The Human Cancer Genetics Programme is currently composed of three Research Groups: Human Genetics, Endocrine Cancer, and Genetic and Molecular Epidemiology Groups; and three Units: Human Genotyping CEGEN and Molecular Cytogenetics and Genome Editing Units, and the Familial Cancer Clinical Unit. In addition, there is a Familial Cancer Consultancy for the evaluation of families with cancer and the provision of genetic counselling. The Consultancy is located in the Hospital Universitario de Fuenlabrada and works in close collaboration with the Oncology Service at that Hospital. The number of consultancy days and amount of families attended have increased since we set it up five years ago. Currently, we are attending to around 350 families/year. This increase of families has resulted in a higher number of genetic and genomic diagnosis studies, which have been made possible thanks to the incorporation of a massive sequencing platform. This platform has been operational over the past year.

The Programme’s core goals are centred on research, training and diagnosis. The genetic and cytogenetic study of tumours, genome editing, genetic interactions, data integration, the search for diagnostic and prognostic markers, the discovery of novel cancer-related genes and environmental factors that confer cancer susceptibility, are our main research priorities. A further complementary area of work is the application of Pharmacogenetics and Pharmacogenomics to identify genes that modify drug response. This research line focuses on a wide variety of tumours, taking advantage of the high-throughput genotyping technologies provided by the Genotyping Unit.

The Programme collaborates closely with the clinical community, not only to foster cooperation in genetic diagnosis but also to promote in training and education. During this year, the Programme’s groups have hosted 8 residents from different Spanish hospitals for 3-month periods. We also offer professionals from different international research centres the opportunity to join us, either as visitors or for longer training visits consisting of short-term stays of 1-3 months (a total of 3 international visitors from Latin America were hosted in 2016).

In terms of education, since the beginning of 2016, 1 foreign and 3 national Erasmus Master’s students and 9 national and 2 international PhD students have worked on their research projects, 1 of whom has already successfully defended her thesis. We participate in many international and national consortia. This enables us to apply for international projects, hold international meetings and publish in the best journals. Likewise, a good collaboration with other CNIO Groups and Units is one of our main characteristics, allowing us to benefit from the internal exchange connecting people, techniques, technology and knowledge.

Milestones and major achievements of the Programme in 2016 include:

→ The co-organisation of the 6th Familial Cancer Conference in collaboration with the European School of Oncology and Nature Reviews Clinical Oncology.
→ Mercedes Robledo, Head of the Hereditary Endocrine Cancer Group, was awarded the International Medal bestowed by the Society for Endocrinology.
→ Núria Malats, Head of the Genetic and Molecular Epidemiology Group, was elected Chair of the EUPancreas COST Action.
→ The co-direction of the CNIO Canceromatics III - Tumor Heterogeneity Conference, November 2016.

“...The programme continues with carrying out its translational work, connecting clinicians with scientists, studying human cancers and helping and advising professionals about the new genetic results generated by novel technologies...”
OVERVIEW

The Human Genetics Group is working on the study of human cancer from a genetic, cytogenetic and epidemiologic point of view. We want to understand why the inherited susceptibility to cancer doesn’t follow a mathematical model in people, but rather ‘an apparently random model’, and why there are families with a large number of members suffering from cancer.

For these studies we work with individuals, families and the affected and normal population, trying to perform a correct diagnosis with known genes as well as looking for new genes that could explain cancer susceptibility in specific families. Our main objective is to work with every family by raising their awareness in regards to their own risk of developing cancer and how to prevent it. To this primary level, we have to add a secondary level of prevention, which will facilitate an important risk reduction in the population, through the development of non-invasive and non-genetic but yet extremely effective measures.

“During 2016, we showed how inhibitors, other than PARPi, could be used in patients with BRCA mutations. We started working on the identification of new treatments for cardiac tumours based on transcriptome analysis, and finally, we are also exploring a polygenic inheritance model in families with testicular cancer that is based on more than 25 identified genes associated with this disease.”

