A 1000-year-old case of Klinefelter's syndrome diagnosed by integrating morphology, osteology, and genetics

Xavier Roca-Rada, Sofia Tereso, Adam B Rohrlach, André Brito, Matthew P Williams, Cláudia Umbelino, Francisco Curate, Ira W Deveson, Yassine Souilmi, António Amorim, Pedro C Carvalho, Bastien Llamas, João C Teixeira

The skeleton of a human being buried over 1000 years ago, in the medieval archaeological site of Torre Velha, in north-eastern Portugal, was referred to us for analysis and investigation (appendix).

On examination, we found the outstandingly well preserved skeleton of an adult who was likely to have been more than 25 years old at the time of death. The person was approximately 1·80 m tall. Notably, out of the six skeletons examined, where height could be ascertained, this individual was the tallest (appendix). Based on pelvic morphology and metric analysis of the skeleton, we concluded that the individual was a man. However, the bi-iliac width of our person was 289 mm, considerably larger than the average width previously reported for ancient Portuguese men (mean 261·8 mm; SD=13·4; Student's *t* test: t=22·6944; df=124; p<0·001). Additionally, the individual's teeth were worn asymmetrically, indicating probable malocclusion of the jaw and maxillary prognathism (figure; appendix). Bone densitometry analysis showed normal bone mineral density values (0·792–0·999 g/cm²).

Genetic analysis was undertaken using a variety of different methodologies—including the calculation of X chromosome to Y chromosome autosome ratios (0·902 and 0·298, respectively), X chromosome and Y chromosome dosages (approximately 2 and 1, respectively), and X chromosome heterozygosity (approximately 0·2). We also determined the Y chromosome haplogroup to be R1b-P310 (R1b1a1b1a1), a frequent western-European and pan-European lineage, in accordance with the individual's Iberian genetic ancestry (appendix).

Furthermore, using a novel Bayesian method, allowing us to probabilistically assign individuals to karyotypes based on the number of sequencing reads—or DNA fragments sequenced—mapping to the X, Y, or autosomal chromosomes, we concluded our individual's karyotype to be 47,XXY, and rejected models of contamination of XXY and XX or XY (figure; appendix). The closeness of the observed position of the studied individual and theoretical position for an XXY karyotype (figure) strongly agrees with our posterior probability of approximately 1 for this individual having a Klinefelter's syndrome karyotype. Considering the morphological findings—specifically the height, the bi-iliac width, the possible jaw malocclusion, and the maxillary prognathism—the genetic findings—indicating a karyotype of 47,XXY—we concluded that the studied individual had Klinefelter's syndrome.

The prevalence of Klinefelter's syndrome in the general population has previously been reported to be 0·1–0·2%, but many patients remain undiagnosed.

Typically, people with Klinefelter's syndrome are tall, have broad hips, sparse body hair, small testes, and gynaecomastia; they may have mandibular prognathism. Obesity, low glucose tolerance, and diabetes are observed, as well as osteoporosis because of androgen deficiency. Notably, the absence of osteoporosis in our case does not preclude a diagnosis of Klinefelter's syndrome; osteoporosis is only present in approximately 10–40% of cases. Genetically, about 80% of cases are 47,XXY.

DNA from osteological remains is often scarce, degraded, fragmented, and unsuitable for analysis using commonly available techniques for clinical diagnosis of chromosomal aneuploidies. To overcome these limitations, we have devised a novel Bayesian method,

Lancet 2022; 400: 691–92

Australian Centre for Ancient DNA, School of Biological Sciences, University of Adelaide, Adelaide, SA, Australia (X Roca-Rada MSc, M P Williams PhD, Y Souilmi PhD, B Llamas PhD, J C Teixeira PhD); **Research Centre for Anthropology and Health, Department of Life Sciences, University of Coimbra, Coimbra, Portugal** (S Tereso MSc, A Brito MSc, Prof C Umbelino PhD, F Curate PhD); **Institute for Medieval Studies, Faculty of Social and Human Sciences, NOVA University of Lisbon, Lisbon, Portugal** (S Tereso); **Department of Archaeogenetics, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany** (A B Rohrlach PhD); **School of Mathematical Sciences, University of Adelaide, Adelaide, SA, Australia** (A B Rohrlach); **Interdisciplinary Center for Archaeology and Evolution of Human Behaviour, University of Algarve, Faculdade das Ciências Humanas e Sociais, Universidade do Algarve, Faro, Portugal** (Prof C Umbelino); **Genomics Pillar, Garvan Institute of Medical Research, Sydney, NSW, Australia** (I W Deveson PhD); **Centre for Population Genomics, Garvan**

