## The "powerful amelogenin": a peptide at the service of paleoanthropology

Andrea Cozza<sup>1</sup>, Alberto Zanatta<sup>2</sup>, Fabio Zampieri<sup>1</sup>, Maurizio Rippa Bonati<sup>1</sup>

<sup>1</sup>Medical Humanities Group, Department of Cardiac, Thoracic and Vascular Sciences - University of Padua; <sup>2</sup>University Museums Centre (CAM) - University of Padua

**Abstract**. An international research group, from the Brighton (UK), São Paulo (Brazil), and Durham (UK) universities, has recently developed a versatile method for sex definition in human bioarchaeological remains by analysing the sex-specific isoforms of amelogenin drawn from dental enamel.

Key words: amelogenin, dental enamel, sex determination, osteo-archaeological remains

Dear Editor,

We are writing to propose the Italian Society for the History of Medicine (SISM) members, especially those who don't operate within the palaeopathology branch, a brief update on sex determination in human bioarchaeologic remains.

As is well known, determining sex of a dead body represents one of the main required parameters in an anthropological research. It can be done by a traditional procedure or by advanced DNA analysis techniques.

On the first case, given a sufficient and adequate amount of osteoarchaeological material, it is possible to determine sex by analysing morphological and metrical features in bones, which represent sexual dismorphism characteristics. Areas showing higher dismorphism characters, and therefore more versatile in determining biological gender, are the skull (for example supraorbital ridge, zygomatic arch, mastoid process, mandible, chin) and the pelvis (for example coxal bones, greater sciatic notch, preauricular sulcus, pubis), followed by other anatomical districts with less discriminating value such as the spine or femur (see Minozzi and Canci, 2015 pp 103-111) (1).

The second procedure is developed by DNA analysis, after its extraction, amplification and sequencing.

The major limits of this technique are the specimen contamination, nucleic acid degradation and high economic costs.

For severely fragmented, deteriorated or contamined remains from which it is not possible to extract DNA it is hard, if not impossible, to identify biological gender. In those cases, dental elements, if present, may be useful to obtain the missing biological information.

Teeth, in general, are considered a research material able to provide several information: sex identification, age of death, health or disease status, food habits and stress and, in some occasions, job or cultural practices. It can also provide data on geographical origin, migrations, phylogenesis, taxonomy, and on population variability (Minozzi and Canci, 2015 p. 185) (1).

By the end of 2017, on *PNAS*, an international research team - from the Brighton (UK), São Paulo (Brazil), and Durham (UK) universities - published an article where they presented a method particularly versatile for sex determination by analysing dental enamel (Stewart et al., 2017) (2).

As is well known, enamel is the hardest tissue in the human body and, consequently, the most resistant to decomposition. By carrying out small abrasions with different acids (among which hydrochloric, formic and trifluoroacetic acids) on enamel surface, it is possible to obtain amelogenin, a production protein for enamel itself. During enamel maturation stage, enamelin, ameloblastin and amelogenin are processed by proteases and certain peptide portions deposit in enamel.

Amelogenin processing eliminates the central portion, leaving C and N terminals instead. These peptide chains present different aminoacidic composition depending on sex, and they distinguish AMELX and AMELY isoforms, the chains conserved in enamel after proteolysis. Analysing these enamel extracted peptides through nanoscale liquid chromatography and mass spectrometry (nanoLC-MS) (3) leads to biological gender identification. Therefore, finding both AMELX and AMELY together indicates a male sample, while finding only AMELX isoforms indicate a female sample. It is not possible though to identify sexual aneuploidy.

Besides, the amelogenin analysis allows to identify gender in young individuals, an extremely difficult task when using traditional anthropometric methods.

Cost difference between DNA analysis and amelogenin analysis for sex determination is also noteworthy: the first one costs approximately 500 euros, while the second, around 20 euros.

For those reasons it is a reliable, minimally invasive and cost-effective method, able to give answers to a main query in anthropological research.

## References

- 1. Minozzi S, Canci A. Archeologia dei resti umani. Dallo scavo al laboratorio Nuova edizione. Roma: Carocci editore; 2015.
- 2. Stewart NA, Gerlach RF, Gowland RL, Gron KJ, Montgomery J. Sex determination of human remains from peptides in tooth enamel. PNAS, 2017; 114 (52): 13649-54.
- 3. http://www.chromatographyonline.com/nano-lc-principles-evolution-and-state-art-technique.

Correspondence: Andrea Cozza MD University of Padua, Italy E-mail: andrea.cozza87@gmail.com