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RESEARCH LETTER

Deciduous Teeth as an Alternative DNA Source for Postmortem Genetic Testing

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Postmortem genetic testing, for example, in the setting of sudden unexpected death (molecular autopsy), may allow identification of the genetic cause of death in the proband.[1] This enables tailored clinical and genetic evaluation of relatives and provides clarity regarding the cause of death of their deceased family member. However, in some cases, no blood or tissue is available for postmortem genetic testing. Here, we present an illustrative case that shows that deciduous teeth are an alternative source for DNA in this setting, when available, with important implications for family members.

The data that support the findings of this study are available from the corresponding author upon request. The study complied with the Declaration of Helsinki, and informed consent was obtained from all participants.

The mother and sister of a 39-year-old male who suffered sudden unexpected death were referred for genetic counseling to the Department of Genetics, University Medical Center Groningen, the Netherlands. The proband was found dead in his chair watching television. He had no medical history of cardiovascular disease and had a healthy and athletic lifestyle. Family history revealed no suggestions for inheritable heart disease (Figure). Comprehensive cardiological examination was performed in the mother and sister. The sister's ECG showed low R wave amplitudes. A left bundle branch block was observed on the mother's ECG. Blood from both mother and sister was taken for DNA storage, in case broad (next-generation sequencing-based) genetic testing failed in the proband. We did start targeted genetic analysis of the *PLN* gene in the sister because of the low-voltage ECG and the family's Frisian roots. This showed no genetic predisposition for cardiomyopathy.

Autopsy was not preformed in the proband. No blood or tissue from him was available for genetic testing. However, his mother had several of his deciduous teeth at home. DNA was isolated from one deciduous tooth in the Forensic Laboratory for DNA research, Department of Human Genetics, Leiden University Medical Center, the Netherlands. To remove surface contamination, this tooth was disinfected with Sanadep alcohol-tissues and irradiated with ultraviolet C (45 minutes each side). The cleaned tooth was placed in a mill-container, cooled in liquid nitrogen (20 minutes N2, ±1 minute room-temperature, 15 minutes N2), and pulverized in a Retsch Mixer Mill 400 (frequency 30, three 10 second cycles). DNA was isolated from 0.4 grams of toothpowder using the following steps: (1) overnight incubation (thermomixer, 1000 rpm, 56°C) in 1 mL 0.5 M EDTA pH 8.0, containing 5 % sarcosyl, and 90 µL proteinase K; (2) centrifugation at 13000 rpm for 3 minutes; (3) purification of the supernatant using the centrifuge protocol of the QIAquick PCR Purification Kit; and (4) elution in 80 µL nuclease-free water in a DNA-Lo-Bind tube by centrifuging for 2 minutes at 8000 rpm in soft mode.

Targeted next-generation sequencing was then performed according to established techniques to search for variants in 61 known cardiomyopathy-associated genes. Identified variants were classified according to established American College of Medical Genetics and Genomics criteria.

In the proband, we found a previously described Dutch founder mutation, the pathogenic missense variant c.992G>A, p.(Arg331Gln) in the *LMNA* gene.[2] No other (likely) pathogenic variants were found. Therefore, a potential cause of death may be malignant ventricular

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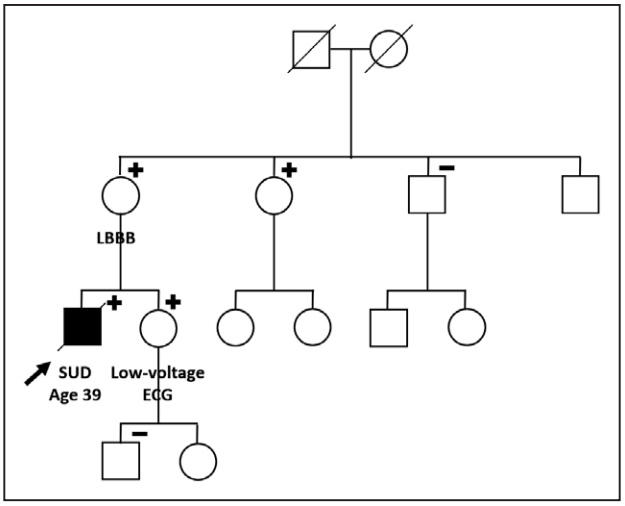

Figure. Pedigree of the reported case with the pathogenic variant c.992G>A, p.(Arg331Gln) in the *LMNA* gene.
Proband is indicated with an arrow. Square symbols indicate male, and circles indicate female. Diagonal lines through symbols indicate deceased. Solid symbol indicates sudden unexpected death. + indicates an individual harboring the pathogenic c.992G>A *LMNA* variant. — indicates a family member who was tested but does not carry the variant. LBBB, left bundle branch block; and SUD, sudden unexpected death.

arrhythmia, which is more likely to occur in individuals harboring a pathogenic *LMNA* variant, especially in males.[2,3] The pathogenic *LMNA* variant was also found in his sister, mother, and aunt. They were advised to be regularly reevaluated by their cardiologist according to current guidelines.[4] First-degree relatives were advised via family letter to seek a referral to a genetics department to be tested (from age 12 onwards) for the pathogenic *LMNA* variant.

To conclude, deciduous teeth are an alternative source of DNA in the postmortem setting when no blood or tissue is available. It may allow the identification of the underlying genetic cause of death, providing clarity and enabling targeted genetic and clinical evaluation in relatives. This avoids unnecessary broad genetic workup in relatives, with possible findings of unknown significance. To the best of our knowledge, we are the first to describe this alternative approach. However, for forensic purposes, like identifying disaster victims, missing person cases, and crime resolution, postmortem genetic testing on teeth has been successful for decades.[5] Preserving deciduous teeth in a decorative box is a widespread tradition in the Western world. It is important clinicians are aware of this and, as a practical implication, ask family members if there are any deciduous teeth available from the deceased family member when genetic testing is warranted, and no blood or tissue is available.

ARTICLE INFORMATION

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Disclosures

None.

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