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

Bigger picture

20 years ago on the 15th February 2001, Nature published the first draft of the human genome followed swiftly by a special edition of Science. So let's take a moment to reflect on how this major milestone in genomics has impacted on the delivery of cancer genetics since then. Well for start, we now know of over 100 cancer predisposition genes many of which we regularly test for in clinical practice with a view to implementing Screening, Prevention and Early Detection for genetically high risk patients. We learnt how to interrogate the cancer genome, pulling out mutational signatures giving clues to the aetiology of the cancer and opening the door to agnostic therapeutic approaches. We can assess tumour mutational burden indicating possibility of response to immune checkpoint inhibitors and we know of an increasing number of driver mutations facilitating access to targeted therapies and clinical trials. We can look at single cell genomics and begin to understand tumour heterogeneity at the single cell level. We have technology to identify cf-DNA and use this in early detection of cancers, screening and monitoring of cancer. Not to mention all the other OMICS which are like to have increasing clinical relevance in the near future.

So take a moment to reflect then on how far we've come in the last 20 years since that first genome sequence was published and place your bets on what the next 20 years may bring...

Translational science

3DIV update for 2021: a comprehensive resource of 3D genome and 3D cancer genome.
Kim *et al.* (2021). *Nucleic Acids Research*; 49(D1): D38-D46.
https://doi.org/10.1093/nar/gkaa1078

- Hi-C (high-throughput chromatin conformation capture), which captures genome-wide all-to-all chromatin contacts in an unbiased manner, enables visualization of the genome organization in three-dimensional (3D) nuclear space in the form of a Hi-C contact map
- The usefulness of Hi-C data in the interpretation of noncoding structural variations has recently been highlighted, revealing the regulatory effects of complex genomic rearrangements in cancer.
- As the Hi-C protocol and 'C'-technologies continue to evolve with the production of various datasets, the 3DIV database will continue to expand in the future to support more experimental results with unique visualization tools.
- The current version of the 3DIV has focused on large-scale structural variations as they greatly impact on the 3D cancer genome; thus, researchers could not fully address the effect of all types of genetic variations such as copy number alterations.

- In this study the authors aimed to resolve issues related with the already existing interaction viewer database 3DIV. They updated the 3DIV and developed new browsing tools specializing in the 3D cancer genome.
- They used one hundred and sixty-eight cancer cell line/tumor and 52 unpublished colorectal cancer Hi-C datasets and added them together with the lists of sample-specific or pan-cancer defined WGS structural variations (SVs).
- They also developed unique live manipulation and visualization tools for the disorganized 3D cancer genome. These unique features enable users to perform multiple tasks, such as examining the impact of SVs on the 3D genome, configuring rearranged 3D chromatin structure, or simulating chromatin contacts of highly rearranged genomes under user-specified order.
- In summary, the 3DIV database was updated with 3D cancer genome data based on most of the publicly available Hi-C data with powerful visualization and manipulation functions, which are expected to be highly useful in understanding the complexity of cancer biology.

FAK displacement from focal adhesions: a promising strategy to target processes implicated in cancer progression and metastasis. Antoniades *et al.* (2021). *Cell Communication and Signaling*; 19(3). https://doi.org/10.1186/s12964-020-00671-1

- FAK is a non-receptor tyrosine kinase that is overexpressed or activated in several advanced-stage solid cancers. It is known to play both kinase-dependent and -independent roles in promoting tumor progression and metastasis
- Here, the authors describe a novel approach to site-specifically target both kinase-dependent and -independent FAK functions at focal adhesions (FAs), the primary sites at which the kinase exerts its activity.
- They took advantage of the well-characterized interactions between the paxillin LD motifs and the FAK FAT domain and generated a polypeptide (LD2-LD3-LD4) expected to compete with interactions with paxillin.
- The authors used co-immunoprecipitation experiments to examine the interaction between the LD2-LD3-LD4 polypeptide and FAK.
- They also evaluated the effects of LD2-LD3-LD4 in the localization and functions of FAK, as well as FA composition with quantitative immunofluorescence, cell fractionation, FA isolation and Western Blot analysis.
- Lastly, the utilized live cell imaging, as well as 2-D migration and cell invasion assays in order to examine the effects on FA turnover and tumor cell migration and invasion.
- They showed that the expression of the LD2-LD3-LD4 polypeptide prevents FAK localization at FAs, in a controlled and dose-dependent manner, by competing with endogenous paxillin for FAK binding.
- They further showed that LD2-LD3-LD4 expression markedly reduces FA turnover and inhibits tumor cell migration and invasion.

- Lastly, they showed that dimers of a single motif, linked through a flexible linker of the proper size, are sufficient for the displacement of FAK from FAs and for inhibition of tumor cell migration.
- Overall, this works shows that FAK displacement from FAs is a promising new strategy to target critical processes implicated in cancer progression and metastasis.

