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

## Bigger picture

Last month in *Science*, Prof. Nik-Zainal and colleagues presented their exciting work on cancer mutational signatures. They analysed the mutational signatures of 12,222 whole-genome-sequenced cancers which were collected through the 100,000 Genomes Project, a British initiative to sequence 100,000 genomes from around 85,000 NHS patients affected by rare disease or cancer. Their analyses reinforced the understanding that mutational signatures are tissue specific, and they also revealed a number of previously unidentified signatures: 40 new single-base substitution signatures, and 18 double-base substitution signatures. The team have also developed an algorithm called Signature Fit Multi-Step (FitMS) which detects both common, organ-specific signatures, as well as additional rare signatures.

## Translational science

**Predictive functional assay-based classification of *PMS2* variants in Lynch syndrome.**
Rayner *et al.* (2022). *Hum Mutat*; doi: 10.1002/humu.24387.

- Lynch syndrome (LS) is an autosomal dominant cancer predisposition, caused by a heterozygous inactivating germ-line defect in one of the major mismatch repair (MMR) genes: *MLH1*, *MSH2*, *MSH6*, or *PMS2*.
- Most germline alterations identified in the DNA mismatch repair (MMR) gene *PMS2*, a low-penetrance gene for the cancer predisposition Lynch syndrome, represent variants of uncertain significance (VUS). The inability to classify most VUS interferes with personalised healthcare.
- The authors of the paper describe an in vitro MMR activity (CIMRA) assay, that only requires sequence information on the VUS, provides a functional analysis-based quantitative tool to improve the classification of VUS in MMR proteins. They then derived a formula that translates CIMRA assay results into the odds of pathogenicity (OddsPath) for VUS in *PMS2*.
- Initially the authors generated Pms2-hemizygous mouse embryonic stem cells using CRISPR-Cas9 using cell cultures.
- Then they generated a set of pathogenic *PMS2* missense variants using an in cellulo genetic screen. These methods have been described before but the authors here attempted to introduce random missense substitutions in the genome. They chose cells that efficiently became MMR-deficient presumably by an inactivating mutation

in monoallelic Pms2 and then these cells were selected using he Guanine analog 6-Thioguanine. The surviving clones were treated and then screened against loss of heterozygosity at Pms2 by intragenic allele-specific PCR. From remaining clones, Pms2 cDNA was generated and the critical, conserved, domains were sequenced using Sanger sequencing to identify the inactivating amino acid substitution.

- Then, they selected the missense substitutions for CIMRA assay calibration and validation. They created PMS2 cDNA using mutagenic PCR followed by IVT and translation of the protein variants. They used CRISPR-Cas9 gene targeting and a mismatch-containing fluorescent substrate and observed the ability of repair of deficiency. Repair deficiency resulting from an inactivating VUS causes the absence of the repair-diagnostic fluorescent fragment, indicating that the VUS is cancer-predisposing.
- Following all these, they performed a regression for CIMRA assay calibration and validation.
- Lastly, the authors used genetic screening for the identification of inactivating PMS2 missense variants and calibrated and validated this method using other human PMS2 substitution variants that have been previously characterized as benign or VUS.
- The authors conclude that the OddsPath provides an integral metric that, following the other, higher penetrance, MMR proteins MSH2, MSH6 and MLH1 can be incorporated as strong evidence type into the upcoming criteria for MMR gene VUS classification of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP).

## In the clinic

**Germline *MBD4* deficiency causes a multi-tumor predisposition syndrome**. Palles *et al*. (2022). *Am J Hum Gen.* 109: 953-960. https://doi.org/10.1016/j.ajhg.2022.03.018.

- The authors show that bi-allelic loss-of-function germline variants in the base excision repair (BER) gene *MBD4* cause an autosomal recessive, multi-organ tumour predisposition syndrome.
- *MBD4* encodes a glycosylase involved in repair of G:T mismatches resulting from deamination of 50-methylcytosine. The colorectal adenomas from MBD4-deficient individuals showed a mutator phenotype attributable to mutational signature SBS1, consistent with the function of MBD4. MBD4-deficient polyps harboured somatic mutations in similar driver genes to sporadic colorectal tumours.
- Through WGS and WES in a cohort of 309 individuals with multiple colorectal adenomas or familial CRC (198 unrelated families), targeted sequencing of *MBD4* in 1,611 individuals (with at least 10 colorectal adenomas, familial or early onset CRC, or CRC in combination with other tumours), and cascade testing, the authors identified five individuals within four families with bi-allelic *MBD4* variants, and these

individuals had a personal and/or family history of adenomatous colorectal polyposis, acute myeloid leukemia, and uveal melanoma.

