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 HCGP works in close collaboration with the clinical community. In 2020, the Human Cancer Genetics Programme was composed of 3 Research Groups: Hereditary Endocrine Cancer, Genetic and Molecular Epidemiology and Human Genetics; 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 de Fuenlabrada.

The Programme collaborates closely with the clinical community, not only to foster cooperation in genetic diagnosis but also to promote training and education. In 2020, the Familial Cancer Consultancy carried out around 75 consultancies, and the HCGP performed 622 genetic diagnoses and 605 cytogenetic studies. In addition, the HCGP’s Groups hosted 10 residents from different Spanish hospitals for a 3-month training. The HCGP also offers short-stay opportunities of 2-6 weeks for professionals from different international research centres; a total of 15 national visitors and students were hosted. In terms of education, 14 national PhD students worked on their research projects, 3 of whom already successfully defended their theses.

The Programme participates in many international and national Consortia. In 2020, the HCGP collaborated with 18 international consortia and led or participated in 17 national and 5 international European projects. Milestones and major achievements of the HCGP in 2020 include: “Ideas Semilla” project from the AECC (Spanish Association Against Cancer), granted to Cristina Rodríguez; ATA (The American Thyroid Association) research grant for young scientists, awarded to Cristina Montero Conde; and H2020 project PANCAIM, Pancreatic cancer AI for genomics and personalised Medicine, awarded to Núria Malats. These projects were awarded for their highly innovative projections.

We would like to take this opportunity to thank our former Programme Director, Javier Benítez, who has served as Director of the Human Cancer Genetics Programme since 2005. We are thankful to him for his important contributions as well as for his inestimable dedication and commitment to the Programme. Thank you Javier for having been a part of our CNIO community and we wish you the best on your retirement!

A new Director for the Human Cancer Genetics Programme will be appointed soon. We are confident that, under a new leadership, the Programme will continue to grow and further develop translational research with the overarching goal of improving the diagnostics, prevention and treatment of cancer.

Maria A. Blasco, Director
Óscar Fernández-Capetillo, Vice Director
Our work focuses on deciphering the genetic bases of hereditary and sporadic breast and ovarian cancer and other rare tumours. During 2020, we contributed to the definition of the main bona-fide breast cancer (BC) susceptibility genes through our substantial participation in an ambitious international collaboration (BRIDGES, a European project). In addition, we obtained funding to develop two new projects focused on breast and ovarian cancer genetics. We have also been exploring the currently unknown genetic basis of testicular cancer. More recently, we started a study to elucidate the common genetic origin of different autoimmune endocrinopathies, such as chronic gastritis atrophy, thyroiditis, diabetes, or arthritis, and we identified several genes that open a new scenario for diagnosis and treatment. Finally, we found interesting results suggesting a synthetic lethal interaction between BRCA1 and OGG1 and a synergistic effect between OGG1 and PARP1 inhibitors.

"In 2020, we contributed to determining the most relevant genes in BC susceptibility, we found several candidate genes explaining the possible common origin of autoimmune endocrinopathies, and we identified a synergistic effect between OGG1 inhibitors and PARP1 inhibitors."

**RESEARCH HIGHLIGHTS**

**Hereditary breast and ovarian cancer**

**Deciphering the role of rare variants in BC**

The first publication reporting results obtained by the European project BRIDGES, to which we made a major contribution, was released this year. Thirty-four confirmed or putative BC susceptibility genes were sequenced in 60,466 cases and 53,461 controls. Nine genes were confirmed as bona-fide BC susceptibility genes, and the risk conferred by their mutations to develop the disease was refined. These results, published in the *New England Journal of Medicine* (Dorling et al., 2020), are highly relevant to improving the genetic diagnosis, counselling and follow-up of BC patients and their families. In addition, we participated in the largest collaborative international study evaluating the risk conferred by mutations in the RAD51C and RAD51D genes to develop breast and ovarian cancer (Yang et al., 2020).

Identification of 14 new candidate BC susceptibility genes

As a complementary approach to the large collaborative projects, we are conducting a study using next-generation sequencing (NGS) technologies in a few, very well selected families, to identify new BC susceptibility genes. This approach led to the identification of RECQL5, a member of the RECQ-like helicases family, as a new BC susceptibility candidate (Tavera-Tapia et al., 2019). Besides RECQL5, we identified 13 additional candidate genes that are extremely interesting because of their function, the potential deleteriousness of the variants, and their rarity in the general population. We developed an NGS panel containing this set of genes and will evaluate their role in BC by sequencing a large set of 3000 Spanish BC families.

**Modifier genes in BRCA1/2 genes**

We continue to play an active role in the CIMBA consortium (Consortium of Investigators of Modifiers of BRCA1 and
mismatch repair (MMR) and microsatellite instability (MSI).

