CGG/ERN GENTURIS Monthly Journal Round-Up – April 2021

Bigger picture

Universal immunohistochemistry for Lynch Syndrome: a systematic review and meta-analysis of 58,580 colorectal carcinomas. Eikenboom *et al.* (2021). *Clinical Gastroenterology and Hepatology.* Available: https://www.cghjournal.org/article/S1542-3565(21)00455-9/fulltext

Background

Lynch Syndrome is the most common hereditary colon cancer predisposition syndrome, caused by constitutional pathogenic variants in MLH1, PMS2, MSH2, MSH6 or rarely EPCAM. Universal immunohistochemistry to detect MMR deficiency is increasingly being applied to colorectal cancers, to identify those patients who may require germline testing to confirm a diagnosis of Lynch Syndrome, as well as to direct systemic chemo- and/or immunotherapy. The aim of this review was to determine the proportions of MMRd colorectal cancer that ultimately can be confirmed to be accounted for by Lynch Syndrome, by somatic MMR aberrations, and those that are unexplained.

Methods

The authors performed a systematic review and metanalysis. A number of sources (Ovid Medline, Embase, Cochrane Central) were interrogated for studies published before 20th March 2020 on the topic of universal MMR IHC in colorectal cancer.

Data related to MMR germline investigations was enumerated, and proportions of Lynch Syndrome-associated, sporadic and unexplained MMRd cancers calculated. Subgroup analyses by age and diagnostic approaches were undertaken.

Results

The authors identified 2723 articles of potential relevance, of which, 56 were included in the final analyses- comprising information related to 58,580 colorectal cancers. Of these cancers, 45776 (78.14%) were deemed MMR proficient, and 5884 (10.04%) MMR deficient with missing data in 6920 (11.81%).

MLH1 and PMS2 loss was the most common pattern identified among MMRd cancers (4075 (69%)), of which 52% was deemed sporadic following detection of a pathogenic somatic BRAF variant or hypermethylation of the MLH1 promoter.

MSH2 and MSH6 loss was reported in 982 tumours (17% MMRd cancers), isolated MSH6 deficiency in 337 tumours (6% MMRd tumours), and isolated PMS2 deficiency in 277 tumours (4% MMRd cancers). Other atypical patterns were demonstrated in 167 tumours (3%), while patterns were not defined in 46 MMRd tumours (<1%).

Germline genetic investigations were undertaken in 76% of eligible patients. Of those tested, 1198 (37.9%) patients were found to carry a pathogenic MMR gene variant, accounting for 2% of all cancers.

Pathogenic constitutional variants were most commonly identified in *MSH2* (n=437) and MLH1 (n=398), and less commonly in MSH6 (n= 197) and PMS2 (n=125), with a small proportion of affected patients carrying a pathogenic deletion in 3' end of EPCAM (n=35). Per protein-analysis demonstrated that, although MSH2/MLH1 variants accounted for the majority of Lynch Syndrome; the likelihood of identifying a germline variant was greatest in patients with tumours demonstrating isolated loss of MSH6 or PMS2.

Sub-analyses by age indicated higher yield of diagnostic testing, with constitutional MMR gene variants found to account for 7.27% (57/784) cancers among patients diagnosed before age 50, and 5.06% (101/1998) in those diagnosed before 70.

MMR deficiency was deemed unexplained in 2489 colorectal cancers (4.24% of total cohort). Biallelic somatic inactivation of MMR genes (biallelic variants or single variant with evidence of loss of heterozygosity) were reported in 140 tumours, with MLH1 (n=95) most commonly implicated, followed thereafter by MSH2 (n=35), MSH6 (n=7) and PMS2 (n=3). However, not all studies included full information regarding somatic diagnostics, with only 7 studies describing complete somatic analyses. These studies together included 6848 tumours, of which 9.21% were MMRd. The majority of MMRd tumours (64.71%) demonstrated a pathogenic BRAF variant and/or MLH1 hypermethylation. 94.33% of eligible patients underwent germline testing, of whom 56.82% had a pathogenic variant (3.01% total cohort). The majority of MMRd tumours in patients in whom no germline pathogenic variant was identified demonstrated biallelic somatic variants (119, 68.79%). Of those tumours for which complete diagnostic investigations were undertaken, 42 tumours (11.11% of MMRd tumours without MLH1 hypermethylation/BRAF variant) were found to have no/only one somatic MMR gene variant or had unsuccessful tumour-based sequencing.