- They propose the name *MBD4*-associated neoplasia syndrome (MANS), and suggest 2-yearly colonoscopies from age 18-20, or the date of diagnosis. They suggest regular follow-up full blood counts for individuals with MANS if initial presentation is with adenomatous polyposis. They also suggest annual ophthalmological surveillance may be appropriate.
- Heterozygotes for *MBD4* LOF variants appear to be at a 4- to 20-fold increased risk of uveal melanoma. The risk of CRC and/or polyposis in heterozygotes is currently unknown.
- Inclusion of *MBD4* in genetic testing for polyposis and multi-tumour phenotypes is warranted to improve disease management. The identification of remaining polyposis genes is important in order to plan appropriate tumour surveillance for affected individuals and their relatives.

**Survival of *BRCA1/BRCA2*-associated pT1 breast cancer patients, a cohort study**. Barele *et al*. (2022). *Breast Cancer Research and Treatment.* https://doi.org/10.1007/s10549-022-06608-1

- It is known that tumour size and lymph node involvement are positively correlated and both are independent predictors for BC-related mortality. However there is debate as to whether the association between tumour size and outcome is as strongly present in *BRCA1/*2-association BC, and the correlation between tumour size and lymph node involvement in *BRCA1* mutation carriers has been reported to be weaker than for sporadic BC or *BRCA2*-associated BC.
    - Together, this gives uncertainty as to the survival benefit from BC screening in *BRCA1/2* mutation carriers
- *BRCA1/2*-associated BC patients were selected from a nationwide cohort. The authors looked at 10-year overall survival (OS) depending on tumour size (pT1a (0.1-0.5cm), pT1b (>0.5-1.0cm), and pT1c (>1.0-2.0cm)), the effect of chemotherapy on prognosis of node-negative BC and lymph node involvement per pT1a-b-c group.
- After median follow-up of 10.5 years, 10-year OS in patients without chemotherapy was 91.4% in pT1aN0, 90.8% in pT1bN0, and 77.1% in pT1cN0
- OS was better with than without chemotherapy for pT1c (91.6% vs. 77.1%) and pT1b (100% vs. 90.8%)
- 10-year OS of pT1a patients with chemotherapy was 69.4%..
- Lymph node involvement increased with larger tumour size (24.9% in pT1c, 18.8% in pT1b, and 8.6% in pT1a)
- In conclusion, smaller tumour size is associated with better OS and less lymph node involvement in pT1 *BRCA1/2*-associated BC patients. The results suggest that early detection in *BRCA1/2* mutation carriers of pT1a/b BC may reduce mortality and the need for systemic therapy.

## Counselling and ethics

**Outcomes of support groups for carriers of BRCA 1/2 pathogenic variants and their relatives: a systematic review.** Bertonazzi *et al*. (2022). Eu *J Hum Genet.* 30: 398-405. https://doi.org/10.1038/s41431-022-01044-7.

- This systematic review assessed studies exploring outcomes of support groups for *BRCA1* or *BRCA2* pathogenic variant carriers.
- 34 papers were reviewed, published between January 1995 and February 2021
- The review aimed to answer the questions "Are support groups helpful for people tested positive for BRCA1/2 and their relatives? What are the outcome of these interventions on this specific population?"
- The authors chose to begin their search from 1995 because *BRCA1/2* testing was available from around this time.
- Three major themes emerged from the analysis of the studies:
    - Risk management decision: groups were helpful to women's decision making on risk-reducing surgery or screening. In some cases, prior preference was reinforced by attending a support group and those attending were more likely to proceed with their choice within two years, compared to those who didn't attend a group. Many asked for real examples of breast reconstruction and talked about emotional implications
    - Family dynamics and risk communication: groups generally allowed for an environment which encourage sharing of personal and family experience. However, a benefit on familial communication was not clearly demonstrated and was sometimes even reduced. Attending a group did not modify attitudes to disclosure of results. However, groups allowed many thoughts and feelings around these issues to be explored
    - Psychosocial functioning: groups allowed sharing of reactions and feelings, particularly if these were unexpected. Participations appeared to reduce distress, depression and anxiety. Issues around guilt and couple relationships were also significantly discussed
- The authors note that this systematic review was limited to a number of countries, meaning certain sociocultural backgrounds were not explored. Different interventions were used meaning direct comparisons can't be made
- Overall, support groups were well-received and no studies reported negative consequences of support groups. Support groups could offer an alternative to individual follow-ups which can be difficult to offer based on clinical resources.

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