HUMAN CANCER GENETICS PROGRAMME

The Human Cancer Genetics Programme (HCGP) is a translational research programme working on areas related to genetics, genomics, pharmacogenetics, molecular cytogenetics and the environmental bases of human cancer.

The Programme's interests focus on: (1) identifying new genetic and non-genetic factors related to cancer predisposition and risk in order to guide risk-stratified screening strategies towards personalised cancer prevention and treatment; and (2) understanding the molecular mechanisms involved cancer to facilitate the development of targeted therapies and early diagnostics.

In 2022, HCGP was composed of 2 Research Groups: Hereditary Endocrine Cancer (HECG) and Genetic and Molecular Epidemiology (GMEG); and 3 Units: Human Genotyping-CEGEN, Molecular Cytogenetics and the Familial Cancer Clinical Unit. In addition, the Programme includes a Familial Cancer Consultancy for the evaluation of families with cancer and the provision of genetic counselling, which is located at the *Hospital Universitario de Fuenlabrada*.

The Programme works in close collaboration with the clinical community to foster cooperation in genetic diagnosis and research, and to promote training and education. In 2022, 729 patients visited the Familial Cancer Consultancy at the *Hospital de Fuenlabrada*, and the HCGP performed 1,884 genetic diagnoses and carried out 2,102 cytogenetic studies. In terms of research, the Programme's members participated in collaborative studies involving a network of more than 30 hospitals from our National Health System, not only to conduct collaborative studies but also to facilitate clinical translation. The Programme also offers professionals and students from different national and international research centres the opportunity to join, either as visitors or for training visits consisting of short-term stays of 1-3 months; a total of 27 national visitors and students and 4 medical residents from different Spanish hospitals were hosted in 2022. The Programme's education activities in 2022 comprised a total of 17 national and 2 international PhD research projects, including the successful defence of 3 doctoral theses.

The Programme has established research collaborations with national and international groups; this is well demonstrated by its publication record as well as the key roles held by several of the Programme's members in consortia and international projects. In this regard, in 2022, several of the GMEG members, led by Núria Malats, and the European Molecular Biology Laboratory (EMBL) in Heidelberg, led by Peer Bork, in collaboration with the CNIO Epithelial Carcinogenesis Group led by Paco Real, and the CNIO Molecular Cytogenetics Unit led by Sandra Rodríguez-Perales, conducted an international comprehensive study on the influence of the microbiome on pancreatic cancer, published in the prestigious Journal *Gut*.

Also, especially noteworthy is the contribution of several of the HCGP's Group members to IMPaCT, an initiative of the *Instituto de Salud Carlos III* to Promote Precision Medicine in our country.

Milestones and major achievements of the HCGP in 2022 include:

- Mercedes Robledo: Within the field of personalised precision medicine co-funded by NextGenerationEU, "Development and implementation of a functional genomics validation platform for undiagnosed hereditary cancer."
- Núria Malats: Chairs ALIPANC, the Alliance of Pancreatic Cancer Research in Spain with 50 scientific groups.
- Núria Malats has contributed to (1) the pancreatic cancer field by proposing a high accuracy faecal metagenomic classifier and (2) the methodological field by proposing a new Mendelian randomization to avoid collider bias.
- Anna González-Neira, Javier Benítez, Ana Osorio: Two multicentre studies in breast cancer as a result of the "Breast Cancer Risk after Diagnostic Gene Sequencing" BRIDGES H2020 Project – "Pathology of tumors associated with pathogenic germline variants in 9 breast cancer susceptibility genes" (*JAMA Oncology*) and "Breast cancer risks associated with missense variants in breast cancer susceptibility genes" (*Genome Medicine*).
- Maria Currás, Ana Osorio: "A large case-control study helps identify a new candidate gene for breast cancer predisposition" (*Cancers*).
- Cristina Rodríguez-Antona: Listed in the "World Ranking Top 2% Scientists", 2022 edition of the Stanford University list of World Top 2% scientists.

One of the main strategic initiatives of the CNIO at present is the consolidation of a new Cancer Genomics Programme. HCGP groups will become part of the new Programme. I am confident that, under this new strategic action, the Programme will continue to grow and further develop research in the field of cancer genomics.

Lastly, I would like to take this opportunity to thank our former Head of the Familial Cancer Clinical Unit, Miguel Urioste, for his dedication and for placing the CNIO as a reference in the field of familial cancer genetic diagnostics. Thank you, Miguel, for having been a part of our CNIO community and we wish you the best on your retirement! I would also like to thank Ana Osorio and Alicia Barroso for their outstanding work on the genetic diagnosis of breast cancer over the last 20 years.