BRCA2 is a protein that is involved in the repair of DNA. This protein is also associated with the development of various types of cancer, including breast and ovarian cancer. In particular, the global burden of mutation has been associated with survival in other tumour types. We compiled more than 200 EOCs and CCOCs and assessed the global load of microsatellite instability and identified mutational burden “hotspots” in 97% of 150 breast cancer and 191 likely target regions. Overall, these results show a good correlation between global instability and mutation burden and suggest that the association between the extent of both indicators and improved disease evolution in patients is relevant.

Altogether, we expect to find markers that will enable more rational therapeutic decision-making for EOC and CCOC patients.

**Familial cancer exome project**

**Autoimmune polyendocrine syndrome (APS)**

In 2015, we published research identifying the ATP3A4 gene as being responsible for familial asthma and chronic atrophic gastritis, as well as of the OGG1 glycosylase inhibitor TH5487 and its post-translational modification that consists of a carbohydrate attachment to different functional groups, was found to be over-represented in all cohorts of patients and thus might be involved in the development of the disease. In addition, the glycosylation with variants uncovered by WE are significantly located in the same cytoplasm as the susceptibility loci previously described by GWAS studies.

**FIGURE**

The inactivation of OGG1 with TH5487 inhibitor causes mismatch repair deficiencies (A) NS (N) Fine-Mapping of mismatch repair deficiency from human colon cancer and breast cancer patients. The correlation between the extent of both indicators and improved disease evolution in patients is relevant. An example of a telomere loss (orange arrowhead) and a fragile telomere (white arrowhead) is indicated in each image. (B) Quantification of telomere signal-free ends for the conditions shown in (A). (C) Quantification of telomere loss (including FR), compared with healthy controls. (D) Representative analyses of the frequency of multi-telomeric signals for the conditions shown in (A). (E) Each dot represents a different metaphase. Metaphase D shows telomere loss that was confirmed by two independent experiments.
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 a miR-483-5p/ALCAM axis as a new player in pheochromocytoma at the metastatic niche and showed that mTOR pathway mutations correlate with poor prognosis in chromophobe renal cell carcinoma.”
mTOR pathway alterations in chromophobe renal carcinoma associated with poor outcome

Chromophobe renal cell carcinoma (cRCC) is a histologically and molecularly distinct class of renal tumour. Knowledge on drug targets is limited and treatments do not follow a common treatment paradigm (p=0.992) so far, we performed an in-depth characterisation of the mTOR pathway through targeted NGS and immunohistochemistry (Roldán-Romero et al. 2020). Furthermore, we investigated mutations in the electron transport chain Complex I genes by developing a bioinformatics approach able to identify mitochondrial variants using NGS off-target data (Lanillos et al. 2020). Mutations in key components of the mTOR pathway (MTOR, TSC1, TSC2) occurred in 17% of primary tumours and were associated with the immunohistochemical staining of phospho-36 and PTEN, and with cRCC eosinophilic variant, supporting their biological relevance. Patients with mTOR pathway mutations had worse outcomes (overall survival: HR=3.5 and P=0.027), confirmed in TCGA with HR=10.3, P=0.006, and mutations in TPSD and telomerase genes were enriched in metastatic cases. Overall, we showed that mTOR pathway mutations correlate with poor prognosis in cRCC, suggesting that mTOR inhibitors might be a good therapeutic option for patients with these alterations.

mTORC1 is a key complex of mTOR that controls protein synthesis. However, mTORC2 is a second complex of mTOR that controls cell growth and survival. The activation of mTORC2 is required for the full activation of mTORC1 and vice versa. The two complexes are connected by the TOS (target of rapamycin) motif in mTOR that is able to modulate the activity of both complexes. In this study, we investigated the role of mTORC2 in chromophobe renal cell carcinoma (cRCC).

One of the key findings of this study was the identification of a novel player in the metastatic niche of cRCC. The researchers found that a protein called ALCAM (activation leukocyte cell adhesion molecule) is overexpressed in cRCC cells that have metastasized to the liver. ALCAM is a molecule that is involved in the processes of cell adhesion and migration. It promotes tumor cell activation and proliferation via its interaction with CD6 and is involved in the processes of cell adhesion and migration. It promotes tumor cell activation and proliferation via its interaction with CD6 and is involved in the processes of cell adhesion and migration. In the context of cRCC, ALCAM expression is important because it helps to build a malignant phenotype by disrupting cell-cell adhesion and enabling tumor cell invasion and metastasis. In fact, the researchers observed a lower expression of ALCAM in metastatic than in primary tumors (as shown in figure 8). This observation is important because it suggests that ALCAM might be a potential therapeutic target for treating cRCC.

In conclusion, the study highlights the importance of mTOR pathway alterations in cRCC and indicates that targeting mTORC2 may be a promising strategy for the treatment of this disease.
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, its 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.

“The integrative effort of the epidemiology, statistics, bioinformatics, and molecular biology fields has allowed us to gain additional insight into the inherited basis of pancreatic cancer.”
Regarding bladder cancer (BC), GEMG participated in a European-based effort to subclassify non-muscle-invasive bladder cancer (NMIBC) through a large integrative multi-omics analysis including gene expression, chromosomal instability, and spatial proteomics. The integrated classifier had independent prognostic value beyond established prognostic clinicopathological parameters. Under the umbrella of the AECC muscle invasive bladder cancer (MIBC) study, we reported that patients with BSAQ-like tumours have a higher likelihood of experiencing a pathologic complete response non-adjuvant chemotherapy. In addition, through an international collaboration, we observed that FGFR3 plays a functional role distinct from FGFR3 overexpression, pointing to the possibility that patients with FGFR3 mutations may be more likely to benefit from anti-FGFR3 therapy than patients with over-expression only.

Methodological Contributions

GEMG continued to explore the analytical strategies and tools required to integrate omics and non-omics data into the cancer risk models, and started considering the integration of medical image information (radiomics and digital pathology) through an H2020-funded project (PanCain).

Translational Activities

GEMG actively continues support in several clinical trials on immunotherapy and vitamin D in BC at the methodological level. We continue to sustain the Spanish Familial PC Registry (Pancre-FAM) and the European Registry of PC (PancreOS).

We launched a PC research platform (PC-CAMEO) to accelerate the translation of research results into the clinical domain. We lead the Research Work Stream of the Pancreatic Cancer Europe (PCRE) multi-stakeholder platform and we moved forward in increasing awareness of PC among health policy makers and translating the urgent need to invest on PC research by joining efforts with EC IPAAC Joint Action and with EAPM.
Since the summer of 2020, the Unit has expanded with 3 new people joining from the Human Genetics Group: Ana Osorio, Alicia Barroso and Victoria Fernández, all 3 involved in the research and diagnosis of hereditary forms of breast cancer. This is the most important change since the Unit’s creation and represents a huge reinforcement for our diagnostic and research activity.

Clinical and diagnostic activity during 2020 was also disrupted by the Covid pandemic. For several months the Consultancy in the Puenteblanca University Hospital had to remain closed. Even so, throughout the year we saw a total of 365 patients (32.1% decrease over 2019). Also, because of the pandemic, the number of genetic studies carried out decreased from over 572 performed in 2019 to 344 during 2020 (40.8% decrease).

Laura Pena left the Unit in November 2019. However, she defended her doctoral thesis in January of 2020. In her work — “Clinical and genetic characterisation of 345 Spanish patients diagnosed with PTEN hamartoma tumour syndrome” — she characterised the disease in a wide series of Spanish patients, at both genetic and clinical levels, reviewing the patients’ features, comparing them with other studied populations, and assessing the usefulness of the diagnostic criteria. The second objective of the work was to look for other genetic factors that could be involved in the phenotype of patients with PHHTS who do not harbour PTEN mutations. The results of this work were formulated to suggest several recommendations: for the diagnosis, the selection of the most useful clinical features to detect genetic testing; and for the follow-up, obesity check-ups and anticipation of cancer screenings. Overall, this work contributes to accelerate and improve the diagnosis and management of PHHTS patients.

During 2020 we continued our work on early-onset colorectal cancer (EOCRC). Our goal is to build partnerships with patients, clinicians, and researchers. The increase in EOCRC incidence, its global dimension, and the many aspects distinguishing it from colorectal cancer that develops at older ages, make it necessary to bring attention to this problem and understand the causes of this striking increase. In June we launched the 2nd International Symposium on EOCRC in collaboration with Fight Colorectal Cancer (Fight CRC), a leading patient-empowerment and advocacy organisation in the United States. This collaboration was established based on the priorities that emerged from the 1st EOCRC Working Group held in Denver, CO (USA) in February 2019, to align research priorities in exploring the causation and aetiology of sporadic EOCRC and to support their ongoing work in convening a workgroup of 50 active participants. As a result of these efforts, this has been the implementation of the Spanish EOCRC Group and the European Study of EOCRC Group (see in publications list Perea et al., 2020). We believe that these initiatives will help to better develop the fight against EOCRC.
MOLECULAR CYTOGENETICS UNIT

Sandra Rodríguez-Perales
Unit Head

Staff Scientist
Raul Torres

Graduate Student
Pilar Puig

RESEARCH HIGHLIGHTS

In vivo CRISPR/Cas9 targeting of fusion oncogenes for selective elimination of cancer cells