RESEARCH HIGHLIGHTS

Breast cancer: PARPi and OGG1 inhibitors in BRCA1 mutation carriers

We have demonstrated that certain missense mutations in BRCA1 seem to make cells more sensitive to Poly (ADP-ribose) Polymerase (PARP) inhibitors than those mutations that give rise to the absence of the protein (frameshift mutations) (T. Valclová, Hum Mol Genet 2016). We are currently investigating the mechanisms underlying these differences with the aim of identifying new markers of sensitivity or resistance to these agents.

In parallel, we recently showed that the Single Nucleotide Polymorphism (SNP) rs2304277, located in the 3’ untranslated region (UTR) of the OGG1 DNA glycosylase gene of the Base Excision Repair pathway (BER), modified cancer risk in patients harbouring mutations in BRCA1 (Osorio A. et al., Plos Genetics, 2014). We have identified that the SNP is associated with a constitutive HOOG1 transcriptional downregulation, which leads to a high genome and telomere instability in those patients harbouring BRCA1 and BRCA2 mutations, thereby explaining the contribution of this polymorphism to cancer risk. This association is most likely explained by a synthetic lethal/sick interaction between these 2 genetic events. (Benitez-Buelga C. et al., Oncotarget, 2016). In order to take an in-depth look at the biological link between BER and the homologous recombination (HR) DNA repair pathway, we tested the pharmacological inhibition of OGG1 in a set of BRCA1 and BRCA2 deficient cancer cell lines. We found that OGG1 inhibition is effective, leading to 1) an accumulation of telomere oxidation (genomic instability), and 2) an alteration in the normal proliferation of BRCA1 deficient cell lines, pointing to a synthetic lethal interaction between OGG1 and BRCA1 (FIGURE 1).

Familial cancer exome project

This project started several years ago with the objective of identifying new high susceptibility genes that explain families...
with rare tumours as well as deciphering the genetic heterogeneity present in some of them:

- In 2015, we identified ATM as being responsible for families with gastric neuroendocrine tumours. We are currently searching for new genes in two families that cannot be explained by mutations in ATM.

- A second gene, POT7, which was published in 2015 as being associated with familial cardiac angiosarcoma, also explains some families with Li Fraumeni-like syndrome. Analysis of tumour samples from Next Generation Sequencing (NGS) has shown an over-representation of the anogenic pathway, which makes it useful in clinical trials. In collaboration with M. Blasco’s Telomeres and Telomerase Group, we are generating a knock-in mouse with the aim of recapitulating the disease, as well as enabling us to work with antiangiogenic drugs.

- We are currently investigating a large family with meningiomas across 3 generations. Analysis of the data generated using bioinformatics tools has shown the existence of 2 candidate genes which could be responsible for this family. We are starting functional studies and are recruiting more families.

- Ovarian cancer families are rare and are usually associated with breast cancer. We sequenced the exomes of 9 patients from 5 families and identified 15 rare variants in 28 genes potentially implicated in ovarian cancer risk. By conducting a case control association study we narrowed down the number of candidate missense variants to 10. Three, together with 5 high-impact variants (protein truncating or splicing variants), will be evaluated in a larger international case control study to finally define their role in ovarian cancer susceptibility. In parallel, we selected a non-described RAD51C missense variant among the identified candidates, and through functional characterisation we were able to determine its pathogenicity and its probable involvement in ovarian cancer risk in one of the families. This finding not only has implications for genetic counselling but also for the potential treatment of affected carriers with PARPi.

- Breast cancer. We have performed whole-exome sequencing (WES) in 5 BRCA1 families (familial breast cancer families negative for mutations in BRCA1). One of these families was found to harbour a deleterious mutation in the known breast cancer susceptibility gene ATM. A complete screening of this gene in a set of 400 Spanish breast cancer families showed a prevalence of almost 2% of mutations in ATM, higher than that reported in other populations (Taveras-Tapia et al., BCRIT 2016). In another family, we found an excellent candidate breast cancer gene that is currently being screened by targeted NGS in a series of 700 BRCA1 families and 700 controls. The third family is still under analysis.

