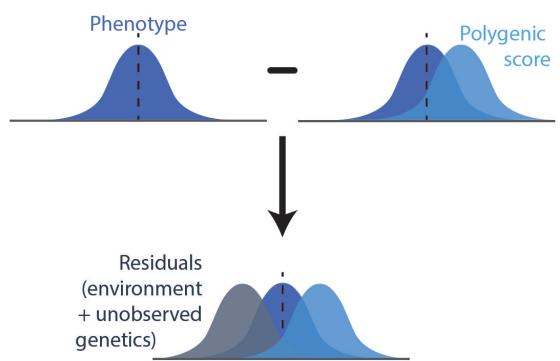
Table 1: Comparison of  $R^2$  values with different PRS and imputation methods; \*GWAS unconfounded by European population stratification; red: highest European PRS  $R^2$ ; blue: highest unconfounded PRS  $R^2$

## 129 2.2 The Neolithic transition had limited effect on stature

130 A long-standing hypothesis states that Neolithic populations were shorter than both the preceding  
131 and subsequent populations due to the negative health consequences of the transition to agricul-  
132 ture [1, 29]. This suggests that higher rates of population growth in early agricultural populations  
133 came at the cost of impaired individual health. However, phenotypic changes cannot necessarily be  
134 attributed to agriculture since the Neolithic transition in Europe coincides with major transitions  
135 in ancestry which could also contribute to changes in stature [30, 10, 14]. In principle, this can  
136 be resolved by comparing genetically predicted and directly measured stature. A previous study  
137 reported that Neolithic individuals were shorter than predicted from PRS, supporting the hypoth-  
138 esis of an environmental decrease in stature [11]. However, because they used PRS derived from  
139 European ancestry GWAS, their result can be confounded by stratification [26, 27]. Indeed, when  
140 they include ancestry in their regression models to control for the effects of population stratifica-  
141 tion, Neolithic individuals are no longer significantly shorter than expected. On the other hand,  
142 it is possible that including ancestry as a covariate, or even as a control for stratification at the  
143 GWAS level, could over-correct — removing real associations between SNP effects and ancestry.  
144 Results based on European GWAS are therefore difficult to interpret since they can lead to both  
145 false positive and false negative results.

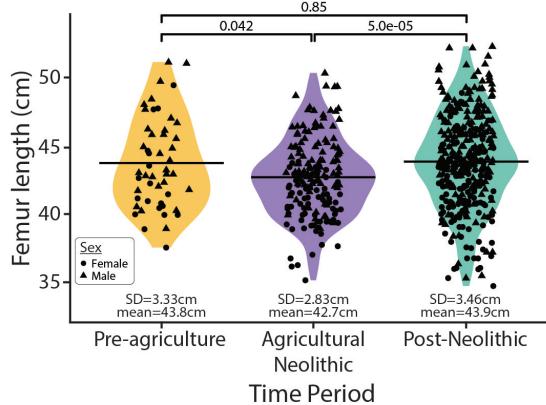
146 To perform an unconfounded test of the Neolithic stature hypothesis, we used the East Asian  
147 derived PRS to compare differences in genetically predicted height across populations [31]. As  
148 well as being unconfounded, our analysis has a larger sample size than previous analyses ( $N=568$   
149 compared to  $N=167$  in [11]). Following previous work[11], we estimate the environmental effects  
150 as the residuals from a regression of femur length on PRS and sex (Fig. 2A), although we note  
151 that these could also represent genetic effects not captured by the PRS. We divide our sample  
152 into pre-agriculture, agricultural Neolithic, and post-Neolithic time periods. We have defined these  
153 categories based on archaeological assessment of subsistence strategy rather than date or genetic  
154 ancestry (Methods), though we recognize that no classification system can fully categorize the

