

# XXIII Encuentro de Cooperación Farma-Biotech

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28 de noviembre de 2023

**Thyromethyl, a method for the diagnosis of thyroid cancer  
based on epigenetic biomarkers**



***Mario Fernández Fraga***



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## The Institution

# Cancer Epigenetics and Nanomedicine



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## Endocrinología, nutrición, diabetes y obesidad

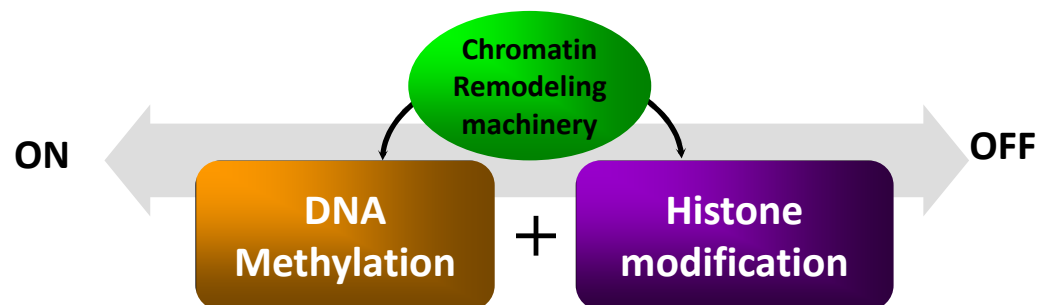
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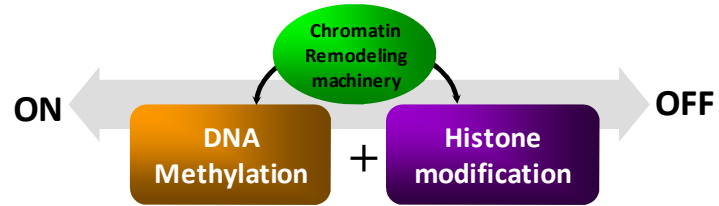
# Epigenetics

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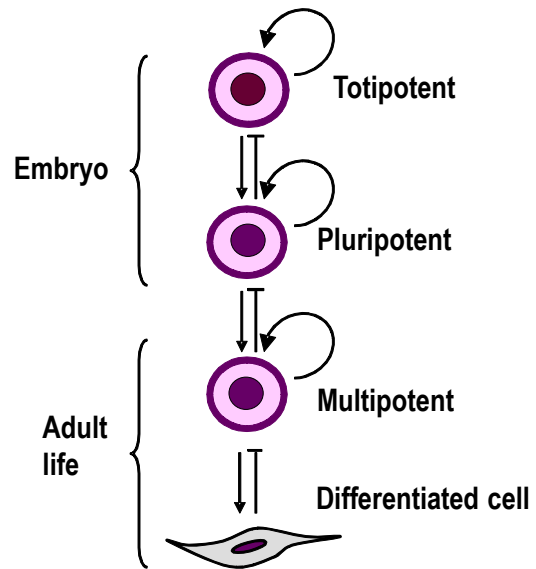
“The study of stable genetic modifications that result in changes in gene expression and function without a corresponding alteration in DNA sequence”



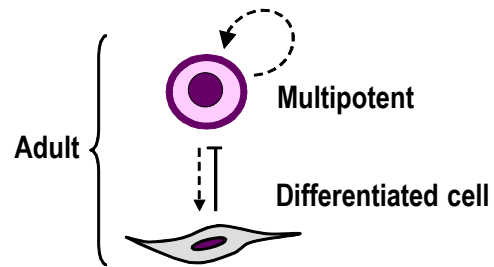
# Epigenetics



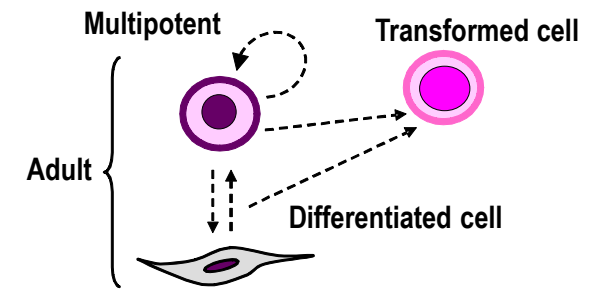
## Development



## Aging (age and external factors)



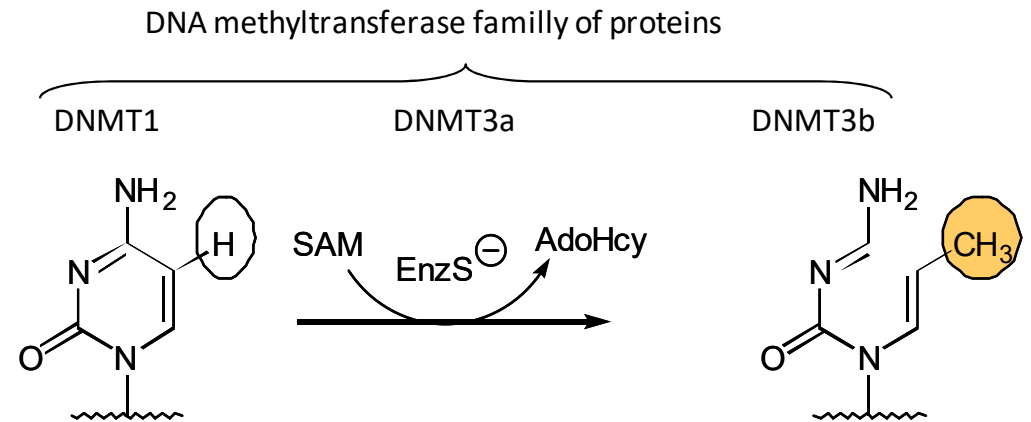
## Cancer



# DNA Methylation

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- DNA methylation is a process by which methyl groups are added to the DNA molecule.
- The reaction is catalyzed by a family of proteins called DNA methyltransferases.

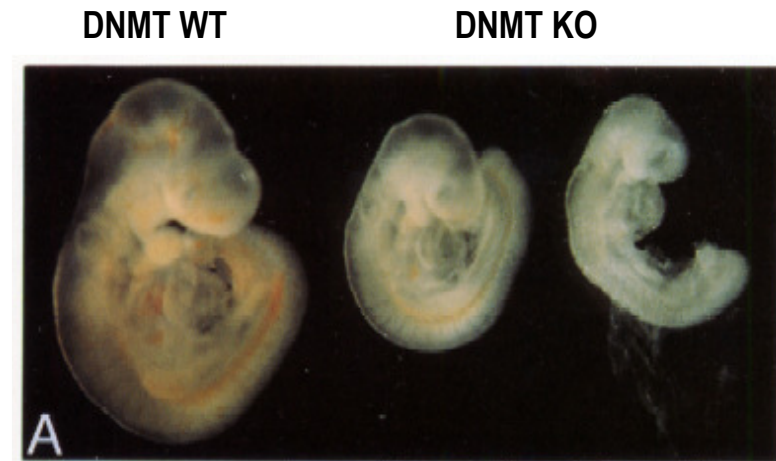


Fraga et al., 2009. Curr Opin Immunol.

## DNA Methylation is essential for mammalian life

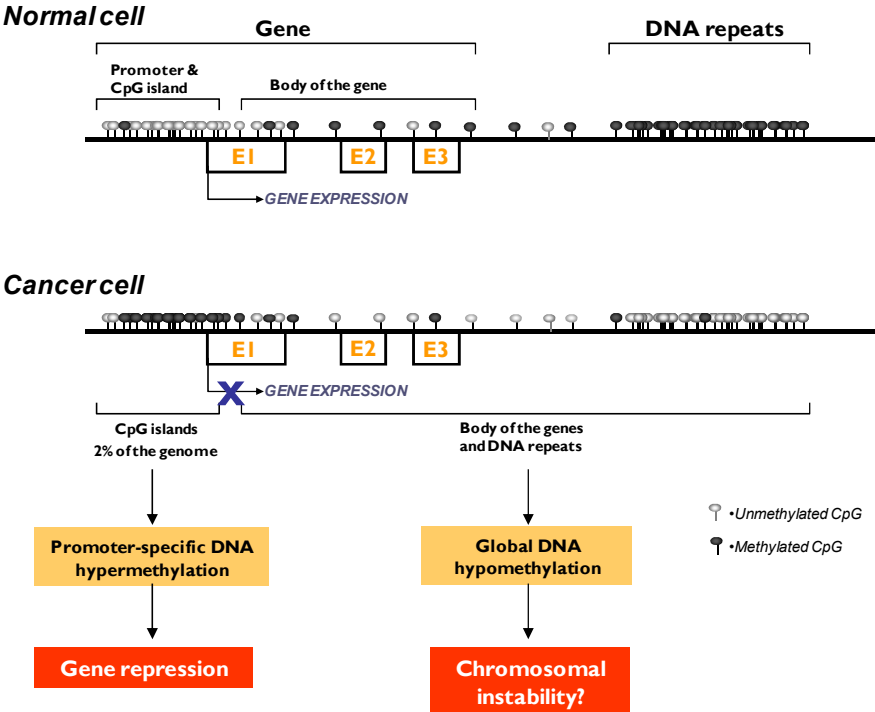
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- Genomic DNA methylation is essential for life in mammals
- Mice deficient in DNA methyltransferases die during the first stages of embryonic development.



# DNA Methylation is altered in cancer

- Global DNA hypomethylation
- CpG island promoter hypermethylation
- **Aberrant patterns of DNA methylation are specific to tumor type and subtype**

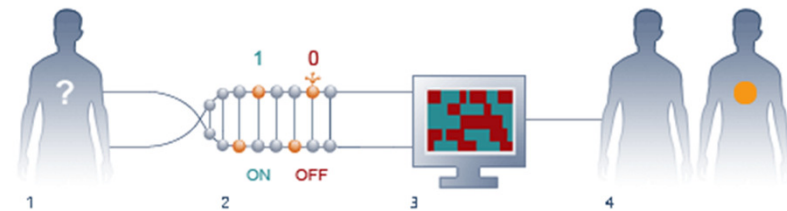




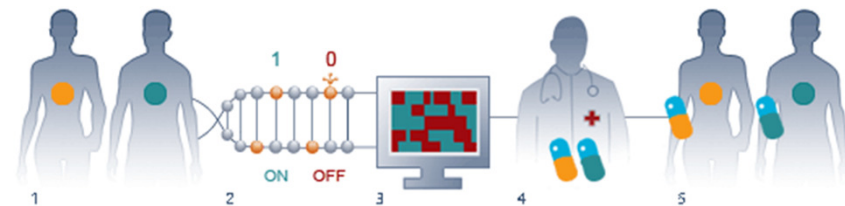
# DNA Methylation biomarkers in cancer

- Aberrant DNA methylation in cancer has clinical applications in diagnosis and pharmacodiagnosis

