

Supplemental Materials

Skeletal sample:

Details of the subject's medical history were assembled from published sources and the original medical case records, related documents and correspondence held at the Muséum régionale des Sciences naturelles, Mons, Belgium (Dufrane 1902, 1904; Dufrane *et al.* 1903; Launois 1903; Launois & Roy 1904; Marc 1905).

Height measures:

In Germany of the era of J.K.'s youth, height records began to be compiled in young adult men (at the age of 20 years) following the military draft laws of 1871; only those records relating to the region of Württemberg survive intact today (Twarog 1997). In the birth cohort that included the subject's birth year of 1872, the mean adult male height was 164 cm.

DNA extraction and library preparation:

During extraction of DNA, to avoid contamination with modern DNA from other sources, a 1 cm oval area of superficial bone was removed first before drilling (Figure 1C). The right petrous temporal bone was targeted and drilled using a Dremel rotary drill with clean spherical drill bits (1.5 mm and 3 mm bits), as previously described (Pinhasi *et al.* 2015). Approximately 300 mg of drilled powder was obtained from the cochlear core and stored in a 1.5 mL sterile tube. The DNA was extracted from the bone powder following the protocol outlined in (Dabney *et al.* 2013). Libraries for Illumina sequencing were then prepared as described previously (Meyer & Kircher 2010; Gamba *et al.* 2014). After being analysed for DNA concentration and length on an Agilent Bioanalyzer 2100, the DNA was sequenced on an Illumina MiSeq platform at the UCD Conway Institute for Biomolecular and Biomedical Research, using 65 base pairs and single-end sequencing. A custom ancient DNA bioinformatics pipeline was used to process raw sequencing data. Cutadapt v1.5 (Martin 2011) was used to trim adapter sequences with minimum overlap set to 1 and minimum length to 17bp. All reads were aligned to the hg19 build of the human genome using BWA v.0.7.5a-r405 (Li & Durbin 2009) with disabled seed (-l 1000) and a minimum QC quality filter of 30 was then applied. Duplicated sequences were later removed using Samtools v0.1.19-96b5f2294a (Li *et al.* 2009). Out of a total of 383120 reads, 158611 were aligned to the human genome with a minimum quality score of 30, corresponding to an endogenous DNA content of 41.4%. We then used the mapDamage2 tool (Jónsson *et al.* 2013) to verify deamination patterns and frequencies. This was done to assess the ancient origin of the DNA, we analysed length distributions and coverage using GATK v.3.3-0-g37228af and bedTools2 (Quinlan & Hall 2010). The DNA showed characteristic deamination patterns consistent with that of a sample just over 100 years old, with frequencies of substitution of 0.06 on the 5' end (C>T) side, and 0.05 on the 3' end (G>A) side. The weighted mean read length was 53bp, with a standard deviation of 12bp. These patterns and values are all within the expected ancient DNA ranges, where the molecules are shorter than 100bp and should present some damage. Concentrations of DNA were insufficient to permit array comparative genome hybridization to be reliably performed. The recovered DNA reads showed no pathological variants in *AIP*.

Digital droplet PCR

Previous studies with the current ddPCR methodology were performed on DNA from whole blood, biopsy tissue and pituitary tumors; in the current study the recovered DNA from the petrous temporal bone was studied. As previously described, the ddPCR experiment was performed with the following reaction conditions: the 21- μ L total reaction mixture contained 5 μ L of DNA template, 2 \times ddPCR supermix for probes (without dUTP), and *GRP101* and *ZIC3* exon 1 primers and probes assays (Bio-Rad Laboratories). Further details on primers, probes and reaction conditions are reported in (Daly *et al.* 2016). The samples were processed on an eight-channel droplet generator cartridge (Bio-Rad Laboratories) and the PCR reaction mixtures a 15,000 droplet emulsion that underwent conventional thermal cycling (PRoFlex PCR systems, Life Technologies); the reactions were read on a QX100 droplet reader (Bio-Rad Laboratories)

and analyzed using QuantaSoft software (Bio-Rad; version 1.7.4.0917). To generate the 95% confidence intervals and the Poisson distribution of CNV data, results from 91 separate samples of DNA from known normal and duplicated individuals were assessed as described previously (Daly *et al.* 2016).

References

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