In the clinic

Time trends in receipt of germline genetic testing and results for women diagnosed with breast cancer or ovarian cancer, 2012-2019. Kurian *et al*. (2021). *Journal of Clinical Oncology*. https://doi.org/10.1200/JCO.20.02785

- Looked at testing trends, rates of VUS, and pathogenic variants (PVs) in women age ≥20 years diagnosed with breast or ovarian cancer (n = 187,535 and 14,689, respectively)
- 25% of the BC patients and 34% of the OC patients were tested
    - Testing gap persists for OC patients (34% tested vs 100% recommended)
- Annually, the rate of testing increased by only 2%, whereas the number of genes tested increased by 28% annually - reflecting a move from testing just *BRCA1/*2 to panel testing
- Proportion of patients with PVs in *BRCA1/2* decreased from 7.5% to 5.0% for BC patients and decreased from 15.7% to 12.4% for OC patients – increase in testing in older patients which likely reduces pre-test probability of PV
- Increase in proportion of both BC and OC patients with PVs in breast cancer-associated or ovarian cancer-associated genes (ATM, BARD1, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, NBN, NF1, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11, and TP53) or PVs in any other actionable gene (APC, BMPR1A, MEN1, MUTYH, NF2, RB1, RET, SDHAF2, SDHB, SDHC, SDHD, SMAD4, TSC1, TSC2, and VHL)
- VUS rates increased markedly, from 8.5% to 22.4% for BC patients and from 8.1% to 28.3% for OC patients
- VUS rates were substantially higher in Asian, Black and Hispanic patients, and the racial or ethnic VUS gap widened over time
- The authors suggest a panel of 20 genes could maximise clinically relevant PV yield while minimising VUS results

Update: variable implementation of the 2018 UKCGG/UKGTN guidelines for breast cancer gene panel tests offered by UK genetics services. Wedderburn *et al*. (2021). *Journal of Medical Genetics.* http://dx.doi.org/10.1136/jmedgenet-2020-107529

- UKCGG and UKGTN held a workshop in 2017 which led to a consensus for UK cancer gene panel testing

- Agreed breast cancer panel: *BRCA1, BRCA2, PALB2, ATM, CHEK2, PTEN, STK11* and *TP53*.
    - *NBN, BRIP1, BARD1* and *CDH1* were discussed, but excluded from the panel
- Agreed ovarian cancer panel: *BRCA1, BRCA2, BRIP1, MLH1, MSH2, MSH6, RAD51C* and *RAD51D.*
- UKCGG conducted a review in March-May 2020 of BC panel testing offered in the UK, with all 24 centres responding
- Still some inconsistency on testing offered, but continued trend towards gene panel testing
- Centres also asked what testing they planned to do following introduction of the National Genomic Test Directory.
    - 33% of centres said they will offer *BRCA1/BRCA2/PALB2* and any combination of *TP53, CHEK2, ATM, STK11* or *PTEN*
    - 38% said they will offer only *BRCA1/BRCA2/PALB2*
    - 29% planned to offer an alternative option for inherited breast cancer
- Ongoing differences in gene testing offered between centres continues to raise concerns about current equity of service for patients and their families across the UK
    - Will be interesting to see how the implementation of the National GTD in the UK impacts variation between centres

How Have Multigene Panels Changed the Clinical Practice of Genetic Counseling and Testing. Pilarski, R. (2021). *Journal of the National Comprehensive Cancer Network.* https://doi.org/10.6004/jnccn.2020.7674

- The article focuses on the challenges that NGS brought in clinical practice of Genetic counseling and testing.
- The main challenges discussed in this work include the difficulties in choosing the appropriate panel test for a given patient, the assessment of the significance of identified genetic variants (including variants of uncertain significance [VUS]), and the understanding of the disease risks and management associated with pathogenic variants in a given gene.
- Initially the author describes the differences between different types of panels: disease specific panels, guidelines specific panels and comprehensive cancer panel. Then he elegantly describes the advantages and disadvantages of these panels in terms of management of a case and diagnosis.
- Then he goes on and focuses on the challenges of VUS both in the laboratory level and in the clinical management of such result. The important issue that the author raises here is the different interpretation that laboratories have on the same genetic variant.
    - He also elegantly states that, in some cases, this can lead to various family members receiving different test interpretations of the same variant, depending on the laboratory that performed their testing
- Then he moves on and raises the management issues in cancer genetic testing giving as an example the early diffuse gastric cancer. This type of cancer has been identified

in such patients who do undergo prophylactic gastrectomy, however, there is still some uncertainty regarding how best to manage these families.
- Lastly the author comments on the financial and insurance challenges, the laboratory choice and the somatic tumor testing.

Counselling and ethics

Surgical decision making in premenopausal *BRCA* carriers considering risk-reducing early salpingectomy or salpingo-oophorectomy: a qualitative study. Gaba *et al*. (2021). *Journal of Medical Genetics*. doi: 10.1136/jmedgenet-2020-107501

- Pre-menopausal risk-reducing salpingo-oophorectomy (RRSO) leads to surgical menopause which has detrimental long-term health sequelae (increased risk of coronary heart disease, osteoporosis, vasomotor symptoms, sexual dysfunction, neurocognitive decline) particularly in women unable to use HRT
- Risk-reducing early-salpingectomy with delayed oophorectomy (RRESDO) proposed as attractive alternative to RRSO (Offered in the UK in the PROTECTOR study)
    - Previously shown that 70% of occult lesions identified in high-risk women undergoing RRSO occur in the fallopian tube, and that salpingectomy may reduce OC risk in low-risk women by 42-65% - figures for high-risk women currently unknown
- