

## Supplementary Information for Bone density and genomic analysis unfold cold adaptation mechanisms of extinct inhabitants of Tierra del Fuego

Mikiko Watanabe<sup>1§\*</sup>, Renata Risi<sup>1§</sup>, Mary Anne Tafuri<sup>2</sup>, Valentina Silvestri<sup>3</sup>, Daniel D'Andrea<sup>4</sup>, Domenico Raimondo<sup>3</sup>, Sandra Rea<sup>5</sup>, Fabio Di Vincenzo<sup>2</sup>, Antonio Profico<sup>2</sup>, Dario Tuccinardi<sup>6</sup>, Rosa Sciuto<sup>5</sup>, Sabrina Basciani<sup>1</sup>, Stefania Mariani<sup>1</sup>, Carla Lubrano<sup>1</sup>, Saverio Cinti<sup>7</sup>, Laura Ottini<sup>3</sup>, Giorgio Manzi<sup>2#</sup> and Lucio Gnessi<sup>1\*#</sup>

<sup>1</sup> Department of Experimental Medicine, Section of Medical Pathophysiology, Food Science and Endocrinology, Sapienza University of Rome, Rome, Italy.

<sup>2</sup> Department of Environmental Biology, Sapienza University of Rome, Rome, Italy.

<sup>3</sup> Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy

<sup>4</sup> MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University, Cardiff, Wales, UK

<sup>5</sup> Nuclear Medicine Unit, IRCCS Regina Elena National Cancer Institute, Rome, Italy

<sup>6</sup> Unit of Endocrinology and Diabetes, Campus Bio-Medico University of Rome, 00128 Rome, Italy

<sup>7</sup> Department of Experimental and Clinical Medicine, Center of Obesity, Università Politecnica delle Marche, Ancona, Italy

§ These authors contributed equally

# These authors contributed equally

\*Corresponding author

### Correspondence to:

Lucio Gnessi MD PhD, Department of Experimental Medicine, Section of Medical Pathophysiology, Food Science and Endocrinology, Sapienza University of Rome, Rome, Italy. Tel. +39 06 499 70 721

**Mail:** lucio.gnessi@uniroma1.it

Mikiko Watanabe MD PhD, Department of Experimental Medicine, Section of Medical Pathophysiology, Food Science and Endocrinology, Sapienza University of Rome, Rome, Italy. Tel. +39 06 499 70 542

**Mail:** mikiko.watanabe@uniroma1.it

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Legends for Datasets S1

**Other supplementary materials for this manuscript include the following:**

Datasets S1

**Table S1. Adjusted Pearson correlations between bone geometry and BAT parameters.**

<b>A</b>					
<i>Height-adjusted</i>	<b>CBA</b>	<b>CSA</b>	<b>MA</b>	<b>Bat Volume</b>	<b>Bat Activity</b>
<b>CBA</b>	1	0.896***	.558**	.392*	.417*
<b>CSA</b>		1	.359*	.294 <sup>^</sup>	.290
<b>MA</b>		.	1	.173	.230
<b>Bat volume</b>				1	.903***
<b>Bat activity</b>					1

  

<b>B</b>				
<i>Height &amp; MA- adjusted</i>	<b>CBA</b>	<b>CSA</b>	<b>Bat Volume</b>	<b>Bat Activity</b>
<b>CBA</b>	1	0.898***	.362*	.357*
<b>CSA</b>		1	.252	.228
<b>Bat volume</b>			1	.900***
<b>Bat activity</b>				1

A positive unadjusted correlation trend of CBA with BAT volume (p 0.07, r 0.307) and activity (p 0.07, r 0.315) was found. Adjusting the analysis for anthropometric parameters likely affecting femoral measures the following results emerged: **(A)** Height-adjusted partial correlation between CBA and BAT volume and activity in a BAT expressing living population (n=34) became significant, and a positive trend between CSA and BAT volume emerged. **(B)** Further adjusting by both height and muscle area (MA), the positive correlation trend between CSA and BAT indexes vanished, whereas the one between CBA and BAT volume and activity was maintained, suggesting that muscle plays a central role in bone remodeling. CSA, Cross-Sectional Area; CBA, Cortical Bone Area; MA, Muscle Area. \*\*\*P<0.001; \*\*P<0.01; \* P<0.05 level; <sup>^</sup> P<0.1

