A 12,000-Year-Old Case of NPR2-Related Acromesomelic Dysplasia

To the Editor:

We used ancient DNA (aDNA) techniques to genetically diagnose a rare disease in a >12,500-year-old individual with short stature. The skeletons of two persons, a short adult (Romito 1, 145 cm; 57.1 inches) and an adolescent (Romito 2, 110 cm; 43.3 inches), were unearthed in 1963 from an unusual Late Upper Paleolithic double grave in the Romito cave, Italy (**Figure S1 Supplementary Appendix**). The bodies had been buried contemporaneously in an embraced position. Romito 2 had disproportionate short stature and severe acromesomelia (**Figure 1 a-d**). The pathology was ascribed to various conditions, including acromesomelic dysplasia-Maroteaux-type (AMDM), a rare, autosomal recessive disorder due to germline *NPR2* gene variants[1,2]. The sex, relatedness, population genetics and paleopathologies of Romito 1 and 2 remained unresolved.

Genetic material from each left inner ear was extracted and analyzed. The molecular sex of both individuals was female, and they were first-degree relatives (i.e. mother and daughter, or sisters; **Figure 1e**). They were from the Villabruna genetic cluster, a Hunter-Gatherer population that expanded from

Southern into Central/Western Europe around 14,000 years ago[3] (**Figure 1f**). There was no evidence of close-relative endogamy, but they exhibited background relatedness consistent with a small population size (**Figure S2 Supplementary Appendix**).

Through sequencing a panel of 38 skeletal pathology-related genes, we identified a homozygous variant in *NPR2* ((chr9:35802590-C-T; c.1801C>T, c.1801C>T (OMIM: 108961)) in Romito 2 (**Table S1**). Average coverage at *NPR2* was 17X and 16X for the c.1801C>T variant (**Figure S2**). We obtained lower coverage (5X) for Romito 1, but nonetheless found evidence for heterozygosity for the c.1801C>T variant. That we obtained lower coverage for Romito 1 reflects challenges in sequencing aDNA, which include fragmentation and degradation over time due to environmental and other effects[3]. Such challenges are particularly relevant to diseases caused by variation at the gene level (as opposed to chromosome level, such as trisomy 21.)

The c.1801C>T variant encodes a missense change, p.Arg601Cys (rs1563988849) that is extremely rare in population databases with no homozygotes reported (**Supplementary Appendix**). Arg_{601} is a highly conserved residue in the NPR2 kinase homology domain, itself highly conserved among receptor guanylyl cyclases (**Figure S3**). Homozygous pathogenic missense *NPR2* variants affecting Arg_{601} cause AMDM [4], and heterozygous *NPR2* variants (including p.Arg601Cys) have been were reported in patients with short stature (**Table S2)** . The variant was classified as Likely Pathogenic according to

criteria of the American College of Medical Genetics. Further supporting a pathogenic effect, a bioinformatic analysis showed damaging/deleterious effects in 85.3% of tools, indeterminate/uncertain in 5.9% and tolerated/low impact in 8.8% (**Table S3**).

Romito 2, a Hunter-Gatherer, would have faced challenges in displacement over distances and terrain, while movement limitations at the elbow and hands would have affected her daily activities. She survived, however, until late adolescence with a similar diet and nutritional stress as other Romito individuals, suggesting that the challenges she faced were met by the provision of care in her family group[5]. Our approach, through which we have made a genetic diagnosis using a prehistoric sample, could be used to study the burden of rare genetic diseases in human groups in antiquity.