Conclusion
Pattern of MMR deficiency, age at diagnosis, and completeness of diagnosis influenced the yield of MMR germline/somatic variants in the studies under review, and, consequently proportion of unexplained MMRd. This will obviously have knock-on implications in surveillance and management of families who may be inappropriately labelled “lynch-like syndrome” as a consequence of incomplete work-up.

Translational science

Evaluating the utility of tumour mutational signatures for identifying hereditary colorectal cancer and polyposis syndrome carriers. Georgeson *et al*. (2021). *Gut*. http://dx.doi.org/10.1136/gutjnl-2019-320462

- The identification and classification of germline pathogenic variants in DNA mismatch repair (MMR) genes and *MUTYH* are still a challenge.
- The authors evaluated tumor mutational signatures in colorectal tumors from variant carriers in order to obtain valuable data for the classification of variants.
- They performed whole-exome sequencing of DNA extracted from formalin-fixed paraffin-embedded CRC tissue from 33 MMR germline pathogenic variant (PV) carriers, 12 biallelic *MUTYH* germline PV carriers, 25 sporadic *MLH1* methylated, MMR-deficient CRCs (MMRd controls) and 160 sporadic MMR-proficient CRCs (MMRp controls). In addition, 498 TCGA CRC tumours (fresh-frozen tissue) were included as non-hereditary CRCs.
- Single base substitutions (SBS) and indels (ID) were called with Strelka. COSMIC v. 3 was used to calculate tumour mutational signatures (TMS). The authors assessed the ability of mutational signatures to differentiate CRCs developed from PV carriers.
- The authors showed that mutational signatures SBS18 and SBS36, with a threshold >30%, were able to discriminate biallelic *MUTYH* carriers from all other non-carrier control CRCs with

100% accuracy (AUC=1.0). [A minimum VAF of 10% and a minimum depth of coverage of 50 reads were selected to maximize the capacity of TMS to identify CRCs from biallelic *MUTYH* carriers].

- Mutational burden was higher in CRC developed by biallelic *MUTYH* carriers compared with MMRp controls, but no association was observed for the indel-derived mutation burden.
- Tumor mutational signatures SBS18 and SBS36 were associated with specific *MUTYH* variants p.Gly396Asp and p.Tyr179Cys, respectively.
- The authors also found that combination of ID2 and ID7 could discriminate the 33 MMR PV carrier CRCs (Lynch syndrome) from the sporadic MMRp control CRCs (AUC 0.99); but, SBS and ID mutational signatures, are less effective at discriminating Lynch syndrome-related CRC from sporadic MMR-deficient CRC (MMRd controls) resulting from *MLH1* gene promoter hypermethylation (AUC 0.79).
- In conclusion, the application of mutational signatures has demonstrated their utility as a potential diagnostic and variant classification tool, leading to improved clinical management and CRC prevention.

Integrated molecular characterisation of the MAPK pathways in human cancers reveals pharmacologically vulnerable mutations and gene dependencies. Sinkala *et al*. (2021). *Communications Biology.* https://www.nature.com/articles/s42003-020-01552-6

