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. Our main objectives are to: (i) discover genes responsible for familial cancer; (ii) decipher the genetic bases of sporadic cancer; (iii) analyse the role of modifier factors (genetic and non-genetic) in cancer development; (iv) understand the relationship between genes and drug response; (v) implement new strategies for the cure of genetic disorders, e.g., genome editing; and (vi) translate this knowledge into clinical practice through genetic diagnostic studies and genetic counselling.

Currently, the HCGP is composed of 3 Research Groups and 3 Units: the Human Genetics Group, led by Javier Benítez; the Hereditary Endocrine Cancer Group, led by Mercedes Robledo; and the Genetic and Molecular Epidemiology Group, led by Núria Malats. The Genotyping Unit, headed by Anna González-Neira, supports our 3 research groups from a technical point of view, and provides support to other CNIO groups as well as to external users. The Molecular Cytogenetics and Genome Editing Unit, headed by Sandra Rodríguez-Perales, contributes to this provision of technical support. Finally, the Familial Cancer Unit coordinates the clinical part of the HCGP through the CNIO Familial Cancer Consultancy, which is located at the Hospital de Fuenlabrada. Miguel Urioste is responsible for these activities. In addition, the 3 Units conduct their own research activities.

The HCGP collaborates closely with the clinical community, not only to foster cooperation in genetic diagnosis but also to promote training and education. In 2019 the Familial Cancer Consultancy carried out around 570 consultancies, and the HCGP performed 1,410 genetic diagnoses and 1,230 cytogenetic studies. In addition, the HCGP’s Groups hosted 10 medical residents from different Spanish hospitals, who rotated between the Groups and Units for 3-month intervals. We also offer professionals from different national and international research centres the opportunity to join us, either as visitors or for training visits consisting of short-term stays of 1-3 months; a total of 6 international and 14 national visitors and students were hosted in 2019. In terms of education, 14 national PhD students worked on their research projects, 2 of whom already successfully defended their theses.

Finally, one of the main objectives of the HCGP is to establish research collaborations with national and international groups; this is well demonstrated by our publication record as well as the key roles held by several of the Programme’s members in consortia and projects, both nationally and internationally. Currently, we collaborate with 14 international consortia representative of the main tumour types on which we focus. In addition, we lead or participate in 15 national and 3 international European projects.

Milestones and major achievements of the HCGP in 2019 include:

- Javier Benitez: the discovery of a group of solute carrier genes that explain several autoimmune pathologies.
- Mercedes Robledo: Group Leader of the 706 Unit, CIBERER (Centro de Investigación Biomédica en Enfermedades Raras).
- Javier Benitez: became member of the International Consortium of Testicular Cancer.

“We are entering a new era of medicine: the study of healthy people based on risk algorithms, in order to identify groups at high-risk of developing cancer and to perform individualised follow-up.”
We continue to decipher the genetic bases of hereditary and sporadic breast and ovarian cancers. In addition, we have participated in a project that combines the genotype and phenotype in order to stratify and select women at high risk of developing breast cancer (BC). Other families with rare tumours are also object of our studies, for example, testicular cancer whose genetic bases are unknown. More recently, we started a study to elucidate the common genetic origin of different autoimmune-polyendocrinopathies (APs), such as chronic gastritis atrophy, thyroiditis, diabetes or arthritis. We have identified several genes (most of them solute carriers) opening the way for new diagnoses and treatments. Finally, we have progressed in understanding the role of glycosylase genes as modifiers of hereditary breast cancer and their role along the cell cycle.

“We continue working to solve new challenges associated with hereditary BC diagnosis and treatment, but in parallel have extended our studies to discover new genes that explain families with rare tumours. In this regard, a whole pathway, including solute carrier genes associated with APs, has been identified.”
RESEARCH HIGHLIGHTS

Looking for new breast cancer susceptibility genes

Deciphering the role of rare variants in breast cancer

The European project BRIDGES, aims to build a panel of high and moderate susceptibility genes to identify families with breast cancer having a genetic aetiology. For this purpose, we have analysed 36 candidate genes in 60,000 breast cancer cases and controls and have confirmed 20% of them as susceptibility genes. The second step comprises the whole exome sequencing of these 60,000 cases, aiming to discover new susceptibility genes. This part of the work was conducted throughout the year. We will translate this knowledge – the new genes plus the previous (already confirmed) genes - to several cancer consultancies throughout Europe in order to analyse their value in clinical practice.