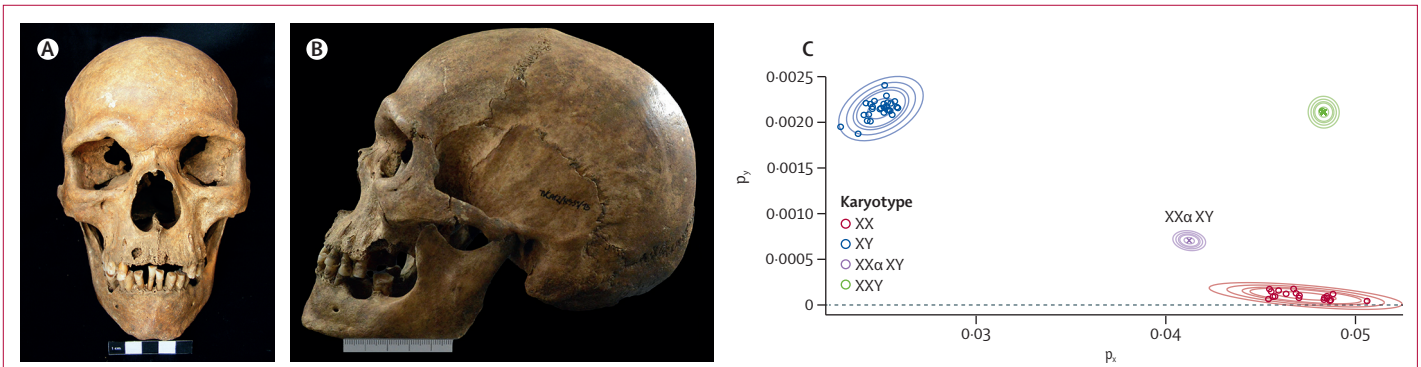

Figure: **Diagnosing Klinefelter's syndrome in a 1000-year-old skeleton**
Photographs of the skull show anterior (A) and lateral (B) views with a probable malocclusion and maxillary prognathism denounced by atypical dental wear. (C) Graph shows the observed proportion of reads mapping to the X chromosome (x-axis) and Y chromosome (y-axis), coloured by karyotype: XX (red), XY (blue), XXY (green), and the highest likelihood contaminated XXα XY (purple). Real data are indicated by circles; theoretical positions (for XXY and contaminated) are indicated by crosses. Ellipses indicate 50%, 90%, 95%, 99%, and 99·9% confidence.

which, we believe, is a potentially efficient statistical way of analysing fragmented DNA from a variety of sources (eg, ancient DNA, cell-free DNA, and DNA from forensic cases).

Contributors

XR-R and JCT designed the project. ST, AB, and PCC conducted the archaeological excavations. ST, CU, and FC conducted morphological and osteological analyses. XR-R, MPW, BL, and JCT performed the ancient DNA experiments. IWD generated the DNA sequencing data. XR-R, YS, BL, and JCT analysed the data. ABR designed and implemented the Bayesian method to the genetic data. XR-R and JCT wrote the manuscript with input from all authors. Written consent was not available from the patient.

Declaration of interests

We declare no competing interests.

Data sharing

The genetic data presented in this Clinical Picture will be made available upon publication.

Acknowledgments

We thank the Municipality of Bragança, the University of Coimbra, the University of Adelaide, the Max Planck Society, and the Calouste Gulbenkian Foundation for the support provided. ST is supported by the Fundação para a Ciência e a Tecnologia (SFRH/BD/116363/2016). BL (FT170100448) and JCT (DE210101235) are supported by the Australian Research Council. ABR is supported by the European Research Council (771234-PALEoRIDER).

Institute of Medical Research and Murdoch Children's Research Institute, Parkville, Vic, Australia (I W Deveson); **School of Clinical Medicine, Faculty of Medicine and Health, University of South Wales, Sydney, NSW, Australia** (I W Deveson); **National Centre for Indigenous Genomics, Australian National University, Canberra, ACT, Australia** (Y Souilmi, B Llamas); **Environment Institute, University of Adelaide, Adelaide, SA, Australia** (Y Souilmi, B Llamas); **Institute of Investigation and Innovation in Health, University of Porto, Porto, Portugal** (Prof A Amorim PhD); **Institute of Molecular Pathology and Immunology, University of Porto, Porto, Portugal** (Prof A Amorim); **Faculty of Sciences, University of Porto, Porto, Portugal** (Prof A Amorim); **Faculty of Arts and Humanities, University of Coimbra, Coimbra, Portugal** (Prof P C Carvalho PhD); **Centre of Interdisciplinary Studies, University of Coimbra, Coimbra, Portugal** (Prof P C Carvalho); **Telethon Kids Institute, Indigenous Genomics Research Group, Adelaide, SA, Australia** (B Llamas); **Centre of Excellence for Australian Biodiversity and Heritage, University of Adelaide, Adelaide, SA, Australia** (B Llamas, J C Teixeira); **Evolution of Cultural Diversity Initiative, Australian National University, Canberra, ACT, Australia** (J C Teixeira)

Correspondence to:
Dr João C Teixeira, Evolution of Cultural Diversity Initiative, School of Culture, History and Language, Australian National University, Canberra ACT 2601, Australia
joao.teixeira@anu.ed.au

See **Online** for appendix