- Male breast cancer. We performed WES in a male breast cancer family with an apparently recessive model of inheritance. We have found 7 candidate variants that are currently being validated in a series of 1000 male breast cancer cases and 1000 controls; this is undertaken in collaboration with Nick Orr’s lab at the Institute of Cancer Research in London.

- Testicular cancer. Testicular cancer follows a polygenic model of inheritance. We have studied, by NGS, 15 families with 2 or 3 first degree relatives affected by the disease. The results have been classified according to different inheritance models, different methods of analysis have been conducted in order to select some candidate genes (FIGURE 2). The candidate variants are currently being genotyped in a set of more than 500 sporadic testicular cancer cases and 500 controls in order to know how many of them could be considered as candidates to be associated with the disease.
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 new major susceptibility gene for paraganglioma, a gene-net regulated by methylation in medullary thyroid carcinoma, and germline variants and tumour microRNAs that predict outcomes in cancer therapies.”
A gain-of-function mutation in DNMT3A causes paraganglioma

The high percentage of patients carrying germline mutations makes phaeochromocytomas (PCC) and paragangliomas (PGL) the most heritable of all tumours. However, there are still cases that are not explained by mutations in the known susceptibility genes. We aimed to identify the genetic cause in patients strongly suspected of having hereditary tumours. Whole-exome sequencing was applied to the germline of a parent-proband triad (FIGURE). Genome-wide methylation analysis of mutated tissues and targeted deep sequencing of 112 additional samples were also performed. Enzyme sequencing identified a single, novel de novo mutation in DNMT3A, DNA (Cytosine-5-) Methyltransferase 3 Alpha, affecting a highly conserved residue located close to the aromatic cage responsible for binding the protein to the trimethylated histone H3. DNMT3A-mutated tumour and blood tissue from the patient exhibited significant DNA methylation changes.

Remarkably, the inhibition of STAT3 increased the sensitivity of class-specific proliferative dependency on STAT3 activity. RET M918T genetic predisposition. A gain-of-function mutation in DNMT3A causes paraganglioma.

Identification of germline genetic variants and tumour microRNAs to predict outcomes in cancer therapies

Personalised cancer treatment is of enormous clinical and social relevance since it can lead to safer and more efficient therapies. This year we focused our efforts on applying next generation sequencing to: (i) understand how low frequency genetic variants impact paclitaxel-induced neurotoxicity, and (ii) identify microRNAs predictive of the antiangiogenic drug response in renal cancer patients. Peripheral neuropathy diminishes the quality of life of many cancer patients, sometimes permanently, and limits the dose and efficacy of many cancer drugs. We found that low frequency variants in EPHA16, EPHA5 and EPHA8 genes contribute to the susceptibility to paclitaxel-induced neurotoxicity. Furthermore, EPHA8 neuronal injury repair function suggests that these genes might constitute important neurotoxicity markers for many neurotoxic drugs. Regarding antiangiogenic therapies, these have drastically improved the survival of kidney cancer patients, however, a fraction of the patients are refractory to these drugs. The first single miRNA deep sequencing study on an exceptional series of patients treated with sunitinib revealed microRNAs predictive of sunitinib response. Furthermore, a two microRNA-based classifier discriminated individuals with progressive disease upon sunitinib treatment (P=1.3E-04) with better predictive value than the commonly used clinicopathological risk factors. Thus, we provide new relevant markers that can help rationalise cancer treatment.

RESEARCH HIGHLIGHTS

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OVERVIEW

The scope of research carried out by our Group ranges from the identification of aetiological 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, prediction, and clinical course of patients with cancer by integrating epidemiologic with omics information.
- Assess clinical and public health strategies for cancer control using current genomic tests and data.

