KRAS GENE GENOTYPING IN TATTOOED INDIVIDUALS

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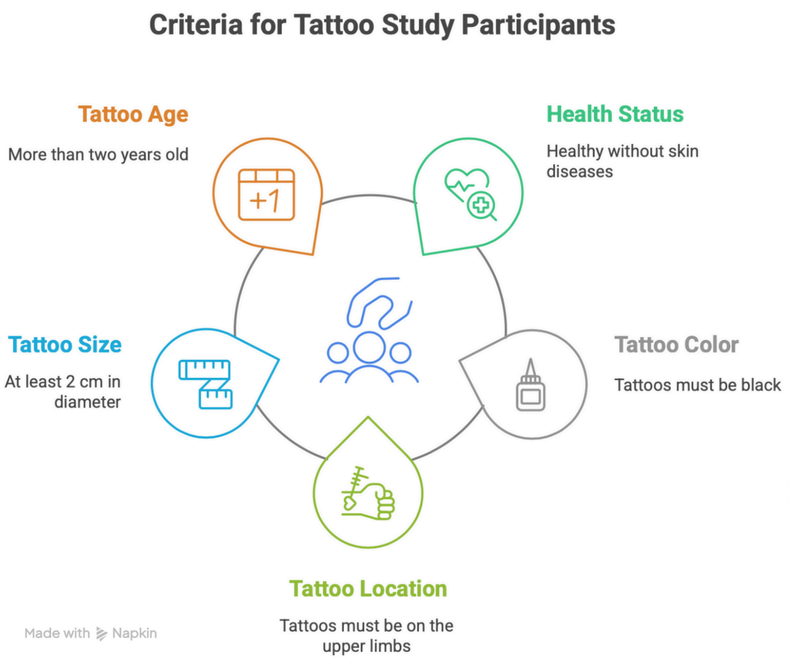
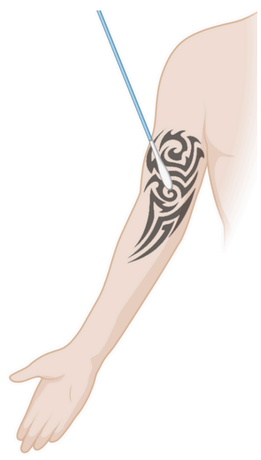
INTRODUCTION AND AIMS

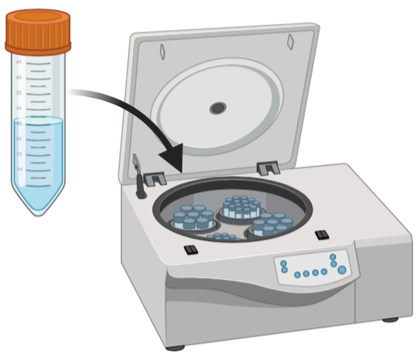
Mutations in the ***RAS* family** of proto-oncogenes, particularly the ***KRAS* gene**, have been identified as key factor in the development of various **skin cancers**, including melanomas and squamous cell carcinomas (1). The most common mutation occurs in **codon 12**, altering the functionality of the GTPase protein, which is involved in cell signal transduction. Cases of skin neoplasms have also been reported in **tattooed individuals**, raising questions about whether the ink, the mechanical trauma of the needle or photoaging of the skin could be triggering factors. However, despite these observations, there is still a lack of robust scientific evidence directly linking tattoos to specific genetic mutations (1)(2). This study aimed to investigate the presence of codon 12 mutations in the *KRAS* gene in tattooed students at the **Egas Moniz School of Health and Science**, and to assess the **feasibility of detecting them in a laboratory setting**.

MATERIALS AND METHODS

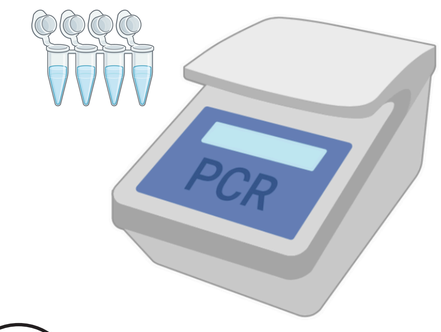




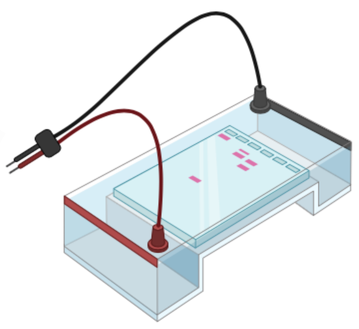
1 DNA was collected by scraping the skin with **wet swabs**.



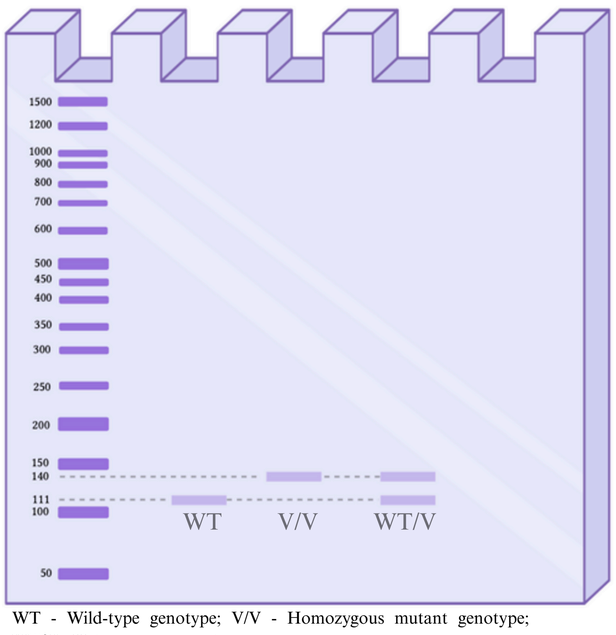
2 In order **to extract a pure sample**, it was necessary to use a **DNA isolation kit** (NZYtech), whose methodology is based on silica-coated centrifugation columns.



