Human Genetics *Group*

Summary

Our Group is interested in understanding the genetic bases of familial breast, ovarian and colorectal cancer, and to apply such knowledge to clinical practice through genetic counselling.

Our approaches range from individual to population levels and from constitutional to somatic tissue using classical genetic approaches, new high throughput technologies and often functional studies in order to interpret all the generated data.

Strategic Goals

- Further insight into familial and sporadic breast cancer
- Molecular, immunohistochemical (IHC) and genomic characterisation of familial ovarian tumours
- Establish the genetic bases of hereditary polyposis and non polyposis-colorectal cancer syndromes

Javier Benítez *Group Leader*

Javier Benítez obtained his PhD in Biology (Human Genetics) from the *Universidad Complutense de Madrid* in 1982. He completed postdoctoral stays at the Department of Physiopathology, University of Ulm, Germany, 1985; and then at the Department of Human Genetics, University of Munster, Germany, 1990; as well as at the Department of Ophtalmogenetics, University of Amsterdam, The Netherlands. He was appointed as Honorary Consultant at the Royal Marsden Hospital in London, UK, in 2004.

Benítez spent several years working on the cytogenetics of human tumours at the *Fundación Jiménez Díaz*, Madrid, and from 1995 his Group focused specifically on hereditary breast cancer and the characterisation of genes responsible for hereditary breast/ovarian cancer (BRCA1 and 2) and the search for new genes (BRCAX).

In 1997 he was appointed as Head of the Human Genetics Service at the *Fundación Jiménez Díaz*. He joined the CNIO in the year 2000 as Director of the Human Genetics Department and in 2005 was appointed Director of the Human Cancer Genetics Programme.

Over the past few years his research has focused on familial cancer in general as well as the search for low penetrance genes associated with cancer using high throughput technologies.

Benítez has been President of the Spanish Society of Human Genetics and is currently an active member of various scientific societies and reviewer for various scientific publications and funding agencies. He has been Professor of Human Genetics at the *Francisco de Victoria* University in Madrid, and is Honorary Professor at the *Universidad Autónoma de Madrid*. He is also Director of the Spanish National Genotyping Centre (CEGEN, Madrid).

Staff scientists: M. José García, Beatriz Martínez, Ana Osorio, Gloria Ribas (until May), Miguel Urioste. Post-doctoral fellows: Francisco J. Gracia, Iván Muñoz. Graduate students: Lara P. Fernández, Maider Ibarrola (until August), Marta Kamieniak (since October), Ricardo Ramires, Bárbara Rivera, Juan M. Rosa, Eva Sánchez, Laura P. Saucedo, Miljana Tanic, Magdalena B. Zajac. Technicians: Alicia Barroso, Samuel Domingo (since March), Fernando Fernández, M. Victoria Fernández, M. Carmen González, Fátima Mercadillo.

Highlights

Breast cancer

Searching for the genetic bases of familial breast cancer: During the past few years we have demonstrated that non BRCA1/BRCA2 tumours (BRCAX) are heterogeneous from an immunohistochemical and genetic point of view. Searching for BRCAX genes, we studied all the genes from the Fanconia Anemia pathway and found that in addition to BRCA2 (FANCD1), there are two more genes – FANCJ (BRIP1) and FANCN (PALB2) – whose mutations explain about 2 – 3% of BRCAX families. By linkage studies in a large set of 41 BRCAX Spanish families we concluded that probably not more than 10 – 20% of the BRCAX families contain BRCAX genes located in candidate regions using this strategy.

Our search for low penetrant genes (LPG) within the BCAC Consortium in collaboration with the CNIO Genotyping Unit and the Genetic Epidemiology Group has concluded that these new genes currently explain about 7% of familial breast cancer risk. These results together with data from other groups suggest that there still exists about 50% of breast cancer families without a known aetiology. Environmental factors, other LPGs or new factors can explain breast cancer risk in some of these families (Figure 1). Along these lines, we are also conducting a large study in collaboration with M. Pollan from the National Epidemiology Centre, looking for genes responsible for mammographic density – one of the main risk factors for breast cancer.