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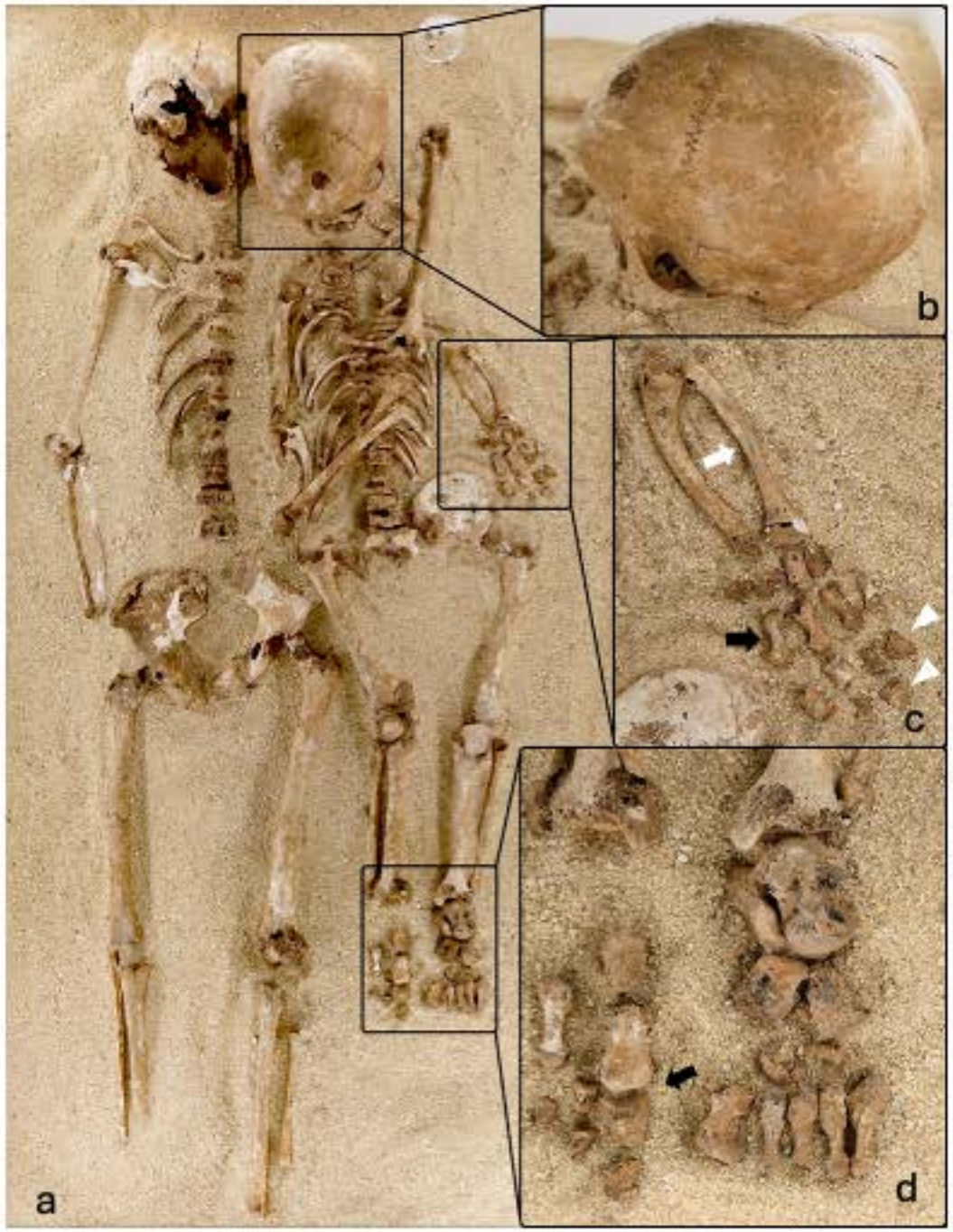
Figure 1. Skeletal Remains, Biologic Kinship, and the Villabruna Cluster

a) Skeletons of the Romito 1 (left) and 2 (right) double burial showing the embraced position with the left arm of Romito 1 around Romito 2 and demonstrating acromesomelia in Romito 2. Panels b-d illustrate typical features of AMDM in Romito 2. b) There is doming and bulging of the upper cranium above a shallow nasal root. c) The radius and ulna are shortened, and radial bowing is evident (open arrow), these characteristics limit elbow joint range of movement due to subluxation/dislocation. The metacarpals are shortened and broadened (filled arrow), and the phalanges are short with cone shaped epiphyses (open arrowheads). d) The feet also demonstrate short, thickened metatarsals and phalanges, apart from the hallux, which is typically prominent in AMDM (filled arrow). e) Biological kinship results demonstrating first-degree relatedness between Romito 1 and 2. Labels on the X axis show the pairs of individuals and datasets used for comparison using the TKGWV2 pipeline (for details see **Supplemental Appendix**). f) Principal component analysis (PCA) highlighting the position of Romito 1 and 2 within the Villabruna cluster of Hunter-Gatherers.


