SUMMARY

The challenge of genetic counselling for persons at high risk of breast and/or ovarian cancer is individualized risk estimation and management. Although other factors may be involved, personalized counselling will obviously not be possible until identification of the genetic cause of the disease for all the HBOC families. During the genetic screening of the major susceptibility *BRCA1* and *BRCA2* genes, several variants of undetermined clinical significance (VUS) are identified. Their clinical relevance must be assessed. Secondly, risk factors other than the mutation in the *BRCA1* or *BRCA2* genes should be integrated in the risk estimation. This thesis compiles several studies aiming at improving the risk assessment of individuals from breast and/or ovarian cancer families with BRCA1/2 genetic variants using genomic technology and transcriptional studies.

Chapter 2 describes the mutations identified during the genetic screening of Portuguese families, who attended the Oncology Centre of Lisbon Francisco Gentil for genetic counselling. The identification and characterization of a Portuguese founder mutation, *BRCA2* c.156_157insAlu, is reported in detail. This mutation, present in 8% (17/210) of the families, was estimated to have occurred many centuries ago. Additionally, in a group of 53 patients, of which 3 carried the founder mutation, other 15 different mutations were identified in 16 individuals. Screening Portuguese high-risk families and their descendants for the founder mutation, prior to the screening of the complete *BRCA1/2*-coding regions is rapid and cost-effective.

Prediction of the cancer-site risk is not possible, even for individuals with the same mutation, irrespective of whether they belong to the same family, although a certain tendency could be observed within each family. Several studies have suggested the existence of additional genetic modifying factors. Since cancer sites affected by *BRCA1/2* mutations mainly involve organs affected by steroid hormone metabolism, we hypothesised that functional polymorphisms in genes involved in steroid hormone metabolism could influence cancer-site risk. The pilot study described in **chapter 3.1** included women from families with a *BRCA1* or *BRCA2* mutation diagnosed at our centre. Two of those genes, the progesterone receptor (*PR*) gene and *FGFR2*, a PR activator, were analysed as putative genetic risk modifiers. Additionally, three polymorphisms in *TNRC9* (*TOX3*) and *CASP8*, previously described to modify the risk of sporadic and inherited breast cancer, were also investigated. The results of this study indicated that the minor allele of *FGFR2* (rs2981582) is protective against ovarian cancer, but increases the risk of bilateral breast cancer. The other polymorphisms did not show or confirm an association with breast cancer or ovarian cancer, probably due to lack of power. In **chapter 3.2** we have analyzed the risk of *FGFR2* (rs2981582) for ovarian cancer in an international and much larger population of more than 20.000 *BRCA1/2* female carriers. This study confirmed that *FGFR2* is indeed protective for ovarian cancer (HR= 0.86, p= 0.090 and HR= 0.67, p= 0.005 for BRCA1 and BRCA2, respectively), especially among *BRCA2* carriers. These results may have a clinical impact in the risk-reducing strategies of women with a *BRCA1/2* mutation.

A large proportion of the genetic variants identified during genetic screening are variants of unclear clinical relevance (VUS), which pose problems to pre-symptomatic DNA-testing and accurate genetic counselling. **Chapters 4.1 and 4.2** evaluate and characterise the effect of a set of *BRCA1/2* VUS predicted by *in silico* models to have an effect on mRNA splicing, using a combination of allele- and transcript-specific primers. The results determined conclusively the pathogenicity of six (out of 14) variants: three were considered pathogenic and three non-pathogenic. From the remaining variants: one variant was determined to be likely pathogenic; three other variants were found to increase the expression of naturally occurring isoforms, without decreasing the expression of the full-length transcript, and therefore remain unclassified; four variants had no effect on splicing, however their pathogenicity could not be excluded, since they lead to missense changes that might affect the protein function. Furthermore, this work allowed to suggest a likelihood threshold that may be used for the software models for future selection of variants with a putative effect on splicing. In summary, except for variants affecting a non-canonical splice site, three algorithms must predict at least 10% decrease or

two algorithms must predict 20% decrease of the splice site score. In **Chapter 4.2** an *ex vivo* minigene splicing assay was used as a complementary technique to those used in the previous study. This approach might substitute the allele-specific analysis when allele-specific amplification cannot be used. The study provided definite evidence that the full-length transcript was not expressed from the allele with the variant c.4987-3C>G and enabled to conclude that it is a pathogenic variant.

Chapter 5 evaluates the potential of MLPA (Multiplex Ligation-dependent Probe Amplification) to detect complete exon skipping events in mRNA from *BRCA1*. The intended use of the commercial *BRCA1* MLPA kit is the detection of genomic rearrangements. Currently, there is no routine technique available to detect complete exon skipping events in mRNA without using specific primers. The availability of such a technique would be very useful in the diagnostic setting. Although after several optimization steps the technique could detect exon skipping events, the results were not fully satisfactory for routine RNA analysis in a diagnostic setting since it was not always reproducible.

Chapter 6 explores the transcriptome of cultured BRCA1$^{+/-}$ lymphocytes after irradiation, aiming at the identification of biologically relevant genes that could be incorporated in a genetic classifier able to distinguish between *BRCA1* mutation-carrier and non-carrier samples. We identified many differentially expressed genes associated with biological processes in which *BRCA1* is known to play important roles. More specifically, we showed that BRCA1$^{+/-}$ lymphocytes, in response to irradiation, had deficient cell cycle arrest, decreased apoptosis, decreased immune response, increased chromosomal instability, and decreased mitotic spindle assembly and chromosome separation, leading to increased micronuclei induction. A gene signature including 25 genes from these pathways and from 141 overlapping genes between two independent groups of samples allowed to cluster separately samples from *BRCA1*-mutated and normal lymphocytes. This approach was more robust than using just the most statistically different expressed genes, as generally used in class prediction approaches. The hierarchical clustering results obtained with this gene signature in datasets from independent studies, which used different cell types and DNA-damage agents, supported this observation.

In conclusion, the studies compiled in this thesis lead to the identification and characterization of several *BRCA1/2* pathogenic variants. A polymorphism in *FGFR2* was found to modify the cancer-site risk among *BRCA1/2*-carriers. However, the result of the combination with other risk and protective factors remains unclear. It is necessary to understand this before the test can be used in a clinical setting. A genetic classifier able to determine the clinical relevance of *BRCA1*-VUS was developed and the results obtained in independent datasets were very promising. Further analysis of different datasets would allow refining the gene list and test its reproducibility. The studies in this thesis contributed to the long and challenging process to get to personalized risk assessment for *BRCA1/2*-mutation carriers.