Maria A. Blasco, Director

HEREDITARY ENDOCRINE CANCER GROUP

Group Leader
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Research Scientists
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Post-Doctoral Fellows
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Gradutate Students
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Technicians
Bruna Calsina (until March) (TS)*, Eduardo Gil (until December), Rocío León, Gabriela Roberta Radu (since December) (PEJ, CAM)**

**Titulado Superior* (Advanced Degree)

*** Plan de Empleo Joven de la Comunidad de Madrid* (Youth Employment Plan, Community of Madrid)

Student in Practice
Sara Gil (January-September)

(Master's Thesis, *Universidad Complutense* de Madrid, Spain)

Visiting Scientist
Noelia Herradón (*Hospital 12 de Octubre*, Madrid, Spain)

OVERVIEW

Our Group is mainly interested in identifying genetic risk factors involved in endocrine tumour susceptibility. Through a comprehensive analysis of tumour genomic features, we have been able to propose diagnostic and prognostic markers, to identify altered pathways that could be therapeutically targeted, and to identify new major susceptibility genes.

We are also interested in defining markers associated with differences in anticancer drug response and toxicity. We are applying targeted and whole-exome next-generation sequencing to a large series of clinically well-characterised patients. The aim is to identify new therapeutic approaches to personalise cancer treatment. These efforts will collectively improve the diagnosis, prognosis, and treatment of patients.

"We identified *PARP1* expression and *PBRM1* mutations as predictive markers of progression free survival in patients with clear cell renal cell carcinoma. In thyroid cancer, telomere shortening leads to a reorganisation of the 5p subtelomeric region, facilitating the accumulation of alterations at the *TERT*-locus."

RESEARCH HIGHLIGHTS

Massive sequencing technologies to advance pharmacogenomics knowledge

Next generation sequencing technologies have boosted the discovery and clinical implementation of novel markers of drug treatment response. We illustrate this with 2 contributions:

i) Generation of a comprehensive germline landscape of pharmacogenetic actionable variants contained in diagnostic exomes. In this study, we analysed data from 5001 individuals who underwent exome sequencing for genetic diagnosis to provide population frequencies of clinically relevant pharmacogenetic alleles and to estimate the contribution of novel loss-of-function variants.

ii) Identification of *PARP1* expression and *PBRM1* mutation as predictive biomarkers in patients with clear cell renal cell carcinoma. Through analysis of genomic, transcriptomic, and clinical data of the IMmotion151 trial in patients treated with atezolizumab plus bevacizumab or sunitinib, we found that tumour *PARP1* expression was a predictor of progression-free survival regardless of treatment arm, while *PBRM1* mutations exerted an interaction only with sunitinib treatment (FIGURE 1).

Comprehensive molecular analysis of immortalisation hallmarks in thyroid cancer reveals new prognostic markers

Around 1 in 2000 individuals in Spain develop thyroid cancer fatal disease. Comprehensive molecular studies on thyroid tumours are needed to identify prognostic molecular biomarkers that will allow the early detection, and thus the personalised management and follow-up, of this rare but life-threatening cancer. We extensively characterised cancer immortalisation-related alterations in a series of 106 thyroid tumours enriched with clinically-aggressive carcinomas to define disease prognostic markers. Using a custom-designed RNA-seq panel, we identified 5 telomerase holoenzyme-complex genes over-expressed in clinically-aggressive tumours compared to tumours from long-term disease-free patients, with *TERT* and *TERC* denoted as independent prognostic markers by multivariate regression model analysis. Characterisation of alterations related to *TERT* re-expression revealed that promoter mutations, hypermethylation and/or copy gains exclusively co-occurred in clinically-aggressive tumours. Quantitative-FISH analysis of telomere lengths showed a significant shortening in these carcinomas, which matched with a high proliferative rate measured by Ki-67 immunohistochemistry. RNA-seq data analysis indicated that short-telomere tumours exhibit increased transcriptional activity in the 5 Mb-subtelomeric regions, site of several telomerase-complex genes. Gene upregulation enrichment was significant for specific chromosome-ends such as the 5p, where *TERT* is located. Co-FISH analysis of 5p-end and *TERT* loci showed a more relaxed chromatin configuration in short telomere-length tumours compared to normal telomere-length tumours. Overall, our findings support that telomere shortening leads to a reorganisation of the 5p subtelomeric region, facilitating the transcription and accumulation of alterations at the *TERT*-locus, and unveil a FISH-based assay as a potential cytogenetic tool to predict disease prognosis in thyroid cancer. ■