## •Diagnostic tests (screening, monitoring, classification)



## •Pharmacodiagnostic tests (responder/non-responder)



## The clinical problem: Unnecessary surgery in patients with thyroid nodules

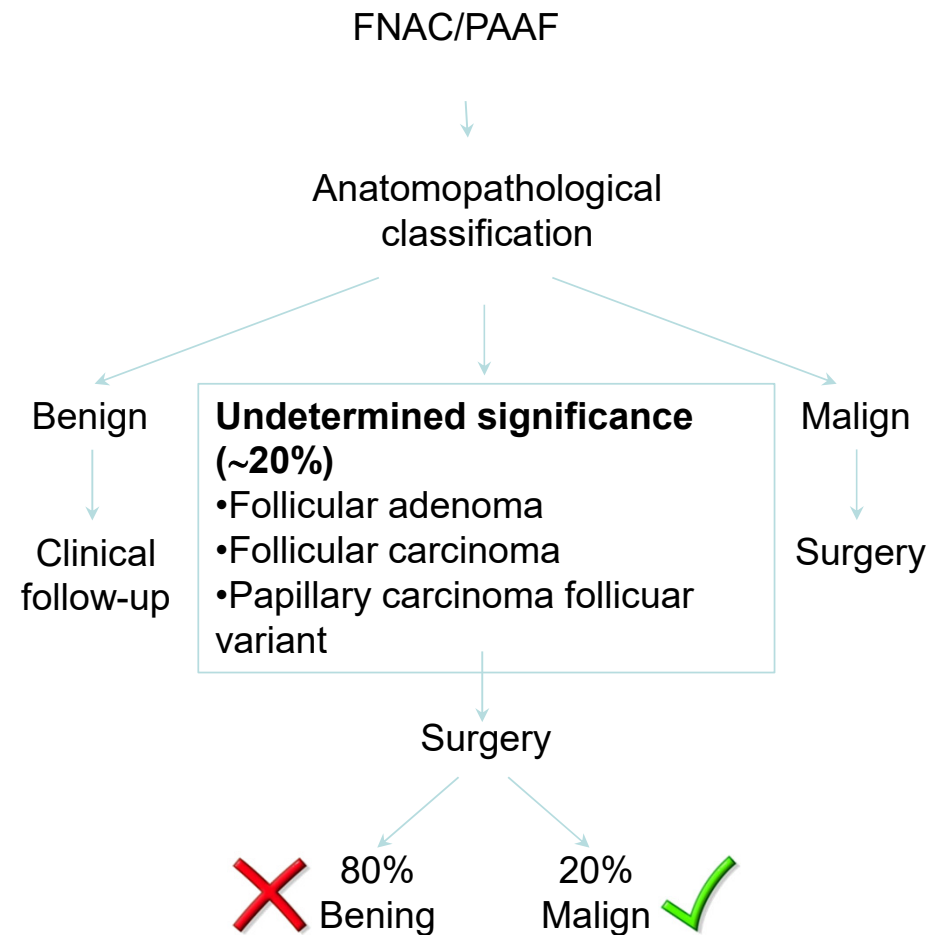
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- Surgical resection of 80% of the thyroid nodules with undetermined anatomopathological significance is unnecessary.
- DNA methylation biomarkers to help anatomopathological classification

## DNA methylation biomarkers in thyroid cancer

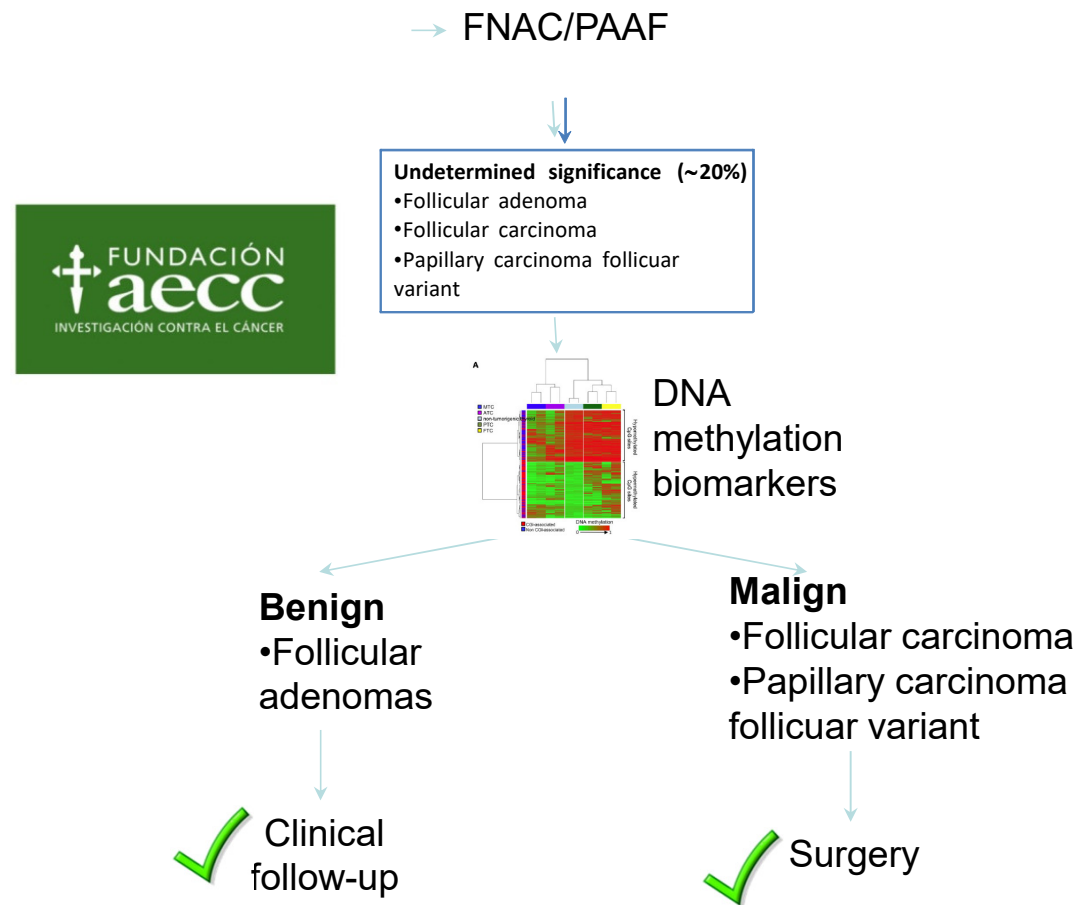
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- Fine needle aspiration cytology (FNAC/PAAF) is commonly used in the diagnosis thyroid cancer.
- Lesions with undetermined cytology are surgically removed. However, surgery biopsies reveal that 80% of them are benign and, consequently, that surgery was unnecessary.