Identification of RECQL5 as a new candidate moderate-risk gene in breast cancer

As a complementary approach to the BRIDGES project, we conducted another study using next-generation sequencing (NGS) technologies to identify new BC susceptibility genes in a few, very well selected families. This approach led to our identifying RECQL5, a member of the RECQL helicase family, as a new breast cancer susceptibility candidate deserving further study. We used a combination of whole exome sequencing in a family negative for mutations in BRCA1/2 (BRCAx) – in which we found a probably deleterious variant in RECQL5 – as well as targeted NGS of the complete coding regions and exon-intron boundaries of the candidate gene in 699 BC Spanish families with BRCAx mutations and 665 controls. Functional characterisation and in silico inference of pathogenicity were performed to evaluate the deleterious effect of detected variants. These results prompted us to propose RECQL5 as a gene that would be worth analysing in larger studies in order to explore its possible implication in BC susceptibility (Tavera-Tapia et al., 2019).

Modifer genes in BRCA1/2 genes

Single nucleotide polymorphisms (SNPs) in DNA glycosylases genes involved in the base excision repair (BER) pathway can modify breast and ovarian cancer risk in BRCA1 and BRCA2 mutation carriers. We previously found that SNP rs434259 in the uracil-DNA glycosylase (UNG) might decrease ovarian cancer risk in BRCA2 mutation carriers. Now, we have validated this finding in a larger series of patients with familial breast and ovarian cancer and gained insight into how this UNG variant exerts its protective effect. We found that rs434259 is associated with significant UNG downregulation and with lower levels of DNA damage at telomeres. In addition, we found that this SNP is associated with significantly lower oxidative stress susceptibility and lower uracil accumulation at telomeres in BRCA2 mutation carriers (Baquer et al., 2019).

Familial cancer exome project

Autoimmune polyendocrine syndrome (APS)

In 2015, we published research identifying the ATP4A gene as responsible for families with gastric neuroendocrine tumours (Calvete et al., 2015). In 2017, we extended this study to a new family that presented the same lesion along with hypothyroidism and arthritis. The family presented 2 mutations in ATP4A and PTHRII following a digenic model that explained the 3 concurrent autoimmune pathologies (Calvete et al., 2017). In 2019, we published a model that involves the uncovered genetic landscape with the acid-base balance malfunction and explained the autoimmune response activation that triggers malignant progression (Benitez et al., 2019).

We are further investigating the apparent relation of gastric autoimmune disease (gastric neuroendocrine tumour or chronic atrophic gastritis) with other autoimmune diseases such as thyroidopathy, arthritis, diabetes type 1, vitiligo, etc. Through targeted sequencing, using a custom panel containing 12 solute carrier genes and some receptors involved in the previously described mechanism, we found several mutations in new genes involved in homeostasis function (solute carriers) – not only in stomach parietal cells but also in other tissues (epithelium, pancreas, thyroid) – altering the acid-base balance and explaining the presence of different autoimmune pathologies (apparently unrelated) in a family or an individual. Several gastroenterologists, pathologists and endocrinologists are collaborating in this project (FIGURE).

Testicular Germ Cell Tumour (TGCT)

During the past year, we completed the first part of our study by performing whole exome sequencing (WES) in familial testicular cancer and described 3 new low susceptibility genes (PLEC, EXO5 and INAP7) that contribute to cancer development (Pasmard et al., 2018). We have revised the data using different selection criteria and filtering of the variants in order to look for moderate-high susceptibility genes. Additionally, we are exploring the genetic landscape and somatic contribution that drives different tumour histologies (seminomas and non-seminomas) in different hereditary models (familial, bilateral and sporadic TGCT). The preliminary results are being validated in newly recruited cases. Finally, we have also joined The Testicular Cancer Consortium (TECAC), led by Dr Katherine Nathanson, to contribute with our data and compare our results in a bigger cohort.

Ovarian cancer

Endometrioid (EOC) and clear cell (CCOC) ovarian carcinomas are considered refractory to chemotherapy and present a poor outcome once disseminated. Defects in mismatch repair (MMR) and microsatellite instability (MSI) are predictors of immunotherapy response. During 2019, we compiled 180 EOC and CCOC cases and CCOC that are being characterised using a comprehensive panel (OvaSeq-MSI) that specifically includes a large set of MS markers to determine MSI status and that generates information about mutations in ovarian carcinoma genes, in ovarian cancer susceptibility genes or therapeutic targets. We have already sequenced 80 tumours of our series. Altogether, we expect to find markers to enable more rational therapeutic decision-making for patients with EOC and CCOC.