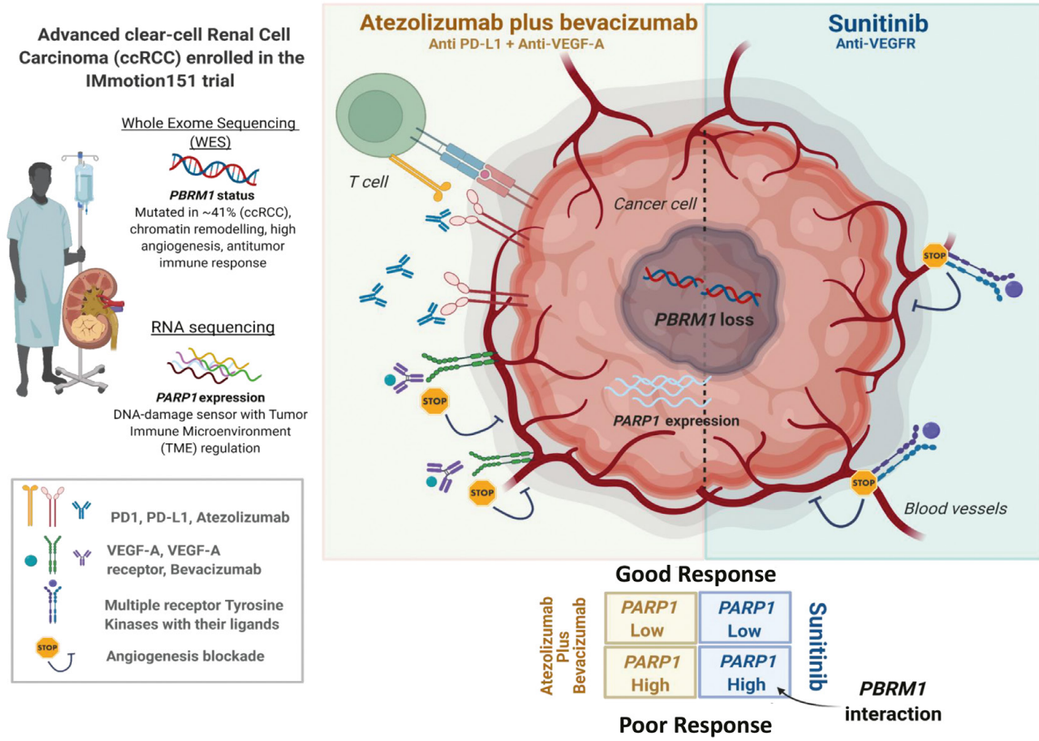

FIGURE 1 Low *PARP1* expression and *PBRM1* loss associate with improved Immunotherapy/antiangiogenic response in clear cell renal cancer patients.

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**Titulado Superior* (Advanced Degree)

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Sol Aletta (since Aug) (Rabdoud University, Nijmegen, The Netherlands), Tania Chadha (March-July) (*ETSIT*, Madrid, Spain), Daniel de San Sebastián (until July) (*UPM*, Madrid, Spain), Soraya Martín (April-June) (*IFP Corredor del Henares*, Spain), Harold Mena (Aug.-Oct.) (Del Rosario Univ., Bogotá, Colombia), Anaëlle Mescam (June-Aug.) (École de Biologie Industrielle, Cergy, France), Nataly Moreu (March-June) (*IES* Mirasierra, Spain)

Visiting Master's Students
Miguel Maquedano (until July) (*UAM*, Madrid, Spain), César Mediavilla (May.-Dec.) and Juana Serrano (since Aug.) (Master's in Bioinformatics, ISCIII-ENS, Madrid, Spain)

Visiting Scientists
Isabel A. Martín (*Univ. CEU San Pablo*, Madrid, Spain), Esther Molina (July-Sep) (*Univ. de Vic*, Barcelona, Spain), Ashwag M. Mukhtar (until May) (Al Neelain University, Sudan, Africa) (Science by Women Programme)

OVERVIEW

The scope of the research carried out by the Genetic and Molecular Epidemiology Group (GMEG) ranges from the identification of aetiological agents and genetic pathways to the translation of the findings into the clinical and public health domains, focusing on bladder, pancreatic, and breast cancers.

We employ a wide variety of biomarkers, including omics data, to better characterise exposures, genetic susceptibility patterns, and cancer outcomes. While omics data provide a unique opportunity in this regard, their integration with non-omics data poses important challenges, and GMEG explores this methodological field in epidemiologic studies.

The strategic goals of the Group are to:

- Identify non-genetic and genetic factors, as well as their interactions, associated with cancer development and progression, and with its molecular/omics subphenotypes.
- Develop and apply statistical/informatics tools to model the risk and course of patients with cancer by integrating epidemiological and clinical data with omics information.
- Assess clinical and public health strategies for cancer control using newly developed biomarkers and algorithms.

"Oral, faecal, and pancreatic microbiome dysbiosis are associated with pancreatic cancer, with stool microbiota-based classifiers that predict pancreatic cancer with high accuracy and specificity."