Searching for modifier factors: Regarding the familial cases that are known to be associated with the high risk susceptibility genes BRCA1 and BRCA2, we are participating in two international consortia that are focusing on the identification of phenotypic modifiers of these two genes: the IBCCS (International BRCA1/2 carrier

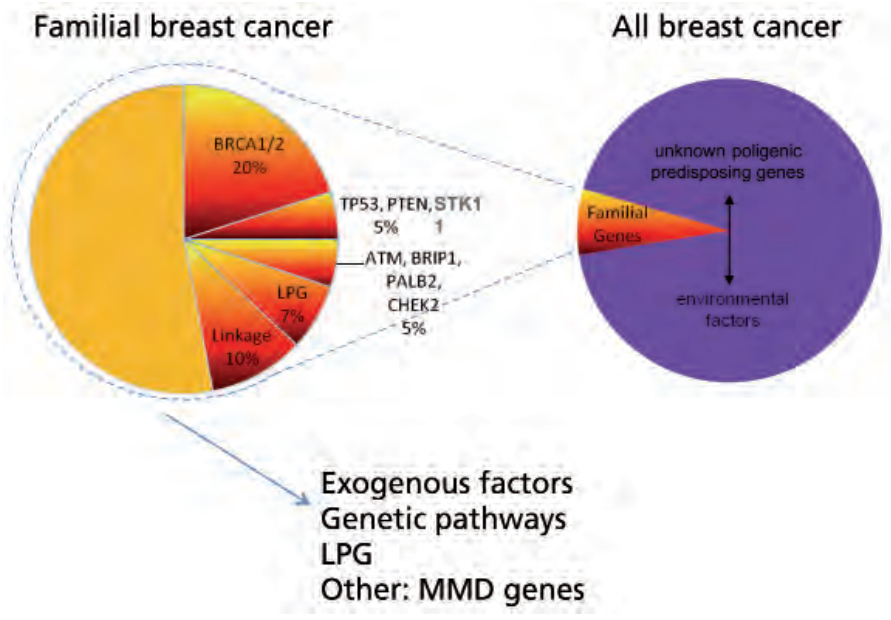

Figure 1: Genetic bases of familial breast cancer. About 25% of familial breast cancer can be explained by mutations in BRCA1/2 or other high susceptibility genes; up to 5% by moderate penetrance genes; low penetrance genes contribute to familial cancer risk by 7%; and no more than 10-15% of the families by genes located in candidate regions through linkage studies.

cohort study) and CIMBA (Consortium of Investigators of Modifiers of BRCA1/2). We are also coordinating a study on the genes involved in the Base Excision Repair Pathway (BER) as potential risk modifiers in a large set of 2000 BRCA1/2 carriers originating from the Spanish Consortium for the study of hereditary breast cancer.

Searching for genetic markers via expression arrays: We have completed an expression microarray study in familial breast cancer and concluded that BRCA1 tumours can be sub classified into two main groups according to their ER status. The ER positive group presented overexpression of cell cycle genes while ER negative group presented overexpression of genes related with the immune response and probably regulated by the REL/NF*k*B transcription factor family. Regarding the BRCAX tumours, we have demonstrated that they are heterogeneous and can be divided into at least two main subgroups, which can be classified as Luminal A and B, considering the intrinsic phenotypes defined for the sporadic breast tumours.