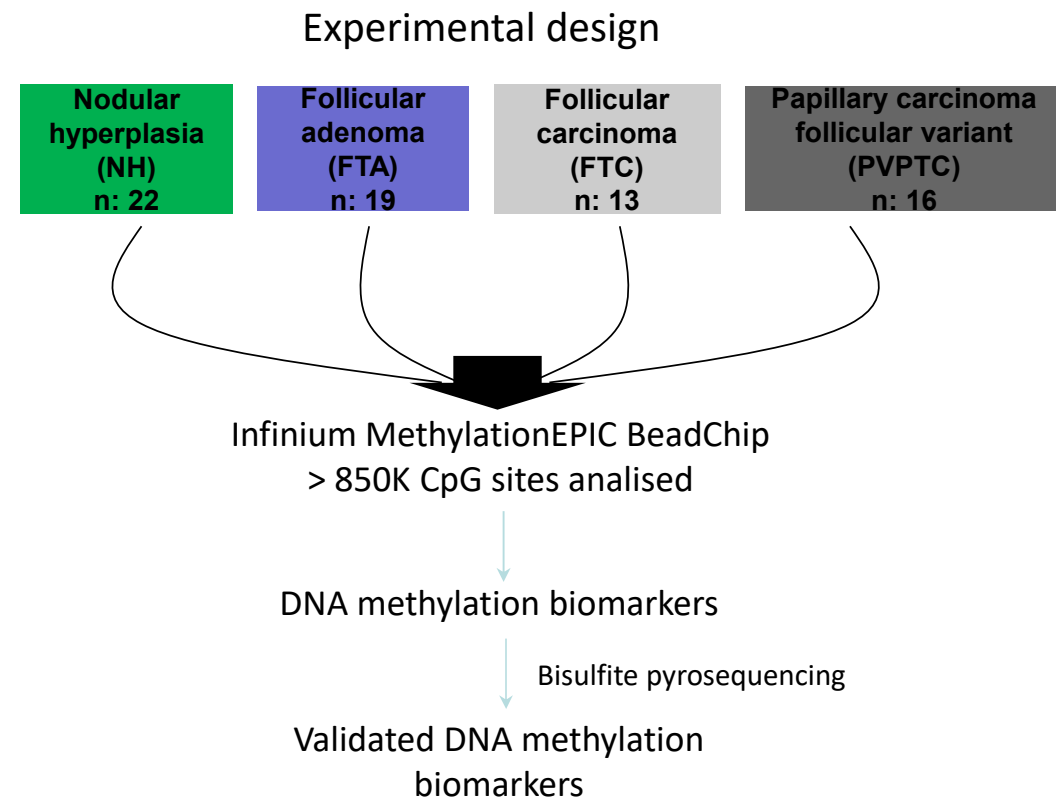
# DNA methylation biomarkers in thyroid cancer

- Improvement of the diagnostic yield of fine needle aspiration cytology (FNAC) samples by the detection of DNA methylation markers, and its application for the early diagnosis of Follicular Thyroid Carcinoma
- AECC grant.



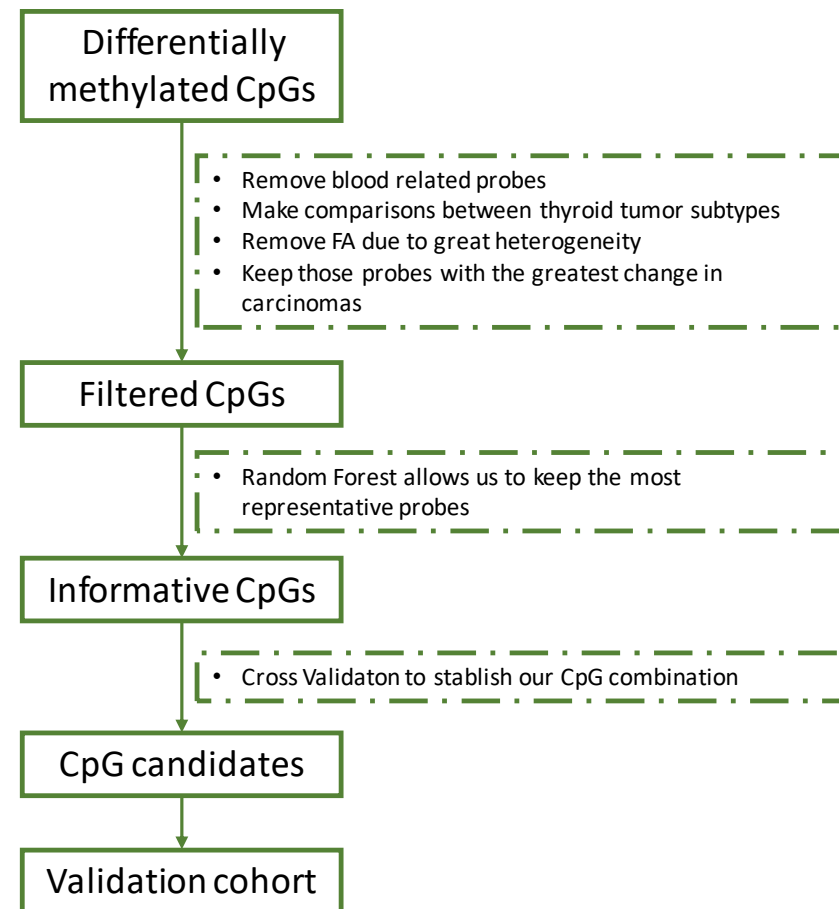
# DNA methylation biomarkers in thyroid cancer

- Discovery cohort: Nodular hyperplasia (n=22), Follicular adenoma (n=19), Follicular carcinoma (n=13), Papillary carcinoma follicular variant (n=16).
- Validation cohort: Nodular hyperplasia (n=84), Follicular adenoma (n=39), Follicular carcinoma (n=26), Papillary carcinoma follicular variant (n=36).



# DNA methylation biomarkers in thyroid cancer

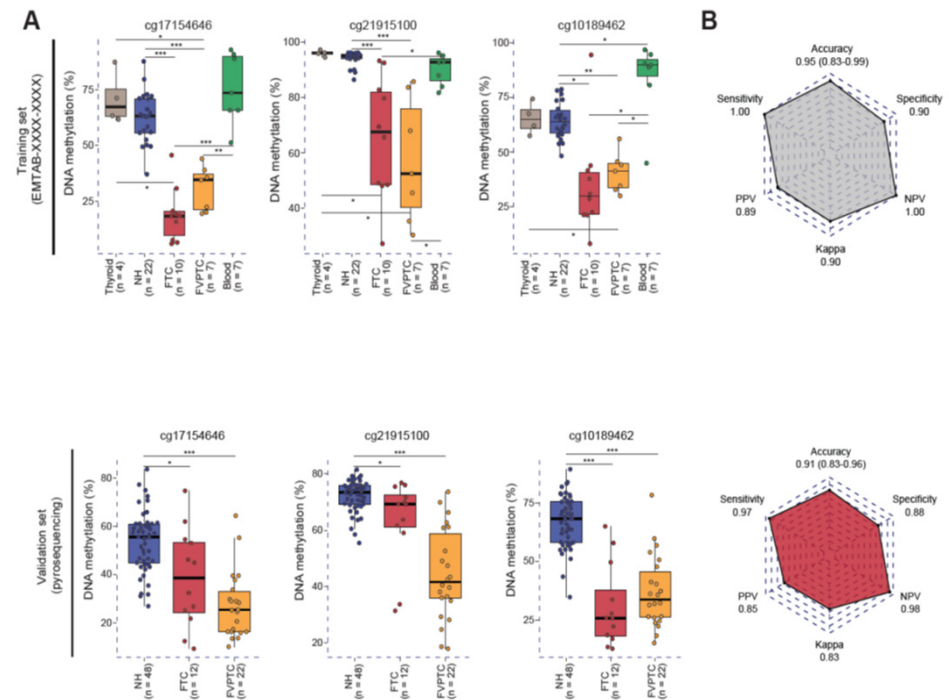
- A two-step data analysis strategy
- Classical approach to identify differentially methylated CpG sites and random forest approach to identify a minimal set of informative CpG sites





# DNA methylation biomarkers in thyroid cancer

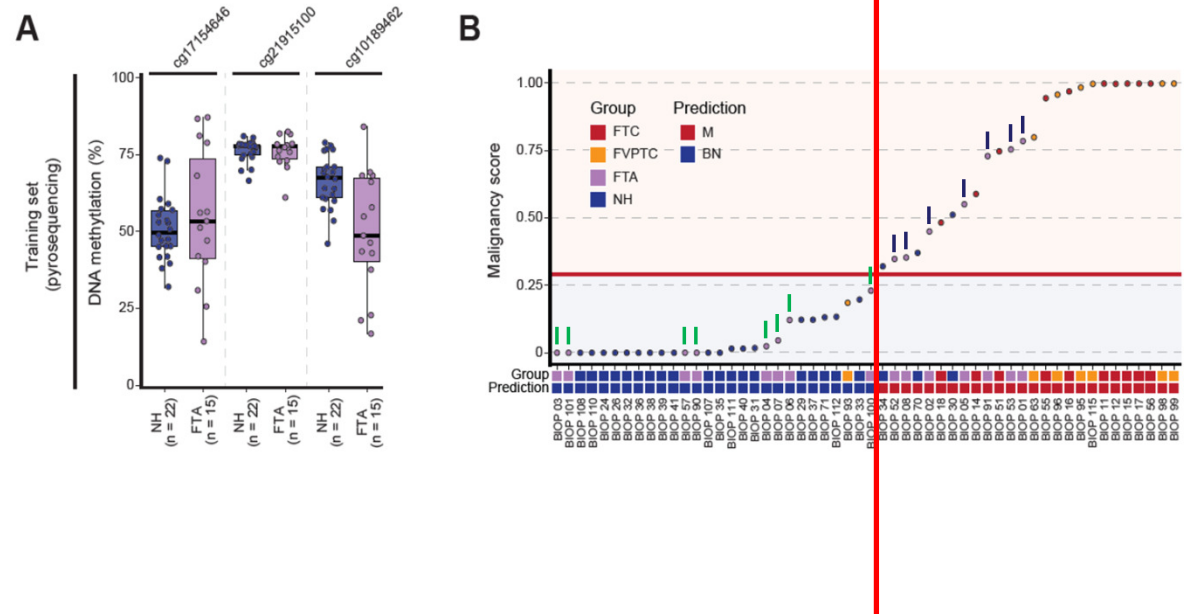
- Identification of the most informative CpG sites using random forest classification.
- Validation of these CpG sites in an independent cohort (Methylation arrays)
- Validation of these CpG sites in an independent cohort (Pyrosequencing)





