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.

Currently, the HCGP is composed of three Research Groups and three Units. The Human Genetics Group, led by Javier Benítez, focuses on contributing to the understanding of the genetic bases of some hereditary tumours. Mercedes Robledo leads the Hereditary Endocrine Cancer Group that aims to identify new major susceptibility genes related to hereditary endocrine tumours as well as to define markers associated with differences in anticancer drug response and toxicity. Both Groups are also involved in the search for low susceptibility alleles that explain sporadic cancers. The Genetic and Molecular Epidemiology Group, led by Núria Malats, works not only from a genetic but also from a non-genetic point of view. She analyses exogenous factors that contribute to explain, together with genetic factors (low susceptibility alleles), the susceptibility to pancreatic and bladder cancer. The Genotyping Unit, headed by Anna González-Neira, supports our three research groups from a technical point of view, and provides support to other CNIO groups as well as to external users. They also work in pharmacogenetics within the framework of their own line of research. The Molecular Cytogenetics and Genome Editing Unit, headed by Sandra Rodríguez-Perales, contributes to this provision of support with classical and molecular cytogenetics techniques and with new genome editing technologies. In addition, her research is focused on the design of human stem cell models carrying cytogenetic alterations. Finally, the Familial Cancer Unit coordinates the clinical part of the Programme through the CNIO Familial Cancer Consultancy, which is located at the Hospital de Fuenlabrada. Miguel Urioste is responsible for these activities and leads a research line focused on hereditary colorectal cancer.

The Programme collaborates closely with the clinical community, not only to foster cooperation in genetic diagnosis but also to promote training and education. This year the Familial Cancer Consultancy attended around 550 consultations, performed 1,417 genetic diagnoses and carried out 1,290 cytogenetic studies. In addition, the Programme’s Groups have hosted 6 resident physicians from different Spanish hospitals who rotated in the Groups and Units for 3-month periods. 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 10 national visitors were hosted in 2018). In terms of education, 1 foreign and 10 national Master’s students and 9 national PhD students have worked on their research projects, 1 of whom has already successfully defended their thesis. Finally, one of the main objectives of the Programme 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 international projects. Currently, we collaborate with 14 international Consortia that are representative of the main types of tumours that we focus on. In addition, we participate in 2 international projects from Europe.

Summary of milestones and major achievements during 2018:

- Mercedes Robledo: the identification of DLST as a new pheochromocytoma and/or Paraganglioma (PGL) susceptibility gene.
- Anna González-Neira: the identification of pharmacogenetic variants predicting response to neoadjuvant single-agent doxorubicin or docetaxel.
- Núria Malats: interaction of FHC and smoking increases pancreatic cancer risk.
- Javier Benítez: the identification of three susceptibility genes PLEC, EXO5 and DNAH7 as novel susceptibility genes in testicular cancer.
- Sandra Rodríguez-Perales: gene editing cancer therapy project, selected by CaixaImpulse Programme in the 2018 edition.
- Mercedes Robledo: became member of the ENS@T Steering Committee (European Network for the Study of Adrenal Tumours).
- Javier Benitez’s Group: was accepted in the international Consortium of Testicular Cancer.

“We use different omics and epidemiologic studies to achieve our goals; this is combined with functional studies that validate our results. Finally, we translate our conclusions into clinical practice.”

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We have continued to decipher the genetic bases of hereditary and sporadic breast cancer. In addition, we participated in a project that combines the genotype and the phenotype in order to stratify and select women at high risk of developing breast cancer. Other families with rare tumours are also the object of our studies, for example, testicular cancer whose genetic bases are unknown. More recently, we started working on a study to elucidate the common genetic origin of different autoimmune-originated pathologies: gastric neuroendocrine tumours or chronic atrophic gastritis plus several immune diseases in other tissues, such as thyroiditis, diabetes or arthritis. We have identified several genes thereby opening up new avenues for new 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 have discovered 3 new genes that confer susceptibility to testicular cancer and a moderate breast cancer susceptibility gene. A whole pathway with several genes associated to gastric neuroendocrine tumours or chronic atrophic gastritis plus several immune diseases, has been identified.”
In a whole-exome sequencing study of 4 BRCAX families, Breast cancer susceptibility genes confirmed 20% of them. The second phase will involve determining their role as possible moderate susceptibility genes in 60,000 breast cancer cases and controls in order to study the role of this and 2 other glycosylases previously studied, OGG1 and NEIL2, across the cell cycle.