- The mitogen-activated protein kinase (MAPK) pathways are crucial regulators of the cellular processes that fuel the malignant transformation of normal cells. The molecular aberrations which lead to cancer involve mutations in, and transcription variations of, various MAPK pathway genes.
- This work focuses on identifying cancer types that harbour mutations in genes that encode MAPK pathway proteins.
- They examined the genome sequences of 40,848 patient-derived tumours representing 101 distinct human cancers to identify cancer-associated mutations in MAPK signalling pathway genes (from cBioPorta).
- Mutations were identified in 42% of all tumours (58% when TP53 mutations are included)
- The authors also integrated information extracted from various large-scale molecular datasets such as: the Cancer Genome Atlas (TCGA18) project, LINCS project, other consortia and compiled by the cBioPortal project19 and Achilles project.
- They used CRISPR gene knockout of MAPK pathway genes and investigated their dose-responses to MAPK pathway inhibitors.
- Their results suggest that patients with tumours that have mutations within genes of the ERK-1/2 pathway, the p38 pathways, or multiple MAPK pathway modules, tend to have worse disease outcomes than patients with tumours that have no mutations within the MAPK pathways genes.
- In contrast, patients with mutations in JNK pathway genes were found to exhibit significantly better disease outcomes than patients with tumours that have mutations in other MAPK pathway modules.
- They also provided information about a link between the most frequently mutated oncogenes in various cancer types, e.g., KRAS mutations in pancreatic cancer (Fig. 4e) and BRAF mutations

in skin cancer (Fig. 4g) and the degree to which cancer cells depended on functional versions of these genes for survival.

- Aside these, they performed CRISPR-derived gene dependencies of cancer cell lines, together with the drug responses of these same cell lines and through this they indicated that the mutations, and expression signatures of, MAPK pathway genes are associated with the responses of the cell lines to various MAPK pathway inhibitors.
- Altogether, this study provides new insights into MAPK pathways, unearths vulnerabilities in specific pathway genes that are reflected in the responses of cancer cells to MAPK targeting drugs: a revelation with great potential for guiding the development of innovative therapies.

In the clinic

Mutations in *BRCA1/2* and other panel genes in patients with metastatic breast cancer – association with patient and disease characteristics and effect on prognosis. Fasching *et al*. (2021). *Journal of Clinical Oncology*. https://doi.org/10.1200/JCO.20.01200

- Prospective cohort study of 2,595 patients with metastatic breast cancer (mBC) evaluated for mutations in cancer predisposition genes compared to patients with non-metastatic BC
- Germline mutations in 12 established BC predisposition genes were detected in 271 (10.4%) of mBC patients (vs 6.6% of non-mBC patients)
    - A mutation in *BRCA1/2* was seen in 129 (5%) of mBC patients
- *BRCA1* mutation carriers had a higher proportion of brain metastasis compared with non-mutation carriers, and the association between *BRCA1* and brain metastases was mainly seen in patients with TNBC
- Patients with mutations in homologous recombination deficiency genes had a higher frequency of luminal B-like tumours (G3, HER2+), whereas patients with mutations in *BRCA1/2* more frequently had a TNBC
- Mutations did not significantly modify progression-free survival or overall survival for patients with mBC
- The authors suggest multigene panel testing may be considered in all patients with mBC because of the high frequency of mutations, potential eligibility for clinical trials for targeted therapies, and to inform cascade testing in family members
- The authors also suggest that mutations in specific genes may promote progression from early to metastatic BC

High likelihood of actionable pathogenic variant detection in breast cancer genes in women with very early onset breast cancer. Evans *et al*. (2021). *J Med Genet*. doi:10.1136/jmedgenet-2020-107347

- Assessed contribution of known breast cancer-associated genes to very early onset disease by testing 379 women with breast cancer aged ≤30 years
    - 75 PVs (19.79%) in BRCA1, 35 (9.23%) in BRCA2, 22 (5.80%) in TP53, 2 (0.53%) CHEK2 c.1100delC
    - At least 7 of the *TP53* PVs wouldn't have been suspected based on personal or family history, therefore the authors suggest *TP53* should be included in first-line testing for women with invasive or *in situ* BC aged ≤30 years