PUBLICATIONS

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-21-3p/TSC2/mTOR axis with predictive value for mTOR inhibitor sensitivity in pheochromocytoma and showed that PTEN expression and TSC1/TSC2/mTOR mutations predict response to rapalogs in renal cancer.”
Integrative multi-omics analysis identifies a prognostic miRNA signature and a targetable miR-21-3p/mTOR axis in metastatic pheochromocytoma Metastatic pheochromocytoma and paraganglioma (mPPGL) represent major clinical challenges due to the limitations in accurate diagnosis and effective treatments. We aimed to identify reliable metastatic risk miRNAs for mPPGL, and to decipher their role in progression through an integrative multi-omic data analysis. For this purpose, we carried out a comprehensive analysis of miRNomes from 443 patients, the largest discovery cohort explored so far thanks to the participation of an International Consortium of Reference Centres with expertise on this disease. First, we succeeded in identifying in tumour tissues a miRNA signature related to metastatic risk and to shorter time to progression. Higher levels of these miRNAs were also detected in mPPGL patients’ liquid biopsies compared with controls. Using a prognostic predictive model, we built a miRNA-based classifier, which was further studied in an in vitro model, confirming that the classifier’s miRNA overexpression induces aberrant levels of mesenchymal and neuroendocrine markers and enhances cell migration capacity. The miRNA/miRNA data integration of the same tumors revealed a link between miR-21-3p and TSC2. Moreover, a pan-cancer TCGA integrative study, also including proteomics data, further elucidated TSC2 as a potential target for miR-21-3p, being able to uncover a non-previously identified regulatory role of a key tumor suppressor gene in the mPPGL pathway. Further analysis of the miRNA predictions in mPPGL patients’ sera, shed light upon how to identify patients at risk of metastatic progression in a clinical setting. In conclusion, our findings suggest that PTEN is a potential target for miRNA-21-3p, and enhances cell migration capacity.

PTEN expression and miTORS, TSC1 and TSC2 mutations predict response to rapalogs in renal cancer The mammalian target of rapamycin (mTOR) pathway, also known as raptor, is key for the treatment of various types of cancer, however, some of these pathways are still predictive biomarkers in clinical use. To address this challenge, we performed a molecular and immunohistochemical characterisation of key mTOR pathway components in a series of 105 renal cell carcinoma patients treated with rapalogs, and recruited by the Spanish Oncology Genitourinary Group (SOGUG) and the University Hospitals Leuven. We demonstrated that response to these drugs occurred more frequently in cases with mTOR pathway mutations than in those without mutations (p=0.030). Negative PTEN immunohistochemistry staining was detected in 58% of the tumours, and it was more frequent in rapalogen responder patients (p=0.029). Furthermore, mutations and negative PTEN protein expression were not mutually exclusive events, and their combination improved response prediction (p=0.008). In conclusion, our findings suggest that mTOR pathway mutations, negative PTEN immunohistochemistry staining, and their combination, are potential markers of rapalog response. These results provide a step forward with regard to guiding treatment with mTOR inhibitors and personalising renal cancer treatment.

**Figure** Schematic representation of the integrative omic analysis. miRNA profiles found in primary tumours were defined to describe a prognostic miRNA signature. The integration with transcriptional data revealed a target gene, which was further integrated with proteomic data. This latter identified a miRNA, whose overexpression could be a potential marker to select patients for treatment with mTOR inhibitors. This prognostic signature, also detected in patients’ serum, shed light upon how to improve the disease follow-up.
The scope of the research carried out by our Group ranges from the identification of etiological agents and mechanisms, 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 to better characterise exposures, genetic susceptibility patterns, and cancer outcomes. Omics data provide a unique opportunity in this regard and the Group explores its integration 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 current genomic tests and data.
RESEARCH HIGHLIGHTS

Research findings
In 2019, the Group focused its research on pancreatic and bladder cancers. Regarding pancreatic cancer (PC), we progressed in the characterisation of pancreatic cancer risk factors by investigating the common genetic background of PC and other chronic disease processes. We further evaluated the less explored association between PC and autoimmune diseases (AIDs) through an epidemiological analysis. Finally, we contributed to the discovery and validation of both urine- and tumour-predictive and prognostic markers in large Spanish and European studies of both non-muscle and muscle invasive BC.

Methodological contributions
The Group continues to develop analytic strategies and tools to integrate both omics and non-omics (OnO) data. In this regard, we reported that the efforts to integrate OnO data are scarce, having been done mainly in the epidemiological field. We identified and listed the challenges in OnO data integration and proposed integrated analytic strategies towards its integration (FIGURE 2).