“We have undertaken in-depth analyses integrating omics and non-omics data to predict pancreatic and bladder cancer risk and outcome, and have assessed the challenges that epidemiology faces in this endeavour.”
RESEARCH HIGHLIGHTS

Research findings

During 2016, the Group mainly focused its research on pancreatic and bladder cancers. Regarding pancreatic cancer (PC), we have further analysed the epidemiological and clinical data from the PanGenEU Study and have characterised the risk of PC associated with diabetes, multimorbidity patterns and family history of cancer, among others. We have completed the genome-wide association study (GWAS) and, in collaboration with the international consortia, we are now replicating the primary findings. We are exploring, in collaboration with experts in the field, genome-wide methylation data generated with the Illumina HumanMethylation450 array in cases and controls. We also participated in a study that identified 3 new pancreatic cancer susceptibility signals on chromosomes 1q21.2, 1p13.3 and 8q24.21. Regarding bladder cancer (BC), we showed that common SNPs have a limited role in predicting BC outcomes and reported, for the first time, a heritability estimate for disease outcome by assessing the predictive ability of the models, including up to 177,364 SNPs for tumour recurrence and progression. We have also provided proof of concept for the joint effect of genetic variants in improving the discriminative ability of clinical prognostic models by using innovative analytic approaches, and demonstrated that SNPs in inflammatory-related genes were associated with BC prognosis (FIGURE 1). Through international collaborations, the Group has participated in the exploration of common germline variants in the APOBEC3 region associated with BC and breast cancer risk, and observed a tissue-specific role of environmental oncogenic triggers. In line with this study, mutations in cancer driver genes were primarily found in high-risk BC, together with APOBEC-related mutations. We also participated in the development of a urine-based peptide biomarker and a combined methylation-assay-based peptide biomarker (FIGURE 2).

Methodological contributions

We have proposed an epidemiological-based integration of omics and non-omics data by considering the ‘massive’ inclusion of variables in the risk assessment and predictive models (FIGURE 2). We also discussed the numerous challenges imbedding this type of research and have proposed analytical strategies that allow considering both omics and non-omics data used in the models towards a personalised prevention. Furthermore, we have adapted Bayesian sequential threshold models in combination with LASSO and applied them to time-to-event and the censoring adapted Bayesian sequential threshold models in combination models towards a personalised prevention. Furthermore, we have proposed an epidemiological-based integration of omics and non-omics data by considering the ‘massive’ inclusion of variables in the risk assessment and predictive models (FIGURE 1). Through international collaborations, the Group has participated in the exploration of common germline variants in the APOBEC3 region associated with BC and breast cancer risk, and observed a tissue-specific role of environmental oncogenic triggers. In line with this study, mutations in cancer driver genes were primarily found in high-risk BC, together with APOBEC-related mutations. We also participated in the development of a urine-based peptide biomarker and a combined methylation-assay-based peptide biomarker (FIGURE 2).

Translational activities

We coordinate the COST Action BM2104 EUPanCancers (www.eupancancers.com). This Action includes 250 multidisciplinary members from 22 EU countries, European and nongovernmental organizations, and private companies. Several scientific, training, and dissemination activities have been conducted during 2016. By endorsing the Pancreatic Cancer Europe (PCE) multi-stakeholder platform, we have actively participated in several activities aimed at increasing the awareness of PC in the general population, the medical community, and among health policy makers. The Group has actively participated in setting up a European-based clinical registry of PC (PancO3) jointly with the EPC; the Joint Research Centre from the European Community, and the European Network of Cancer Registries. The Group has also contributed to the development of recommendations for a state strategy for personalised/prediction/medicine, led by the Roche Institute. Another area our Group contributed to was the identification of different sources of big data and the importance of unstructured data for potential future uses in drug discovery; the main practical and ethical challenges to unravelling the full potential of big data in healthcare were discussed. 

**PUBLICATIONS**

7. Martinell P, Carrión-García CJ, Guerra-Hernández S et al (incl. Malats N) (2016). Figure 1. Progression-free survival of the 822 non-muscle invasive BC patients according to CD3G-rs3212262 genotypes. Five-year progression free survival was 92% for AA, 85% for Aa and 77% for aa genotypes (log rank p-value = 6.410E-4; adjusted Cox p-value = 0.023).