3 The *KRAS* gene was amplified using **Nested PCR**.



4 Mutation analysis consisted of digesting the amplified product with the **BstNI restriction enzyme**, followed by **gel electrophoresis**.



WT - Wild-type genotype; V/V - Homozygous mutant genotype; WT/V - Heterozygous genotype.

RESULTS

Only five of the 16 participants had DNA of sufficient quality for molecular analysis. Of the ten valid samples in total, two per participant (5, 6, 7 and 8), eight exhibited a fragmentation pattern characterised by a **single 111 bp band and a wild-type genotype (WT)**. This finding indicates an absence of mutations in codon 12 of the *KRAS* gene.

Samples from participant number 1 exhibited a distinctive pattern, characterised by the presence of a **single 140 bp band and a homozygous mutant genotype (V/V)** in both the arm bearing the tattoo and the non-tattooed arm.

The remaining 22 samples, corresponding to 11 participants, were excluded from the study due to the low concentration of genetic material, its degradation, or its absence, which impeded the interpretation of the results. The negative control, corresponding to well number 2 in the PCR reactions, exhibited no visible bands after amplification and enzymatic digestion.

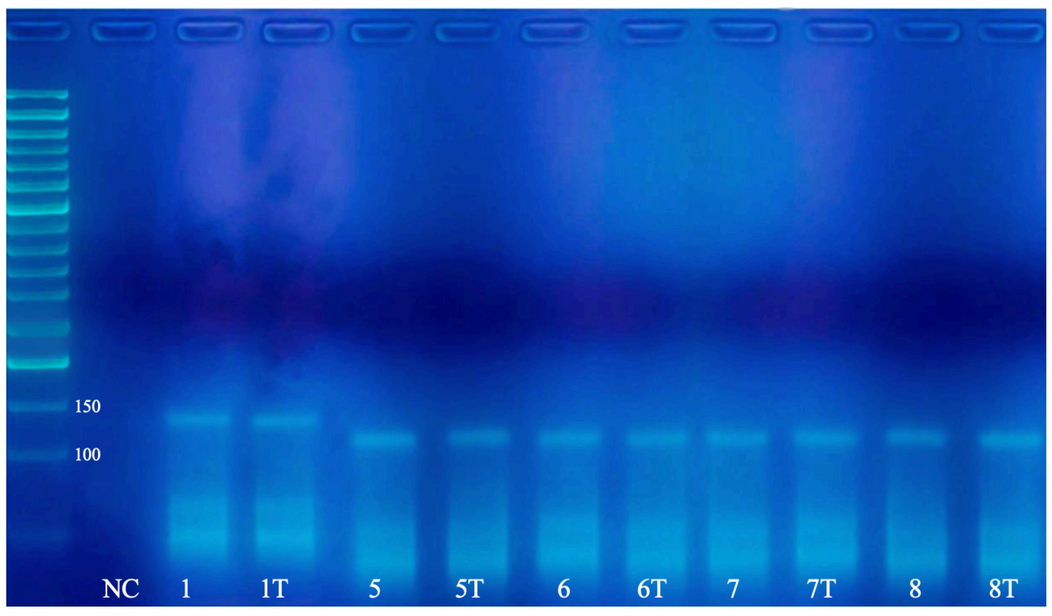


Figure 1 : Band profiles obtained by agarose gel electrophoresis (4%), organised by participant number. The 'T' indicates the sample taken from the tattooed arm and 'NC' indicates the negative control.

CONCLUSION

Although a **homozygous mutation** was identified in one of the participants, this may not be directly related to exposure to tattoo ink, but rather may have a hereditary origin. Notwithstanding the **inherent limitations** of the study, including the **limited number of viable samples** and the **absence of genetic sequencing confirmation**, the preliminary results indicated **the approach's promise as a foundation for subsequent research**. However, the data also demonstrates significant deficiencies in the methodology employed, particularly with regard to the **quality of the extracted DNA**, which may have compromised the efficiency of amplification in a substantial proportion of the samples. In order to overcome these constraints, **alternative approaches could be tested**, such as the use of an alcohol solution to clean the skin in advance in order to remove dead cells, or the collection of hair follicles as a source of DNA instead of skin scraping.

REFERENCES

(1) Emmert, S., Schön, M. P., & Haenssle, H. A. (2014). Molecular biology of basal and squamous cell carcinomas. Advances in experimental medicine and biology, 810, 234–252. https://doi.org/10.1007/978-1-4939-0437-2_13
(2) Wang, Q. J., Yu, Z., Griffith, K., Hanada, K. I., Restifo, N. P., & Yang, J. C. (2016). Identification of T-cell Receptors Targeting KRAS-mutated Human Tumors. Cancer immunology research, 4(3), 204. https://doi.org/10.1158/2326-6066.CIR-15-0188