a
b
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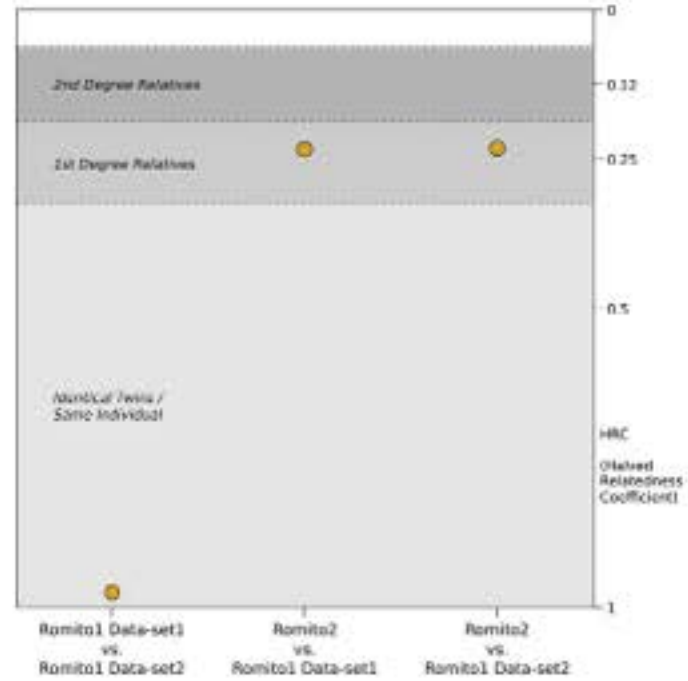
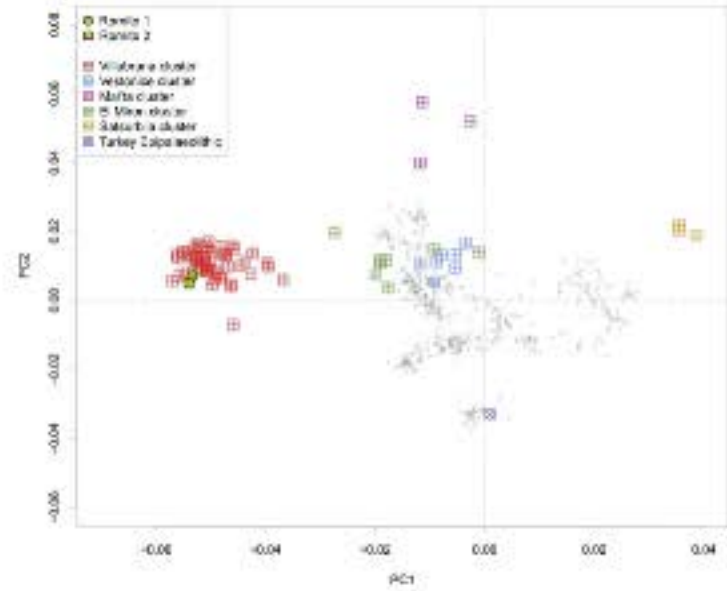

2nd Degree Relatives
1st Degree Relatives
Identical Twins / Same Individual
0
0.12
0.25
0.5
1
HRC
Obtained Relatedness Coefficient
Romito1 Data-set1 vs. Romito1 Data-set2
Romito2 vs. Romito1 Data-set1
Romito2 vs. Romito1 Data-set2

f


PC2
PC1