RESEARCH HIGHLIGHTS

Research findings

In 2022, GMEG contributed to the pancreatic cancer (PC) field by proposing a faecal metagenomic classifier that identifies PC with an accuracy of 0.84 area under the receiver operating characteristic curve (AUROC) in a Spanish cohort, based on 27 species. The accuracy improves up to 0.94 AUROC when combined with CA19–9 serum marker. The classifier was validated in an independent German PC cohort (0.83 AUROC), and PC disease specificity was confirmed against 25 publicly available metagenomic study populations with various health conditions (n=5792). The presence of marker taxa enriched in faecal samples (*Veillonella*, *Streptococcus*, *Akkermansia*) and also taxa with differential abundance in healthy and tumour pancreatic tissues (*Bacteroides*, *Lactobacillus*, *Bifidobacterium*) was validated by fluorescence *in situ* hybridisation (FIGURE 1). The presented PDAC-specific microbiome signatures, including links between microbial populations across tissues, provide novel microbiome-related hypotheses regarding disease aetiology, prevention, and possible therapeutic intervention. In addition, we also collaborated in elucidating that *GATA4* and *GATA6* cooperate to maintain the classical PC phenotype. We further explored the immune repertoire landscape of 9522 tumour and adjacent non-tumour samples across 28 tumour types in the Cancer Genome Atlas project, and performed diversity and network analysis. We identified differences in diversity and network statistics across tumour types and subtypes and observed a trend towards increased clonality in primary tumours compared to adjacent non-tumour tissues. Regarding **bladder cancer** (BC), GMEG participated in 1 study that delivered suggestive evidence for a multiplicative interaction between the most common class of disinfection by-products, trihalomethanes, and a bladder cancer susceptibility variant (rs907611). Furthermore, we contributed to the validation of BlaDimiR, a urine-based miRNA score for accurate bladder cancer diagnosis and follow-up.

Methodological contributions

We proposed an approach allowing Mendelian randomisation estimation in strata of the population while avoiding collider bias (FIGURE 2). This approach constructs a new variable, the residual collider, as the residual from regression of the collider on the genetic instrument, and then calculates causal estimates in strata defined by quantiles of the residual collider. The new approach generated unbiased estimates in all the simulation settings, and can be used to perform Mendelian randomisation studying heterogeneity among subgroups of the population while avoiding collider bias. Furthermore, GMEG continued exploring the analytic strategies and tools to integrate omics and non-omics data into the cancer risk models, and made progress in the integration of medical image information (radiomics and digital pathology).

Translational activities

GMEG actively supports several clinical trials of immunotherapy in BC at the methodological level. We continue to sustain the Spanish Familial PC Registry (PanGen-FAM) and the European Registry of PC (PancreOS). We chair the Spanish Alliance for Pancreatic Cancer Research (ALIPANC) to accelerate the translation of research results into the clinical and public health domains. We lead the Research Work Stream of the Pancreatic Cancer Europe (PCE) multistakeholder platform, and we have moved ahead in increasing awareness of PC. We also contributed to the publication of the UEG position paper on pancreatic cancer. Finally, we joined an initiative of the European Alliance for Personalised Medicine to express concerns that disrupting the current balance of the pharmaceutical legislation to meet objectives that are more precisely targeted could have unintended consequences in the EU, reducing rather than increasing the flow of innovative treatments for rare diseases. ■

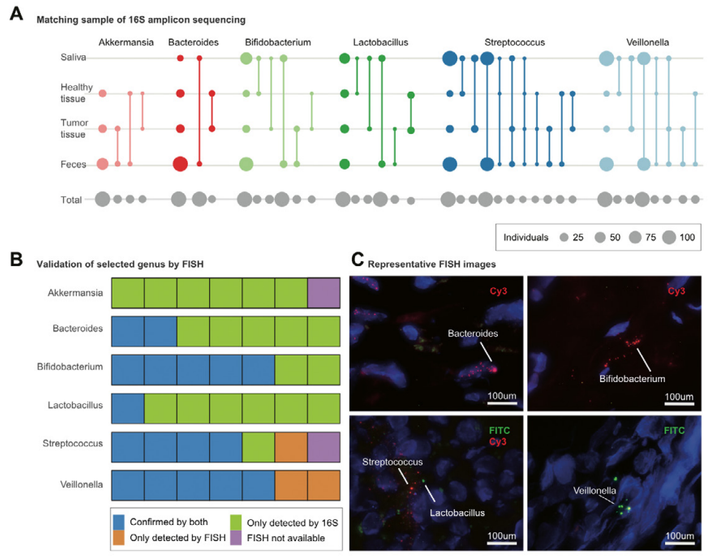

FIGURE 1 Presence of bacteria in 4 different body sites including faecal, saliva, pancreatic tumour and healthy tissue samples (**A**). Bacterial presence/absence with both 16S amplicon and FISH methods in 7 selected pancreatic tissue samples (**B**). FISH microscopy images for *Bacteroides* (intranuclear, tumour tissue), *Bifidobacterium* (extranuclear, tumour tissue), *Lactobacillus* (extranuclear, non-tumour tissue), *Streptococcus* (extranuclear, non-tumour tissue), and *Veillonella* (extranuclear, tumour tissue) (**C**).