### A) Inferring Environmental Effects



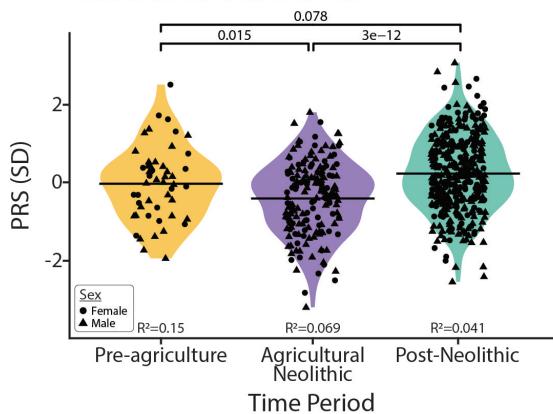
### B) Phenotype

#### Sex-adjusted femur length



### C) Polygenic score

#### East Asian-derived PRS



### D) Residuals

#### Femur ~ EAS-derived PRS + sex (+ LCT)

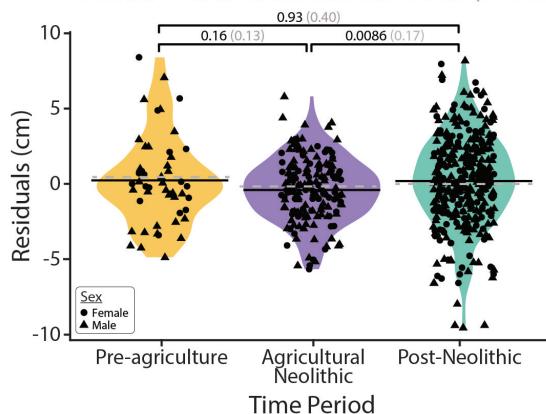


Figure 2: Were Neolithic individuals shorter than expected? A) Schematic illustration of inferring environmental effects. A phenotype is a combination of genetic and environmental effects. When we have known values for two of the three variables (phenotype and polygenic scores), we can reverse the equation to solve for the unknown quantity (environment). This inferred environmental component will also include the contribution of any unknown genetic effects. B) Phenotype effect: Femora are nominally shorter in the Neolithic than the pre-Neolithic and significantly shorter than the post-Neolithic period. Female femur lengths have been adjusted to remove the effect of sex by regressing out this variable; C) Polygenic score effect: PRS show that Neolithic populations are genetically shorter than the pre- and post-Neolithic; D) Residual effect: residuals from the linear regression of sex and East Asian-derived PRS indicate that compared to pre-Agriculture, Neolithic populations are not shorter than would be expected after accounting for these variables. Grey values and dotted lines indicate the effects of also including lactase persistence in the regression. P-values on all plots generated by two-tailed t-test.

complexity of subsistence strategies and transitions across the entirety of Europe.

We find that pre-agricultural femora were significantly longer than in the agricultural Neolithic (P=0.042, Fig. 2B). However, the absolute difference is small (0.9cm), corresponding to a difference of about 2.5cm in standing height. The polygenic score is significantly lower in the Neolithic (P=0.015,  $\beta=0.38\text{SD}$ , Fig. 2C), but the residuals are not significantly lower (P=0.16, Fig. 2D), in contrast to previous results [11]. While we do confirm a small decrease in average stature in the

161 Neolithic, it appears to be at least partially genetic, limiting the potential for environmental effects.  
162 Post-Neolithic populations are also slightly but significantly taller than Neolithic populations  
163 (1.1 cm in femur length, 3cm in stature,  $P=5.0 \times 10^{-5}$ , Fig. 2B). However, in this case, both the  
164 PRS and the residuals are significantly higher (by  $0.63\text{SD}$   $P=3.0 \times 10^{-12}$  and  $0.68\text{cm}$   $P=0.009$ ,  
165 respectively). The contrast in PRS indicates a substantial genetic contribution to the greater  
166 stature of post-Neolithic populations. Although higher residuals could reflect an environmental  
167 contribution, it could also represent the effect of genetic variation not captured by the PRS. Indeed,  
168 if we include lactase persistence in the regression (see below), the difference in residuals is no longer  
169 significant ( $P=0.17$ , Fig. 2D).

170 In summary, we cautiously refute the Neolithic stature hypothesis. Our data support that  
171 agricultural Neolithic populations were slightly shorter than both pre-agricultural and post-Neolithic  
172 populations but we find evidence for a genetic contribution to this difference, and limited evidence  
173 for any residual environmental effect. However, regardless of their origin, these average differences  
174 are small—2.5-3.5cm difference in stature compared to present-day differences of up to 7cm between  
175 European countries [32], global changes of up to 20cm in the past century [18] and differences of  
176 over 5cm between females from different Neolithic European populations [17].

