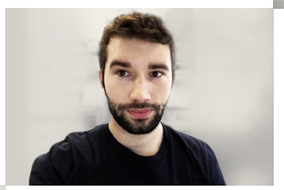
HUMAN GENETICS GROUP

Javier Benítez (until July)
Group Leader

Staff Scientists
Maria José García (until July), Ana Osorio (until July)

Post-Doctoral Fellow
Oriol Calvete (until November)



Graduate Students
Juan Miguel Baquero (until December), Paloma Martín (until December)

Technicians
Alicia Barroso (until July), Victoria Fernández (until August) (TS)*

Titulado Superior (Advanced Degree)

Students in Practice
María Escobar (February-July) (*Universidad Autónoma de Madrid*, Spain), Rocío Mirabet (February-June) (*Universidad Politécnica de Madrid*, Spain), Anne B. Parra (January-June) (*Universidad Complutense de Madrid*, Spain), Eneida Turiján (until April) (*Universidad Autónoma de San Luis Potosí*, Mexico)

OVERVIEW

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

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

RESEARCH HIGHLIGHTS

Hereditary breast and ovarian cancer

Deciphering the role of rare variants in BC

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

Identification of 14 new candidate BC susceptibility genes

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

Modifier genes in BRCA1/2 genes

We continue to play an active role in the CIMBA consortium (Consortium of Investigators of Modifiers of *BRCA1* and

BRCA2), which made important contributions in 2020 i) showing the modification of ovarian cancer risk by the PRS (Polygenic Risk Score) in *BRCA1* and *BRCA2* mutation carriers (Barnes *et al.*, 2020), ii) identifying the spectrum of mutations in male BC patients harbouring mutations in the *BRCA* genes (Silvestri *et al.*, 2020), and iii) associating mutations in different domains of *BRCA1* and *BRCA2* with prostate cancer risk (Patel *et al.*, 2020). We also started collaborating in the ambitious CONFLUENCER project, funded by the National Cancer Institute, the aim of which is to perform new Genome-Wide Association Studies (GWAS).

DNA glycosylase inhibitors as a new therapeutic approach in hereditary BC patients

In 2014, we reported that SNPs located in genes encoding for DNA glycosylases involved in the Base Excision Repair (BER) pathway could act as breast and/or ovarian risk modifiers in *BRCA1/2* mutations carriers (Osorio *et al.*, 2014). Since then, we have been exploring the mechanisms of action of these SNPs (Benítez-Buelga *et al.*, 2016; and 2017; Baquero *et al.*, 2019), as well as of the OGG1 glycosylase inhibitor TH5487 and its possible therapeutic use in BC patients. We found that the inactivation of BER by TH5487 increases the accumulation of oxidised bases at the telomeres, leading to telomere loss and post-mitotic defects (Baquero *et al.*, 2020 under review) (FIGURE). Moreover, we discovered that TH5487 enhances the activity of the PARP1 inhibitor olaparib in BRCA1 deficient cells. These preliminary results might represent the proof-of-concept for new alternative or complementary therapies for hereditary breast and ovarian cancer patients.

Ovarian cancer

Endometrioid (EOC) and clear cell (CCOC) ovarian carcinomas are considered refractory to chemotherapy and present bad outcomes once disseminated. About 15% show defects in mismatch repair (MMRd) and microsatellite instability (MSI). MMRd is a predictor of immunotherapy response, and the global burden of instability has been associated with survival in other tumour types. We compiled more than 200 EOCs and CCOCs and assessed the global load of microsatellite instability and tumour mutation burden using an "*ad hoc*-designed" NGS ovarian cancer panel (OvaSeq-MSI). OvaSeq-MSI includes a large set of MS sequences to improve MSI determination and provides information about mutations in ovarian carcinogenesis genes, ovarian cancer susceptibility genes, or therapeutic targets. Preliminary results show a good correlation between global instability and mutation burden, and an association between the extent of both indicators and improved disease evolution in patients (lack of relapse). Altogether, we expect to find markers that will enable more rational therapeutic decision-making for EOC and CCOC patients.

Familial cancer exome project

Autoimmune polyneuroendocrine syndrome (APS)

In 2015, we published research identifying the *ATP4A* gene as being responsible for families with achlorhydria-mediated gastric neuroendocrine tumours (gNET) (Calvete *et al.*, 2015 and 2016). In 2017, we extended this study to a new gNET family that presented with hypothyroidism and arthritis explained by two mutations in the *ATP4A* and *PTHR1* genes (Calvete *et al.*, 2017). In addition, we explored the pathogenic mechanism underlying tumour progression mediated by mutations affecting achlorhydria (Benitez *et al.*, 2020). We have further investigated the apparent relation of gastric autoimmune disease (gastric neuroendocrine tumour or chronic atrophic gastritis) co-occurring with other autoimmune diseases (hypothyroidism, arthritis, DM1, vitiligo). Using an NGS custom panel including 12 new candidate genes, we found several mutations putatively affecting the cell's internal acid-base balance function, not only in parietal cells from the stomach but also in other tissues (thyroid, epithelium, pancreas, skin), explaining this autoimmune polyendocrine syndrome (Calvete *et al.*, under review).

Testicular Germ Cell Tumour (TGCT)

During the past year, we explored TGCT in familial, bilateral, and sporadic patients, by evaluating their clinical history and by identifying moderate susceptibility genes and pathways involved in the disease. Familial and bilateral patients develop TGCT significantly earlier, and their relatives have a lower percentage of other tumour types compared to sporadic patients, suggesting a more relevant genetic background in their origin. A gene ontology analysis was performed with the variants obtained by WES, and the glycosylation pathway, a post-translational modification that consists of a carbohydrate attaching to different functional groups, was found to be over-represented in all cohorts of patients and thus might be involved in the development of the disease. In addition, the glycosylation genes with variants uncovered by WES were significantly located in the same cytoband as the susceptibility loci previously described by GWAS studies. ■

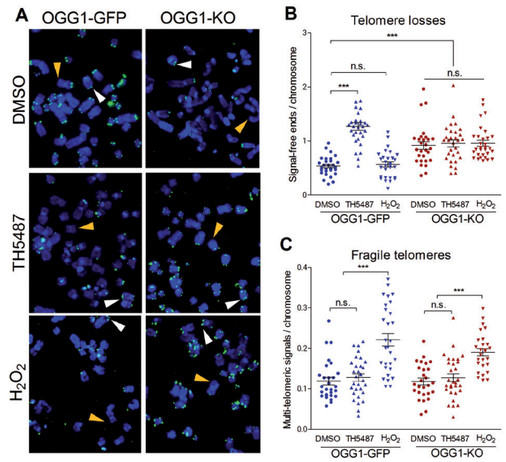


FIGURE The inactivation of OGG1 with TH5487 inhibitor causes telomere losses. (**A**) Telo-FISH images of metaphase chromosomes from U2OS OGG1-GFP or OGG1-KO cells for non-treated (DMSO), TH5487, and oxidative treatment. Chromosomes were stained with DAPI (blue) and telomeres were stained with PNA telomeric probe (green). An example of a telomere loss (orange arrowhead) and a fragile telomere (white arrowhead) is indicated in each image. (**B**) Quantification of telomeric signal-free ends for the conditions shown in (A). (**C**) Quantification of fragile telomeres. Comparative analysis for the frequency of multi-telomeric signals for the conditions shown in (A). Each dot represents a metaphase. More than 30 metaphases per condition from two independent experiments.

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