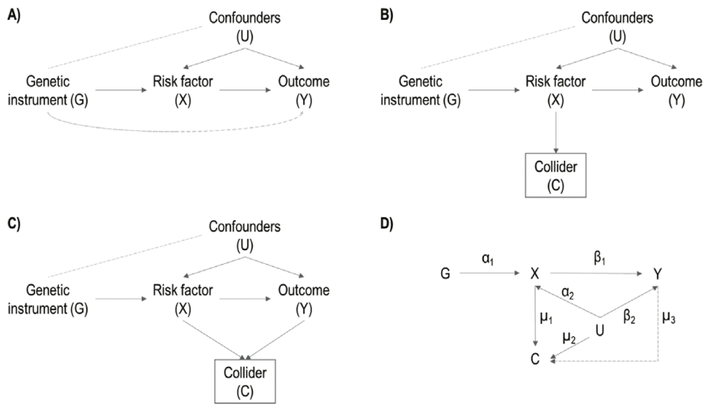

FIGURE 2 Directed Acyclic Graphs (DAGs) illustrating relationships between the variables. (**A**) Mendelian randomisation causal diagram with the instrumental variable assumptions. (**B**) DAG considering a collider variable C, being a common child of risk factor X and outcome Y. (**D**) DAG illustrating the variables and parameters used for the simulation study.

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Unit Head

Research Scientist
Raúl Torres

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Technicians
M. Carmen Martín, Francisco José Moya (TS)*(PEJ)**

Titulado Superior (Advanced Degree)
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Beatriz Álvarez and Daniel Lucena (Sep.-Dec.) (*CIB Margarita Salas*, Madrid, Spain), Carlos Carrasco (July.-Dec.) (*Univ. Complutense de Madrid*, Spain)

OVERVIEW

Recurrent chromosomal rearrangements, changes in the structure of native chromosomes, are very common and well-known hallmarks of cancer. A better understanding of these cancer-causing mechanisms will lead to novel therapeutic regimens to fight cancer. The research activity of the Molecular Cytogenetics and Genome Editing Unit focuses on increasing our knowledge about the role of chromosomal rearrangements in cancer development and progression and discovering new therapeutic targets. With the combined use of CRISPR genome editing and cytogenetic technologies, we are creating models that recapitulate chromosomal and genetic cancer alterations. The goal of the Unit is to provide CNIO and external researchers with the latest technologies used in the fields of molecular cytogenetics and genome editing. The Unit is continuously implementing and developing new technologies in those fields.

"In 2022, we applied genome engineering approaches to reproduce and eliminate chromosome rearrangements and gene alterations. We also provided access to the latest cytogenetic and CRISPR technologies."

We also participate in collaborative projects with clinical and basic science investigators across the CNIO and other institutions.

RESEARCH HIGHLIGHTS

Molecular cytogenetics. The "Optimization Optical Genome Mapping" (OGM, Bionano) technique, based on the analysis of ultra-high long DNA molecules, provides a genome-wide high-resolution analysis of copy number and structural cytogenetic variations. We are optimising the use of OGM technology in our Unit, comparing it with standard techniques (e.g., karyotyping, fluorescent in situ hybridisation) using hematologic and solid tumour fresh and frozen samples. We think OGM represents a promising complementary approach to existing cytogenetic techniques for the characterisation of cancer cells. OGM enables a time and cost-effective analysis allowing the identification of complex cytogenetic rearrangements, including some that are currently inaccessible to standard techniques.

Technological and translational activities. Approximately 20% of human cancers contain specific fusion oncogenes (FOs). Due to their tumour-specific expression, FOs offer unique advantages such as diagnostic and therapeutic targets.

→ FO targeted diagnosis: In the clinic, conventional diagnostic techniques like qRT-PCR, FISH, or NGS are routine. But these methods require specialised machinery and personnel, are expensive, time-consuming, and involve multiple steps.
→ FO targeted therapy: Many currently used treatments are non-selective, leading to severe side effects responsible for prolonged recovery and frequently resulting in relapses.