Translational activities
The Group actively provides methodological support to several clinical trials on immunotherapy and vitamin D in BC. We continue to support the Spanish Familial PC Registry (PanGen-FAM) and the European Registry of PC (PanFeTrac80). We also lead the Research Work Stream of the Pancreatic Cancer Europe (EPC) multi-stakeholder platform. By joining efforts and participating in the European Alliance of Personalized Medicine Annual Meeting, we also made advances in increasing awareness of PC among health policy makers and in discussing the urgent need to invest in PC research.

Figure 2 Challenges found in the integration of omics and non-omics (OnO) data and analytical designs for building hybrid models containing OnO data.

• PUBLICATIONS

• AWARDS AND RECOGNITION
  Stand Up To Cancer (SU2C)/Lustgarten Foundation – Pancreatic Cancer Collaborative Grant, $1 million over 2 years (2019-2021), awarded to Núria Malats and Raúl H2020-funded VISION Project.

ANNUAL REPORT 2019
SPANISH NATIONAL CANCER RESEARCH CENTRE: ONCO
TRANSLATIONAL RESEARCH
HUMAN CANCER GENETICS PROGRAMME | GENETIC AND MOLECULAR EPIDEMIOLOGY GROUP
CRC is the third most frequent type of cancer and the third cause of cancer-related deaths in most developed countries. Age is the main risk factor. The median age at diagnosis is 68 years old (range 14 to 28 years old). The increase was observed in the age range of 40 to 49 years, in individuals over 50 years of age. The greater concern is probably related to the extended use of the faecal occult blood test and colonoscopy, which facilitates the removal of precancerous lesions.


Familial Cancer Unit: A multidisciplinary approach. Miguel Urioste (Head) Laura Pena (Graduate Student) Verónica García (since June), Maika González-Noreña, Fatma Merzañdoi.

Clinical, Diagnostic and Research Highlights

The FCCU’s clinical and diagnostic activities contribute to the subclassification of CRC according to its molecular basis, age-of-onset and tumour location. The analysis of main susceptibility genes enables the selection of those cases of special interest: EOCRC not associated with germline mutations. The analysis of young-onset CRC in this group of patients with CRC has a family history of cancer, usually tumours of the Lynch syndrome spectrum, but no germline mutations in the mismatch repair genes. Another considerable number of cases of young-onset CRC have been identified. Many of these tumours are MAPPs (microsatellite and chromosome stable) that fall into the “Familial CRC type X” category, described years ago, and which constitutes a special interest group for the search of new susceptibility genes.

Our clinical and diagnostic activities in 2019 can be summarised as: 538 patients visited our consultancy at HUF (100% increase in 2018), and 572 genetic diagnostic studies were performed in the FCCU laboratory (12.5% increase). Among these studies, we identified 17 cases of CRC in individuals younger than 45, of whom were patients younger than 30 (14 and 28 years old). The FCCU also focuses its research efforts on less frequent cancer predisposition syndromes. One of these is the PTEN hamartoma tumour syndrome (PHTS), in which the high clinical heterogeneity is a major obstacle to establishing an early diagnosis. We studied this pathology at the clinical and molecular level in the largest series of Spanish patients with PHTS (145 probands). Our findings are consistent with the syndrome descriptions in other populations, with few exceptions such as a higher proportion of carriers of mutations in PHTS exon 1 who apparently have an increased risk of developing renal cancer. We discussed the usefulness of the different diagnostic criteria proposed to date and made recommendations based on our results (FIGURE).

Future directions will focus on unravelling the relevance of these findings. Our study continues to contribute to a better understanding of PHTS and to accelerate its diagnosis.

Noteworthy is that we highlight a novel risk for patients with PHTS, namely the development of cancer at young ages, for which we suggest to anticipate cancer screening in these individuals. Moreover, we demonstrated that the presentation of cancer types within the PHTS spectrum criterion alone is not sufficient to refer patients for PSEN screening, at least as a first measure. In collaboration with the groups of Dr Pulido (Barcelona) and Dr Cid (UCM), we functionally demonstrated the deleterious effect of several PSEN variants of unknown significance. However, about half of our PHTS patients could not be explained by alterations in PSEN, and therefore we also focused our efforts on the search for other genes possibly involved in this disease, using a gene panel (including PIK3 /akt/mTOR) pathway genes and whole exome sequencing.