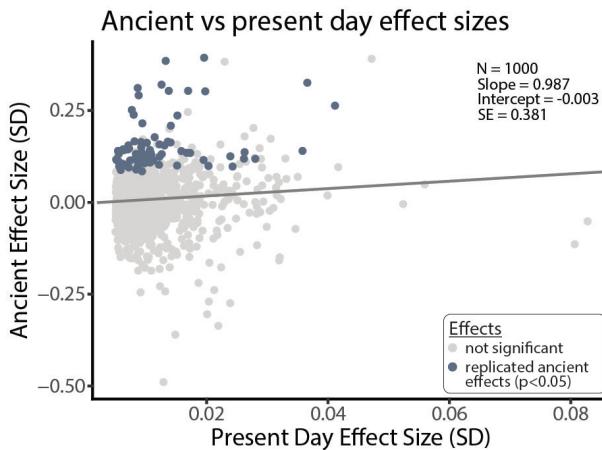


Figure 3: Re-estimating Ancient SNP effects *The relationship between present-day and re-estimated ancient effect sizes. Each point is one of the 1000 SNPs we tested. Grey points are not significant in our re-estimation. Dark points are significant at  $P < 0.05$  with consistent direction. Regression line is based on Deming regression of modern ~ ancient betas which takes into account the uncertainty in both dependent and independent variables*

## 177 2.3 Ancient association tests replicate modern GWAS effects

178 We next measured the effect of individual SNPs on stature in the ancient data. This dataset is  
179 too small to run a GWAS, so we focused on estimating effects at known height associated SNPs.  
180 Restricting to the 1000 most significant independent SNPs in the largest present-day height GWAS  
181 [22], we tested for association with femur length in our data. We reject a model where all 1000 effects  
182 are zero ( $\chi^2_{1000}=1171$ ,  $P=0.0001$ ) and do not reject a model where the effect sizes in our data are

183 identical to those in the present-day data ( $\chi^2_{1000}=1008$ ,  $P=0.42$ ). In fact, the chi-squared statistic for  
184 the second test is very close to the expected value (1000), and the slope from a regression of ancient  
185 on modern effect sizes is close to 1 (Fig. 3), suggesting that genetic effects on stature detected in  
186 present-day GWAS are very similar in ancient individuals. Of the 1000 SNPs, we replicate 81 in  
187 the correct direction at nominal significance ( $P=0.05$ ) (Fig. 3). Consistent with the chi-squared  
188 test, this is significantly more than we would expect to replicate if we had no power (binomial test  
189  $P=3.133 \times 10^{-5}$ ) but is not significantly different than the 67 we would expect to replicate given  
190 the ancient sample size (binomial test  $P$ -value=0.081, Methods).

## 191 2.4 Lactase persistence had a large effect on ancient stature

192 Although SNPs detected in present-day GWAS have similar effects in ancient individuals, we hy-  
193 pothesized that the lactase persistence SNP rs4988235 could be associated with stature in ancient  
194 individuals even though it is not associated today [33]. Lactase persistence was under exception-  
195 ally strong selection between about 4500 and 1000 years BP [34], and must therefore have had a  
196 large phenotypic effect. The selective advantage remains unclear [35, 33], though it could be due  
197 to additional calories gained from digesting lactose [36, 37, 38, 39], or improved bone development  
198 via vitamin D and calcium metabolism [40, 41, 42]. After imputation about 20% of our ancient  
199 sample has either one or two copies of the lactase persistence allele (Fig. 4A). We find that the  
200 allele is significantly associated with longer femur length ( $P=6.01 \times 10^{-7}$ ,  $\beta=0.358\text{sd}$ ,  $se=0.071$ ; Fig.  
201 4A,B), even controlling for ancestry ( $P=2.38 \times 10^{-5}$ ,  $\beta=0.318\text{sd}$ ,  $se=0.075$ ), and polygenic scores  
202 ( $P=3.02 \times 10^{-4}$ ,  $\beta=0.256\text{sd}$ ,  $se=0.071$ ). As an alternative method of controlling for ancestry, we also  
203 analyzed just the post-Neolithic samples to reduce confounding due to the introduction of Steppe  
204 ancestry, with consistent results ( $P=0.0001$ ,  $\beta=0.30\text{SD}$ ,  $se=0.078$ ; Fig. 4B) Confidence intervals for  
205 unimputed data overlap with those from the imputed genomes, indicating that this result is not  
206 due to imputation bias (Fig. 4B). Including lactase persistence in a regression of femur length on  
207 sex, ancestry and polygenic scores increases  $R^2$  by about 2% for both European and East Asian  
208 derived PRS, and removes the residual difference between Neolithic and post-Neolithic populations  
209 (Fig. 2D).

