CGG/ERN GENTURIS/ICARE Monthly Journal Round-Up – September 2022

Translational science

Constitutional *BRCA1* methylation and risk of incident triple-negative breast cancer and high-grade serous ovarian cancer. Lønning *et al*. 2022. *JAMA Oncology.* doi:10.1001/jamaoncol.2022.3846

- Around 25% of all TNBCs and 10-20% of HGSOCs have *BRCA1* promoter methylation.
- While and association between *BRCA1* methylation in normal tissue and TNBC and HGSOC was established previously, these studies were performed on normal tissue obtained after the patients received their cancer diagnoses, therefore the potential role of normal tissue methylation as a risk factor for incident TNBC or HGSOC is unknown
- Case-control study looking at 637 women developing incident TNBC and 511 women developing incident HGSOC and matched cancer-free controls (matched for age at entry, hormone therapy use, race and ethnicity, and DNA extraction method)
- Methylated *BRCA1* alleles were present in 194 controls (5.5%)
- Methylation was significantly associated with risk of incident TNBC (12.4% methylated) and incident HGSOC (9.4% methylated)
- *BRCA1* methylation status was not associated with a self-reported family history of either breast or ovarian cancer
- Across individuals, methylation was not haplotype-specific, which goes against an underlying cis-acting factor (factor located on the same allele as the methylation)
- Within individuals, *BRCA1* methylation was observed on the same allele, indicating that *BRCA1* methylation may have occurred as a single, early event that was followed by clonal expansion of the methylated cell.
- No association found between *BRCA1* methylation and germline *BRCA1/2* PV status.
- The authors suggest this has potential implications for prediction of incident TNBCs and HGSOCs, and warrants further research to determine if constitutional methylation of tumour suppressor genes are pancancer risk factors.
- The study results may serve as proof of concept for early life constitutional methylation as a cancer risk factor

Digenic contribution of Cancer driver genes and *TNFRSF13B* rare variants in malignancies predisposition. E. Pasquinelli et al., abstract accepted by the ESHG Conference 2022 as Poster.

- Mutations in the *TNFRSF13B* gene encoding TACI (Transmembrane Activator and CAML Interactor) were previously associated with common variable immunodeficiency (CVID).

- A normal function of immunity-related genes and their signaling pathways safe-guards against the development of tumors, whereas its impairment might increment the likelihood of their recurrence.
- By Exome Sequencing (ES), a series of 2603 individuals divided into a control group (1937 patients) and cohort affected by different forms of cancer (666 patients) were analyzed.
- The focus was on cancer patients in whom simultaneously at least one pathogenic or likely pathogenic rare variants in *TNFRSF13B* and one in moderate penetrance cancer genes (including many DNA repair genes) were detected.
- Data analysis in these patients identified heterozygous loss-of-function mutation in the *TNFRSF13B* gene:
    - Four stop-variants (c.431C>G, p.Ser144*; c.706G>T, p.Glu236*; c.198C>A, p.Cys66*; c.579C>A, p.Cys193*);
    - One frameshift variant (c.204dupA, p.Leu69Thrfs*12);
    - Two missense-variants (c.310T>C, p.Cys104Arg; c.693C>G, p.Ser231Arg) (CADD>24).
- The significant association of heterozygous rare pathogenic or likely pathogenic variants in *TNFRSF13B* in cancer patients suggests a possible digenic inheritance of cancer driver genes and *TNFRSF13B*.
- These results are in agreement with some observations:
    - a common polymorphism in regulatory region of *TNFRSF13B (*rs4792800) has an impact of prostatic cancer survival through modulating apoptosis;
    - the primary cause of death of patients suffering of common variable immunodeficiency is cancer in different organs.
- Functional studies are ongoing to demonstrate that the combined effect of cancer driver genes and reduced immunity through B cell activation leads to malignancies predisposition.

Towards polygenic inheritance in familial cancer, Lista M. et al., Abstract accepetd by ASHG Conference 2022 as Poster.