**Table S2. Multiple regression analysis to assess predictors of cortical thickness.**

<b>A</b>					
<b>Model</b>		<b>Standardized <math>\beta</math></b>	<b>SE</b>	<b>Sig.</b>	<b>R</b>
<b>1</b>	MA	.723	.004	.000	.723
<b>2</b>	MA	.514	.005	.001	.781
	Height	.363	1.260	.013	
<b>3</b>	MA	.468	.005	.001	.812
	Height	.386	1.202	.006	
	BAT Volume	.224	.207	.047	
<b>4</b>	MA	.412	0.005	.002	.848
	Height	.516	1.211	.001	
	BAT Volume	.276	.195	.011	
	Age	.271	.619	.019	
Dependent Variable: CBA					
Independent variables evaluated: MA, height, Bat Volume, Age					
<b>B</b>					
		<b>Standardized <math>\beta</math></b>	<b>SE</b>	<b>Sig.</b>	<b>R</b>
	MA	.860	.005	.000	.731
Dependent Variable: CBA					
Independent variables evaluated: MA, height, Age					

(A) A stepwise multiple regression in a BAT expressing living population (n=34) including age, weight, height, MA, BAT volume as independent variables showed that BAT volume independently predicted CBA after height and MA, while (B) a stepwise multiple regression in a BAT negative living population (n=34) including the same independent variables showed that MA independently predicted CBA. These findings suggest that, in the absence of BAT, muscle mass closely influences bone morphology, while, when BAT is present, this acquires the strength of independent predictor of bone state. MA, Muscle Area; CBA, Cortical Bone Area.

**Supplementary Table 3. Anthropometric characteristics, bone mineral density and geometry**

parameters	of	selected	Fuegians	bone	remains			
Sample info	Sex	Estimated age at death	CSA mm <sup>2</sup>	CBA mm <sup>2</sup>	EndA mm <sup>2</sup>	LS BMD (g/cm <sup>2</sup> )	TH BMD (g/cm <sup>2</sup> )	FN BMD (g/cm <sup>2</sup> )
Fuegian 1	F	40+	583.68	312.16	271.53	Missing	0.666	0.533
Fuegian 2	F	20-30	472.86	313.69	159.17	0.709	0.893	0.714
Fuegian 3	F	40+	426.91	279.37	147.54	0.776	0.83	0.548
Fuegian 4	F	40+	492.03	259.56	232.48	0.642	0.893	0.721
Fuegian 5	M	40+	645.62	467.64	177.99	0.815	1.024	0.9
Fuegian 6	M	40+	562.22	442.24	119.98	0.788	0.986	0.808
Fuegian 7	F	40+	684.39	484.1	200.3	0.569	0.647	0.478
Fuegian 8	M	30-40	509.01	416.9	92.111	1.022	1.131	0.924
Fuegian 9	F	40+	411.8	321.7	90.104	0.817	1.076	0.873
Fuegian 10	F	20-30	505.57	348.71	156.86	0.877	1.043	0.858
Fuegian 11	F	40+	491.23	295.04	196.2	0.623	0.643	0.490
Fuegian 13a	M	40+	564.13	405.68	158.45	0.955	1.088	0.954

CSA, Cross-Sectional Area; CBA, Cortical Bone Area; EndA, Endocortical Area; LS, LumbarSpine; BMD, Bone Mineral Density; TH, Total Hip; FN, Femoral Neck; F, Female; M, Male.

**Dataset S1 (separate file). Coding and non coding variants identified in the Fuegian population.**