Deciphering the role of rare variants in breast cancer

The European project BRIDGES, in which we participate, has 3 main phases. The first one was the study of 36 candidate genes in 60,000 breast cancer cases and controls in order to determine their role as possible moderate susceptibility genes. The second phase involves 4 homestays and genetic and functional assays, we identified 7 deletions or likely deleterious mutations in the gene in a series of 700 BRCAX cases and only 1 deleterious mutation in 700 controls, suggesting that the gene could actually explain a small percentage of the BRCAX families (Tavares-Tapia et al., submitted).

Breast cancer susceptibility genes

In a whole-exome sequencing study of 4 BRCAX families, we identified a mutation in the moderate susceptibility gene ATM as being responsible for the disease in one of the families (Tavares-Tapia et al., 2017). In a second family, we found a deleterious mutation in an excellent candidate gene, RQCLQ5, that belongs to a family of DNA helicases that have a role in the Homologous Recombination (HR) DNA repair pathway. Using a combination of targeted next-generation sequencing and genetic and functional assays, we identified 7 deletions or likely deleterious mutations in the gene in a series of 700 BRCAX cases and only 1 deleterious mutation in 700 controls, suggesting that the gene could actually explain a small percentage of the BRCAX families (Tavares-Tapia et al., submitted).

SNPs and the BER pathway

We investigated the molecular basis underlying the effect of an SNP in the DNA glycosylase UNG as an ovarian cancer risk modifier in BRCA2 mutation carriers (Baquero et al., submitted). We found that an SNP rs14209 is associated with significant UNG down-regulation and a better performance of the enzyme, measured by a lower accumulation of uracil at the telomeres in BRCA2 mutation carriers. Our findings could help to explain the association of this variant with a lower ovarian cancer risk in BRCA2 mutation carriers. In addition, we want to study the role of this and 2 other glycosylases previously studied, OGG1 and NEIL2, across the cell cycle.

Familial cancer exome project

In 2015, we identified a gene responsible for families with cardiac tumours (POT1) (Calvete et al., 2015). Recently, we described its relation not only to cardiac tumours but also to other types of cancer (POT1) (Calvete et al., 2016). We investigated the molecular basis underlying the effect of an SNP in the DNA glycosylase UNG as an ovarian cancer risk modifier in BRCA2 mutation carriers (Baquero et al., submitted). We found that an SNP rs14209 is associated with significant UNG down-regulation and a better performance of the enzyme, measured by a lower accumulation of uracil at the telomeres in BRCA2 mutation carriers. Our findings could help to explain the association of this variant with a lower ovarian cancer risk in BRCA2 mutation carriers. In addition, we want to study the role of this and 2 other glycosylases previously studied, OGG1 and NEIL2, across the cell cycle.

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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 susceptibility gene for paraganglioma, discovered predictive markers of mTORi response, and uncovered the Hsa-miR-139-5p/HNRNP axis as a critical modulator of thyroid tumour virulence.”
Recurrent germline DLST mutations in patients with multiple pheochromocytomas and paragangliomas (PPGLs). Taking as a starting point the involvement of the TCA cycle in PPGL development, we aimed to identify novel disease-related genes involved in the key metabolic pathway that could explain additional patients lacking mutations in known susceptibility genes. To this end, targeted sequencing of thirty-seven TCA cycle-related genes was applied to DNA from 104 PPGL patients with no mutations in the major known predisposing genes. In order to decipher the role of the identified variants, omics-based analyses, TCA-related metabolite determination and 13C- and 2H-glutamate labelling assays were performed. We identified DLST germline variants in 7% of patients. A recurrent mutation, c.G374A, found in 43% of patients, triggered accumulation of 2-hydroxyglutrate, both in tumours and in a heterologous cell-based assay designed to functionally evaluate DLST variants. p.Gly374Glu-DLST-mutated tumours exhibited loss of heterozygosity as well as consistent methylation and expression profiles. We also found positive DLST immunostaining not only in DLST-mutated tumours, but also in other tumours in which the TCA cycle is deregulated. DLST positive DLST immunostaining not only in tumours and in a heterologous cell-based assay designed to functionally evaluate DLST variants. p.Gly374Glu-DLST-mutated tumours exhibited loss of heterozygosity as well as consistent methylation and expression profiles. We also found positive DLST immunostaining not only in DLST-mutated tumours, but also in other tumours in which the TCA cycle is deregulated.