**CONCEPTUAL MODEL**


**PATENT**


**AWARDS AND RECOGNITION**


**AWARDS AND RECOGNITION**


ANNUAL REPORT 2016

NGS, the utility of testing multiple genes with different modes of inheritance and with varying levels of penetrance has been questioned due to the increasing costs of surveillance and unnecessary treatments, and the uncertain consequences of the identification of variants of unknown significance. More than ever it is necessary to underline that NGS testing should only be offered in the context of expert genetic counselling.

Genetic susceptibility to colorectal cancer is a key area focus for the FCCU’s research activities. Familial or hereditary forms of colorectal cancer, early-onset colorectal cancer (EOCC), and synchronous or metachronous colorectal tumours are our main topics of interest. We have continued the characterisation of EOCC, on the premise that the carcinogenic mechanisms and the progression of these tumours may differ in comparison with late-onset colorectal cancer (LOCC) (FIGURE). The APC gene status, wild-type or mutated, seems to be a marker of prognosis in colorectal cancer with microsatellite stability (MSS), but the prognosis would have a different sign in EOCC and LOCC. In MSS-EOCC, the worst prognosis was associated with APC-mutated tumours and distal location. However, in the MSS-LOCC group, the worst prognosis was observed among proximally located tumours with APC-wild type. These results not only continue to suggest a different behaviour according to the age of onset, but also define different groups in relation to the tumour location.

During 2016 the FCCU has continued a fruitful relationship with AEAS. Several members of the association have received genetic counselling in our consultancy, and the study of sarcoma predisposition genes (mainly TP53, POT1 and CIN82A) was also carried out in our laboratory. These activities are part of our ongoing collaborations with cancer patients associations. Recently, we have designed a new survey that will be distributed among members of the AEAS with the aim of identifying those families with an increased susceptibility to cancer.

**OVERVIEW**

Individuals that present with an uncommon malignancy or with cancer at an early age of onset deserve special attention because they are more likely to harbour an inherited predisposition and may require unique treatment strategies. Identification of a heritable cancer predisposition syndrome is not only essential for genetic counselling and for the design of a surveillance scheme for both the patient and his/her relatives, but also for facilitating the refinement and optimisation of treatment strategies so as to minimise toxicity and maximise efficacy. Vigilance of these syndromes can significantly enhance the quality and comprehensiveness of clinical management of cancer.

In addition, the evaluation of inherited cancer predisposition is changing with the routine use of NGS. Despite the promise of new technologies.

**CLINICAL, DIAGNOSTIC AND RESEARCH HIGHLIGHTS**

The FCCU evaluates individuals and families who are at an increased risk of developing cancer at our cancer genetics consultancy in the Medical Oncology Service of the Hospital Universitario de Fuenlabrada (HUF). The referral system, surveillance and treatment measures are discussed with medical oncologists and other clinicians during the monthly sessions conducted by the hospital’s Hereditary Cancer Clinical Committee. During 2016, our consultancy at HUF was visited by 408 patients, a 21% increase over 2015. Moreover, 152 genetic diagnostic studies were performed in the FCCU laboratory (306 in 2015). We also tested patients with multiple gene panels, this enables us to offer results on genes of interest in just a few weeks’ time. The FCCU has continued to actively contribute to unravel the complexity of genetic cancer predisposition and to help refine tools for a better evaluation of patients and families. FCCU members collaborate with the ‘Lynch Syndrome prediction model validation study group’ to define the most precise tools for the evaluation of families with hereditary colorectal cancer as well as to identify the best candidates for genetic studies. In collaboration with other research groups, the FCCU has defined the role of the UNC5C gene in hereditary forms of colorectal cancer and in polyposis, as well as the role of OGGL as a cancer risk modifier in BRCA1 and 2 mutation carriers.

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**PUBLICATIONS**


**PATENT**

OVERVIEW

Chromosomal translocations are very common events involved in the development of several cancers, especially in sarcomas and haematological malignancies. The research activity of the Molecular Cytogenetics and Genome Editing Unit covers the main topics related to cancer cytogenetics and genome engineering: from classical cytogenetics techniques to new genome engineering tools, including the CRISPR-Cas9 system. We are focusing on the implementation and development of genome engineering tools, including the CRISPR-Cas9 system.