## 210 3 Discussion

211 Our results are consistent with previous findings that the variance explained by PRS for height in  
212 ancient populations is about one fourth of the variance explained in present-day data [19, 11]. This  
213 likely reflects some combination of lower data quality (both genetic and phenotypic data), imputa-  
214 tion accuracy, decline in performance due to genetic distance from the training population [28], and  
215 population stratification [43]. Population stratification is a particular concern when interpreting  
216 differences in polygenic score across populations, and for this reason we restrict our comparisons to  
217 PRS computed using East Asian GWAS summary statistics in which any stratification should be

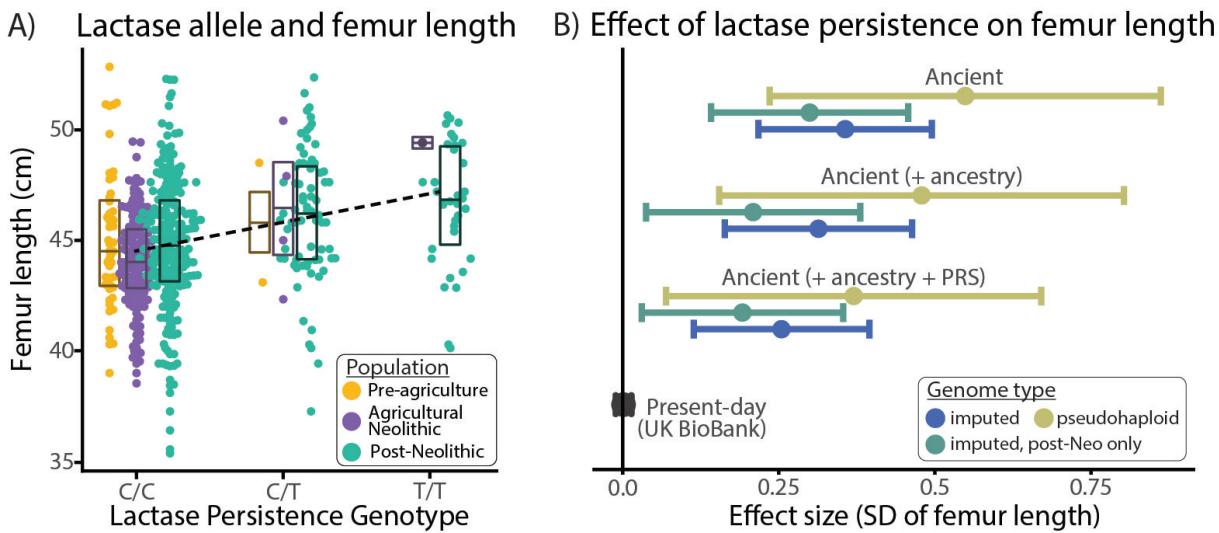


Figure 4: Effects of Lactase Persistence A) *Effect of lactase persistence on femur length.* In present day GWAS there is no association between having the lactase persistence allele and being taller (top row). In our ancient samples, having the lactase persistence allele is significantly associated with having longer femora (second row; imputed:  $P=7.05 \times 10^{-8}$ ,  $\beta=0.420\text{cm}/\text{SD}$ ,  $se=0.077$ ) with some ancestry-related variation (third row; imputed:  $P=3.43 \times 10^{-6}$ ,  $\beta=0.372\text{cm}/\text{SD}$ ,  $se=0.077$ ). The effect is attenuated when we also include PRS in the model (calculated without chromosome 2 to avoid LD with the *LCT* gene) but is still significant (bottom row; imputed:  $P=0.0017$ ,  $\beta=0.243\text{cm}/\text{SD}$ ,  $se=0.079$ ). Bars show standard error. B) Relationship between femur length and the lactase persistence (LP) allele. Having the LP allele significantly increases individual height ( $P=7.05 \times 10^{-8}$ ) and presence of this allele is predominant in the post-Neolithic period. Boxes for each time period show quartiles and mean of femur length during those periods for each genotype. Dashed line is the regression line of femur length on LP allele. Femur lengths on the y-axis have been sex-adjusted by regressing out the effects of this variable.