- The majority of cancer driver genes have moderate to low penetrance. However, the molecular bases of incomplete penetrance have still not been fully understood yet.
- By Exome Analysis (EA) we wanted to explore:
    1) the model of transmission in 40 families in which the DNA of at least two affected members was available (*Not Segregating Pathogenic Variants*)
    2) 80 cases in which only one affected member was available (*Two Pathogenic Variants*)
    3) if the number of rare P-VUS (MAF<0,01) in cancer driver genes of 30 cancer patients differs from that identified in 50 non-cancer subjects (*Towards Polygenic Model*).
- **Not Segregating Pathogenic Variants**: In a relevant percentage of cases (30%) the pathogenic variant (P) identified in one cancer gene did not segregate with the disease in the family. We found another variant of unknown significance (VUS) in another cancer driver gene segregating with the disease.

- **Two Pathogenic Variants**: In a significant fraction of patients (10%) we identified P variants in 2 different cancer driver genes
- **Towards Polygenic Model**: We identified a mean of 2,8 P-VUS in patients and a mean of 1,6 P-VUS in controls with a *p-value* between them of 0,00098 obtained with Mann-Whitney U Test.
- These data can give us the evidence that cancer may have a polygenic inheritance and that, also familial cancer genetic susceptibility, could be the result of more than one variant in cancer driver genes.
- The future studies will be focused on enlarged the statistical study cohort and performed functional studies on VUS variants.

In the clinic

Exome Sequencing in BRCA1-2 Candidate Familias: The Contribution of Other Cancer Susceptibility Genes. *Doddato et al. 2021. Frontiers in Oncology.* https://doi.org/10.3389/fonc.2021.649435

- Hereditary Breast and Ovarian Cancer (HBOC) syndrome is a condition in which the risk of breast and ovarian cancer is higher than in the general population. The prevalent pathogenesis is attributable to inactivating variants of the BRCA1-2 highly penetrant genes, however, other cancer susceptibility genes may also be involved.
- Since 1994, when BRCA1 (MIM#113705) and BRCA2 (MIM#600185) were identified, pathogenic variants (PVs) in these two genes have been known to be the most important cause of HBOC.
- The objective of the current study was to analyze a series of 200 individuals selected for genetic testing in BRCA1-2 genes according to the updated National Comprehensive Cancer Network (NCCN) guidelines. Analysis by MLPA was performed to detect large BRCA1-2 deletions/ duplications.
- This study allowed to go further beyond BRCA1-2 genes and identify variants in additional 22 patients with PVs in canonical and candidate non-canonical HBOC genes.
    - Focusing on BRCA1-2 genes, belonging to homologous recombination pathway, data analysis identified 11 cases with pathogenic variants (5.5% diagnostic yield) and only one case was found with a large BRCA1 deletion (0.5% diagnostic yield).
    - Exome analysis allowed to characterize 22 pathogenic variants in 16 additional genes. Among these variants, 10 are located in 7 genes more traditionally associated to breast and ovarian cancer, of which 4 members of BRCA1-2 pathway (ATM, BRIP1, PALB2 and RAD51C) and 3 within Pathways in cancer (CDH1, PTEN and TP53) (5% diagnostic yield).
    - The remaining 12 PVs are distributed in 9 candidate cancer susceptibility genes (DPYD, ERBB3, ERCC2, MUTYH, NQO2, NTHL1, PARK2, RAD54L, and RNASEL) (6% diagnostic yield). The most frequently mutated was *MUTYH* (4/200; 2%) involved in the base excision repair pathway.

  - 86 variants were classified as VUS (class 3) on the basis of the IARC recommendations: among them 12 variants in BRCA1-2 genes are mainly missense substitutions located in exon 11 (8/12; 66%), while the remaining 74 variants were mostly missense mutations located in ATM and PALB2 genes.
- This study provided a personalized risk assessment and clinical surveillance in an increased number of HBOC families and to broaden the spectrum of causative variants also to candidate "non-canonical" genes.
- ES analysis in a cohort of HBOC suspected patients enabled a total diagnostic yield of a further 11% in non BRCA1-2 genes, although this strategy increases the level of complication of the analysis and the number of VUS per sample, their identification appears to be essential for future definitive classification and the detection of new inheritance patterns.

Monthly Journal Round-Up brought to you by:

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