In this regard, we applied the RNA-targeting Cas13 system to selectively cleave FO transcripts. Contrary to the CRISPR/Cas9 method, RNA targeting with Cas13 results in reversible and temporally controllable alterations without modifying the DNA. Furthermore, compared to shRNAs, the Cas13 method is associated with high knockdown efficiency and no off-target effects, offering unique advantages when used for therapeutic purposes. Diagnostic methods based on Cas13 provide rapid RNA detection with attomolar sensitivity and single-base mismatch specificity. ■

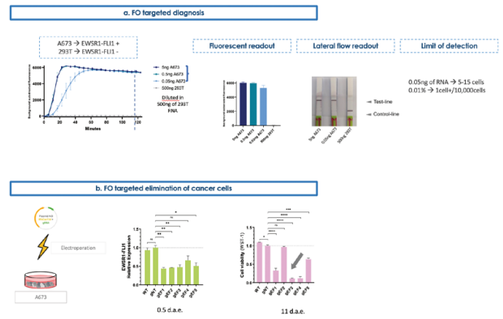

FIGURE 1 We have taken advantage of the versatile CRISPR/Cas13 system to: (a) develop a sensitive, specific, affordable, and instrument-free diagnostic test for FO detection in patient samples; and (b) silence FO RNA inducing efficient and selective elimination of cancer cells.

PUBLICATIONS

- Kartal E *et al.* (incl. Rodríguez-Perales S, Torres-Ruiz R, Real FX, Malats N) (2022) A faecal microbiota signature with high specificity for pancreatic cancer. *Gut* 71, 1359-1372.
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- Montero-Conde C *et al.* (incl. Moya FJ, Currás-Freixes M, Gonzalez-Neira A, Megías D, Blasco MA, Caleiras E, Rodríguez-Perales S, Robledo M) (2022). Comprehensive molecular analysis of immortalization hallmarks in thyroid cancer reveals new prognostic markers. *Clin Transl Med* 12, e1001.
- Baquero JM *et al.* (incl. Torres-Ruiz R, Blanco-Aparicio C, Rodríguez-Perales S, Benítez J) (2022). OGG1 inhibition triggers synthetic lethality and enhances the effect of PARP inhibitor olaparib in BRCA1-deficient TNBC cells. *Front Oncol* 12, 888810.
- Puig-Serra P, Casado-Rosas MC, Martinez-Lage M, Olalla-Sastre B, Alonso-Yanez A, Torres-Ruiz R*, Rodriguez-Perales S* (2022). CRISPR approaches for the diagnosis of human diseases. *Int J Mol Sci* 23, 1757. (*) Co-corresponding authors.

PATENT

- Malats Riera N, Bork P, Kartal E, Molina Montes E, Rodríguez S, Estudillo L, Real FX, Schmidt TSB, Zeller G, Wirbel J, Maistrenko OM. Faecal Microbiota Signature for Pancreatic Cancer. PCT application (2022). *PCT/EP2022/077087. WO2023052486A1.*

AWARDS AND RECOGNITION

- Raul Torres-Ruiz has received the 2022 ESGCT Young Investigator Award from the European Society for Gene and Cell Therapy (ESGCT).

FAMILIAL CANCER CLINICAL UNIT

Maria Currás
Clinical Unit Head

Research Scientist
Ana Osorio (until September)

Gradutate Student
Erik Michel Marchena (PEJ, CAM)*

Plan de Empleo Joven de la Comunidad de Madrid (Youth Employment Plan, Community of Madrid)

OVERVIEW

The Unit's activity is divided into 2 main areas:

1. *Genetic diagnosis of cancer patients, especially those at a young age, with multiple tumours or other family members with cancer.* Elucidating hereditary cancer helps the physician to decide on appropriate treatment and, for risk relatives, to initiate preventive strategies if they are carriers. We work mainly with colorectal cancer/Lynch syndrome and breast/ovarian cancer, but we are also a referral unit for rare genetic-based and cancer-related diseases. We provide genetic diagnosis at the Familial Cancer Consultancy (FCC) of the University Hospital of Fuenlabrada (UHF), but also in other hospitals in Madrid and the rest of Spain.
2. *Research work on the elucidation of genetic factors related to familial breast and colorectal cancer*. We focus on identifying new driver genes and clarifying their role in patient management. In addition, we are interested in genetic risk factors, prognostic markers, and genetic and molecular factors that could affect therapeutics.

"The Familial Cancer Clinical Unit (FCCU) has confirmed *RECQL5* as a novel breast cancer gene and has been involved in understanding the role of OGG1, *BRCA* carrier modifiers, and the first germline biallelic mutation in *MAD1L1*."

Technicians
Alicia Barroso (until September), Victoria Fernández (TS)*, Verónica García (TS)*, Maika González-Neira, Fátima Mercadillo

Titulado Superior (Advanced Degree)

Master´s Student
Milton Eduardo Salazar (Jan.-Sep.) (*Universidad Complutense de Madrid*, Spain)

RESEARCH HIGHLIGHTS

Clinical and diagnostic activity. The catalogue of genes has been updated and expanded to tumours that were not previously covered. 729 patients visited our consultancy at the UHF, and 1884 genetic studies were carried out in the FCCU laboratory.

Elucidating new breast cancer (BC) genes. We found a statistically significant association between loss-of-function variants in the *RECQL5* gene and BC risk in almost 2000 index cases of Spanish BC families, supporting its role as a novel moderate-risk BC gene.