218 independent of European population structure.

219 Our results do not support a substantial decrease in stature caused by the European transi-  
 220 tion to agriculture [1]. First, though Neolithic populations were shorter than both Mesolithic and  
 221 Bronze Age populations, these differences were small in both absolute and relative terms, espe-  
 222 cially when compared to variation among Neolithic [17] and modern [18] populations. Second,  
 223 we find evidence that there was a significant genetic contribution to these changes, which means  
 224 that the environmental effects, if any, were even smaller. Modern secular trends have shown that  
 225 environmentally-driven shifts in stature can be dramatic, and these Neolithic changes were modest  
 226 by comparison. It is therefore hard to support the idea that the adoption of agriculture had a  
 227 substantial systematic effect on stature. On the other hand, the inconsistent results between stud-  
 228 ies [e.g., 6, 1, 4, 3, 2, 14] indicate that large, continent-wide surveys of Neolithic stature may be  
 229 sensitive to sampling strategies and miss heterogeneous local patterns. Addressing these questions  
 230 would then be better served by smaller regional analyses that can specifically focus on the complex  
 231 interactions between hunter-gatherers, early farmers, and their cultural-environmental landscapes  
 232 [17]. Finally, we note that although stature is generally regarded as a good proxy for population  
 233 health [6, 44], there are other metrics of health (dental, paleopathology, demography) that we are

234 not testing and it remains possible that the Neolithic transition was detrimental in ways that are  
235 not captured by stature.

236 We find that the effects of individual SNPs on stature are broadly the same in ancient populations  
237 as they are in the present-day. However, the lactase persistence associated SNP rs4988235 is an  
238 extreme exception. Although it has no effect on height (or any other substantive phenotype) today,  
239 we estimate its effect in ancient populations to be at least 0.25 standard deviations, corresponding  
240 to about 1.5cm in stature. This is an exceptionally large effect for a common variant, around 25  
241 times larger than the most significant variants identified in present-day GWAS. If we were to run  
242 a GWAS for stature in Bronze or Iron Age Northern Europe, rs4988235 would likely be by far the  
243 most significantly associated SNP.

244 This has important implications for understanding the evolution of lactase persistence, one of  
245 the most intensively studied examples of recent human adaptation. Although the lactase persis-  
246 tence allele has been one of the most strongly selected variants in the entire human genome, there  
247 is still considerable debate about the nature of selection. It has recently been argued [33] that the  
248 lack of phenotypic associations with lactase persistence in the UK Biobank suggests the selective  
249 advantage was unrelated to the nutritional benefits of milk consumption. Our results illustrate the  
250 limitations of this argument—the absence of present-day effects cannot be used to reject prehis-  
251 toric selective advantages. Instead, our findings provide support for lactase persistence having a  
252 nutritional advantage due to increased calories from digesting the lactose itself [36, 37, 38, 39] or  
253 improved vitamin D and calcium metabolism [40, 42].

254 In summary, our analysis demonstrates the value of integrating genomic and phenotypic data  
255 from ancient individuals. We hope that future ancient DNA studies will be more systematic about  
256 collating archaeological and anthropological data in order to enable more fine-scale analysis of  
257 phenotypic trends.

## 258 4 Materials and Methods

### 259 4.1 Genetic data

260 We used the genetic data from 568 ancient Eurasian individuals [45, 46, 47, 48, 49, 50, 51, 52, 53, 54,  
261 55, 56, 57, 58, 59, 60, 61, 62, 9, 63, 64, 65, 10, 66, 67, 68, 69, 70, 71, 72, 12, 73, 74, 75, 76, 77, 78, 79,  
262 80, 81, 34, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97]. Most samples are genotyped  
263 by in-solution capture of a set of 1.24 million single nucleotide polymorphisms (SNPs), colloquially  
264 known as the "1240k" [9, 98]. For each individual, a single SNP was randomly selected at each of  
265 the 1240k sites, generating pseudohaploid data. For each sample, we downloaded the aligned bam  
266 files from the sources cited in their publications. For the few samples that did not have aligned bam  
267 files we aligned them using the pipeline from [50]. Calls were generated from aligned bams using the  
268 default parameters in *pileupCaller* (<https://github.com/stschiff/sequenceTools>). As these ancient  
269 samples are low coverage (median 0.51×), it is not generally possible to generate diploid genotypes.