# DNA methylation biomarkers in thyroid cancer

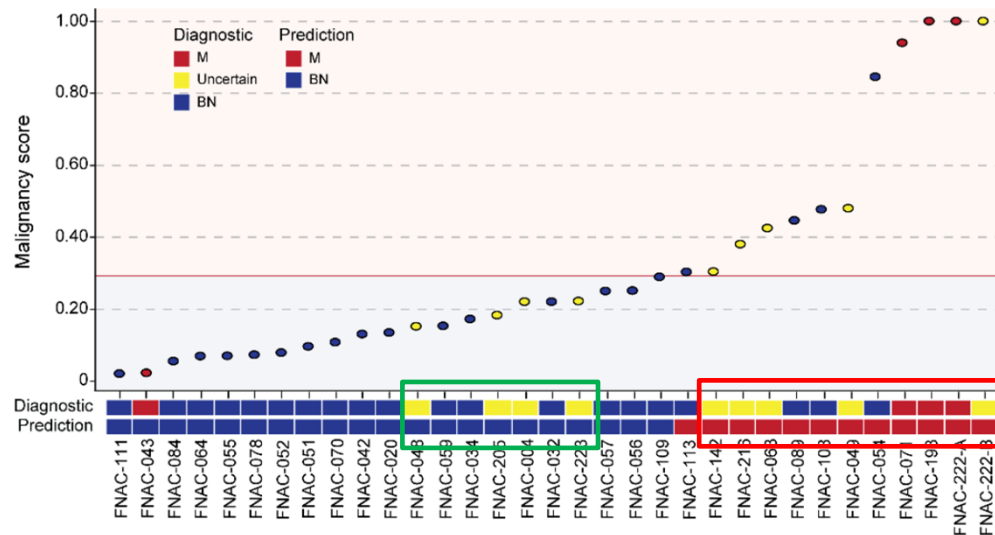
- Follicular adenomas present a heterogenous behavior.
- A malignant score can stratify thyroid lesions accordingly to risk of malignancy.



# DNA methylation biomarkers in thyroid cancer

## Validation of the epigenetic classification system of thyroid lesion malignancy in Thyroid nodules (FNACs)

- 33 FNACs
- 9 Uncertain



### Acknowledgement of receipt

We hereby acknowledge receipt of your request for grant of a European patent as follows:

Submission number	300441836
Application number	EP22382165.3
File No. to be used for priority declarations	EP22382165
Date of receipt	25 February 2022
Your reference	EP1641.1719
Applicant	CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
Country	ES
Title	EPIGENETIC BIOMARKERS FOR THE DIAGNOSIS OF THYROID CANCER



### JOURNAL ARTICLE

## Classification of follicular-patterned thyroid lesions using a minimal set of epigenetic biomarkers [Get access >](#)

Sandra Rodríguez-Rodero, Paula Morales-Sánchez, Juan Ramón Tejedor, Andrés Coca-Pelaz, Cristina Mangas, Alfonso Peñarroya, Iván Fernández-Vega, Luís Fernández-Fernández, Carmen M Álvarez-López, Agustín F Fernández ... [Show more](#)

*European Journal of Endocrinology*, Volume 187, Issue 3, Sep 2022, Pages 335–347, <https://doi.org/10.1530/EJE-22-0012>

Published: 19 July 2022 [Article history](#) ▼

Rodriguez-Rodero et al., 2022  
Patented



## Target Indications

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- Public and private hospitals who treat patients with thyroid tumors
- In Spain about 50,000 PAAFs are performed every year. Therefore, about 10,000 patients per year could benefit from the use of this product.
- In our hospital, about 450-500 PAAFs per year.

## Innovative mechanisms of action

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### *Epigenetic vs Genetic biomarkers*

- Plasticity and Environmental Response
- Dynamic and Temporal Changes
- Better Reflection of Current Cellular Function
- Potential to Identify Modifiable Risk Factors

## Differential features facing the market

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### ThyroMethyl

- Simplicity: 60-70 targets vs 3
- Sensitivity, specificity, etc.
- Availability of Pyrosequencers in public hospitals. E.g. Thyroidprint requires the purchase of costly equipment
- Valorization of national product. None of the similar products in the market have been developed in Spain
- Connections with the Spanish Public Health System, ISCIII, Public Hospitals, CIBER, etc. The product will be used by clinicians throughout Spain who have participated in its development.

## Current status of development

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TLR5: Validated with clinical samples

Biomarker identification (machine learning).

Versatility in multiple technologic environments  
(arrays, pyroseq)

Validation of these results with additional biopsies  
with external cohorts

Proof of concept in a relevant clinical setting (PAAFs)

## Pitfalls & Risks to be considered

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Standardizing protocols for extracting DNA from PAAF biopsies

Standardizing protocols for subsequent DNA methylation analyses,  
implementation in the clinical practice

## Partnering Opportunities

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Expand the methodology in novel clinical trials focused in the implementation of diagnostic biomarkers of thyroid cancer cohorts

Develop molecular extraction kits for the implementation of a standardized conditions across laboratories.

Implementation of an easy-to-use web interface to analyze data from the prospective cases.