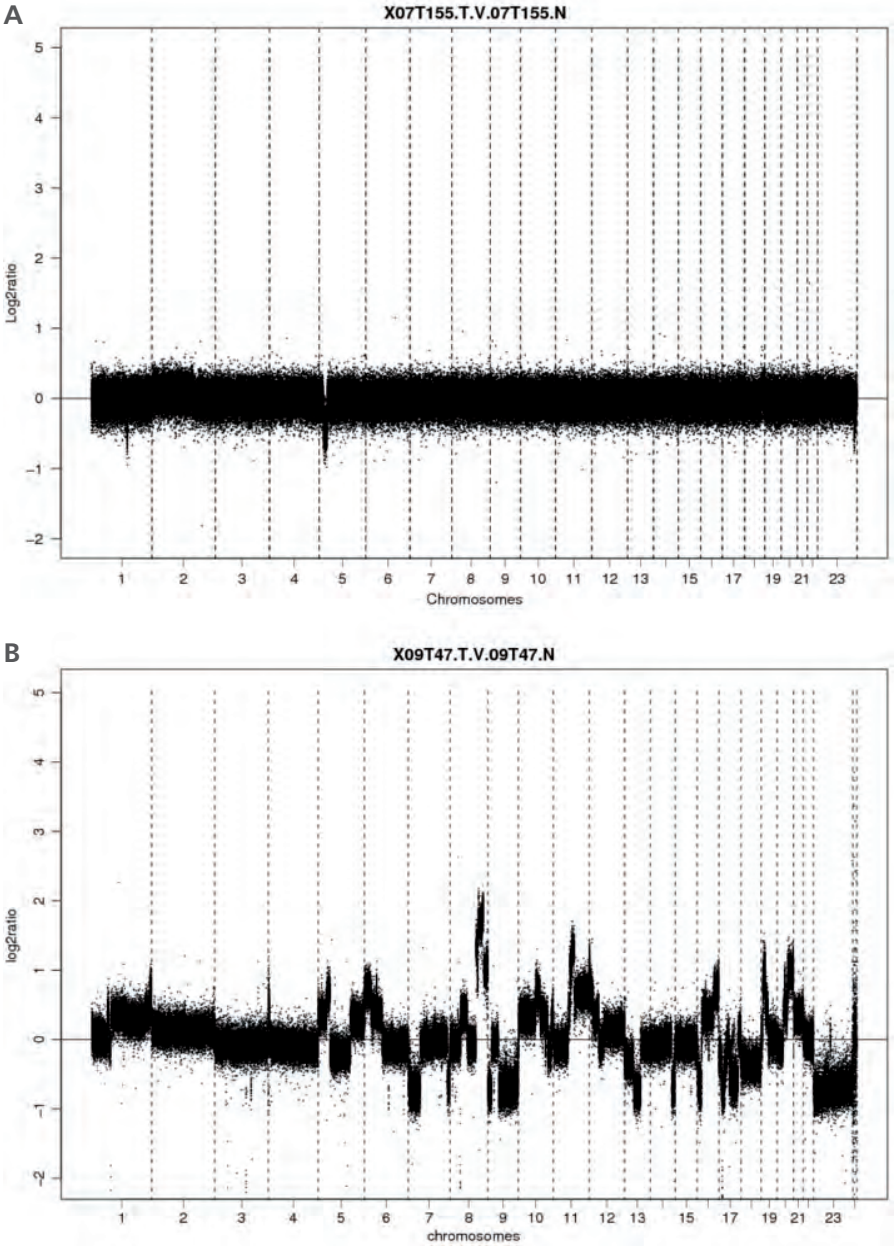

Figure 2: Chromosomal heterogeneity of familial ovarian tumours: aCGH of an ovarian tumour without genomic changes (A); an example with multiple abnormalities (B).

miRNA and familial cancer: We are conducting a study aimed at identifying the molecular miRNA profile associated with BRCA1/2 and BRCAX. The objective is to detect specific markers that can provide us with a faster and better diagnosis of these tumours. We are also trying to identify miRNAs that are regulating the BRCA1 gene. About 80 candidates have been identified through *in silico* studies.

Ovarian tumours

Genomic and IHC classification: During this past year we have collected 75 familial and 125 sporadic tumours with the objective of classifying them from an IHC point of view and defining diagnostic, prognostic and drug response markers. For this study we have built various tissue microarrays (TMAs) that are being analysed with a large set of antibodies from different pathways. We want to combine these studies with data obtained from high-resolution oligo array-based comparative genomic hybridisation (aCGH) of the same cases to generate a common pool of data that will facilitate a wide and integrated interpretation of the results (Figure 2). We are currently analysing the data.

Familial colorectal cancer

a) HNPCC type X genes: We are working with 21 HNPCC type X families in two complementary ways: 1) using linkage studies we have identified three regions of interest in chromosomes 2, 4 and 12 and have started conducting refinement analysis; 2) we have built several TMAs with colorectal cancer (CRC) type X and other types of hereditary and sporadic colorectal tumours in order to compare

the IHC expression of proteins involved in different CRC carcinogenesis pathways.

b) APC/MUTYH negative Familial Adenomatous Polyposis (FAP): We are studying 27 families with classical forms of FAP without detectable mutations in both APC and MUTYH genes and trying to define other mechanisms of APC inactivation such as allelic imbalance or the involvement of other genes in the Wnt pathways.

Human genetics service activity

Our Group is a reference in Spain for genetic studies in familial cancer syndromes. In 2009 we carried out over 600 studies involving more than 25 genes responsible for different types of familial cancer syndromes. About 40% correspond to families with breast and ovarian cancer; 30% to colorectal cancer; 20% to endocrine cancer; and the remaining cases to rare genetic diseases.

Publications

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