270 This potentially limits the performance of the polygenic scores, so we imputed diploid genotypes in  
271 our samples with *GLIMPSE* [20] using the ancient DNA pipeline developed by [21] and the 1000  
272 Genomes Europeans [99] as reference panel.

273 There are a number of large height GWAS available from which we can test our ancient PRS  
274 predictions. We tested the height prediction performance of six different GWAS [23, 25, 24, 22] on  
275 both our imputed and unimputed samples. For each GWAS, we intersected the GWAS SNPs with  
276 those on the 1240k array, with the exception of those from the large [22] meta-GWAS. Due to data  
277 restrictions in the [22] analysis, they only published PGS weights for HapMap3 SNPs [100] from  
278 their full sample (N=approx 5 million), instead of traditional summary statistics. The intersection  
279 between HapMap3 and the 1240k array is low, and once the GWAS SNPs were clumped to calculate  
280 the PRS we did not have enough power to test it using our unimputed 1240k data. As an alternative,  
281 we tested PRS predictions from this GWAS by imputing the missing HapMap3 SNPs. Additionally,  
282 Yengo et al. also released traditional summary statistics from smaller GWAS, with less power, but  
283 fewer restrictions. These were successfully intersected with the 1240k array and clumped for testing  
284 both our imputed 1240k and unimputed data. In total, we tested PRS constructed from European  
285 GWAS from the UK Biobank [23] (n=7,008 SNPs), East Asian GWAS [24](n=3,326 SNPs), within-  
286 family sibling GWAS [25](n=1,115 SNPs), 1240k intersected Yengo Europeans (n=21,616 SNPs),  
287 1240k intersected Yengo East Asians (n=1,940 SNPs), and 1240k intersected Yengo multi-ancestry  
288 (n=22,435 SNPs) SNP sets on both our imputed and unimputed 1240k ancient DNA data, as well  
289 as our HapMap3 imputed ancient DNA on PGS weights from the Yengo European (n=1,099,006  
290 SNPs), East Asian (n=990,793 SNPs) and multi-ancestry (n=1,103,043 SNPs) meta-GWAS SNPs.

291 We constructed PRS as previously described [19] using clumping and thresholding methods in  
292 *plink* v.1.9 (-*-score*, p-value cut-off= $10^{-6}$ ,  $r^2=0.3$ , and 250kb windows). We chose parameters  
293 to maximize prediction based on [19] except for the larger Yengo meta-GWAS, from which the  
294 PRS were constructed without clumping, using the PGS weights published by those authors. For  
295 PRS calculations in the unimputed data, missing sites for each individual were ignored, effectively  
296 shrinking scores towards the sample mean.

297 We conducted principal component analysis (PCA) using *smartpca* [101] to project ancient  
298 individuals in to the PC space of 777 present-day Eurasian individuals [10]. Values from PCs 1-4  
299 for each individual were included in regression analyses to control for ancestry related effects and  
300 are referred to collectively throughout the paper as "ancestry". We included the date in years before  
301 present (BP) for each sample based on what was reported in the original publication for the sample.  
302 Whenever possible, we used  $^{14}\text{C}$  dates, otherwise we took the mid-point of the archaeological time  
303 range, and included genetic sex as reported in the original publications.

## 304 4.2 Osteological data

305 We obtained osteological metric data from publications as well as from direct measurements taken  
306 by the authors [102, 46, 103, 48, 104, 19, 105, 106, 107, 108, 109, 110, 111, 112, 17, 113, 114, 115,

307 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 59, 129, 130, 131, 62, 132, 133, 134,  
308 135, 136, 137, 138, 139, 140, 141, 142, 66, 143, 144, 145, 5, 68, 146, 11, 147, 148, 149, 150, 151,  
309 152, 153, 80, 154, 155, 156, 157, 158, 159, 160, 161, 162, 88, 163, 92, 95, 164, 165, 166, 167, 168,  
310 169, 170, 97, 171, 172, 173]. When only estimated statures have been published, rather than direct  
311 measurements, we reversed the equation of the cited stature estimation method to retrieve the  
312 maximum femur length that is associated with that stature [19]. There are a number of individuals  
313 (n=107) for which the estimation method used to calculate the published stature was not cited in  
314 the publication. In order to include these samples, we chose to estimate the femur length using  
315 the Trotter and Gleser (1952) equation as this is one of the most common methods, especially in  
316 publications predating [174].