Mutations leading to extraordinary responses to mTOR inhibitors. The inhibitors of the mammalian target of rapamycin (mTOR) are key drugs for the treatment of several tumours. However, we lack markers able to identify patients with enhanced therapeutic sensitivity. To discover molecular determinants of drug response and to contribute to the definition of predictive biomarkers, we recruited renal cancer patients with extraordinary responses to these drugs and performed a comprehensive genomic, immunohistochemical and functional characterisation of the tumours. In two young adults with metastatic cancer, a renal epithelioid angiomyolipoma and a renal epithelioid angiomyolipoma, we identified a somatic mutation in the gene encoding a mitogen-activated protein kinase (MAPK) pathway effector, RHEB (C119Y). Somatic mutational analysis of RHEB identified a variant (c.356_357insA; p.Ala119ValfsTer17) in tumours and in a heterologous cell-based assay designed to functionally evaluate RHEB variants. p.Ala119ValfsTer17-RHEB-mutated tumours exhibited loss of heterozygosity as well as consistent methylation and expression profiles. We also found positive RHEB immunostaining not only in RHEB-mutated tumours, but also in other tumours in which the MAPK pathway is deregulated.

Differential alternative splicing (A3SS): Alternative 3′ splice site; RI: Retained intron. SE: Skipped exon; Log2 FC: Log base 2 fold change; A5SS: Alternative 5′ splice site; MXE: Mutually exclusive.

Deep sequencing of small RNAs reveals a prognostic marker functionally associated with alternative splicing modulation in thyroid cancer. It is urgent to identify biomarkers and functional networks associated with aggressive thyroid cancer behaviour in order to anticipate disease progression and facilitate patient-personalised management. The miRNome sequencing of thyroid tumour series enriched for advanced disease patients uncovered miRNA profiles correlated with tumour-specific histopathological and molecular features, such as thyroidal cell infiltration and tumour-driver mutation. Differential analysis considering disease prognosis revealed a significant hsa-miR-139-5p up-regulated in primary extracranial tumours from patients with recurrent/metastatic disease.Exogenous expression of hsa-miR-139-5p significantly reduced migration and proliferation abilities of anaplastic thyroid cancer cell lines. Proteomics analysis pointed to RICTOR, SMAUD3 and HNRNPF as hnrnfp5 upregulated putative targets in vitro.

Significantly, an abundance of hnrnfp5, an alternative splicing factor mainly involved in cryptic exon inclusion/exclusion, showed an anti-correlation with hsa-miR-139-5p expression in human tumours. Analysis of alternative splicing from RNA sequencing data revealed 174 events differentially regulated upon HNRNPF repression in genes and signalling cascades critical for thyroid cancer (FIGURE). These results point at hsa-miR139-5p/HNRNPF-gene transcripts balance as a novel regulatory axis associated with tumour virulence and modulation of major thyroid cancer signalling pathways.
The scope of the 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.
In 2018, the Group mainly focussed its research on pancreatic cancer while building resources for bladder cancer research. For pancreatic cancer (PC), we continued exploiting the data generated by the PanGenEU Study to further characterise pancreatic cancer risk. Two main articles exemplify our contributions to this domain. First, by applying complementary analytical approaches we reported that, regardless of non-genetic risk factors, the risk of PC was 2.5 higher among family members with 2 relatives affected with PC, with this risk being stronger in current smokers (FIGURE 1). Furthermore, we confirmed that PC was diagnosed at younger ages among those subjects with a family history of PC who smoked in non-smokers. In the second article, we reported on the underlying genetic basis behind PC and its associated multimorbidities network through a computational approach using the DisGeNET. This strategy allowed us to identify several autoimmune diseases linked to PC and the shared altered genes (FIGURE 2). These associations were subsequently confirmed at the individual level in the PanGenEU Study population of 1,705 PC cases and 1,084 controls that resulted in a reduced risk of PC in subjects having 2 autoimmune diseases. These findings again pointed to the role of the immunological status in PC carcinogenesis. We also continued to participate in international large-scale investigations to further characterise the genetic susceptibility and somatic alteration landscape of PC. For bladder cancer (BC), the Group reported on the inverse association between asthma and BC using the Spanish Bladder Cancer Epidemiology Study (SpanBCE) resource. This reduced risk of BC was especially observed among aggressive tumours. The Group also participated in the discovery process of both urine and tumour prognostic marker combination in large European studies of non-muscle invasive BC. We also performed a review of the genetic susceptibility to BC risk and progression based on GWAS hits. Most of the variants were common and conferred small and, therefore, they were not clinically actionable at the individual level.

Methodological contributions

The Group made contributions to both integrative analytic approaches concerning omics and omics (OnO) data as well as in the nutrition epidemiological field. Regarding the latter, we compared the antioxidant profiles of 21 a priori-defined Mediterranean diet indexes and reported that the level of dietary antioxidant intake captured through the different indexes differed due to the variation in their construction. As of the data integrative efforts, we observed that only a small number of published studies performed a ‘real’ integration of OnO data, primarily to predict cancer outcomes. We identified the challenges in OnO data integration and presented, discussed, and proposed integrative analytical strategies towards its integration.