RESEARCH HIGHLIGHTS

Optimising CRISPR-Cas9 to model cancer aberrations in primary cells

In vitro modelling of complex tumour-associated chromosome translocations at native loci is feasible with CRISPR. However, the generation of translocations must be optimised, especially for mimicking events in human primary cells. We have optimised our CRISPR protocol to efficiently obtain those cells, thereby enabling the rescue of translocation populations of human primary cells, including induced pluripotent stem (IPS) cells and mesenchymal stem cells (MSCs). These models can surely help us to understand the molecular mechanisms underlying the initiation of human cancers, and can also be used for high-throughput drug screening, toxicological testing and biomarker identification.

From the patient’s chromosome translocations to their functional effects

We have worked on the oncogenic role of the translocation t(8;21)(q22;q22)/RUNX1-RUNX1T1, which occurs in 4% of acute myeloid leukaemia patients. We deciphered a new function for the activation of MAP8, observed in t(6;21)+ cells, which is responsible for the stabilisation of SPI. Our data show the essential role of SP1 in t(8;21)+ cell maintenance through the MAPK8-mediated stabilization of SP1 is responsible for the stabilisation of SPI. The Unit makes available various techniques to the CNIO Research Groups; these techniques provide more sensitive and accurate tools to analyse cancer cells, such as RNA-FISH, chromosome stability studies based on a combined array CGH-FISH approach, or the use of CRISPR libraries to perform high-throughput functional analysis. For gene editing experiments, we have set up a specific FISH analysis to detect genomic integration sites of small constructs including LV particles. In 2016, we carried out over 1,000 assays for experimental and clinically-oriented projects.

Technical and translational activities

We provide state-of-the-art molecular cytogenetics and genome editing services. The Unit makes available various techniques to the CNIO Research Groups; these techniques provide more sensitive and accurate tools to analyse cancer cells, such as RNA-FISH, chromosome stability studies based on a combined array CGH-FISH approach, or the use of CRISPR libraries to perform high-throughput functional analysis. For gene editing experiments, we have set up a specific FISH analysis to detect genomic integration sites of small constructs including LV particles. In 2016, we carried out over 1,000 assays for experimental and clinically-oriented projects.
the promoter of ABC22 as the strongest association with tumour response in patients treated with docetaxel (P=0.009). We also identified a significant association for an intrinsic variant located in CYP1B1 associated with docetaxel tumour response (P=2.15x10⁻⁶). Our integrated pathway-based approach enables the revealing of promising genetic biomarkers of treatment outcome in breast cancer patients.

New low-frequency variant loci associated with anthracycline-induced cardiotoxicity (AIC) in cancer patients by Illumina HumAnalyse BeadXcel

Anthracycline chemotherapeutic agents are widely used in the treatment of cancer; however, chronic anthracycline-induced cardiotoxicity (AIC) is a serious long-term complication leading to substantial morbidity. Our aim was to identify new genes and low-frequency variants influencing the susceptibility to AIC. We studied the association of variants on the Illumina HumAnalyse BeadXcel array in a discovery cohort of breast cancer anthracycline-treated patients. Using gene-based tests (SKAT-O) that have greater statistical power to detect rare variant associations and that can evaluate the cumulative effect of multiple genetic variants, we identified novel significant associations in genes with a major role in mitochondrial fatty acid-β-oxidation and the respiratory chain, involved in anthracycline-related toxicity via an oxidative stress mechanism. We replicated our association results in another cohort of anthracycline-treated paediatric cancer patients from Spain.

Functional characterisation at the 20q11.33 risk locus for capetebitaine-induced hand-foot syndrome (CiHFS)

Capecitabine is a chemotherapy drug widely used in breast and colorectal cancer; the most frequent adverse drug reaction to this treatment (in 10% of the patients) is CiHFS, a cause of dose reductions and dose delays. By genome-wide association studies (GWAS), we identified four linked CDH4 regulatory variants (h²=risk haplotype) associated with the risk of CiHFS appearance (HR=2.48 p=1.43x10⁻⁷). The CDH4 gene encodes R-Cadherin, which is located in the granular layer of the epidermis and is involved in the cohesion of epidermal layers. We demonstrated that these regulatory variants are able to mediate chromatin structural changes in chromatin organisation, which results in the presence of the risk alleles and in decreased expression levels of CDH4 mRNA and R-Cadherin protein. Additional functional experiments are being performed. The study has been carried out in collaboration with CNIO’s Chromosome Dynamics Group and the Epithelial Cell Biology Group.