Fusion oncogenes (FOs) are common alterations found in around 20% of cancer types and are powerful drivers of tumour development. Because their expression is exclusive to cancer cells and their elimination induces apoptosis in FO-driven cancer cells, FOs are attractive therapeutic targets. However, specifically targeting the resulting chimeric products is challenging. Based on CRISPR/Cas9 technology, we devised a gene-editing strategy targeting 2 introns of the genes involved in the rearrangement, allowing for robust disruption of the FO specifically in cancer cells. As a proof-of-concept of its potential, we demonstrated the efficacy of intron-based targeting of FOs in reducing tumour burden/mortality in vivo in various sarcoma and chronic myeloid leukemia models. The FO targeting approach might open new horizons for the selective elimination of cancer cells.

Technological and translational activities

We provide state-of-the-art Molecular Cytogenetic and Genome Editing services. The Unit makes available a complete suite of tools for cellular and genetic manipulation to research groups; these tools can be used interchangeably with an array of delivery vehicles, offering a flexible, modular platform for precision genome manipulation. The Unit offers molecular cytogenetic technology analysis of human and mouse chromosomes, including conventional karyotyping, FISH, SKY and CGH array.

Our Unit offers rapid, precise, and affordable technologies to analyze cancer cells at the chromosome level and to functionally interrogate the cancer genome. In 2020, we carried out over 2,700 assays for experimental and clinically oriented projects.

“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 Unit focuses on increasing the knowledge about the role of chromosomal rearrangements in cancer development and progression and the discovery of 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. We also participate in collaborative projects with clinical and basic science investigators across the CNIO and other institutions.

• Publications


Our Unit offers rapid, precise, and affordable technologies to analyze cancer cells at the chromosome level and to functionally interrogate the cancer genome. In 2020, we carried out over 2,700 assays for experimental and clinically oriented projects.
In the Unit we implement high-throughput methods for detection of genetic variation (single nucleotide variants, indels, structural variants) and methylation analysis using DNA microarray and next-generation DNA sequencing technologies. Complementarily, research focused on identifying predictive biomarkers for precision medicine is undertaken. Our aim is to identify predictive biomarkers in cancer patients in order to implement precision medicine in clinical practice.

### RESEARCH HIGHLIGHTS

Novel predictive genetic markers for adverse drug reactions in breast cancer (BC) patients. Persistent chemotherapy-induced alopecia (pCIA) and capectabine-induced hand-foot syndrome (CHFS) are 2 common adverse drug reactions in cancer treatment. pCIA occurs in its most severe form in up to 10% of BC patients treated with docetaxel-based therapies, having a profound psychological impact on them. CHFS is a dermatological toxicity affecting around 30% of patients, and the main cause of dose reductions and chemotherapy delays. By GWAS, we identified a regulatory variant associated with pCIA appearance in patients; this finding was validated in the replication cohort (ORcombined 4.05; 95% IQR 2.46-6.67; P=3.946 x 10^-5). This variant affects ABCBI mRNA expression, being the risk allele associated with decreased expression. The ABCBI gene encodes P-glycoprotein, an efflux pump responsible for the elimination of docetaxel, and lower expression could cause decreased drug elimination and thus its intracellular accumulation. Carriers of the risk allele would experience high drug exposure in the hair follicle and alopecia may become permanent, owing to the destruction of hair follicle stem cells. In addition, we discovered and replicated a cluster of 4 variants associated with decreased levels of CDH4 mRNA and the protein it encodes, R-cadherin, which localises in the granular layer of the epidermis. This resulted in reduced expression of involucrin, a protein of the cornified envelope, an essential structure for skin barrier function.

Identifying variants of pharmacogenomic interest using CSVS, a crowdsourcing database of the Spanish population genetic variability. Genetic differences between human populations are becoming increasingly recognised as important factors accounting for interindividual variations in drug responsiveness. Using data from the CSVS repository, we addressed how population-specific differences in genes involved in drug absorption, distribution, metabolism, excretion and toxicity (ADMET) could affect the rates and risks of drug inefficacy and/or adverse drug reactions in the Spanish population. We studied the Spanish genetic variability in a total of 421 pharmacogenes and, interestingly, a non-negligible percentage of private variation was observed in genes encoding proteins involved in drug metabolism, transport, and response.

Detection of mutations in liquid biopsies from paediatric CNS tumours. Paediatric CNS tumours are the most fatal cancer diseases in childhood. Due to their localisation and infiltrative nature, some tumour resections or biopsies are not feasible. We conducted the first study to compare different sources of liquid biopsies in paediatric cancers, an unmet need for clinical practice. We found serum to be more promising than plasma for BRAF V600E by dPCR detection in liquid biopsy of CNS paediatric cancers.

### PUBLICATIONS