Translational activities

The Group actively provides support in several clinical trials on immunotherapy and vitamins D in bladder cancer at the methodological level. We continue to sustain the Spanish National Cancer Research Centre, CNIO Familial PC Registry (PanGen-FAM) and the establishment of the European Registry of PC (PanGenEU). We lead the Research WorkStream of the Pancreatic Cancer Europe (EPC) multisite platform, with whom we hosted a session on PC Liquid Biopsy during the 2018 ESMO GI Meeting. To increase awareness of PC among health policy makers and discuss the different urgent need to invest in PC research, we participated and co-organised sessions with MEPs at the European Parliament and with delegates at the Annual Meeting of the European Association of Personalized Medicine.
The clinical and diagnostic activities carried out by the FCCU through the consultancy in the Medical Oncology Department of Puerta del Hierro University Hospital, have contributed to the selection of patients who are good candidates for targeted therapies. In order to extend the study, we apply a multigene panel test to an increasingly larger number of pathologies. Ovarian cancer (OC) for instance, is genetically heterogeneous malignancy that is potentially driven by multiple aberrant molecular pathways. Germline BRCA1/2 mutations account for 65-85% of all hereditary OC, while mutations in Lynch genes (DNA mismatch repair genes) are responsible for 10-15% of these hereditary OC. Germline mutations drive the therapeutic strategy: OC associated to BRCA1/2 mutations have a demonstrated sensitivity to PARP inhibitors, while immune checkpoint inhibitors are indicated for metastatic solid tumours associated with DNA mismatch repair deficiency.

Our clinical and diagnostic activities this year can be summarised as follows: 550 patients visited our consultation at HUF (66% increase over 2017), and 508 genetic diagnostic studies were performed in the FCCU laboratory (18.69% increase). Among these studies, we identified 25 tumours with MSI, all of them potential candidates to be treated with monoclonal antibodies that target PD-1.

Our research in colorectal cancer (CRC) focuses on early-onset forms and multiple primary tumours. We recently reported the largest series of Synchronous Colorectal Cancers (SCRC), in which clonality was analysed by Single-NArticular cancer according to tumor location. Huf et al. (2018). Comment on: Distinct clinical outcomes of two CIMP-positive colorectal cancer subtypes based on a revised CIMP classification system. Br J Cancer 118, 1333-1338.

Ovarian cancer (OC) is a genetically heterogeneous malignancy associated with DNA mismatch repair (MMMR) deficiency. Germline mutations in the mismatch repair genes are responsible for 10-15% of all hereditary OC, while mutations in Lynch genes (DNA mismatch repair genes) are responsible for 10-15% of these hereditary OC. Germline mutations drive the therapeutic strategy: OC associated to BRCA1/2 mutations have a demonstrated sensitivity to PARP inhibitors, while immune checkpoint inhibitors are indicated for metastatic solid tumours associated with DNA mismatch repair deficiency.

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MOLECULAR CYTOGENETICS AND GENOME EDITING UNIT

OverView

Recurrent chromosomal rearrangements are very common and well-known hallmarks of cancer. One of their main consequences is the creation of new chimeric genes as a result of the fusion of the coding sequences of 2 different genes. The research activity of the Molecular Cytogenetics and Genome Editing Unit (MC&GEU) is focused on increasing the knowledge about the genetics of tumours and the discovery of new therapeutic targets. With the combined use of genome editing and cytogenetic technologies, we are creating human in vitro models that recapitulate chromosomal, genetic and epigenetic cancer alterations. The goal of the Unit is to provide the CNIO and external researchers with the latest genome editing and cytogenetic technologies, we are creating new therapeutic targets. With the combined use of CRISPR and gene alterations. We provide state-of-the-art Molecular Cytogenetic and Genome Editing services. The Unit supplies research groups with various techniques that may provide more sensitive and accurate tools to analyse cancer cells, such as 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 PCR-based FISH analysis to detect genome integration sites of small constructs using LAV particles. 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 CRISPR-based cellular platforms offer a rapid, precise and affordable opportunity to functionally interrogate the cancer genome. In 2018, we carried out over 1,500 assays for experimental and clinically-oriented projects.

"We have applied genome engineering approaches for cancer modelling, reproducing chromosome rearrangements and gene alterations. We provide access to the latest Cytogenetic and CRISPR Technologies."