Understanding the role of new variants in moderate-risk BC genes. Through a mutational analysis of the *BARD1* gene, in a cohort of 1946 Spanish patients with BC using NGS, we saw that the prevalence and spectrum of *BARD1* mutations could vary between different regions of Spain and highlighted the relevance of analysing copy number variations.

New BC therapeutic approaches. Osorio was involved in the discovery that TH5487, an inhibitor of 8-oxoguanine DNA glycosylase 1 (OGG1), increases sensitivity to the PARP inhibitor Olaparib, especially in the context of BRCA1 deficiency. She was also involved in the description of TH10785, which increases OGG1 recruitment and repair of oxidative DNA damage that may have therapeutic applications.

Contributions to the diagnosis and clinical follow-up of PTEN hamartoma tumour syndrome (PHTS). In the largest study performed in the Spanish population with clinical features of PHTS (n = 145), we concluded that to improve clinical diagnosis we should focus on macrocephaly, mucocutaneous lesions, obesity, and gastrointestinal polyposis. We pointed out the importance of regular weight control and of considering cancer screening at an earlier age. In addition, we participated in an extensive functional characterisation of variants of unknown significance identified in patients with PHTS.

Mosaic variegated aneuploidy (MVA). Urioste was involved in the description of the first germline biallelic mutation in *MAD1L1* as a novel cause of aneuploidy in an individual with no intellectual disability and an unprecedented number of neoplasias, including 5 malignant tumours before the age of 36. ■

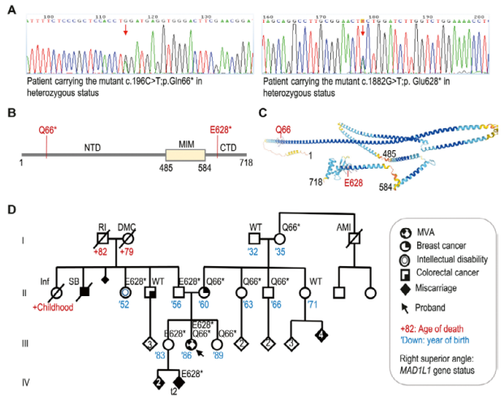

FIGURE 1 Biallelic loss-of-function mutations in *MAD1L1*. (**A**) *MAD1L1* mutations in the proband. (**B**) Schematic representation of the MAD1 protein and the mutations found in the proband. NTD, N-terminal domain; MIM, MAD2 interaction motif. (**C**) Predicted structure of MAD1 and the position of the Q66 and E628 mutated residues. (**D**) Pedigree of the family. AMI, acute myocardial infarction; DMC, diabetes mellitus complications; Inf, infection; RI, renal insufficiency; SB, stillbirth; t2, trisomy chromosome 2.

SELECTED PUBLICATIONS

- Michel M *et al.* (incl. Osorio A) (2022). Small-molecule activation of OGG1 increases oxidative DNA damage repair by gaining a new function. *Science* 376, 1471-1476.
- Breast Cancer Association Consortium *et al.* (incl. Osorio A) (2022). Pathology of tumors associated with pathogenic germline variants in 9 breast cancer susceptibility genes. *JAMA Oncol* 8, e216744.
- Villarroya-Beltri C *et al.* (incl. Osorio A, Mercadillo F, Urioste M) (2022). Biallelic germline mutations in MAD1L1 induce a syndrome of aneuploidy with hightumor susceptibility. *Sci Adv* 8, eabq5914.
- Marchena-Perea EM *et al.* (incl. Currás-Freixes M, Osorio A) (2022). A large case-control study performed in Spanish population suggests that RECQL5 is the only RECQ helicase involved in breast cancer susceptibility. *Cancers (Basel)* 14, 4738.
- Torices L *et al.* (incl. Mercadillo F, Currás M, Urioste M) (2022). Functional analysis of PTEN variants of unknown significance from PHTS patients unveils complex patterns of PTEN biological activity in disease. *Eur J Hum Genet*. PMID: 36543932.
- Grootes I *et al.* (incl. Osorio A) (2022). Incorporating progesterone receptor expression into the PREDICT breast prognostic model. *Eur J Cancer* 173, 178-193.
- Baquero JM *et al.* (incl. Osorio A) (2022). OGG1 inhibition triggers synthetic lethality and enhances the effect of PARP inhibitor olaparib in BRCA1-deficient TNBC cells. *Front Oncol* 12, 888810.