317 We took dates for each sample from the mean of the calibrated  $^{14}\text{C}$  range in the original publi-  
318 cation or the midpoint of the archaeological date range if no carbon date was available. Individuals  
319 were assigned to pre-agricultural, agricultural Neolithic, or post-Neolithic time periods based on  
320 archaeological assessments in publications associated with each sample. The pre-agricultural group  
321 includes all populations who do not yet have archaeological indications of agriculture, including  
322 hunter-gatherers with ceramics in Scandinavia. The post-Neolithic group is defined by cultures  
323 with metal technology and includes early Copper Age settlements. These lifestyle categories were  
324 not defined by genetic ancestry, although they are highly correlated (Fig. 1B).

### 325 4.3 Statistical models

326 **Evaluating PRS performance.** We fit linear models of femur length on PRS, sex, date in years  
327 BP, date-squared, and PCs 1-4 (ancestry) as described in [19]. For each linear model, we calculated  
328 the contribution of the PRS by taking the  $R^2$  of the full model with all variables and subtracting  
329 the  $R^2$  of a reduced model without the PRS variable; we refer to this simply as " $R^2$ " throughout the  
330 text [19]. For the analysis of femur-length as shown in Fig. 2B, we regressed out the effects of sex  
331 on femur length in order to remove the substantial sex effect, adjusting females to have the same  
332 mean as males. All p-values in Fig.2 come from two-tailed pair-wise t-test between time period  
333 groups.

334 **Re-estimating SNP effects.** We re-estimated the effects of the top 1000 [22] European GWAS  
335 SNPs by regressing each of the SNPs on femur length, including sex as a covariate (femur length  
336 SNP + sex). All SNPs have both the effect and other alleles present within the dataset. All missing  
337 1240k SNPs were imputed as summarized above. Power for the re-estimation of PRS SNPs was  
338 calculated as

$$1 - \Phi \left( \Phi^{-1}(0.95) - \frac{|\hat{\beta}| \sqrt{n \cdot f \cdot (1-f)}}{\hat{\sigma}} \right),$$

339 where  $\Phi$  is the standard cumulative normal distribution function,  $\beta$  and  $\sigma$  are the estimated effect  
340 size and standard error of the UK Biobank GWAS, n is the ancient sample size (1136 haplotypes),  
341 and f is the minor allele frequency in the ancient sample. Because the UK Biobank GWAS is for

342 height, not femur length, we adjusted beta and sigma to units of femur length by scaling them using  
343 the slope of the [174] stature estimation equation (2.7).

344 **Evaluating the effect of the lactase persistence allele.** To assess the effect of lactase  
345 persistence, we regressed femur length on sex in all models and also included genetic ancestry (PCs  
346 1-4) and PRS where indicated. Given the number of SNPs involved in the height PRS, there is a  
347 strong likelihood that some of these could be in LD with the lactase gene (LCT) on chromosome 2,  
348 confounding the analysis. To mitigate this effect, we removed all SNPs on chromosome 2 from the  
349 GWAS summary statistics and re-calculated the PRS. For Fig. 4B we again adjusted female femur  
350 length to have the same mean as males.

#### 351 4.4 Code and data Availability

352 All non-genetic data and polygenic scores used in this analysis are provided in Supplementary Table  
353 1. Original ancient DNA data files can be downloaded from the resources provided in their cited  
354 publications and from the Allen Ancient DNA Resource (AADR). Previously published osteological  
355 data can be found in their cited sources which include the LiVES database (doi: 10.17171/2-12-2-1)  
356 and Dr. Christopher Ruff's public dataset (<https://www.hopkinsmedicine.org/fae/CBR.html.>)  
357 R code for each analysis is available at [https://github.com/mathilab/\\*\\*\\*.git](https://github.com/mathilab/***.git).

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