Publications


Research Highlights

Modeling 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 have recently demonstrated the feasibility of utilising gRNA/Cas9 ribonucleoprotein (RNP) complexes to model cancers driven by fusion genes generated by chromosomal rearrangements. We have optimised new strategies to enhance the efficiency of the CRISPR-mediated translocation induction in human stem cells, including mesenchymal and induced pluripotent stem cells. We found that the generation of targeted translocation is significantly increased by using a combination of ribonucleoprotein complexes (Cas9 protein+sgRNA) and ssODNs. The CRISPR-Cas9-mediated generation of targeted translocations in human stem cells opens up new avenues to model cancer.

Technological and translational activities

We provide state-of-the-art Molecular Cytogenetic and Genome Editing services. The Unit supplies research groups with various techniques that may provide more sensitive and accurate tools to analyse cancer cells, such as 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 PCR-based FISH analysis to detect genome integration sites of small constructs using LAV particles. 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 CRISPR-based cellular platforms offer a rapid, precise and affordable opportunity to functionally interrogate the cancer genome. In 2018, we carried out over 1,500 assays for experimental and clinically-oriented projects.
Pharmacogenetic variants and response to neoadjuvant single-agent doxorubicin or docetaxel: a study in locally advanced breast cancer patients participating in the NCT00123929 phase 2 randomised trial. Docetaxel and anthracyclines are widely used in the treatment of breast cancer despite the benefit being limited to a small proportion of patients, and predictive biomarkers predictive of clinical outcome remain lacking. We carried out a pharmacogenetic study in 183 patients with locally advanced breast cancer who were previously enrolled in a phase 2 randomised clinical trial (NCT00123929), in which patients were randomly assigned to receive doxorubicin (anthracycline) or docetaxel (taxane) in neoadjuvance. 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 ABCG2, as having the strongest association with tumour response observed in patients treated with doxorubicin (P=0.009). We also identified a significant association for an intronic variant, located in CYP1B1, associated with doxorubicin tumour response (P=2.15x10^{-4}).

Our integrated pathway-based approach allows revealing promising genetic biomarkers for treatment outcome in breast cancer patients (Ruiz-Pinto et al., 2018).

Genome-wide association study (GWAS) identifies three new loci associated with Ewing sarcoma susceptibility. Ewing sarcoma (EWS) is a paediatric cancer characterised by the EWSR1-FLI1 fusion. Our previous GWAS identified susceptibility loci at 1p36.22, 10q21.3 and 15q15.1, and identifies new loci at 6p25.1, 20p11.22 and 20p11.23. In the analyses of the new loci, there is evidence of informative eQTLs with nearby biologically plausible candidate genes that could be likely target genes for future functional investigations. It is remarkable that 6 independent susceptibility regions with relatively large effect sizes (estimated OR > 1.7) have been discovered in a sample of 775 EWS cases. In conclusion, our study provides support for a strong inherited genetic component to EWS risk and suggests that interactions between germline variation and somatically acquired EWSR1-FLI1 translocations are important etiologic contributors to EWS risk (Machiela MJ et al., 2018).

**New loci associated with risk to develop tobacco-induced lung cancer: genome-wide association study in heavy smokers.** We genotyped 27.3 million SNPs across the genome in heavy smokers that either developed NSCLC at an early age (extreme cases), or did not present NSCLC at an advanced age (extreme controls), selected from a discovery set (n=361). We validated significant SNPs in 133 additional subjects with extreme phenotypes selected from databases including >39,000 individuals. Two SNPs were validated: rs12660420 (p combined = 5.66 x 10^{-5}; OR combined = 2.80), mapping to a noncoding transcript exon of FDX1B; and rs68199797 (p combined = 1.62 x 10^{-4}; OR combined = 2.57), an intronic variant in ATP5D. We assessed the relevance of both proteins in early-stage NSCLC. FDX1B and ATP5D RNA expressions correlated with survival in 821 stage I-II NSCLC patients (p = 0.003 and p = 0.0001, respectively), FDX1B protein expression correlated with survival in 149 patients with stage I-II NSCLC (p = 0.002). In conclusion, we validated 2 novel variants associated with risk of developing tobacco-induced NSCLC in heavy smokers (Fusco JP et al., 2018).

**Publications**


**RESEARCH HIGHLIGHTS**

**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 and drug responses. In this Unit we implement different high-throughput and cost-effective methods to measure from one and drug responses. In this Unit we implement different high-throughput and cost-effective methods to measure from one and drug responses. In this Unit we implement different high-throughput and cost-effective methods to measure from one and drug responses.