HUMAN GENOTYPING-*CEGEN* UNIT

Anna González-Neira
Unit Head

Gradutate Students
Hugo Tejera, Alejandro Velasco

Bioinformatician
Guillermo Pita (TS)*

Technicians
Charo Alonso, Núria Álvarez, Belén Herráez, Rocío Núñez (TS)*

Student in Practice
Javier Pérez (May.-Dec.) (Master's Programme in Bioinformatics, *ENS-ISCIII*, Madrid, Spain)

**Titulado Superior* (Advanced Degree)

OVERVIEW

In the Unit, we offer researchers access to state-of-the-art methods for high throughput genotyping and sequencing for a wide range of applications. We currently have available different genotyping and sequencing platforms to be used according to the scale of analysis required, and we are continuously developing new techniques to cover all research project needs. The research carried out in the Unit is based on identifying genetic risk factors of breast cancer susceptibility and treatment response. Our main goals are to: i) improve individual breast cancer risk assessment, ii) develop novel strategies for breast cancer early detection, and iii) provide cancer patients more accurate and safe treatment.

"Our research on breast cancer will improve breast cancer risk prediction and guide risk-stratified breast screening strategies."

RESEARCH HIGHLIGHTS

Breast cancer risks associated with missense variants in breast cancer susceptibility genes. This study is the result of the European project BRIDGES (Breast Cancer Risk after Diagnostic Gene Sequencing), in which the Unit participates. Protein truncating variants in ATM, BRCA1, BRCA2, CHEK2, and PALB2 are associated with increased breast cancer risk, but risks associated with missense variants in these genes are uncertain. We analysed 59,639 breast cancer cases and 53,165 controls for missense variants in these 5 breast cancer genes, evaluating the risk according to *in silico* prediction-of-deleteriousness algorithms, functional protein domain, and frequency. For ATM, BRCA1, and BRCA2, data were compatible with small subsets (7%, 2%, and 0.6%, respectively) of rare missense variants giving similar risk to those of protein truncating variants in the same gene. For CHEK2, data were more consistent with a large fraction (approximately 60%) of rare missense variants giving a lower risk [OR 1.75, 95% CI (1.47-2.08)] than CHEK2 protein truncating variants. Our results could contribute to the clinical reporting of gene panel testing for breast cancer susceptibility (Dorling L *et al.* 2022).

Pathology of tumours associated with pathogenic germline variants in 9 breast cancer susceptibility genes. The main objective of this study was to determine the distribution of intrinsic subtypes in the 9 confirmed breast cancer genes — *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, *RAD51C*, *RAD51D*, and *TP53* — harbouring rare truncating variants and likely pathogenic missense variants associated with increased breast cancer risk. For this purpose, we used data from the BRIDGES project, including 42,680 patients and 46,387 control participants. The results suggested that variants in the 9 breast cancer risk genes are generally associated with triple-negative and/or high-grade disease. Together, the 9 genes were associated with 27.3% of all triple-negative tumours in women 40 years or younger. (Breast Cancer Association Consortium *et al.* 2022).

Novel genes and sex differences in Covid-19 severity. The study is the result of the Spanish COalition to Unlock Research on host GEnetics on COVID-19 (SCOURGE) consortium, in which the Unit participates. The consortium was launched in May 2020 to find biomarkers of evolution and prognosis that can have an immediate impact on the clinical management and therapeutic decisions in SARS-CoV-2 infections. We conducted a genome-wide study of COVID-19 with patients recruited in Spain from 34 centres in 25 cities. The discovery stage of the study comprised up to 9,371 COVID-19 positive cases and 5,943 population controls. Replication was pursued in an additional 1,598 COVID-19 cases and 1,068 population controls, and in other studies from the Host Genetics Initiative. When we performed sex-disaggregated genome-wide association studies for COVID-19 hospitalisation, genome-wide significance ($P < 5 \times 10^{-8}$) was crossed for variants in 3p21.31 and 21q22.11 loci only among males ($P = 1.3 \times 10^{-22}$ and $P = 8.1 \times 10^{-12}$, respectively), and for variants in 9q21.32 near TLE1 only among females ($P = 4.4 \times 10^{-8}$). The results in the overall analysis revealed 2 novel risk loci in 9p13.3 and 19q13.12, associated with *AQP3* ($P = 2.7 \times 10^{-8}$) and *ARHGAP33* ($P = 1.3 \times 10^{-8}$), respectively. In summary, new candidate variants for COVID-19 severity and evidence supporting genetic disparities among sexes are provided (Cruz R *et al.* 2022). ■

PUBLICATIONS

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- Marchena-Perea EM *et al.* (incl. Tejera-Pérez H, Pita G, Núñez-Torres R) (2022). A large case-control study performed in Spanish population suggests that *RECQL5* is the only RECQ helicase involved in breast cancer susceptibility. *Cancers (Basel)* 14, 4738.
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- Santonja Á *et al.* (incl. González-Neira A) (2022). Role of germline variants in the metastasis of breast carcinomas. *Oncotarget* 13, 843-862.
- Vega T *et al.* (incl. González-Neira A) (2022). Genetic, environmental and lifestyle factors associated with longevity. Protocol and response of the LONGECYL Study. *Gac Sanit* 36, 260-264.