Figure. Proportion of individuals showing each clinical manifestation of in our PHTS series, excluding carriers of variants of unknown significance.
MOLECULAR CYTOGENETICS AND GENOME EDITING UNIT

Sandra Rodríguez-Perales

UNIT HEAD

Molecular Cytogenetics and Genome Editing Unit focuses on increasing our understanding of cancer genomics and epigenetics. We provide the CNIO and external researchers with the latest and most advanced genome and epigenetic cancer alterations. The goal of the Unit is to create in vitro models that recapitulate chromosomal, genetic, and epigenetic changes, and to eliminate chromosome rearrangements. A better understanding of these cancer hallmarks is essential for the development of new therapeutic targets.

with clinical and basic 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 geneticist technology analysis of human and mouse chromosomes, including conventional karyotyping, FISH, SKY and CGH array. As the field of cancer cytogenomics moves forward with the identification and cataloguing of recurrent chromosomal aberrations and gene mutations in a variety of human cancers, our Unit offers rapid, precise and affordable technologies to analyse cancer cells at the chromosome level and to functionally interrogate the cancer genome. In 2019, we carried out over 1,200 assays for experimental and clinically-oriented projects.

RESEARCH HIGHLIGHTS

Modelling cancer using CRISPR/Cas9 genome editing technology

Efficient methodologies for recreating cancer-associated chromosome aberrations and gene mutations are in high demand as tools for investigating how such events initiate cancer. We recently demonstrated, by generating chromosomal rearrangements, the feasibility of utilising gRNA/Cas9 ribonuclease (RNPL) complexes to model cancers driven by fusion genes. We optimised new strategies to enhance the efficiency of CRISPR-mediated translocation induction in human stem cells, including mesenchymal and induced pluripotent stem cells. The CRISPR-Cas9-mediated generation of targeted translocations in human stem cells opens up new avenues to modelling cancer. We are also working on an efficient approach to selectively eliminating cancer-associated fusion oncogenes.

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 cytogenetics technology analysis of human and mouse chromosomes, including conventional karyotyping, FISH, SKY and CGH array. As the field of cancer cytogenomics moves forward with the identification and cataloguing of recurrent chromosomal aberrations and gene mutations in a variety of human cancers, our Unit offers rapid, precise and affordable technologies to analyse cancer cells at the chromosome level and to functionally interrogate the cancer genome. In 2019, we carried out over 1,200 assays for experimental and clinically-oriented projects.
The most abundant types of genetic variation are single nucleotide variants (SNVs) and copy number variants (CNVs). Association studies involving the large-scale analysis of both SNVs and CNVs in thousands of patients can help to identify genes underlying complex diseases such as cancer and drug responses. In this Unit we implement different high-throughput and cost-effective methods to measure from one to millions of responses. In this Unit we implement different high-throughput methods for circulating extracellular vesicle-derived DNA isolation.

Genetic factors underlying the risk of persistent chemotherapy-induced alopecia in patients treated with docetaxel. Persistent chemotherapy-induced alopecia (pCIA) appears in its more severe grade (grade 2) in up to 10% of breast cancer patients treated with docetaxel-based therapies, and has severe psychological impact on these patients. We conducted a genome-wide association study (GWAS) to identify variants associated with the risk to develop this adverse effect. A regulatory variant located in an enhancer element that interacts with the ARCBT promoter was found to be associated with pCIA appearance: this finding was validated in the replication cohort (OR= 4.05; 95% CI 2.46-6.67; P=3.946 x 10^-3). This variant affects ABCB1 mRNA expression and is the risk allele associated with decreased ABCB1 expression levels (P=1.64 x 10^-5). To our knowledge, this is the first study to identify a genetic factor related to this adverse effect.

Mutational characterisation of glioblastomas for the identification of prognostic markers and therapeutic alternatives. Glioblastoma (GBM) is the most common and aggressive malignant brain tumour in adults. Despite the advances in surgical resection, the prognosis for patients with GBM remains poor, with a median survival of 15 months, and tumours generally recur after standard multimodal treatments. We have performed the genomic characterisation of 89 primary tumours from GBM patients recruited from the Hospital Clínico San Carlos and Hospital 12 de Octubre, using a custom NGS panel with 1313 amplitons of 48 genes including 26 genes significantly mutated in GBMs in the Cancer Genome Atlas Project, and also 22 actionable genes to identify clinically relevant mutations in these patients. We identified a total of 3393 pathogenic variants having an average of 4.3 (1-26) mutations per tumour. We found that PTEN mutations are strongly associated with shorter survival in patients with GBM (P=0.000413). We also found mutations in several actionable genes, such as PARP and BRAF genes. Our results demonstrate that the molecular characterisation of GBM tumours using NGS multigene panels could be a good strategy to improve the management of these patients.