OVERVIEW

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 SNVs and CNVs. In addition, epigenetic studies using whole-genome methylation arrays are performed in the Unit. Complementarily, research focused on the identification of biomarkers for precision medicine is also undertaken.

“Advances in understanding patients’ responses to therapy will help to individualise cancer patient care.”

RESEARCH HIGHLIGHTS

Identification of genetic variants associated with docetaxel and anthracycline efficacy

Taxanes and anthracyclines are widely used in the treatment of breast cancer, despite the benefit being limited to a small proportion of patients and that preoperative biomarkers, which are predictive of clinical outcome, still remain lacking. We carried out a pharmacogenetic study in 181 patients with locally advanced breast cancer; previously enrolled in a phase 2 randomised clinical trial (NCT00432929), in which patients were randomly assigned to receive docetaxol (anthracycline) or docetaxel (taxane) neoadjuvant chemotherapy. We assessed whether genetic variants in 15 key transport or metabolism genes relevant to doxorubicin and docetaxel drugs could play a role as predictive biomarkers. We identified a genetic variant located in the promoter of ABC22 as the strongest association with tumour response in patients treated with docorubicin (P=0.009). We also identified a significant association for an intrinsic variant located in CYP1B1 associated with docetaxol tumour response (P=2.15x10⁻⁶). Our integrated pathway-based approach enables the revealing of promising genetic biomarkers of treatment outcome in breast cancer patients.

New low-frequency variant loci associated with anthracycline-induced cardiotoxicity (AIC) in cancer patients by Illumina HumAnalyse BeadXcel

Anthracycline chemotherapeutic agents are widely used in the treatment of cancer; however, chronic anthracycline-induced cardiotoxicity (AIC) is a serious long-term complication leading to substantial morbidity. Our aim was to identify new genes and low-frequency variants influencing the susceptibility to AIC. We studied the association of variants on the Illumina HumAnalyse BeadXcel array in a discovery cohort of breast cancer anthracycline-treated patients. Using gene-based tests (SKAT-O) that have greater statistical power to detect rare variant associations and that can evaluate the cumulative effect of multiple genetic variants, we identified novel significant associations in genes with a major role in mitochondrial fatty acid-β-oxidation and the respiratory chain, involved in anthracycline-related toxicity via an oxidative stress mechanism. We replicated our association results in another cohort of anthracycline-treated paediatric cancer patients from Spain.

Functional characterisation at the 20q11.33 risk locus for capetebitaine-induced hand-foot syndrome (CiHFS)

Capecitabine is a chemotherapy drug widely used in breast and colorectal cancer; the most frequent adverse drug reaction to this treatment (in 10% of the patients) is CiHFS, a cause of dose reductions and dose delays. By genome-wide association studies (GWAS), we identified four linked CDH4 regulatory variants (h²=risk haplotype) associated with the risk of CiHFS appearance (HR=2.48 p=1.43x10⁻⁷). The CDH4 gene encodes R-Cadherin, which is located in the granular layer of the epidermis and is involved in the cohesion of epidermal layers. We demonstrated that these regulatory variants are able to mediate chromatin structural changes in chromatin organisation, which results in the presence of the risk alleles and in decreased expression levels of CDH4 mRNA and R-Cadherin protein. Additional functional experiments are being performed. The study has been carried out in collaboration with CNIO’s Chromosome Dynamics Group and the Epithelial Cell Biology Group.

PUBLICATIONS

- Dunning AM et al. (incl. González-Neira A, Orna, A; Benítez J) (2016). Breast cancer risk variants identified by different phenotypes and regularised EIGENs (GWAS and eQTLs). Nat Genet 48, 68-76.