- Those testing negative (n=184) for the above genes were screened for PVs in a minimum of eight additional BC-associated genes. Only 8 (4.35%) actionable PVs identified: *ATM* = 2, *PALB2* = 4, *CHEK2* = 1, *PTEN* = 1
    - Suggests limited benefit from testing of additional BC-associated genes
- *BRCA1/2* PVs were more common in women aged 26-30 years than in women <26 years. The reverse was true of *TP53* PVs. (Similar trend seen in Ambry genetics data)
- 11/26 of women with DCIS had a PV (8 of the 11 cases were high-grade DCIS): *TP53* = 6, *BRCA2* = 2, *BRCA1* = 2, *PALB2* = 1
- Tumour characteristics:
    - 48.8% of women with TNBC had a PV in *BRCA1, BRCA2* or *TP53*
    - 18.6% of women with HER2+ BC had a PV in *TP53* (& only 6.9% had a PV in *BRCA1/2*)
    - Bilateral breast cancer was also predictive of PVs

Counselling and ethics (with a focus on PRS)

Communicating polygenic risk scores in the familial breast cancer clinic. Gupta *et al.* (2021). *Patient Education and Counselling*. https://doi.org/10.1016/j.pec.2021.02.046

- There are methodological and implementation elements that need to be addressed when using polygenic risk scores (PRS) in clinic settings – such as lack of guidelines for reporting and communicating results, long-term evidence of clinical utility and transferability of results to non-European ancestry groups
- This observational study aimed to describe current practice in communication and information giving behaviours of genetics healthcare professionals (GHPs; genetic counsellors, clinical geneticists, oncologists) providing PRS information for Australian women with no identified pathogenic variant in a breast cancer risk gene
- Patients completed baseline questionnaires before consultation to receive PRS
- Mean age of patient was 50 years, and half of patients had a personal history of breast cancer
- Most GHPs were female, aged 30-39 years. 62% were genetic counsellors
- Consultations began with introduction to the study, followed by exploration of family/medical history, disclosure of result, explanation of PRS and discussion of management options
- GHPs spoke three times more than patients on average, but patients were actively involved and asked questions including clarification of risks, impact of relatives, impact of other lifestyle factors etc
- Extensive summary tables provided on information-giving and process behaviours
- Differences between genetic counsellor and medical practitioner consultations – counsellors more likely to utilise strategies to build rapport

Discussion and conclusion

- Authors have provided key messages for genetic health professionals when communicating PRS, based on data from this study, covering key topics of risk communication, multifactorial nature of breast cancer, personalised risk, disease-specific nature of PRS, key limitations and psychosocial impact
- Some GHPs could benefit from further training in patient communication skills specific to PRS

Breast cancer polygenic risk scores in the clinical cancer genetic counseling setting: Current practices and impact on patient management. McGuinness *et al*. (2021). *J Genet Couns.* doi: 10.1002/jgc4.1347.

- Survey of U.S. cancer genetic counsellors from October 2019 to January 2020
- Looking at current practice with regard to breast cancer PRS, assessing impact of PRS on patient management, and anticipating future genetic counsellor practices with breast cancer PRS
- 65% had discussed BC PRS with a patient, 43% of respondents had ordered BC PRS
- Approximately 1/3 of those that had ordered PRS reported that the PRS had changed their medical management recommendations, i.e. increasing or decreasing screening
- Change in risk estimates due to PRS were also reported to affect patient anxiety level and confusion, and nearly half of respondents expressed regret from ordering PRS
- Reasons for not having ordered PRS included lack of clinical guidelines, insufficient evidence of clinical utility, lack of endorsement from professional societies, and lack of availability for patients of non-European ancestry
- Only 10% of respondents felt they would not order PRS in the future, but a further 49% were unsure if they would order BC PRS in the future

Monthly Journal Round-Up brought to you by:

Izzy Turbin, Genetic Counsellor, Addenbrooke's Hospital, Cambridge
Terri McVeigh, Consultant Clinical Geneticist, Royal Marsden
Alice Coulson, Genetic Counsellor, Guy's Hospital, London
Pilar Mur, Senior Postdoctoral Researcher, Laura Valle's group, Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain
Ouranio Anastasiou, Genturis Project Manager, ERN GENTURIS Affiliated Partner Cyprus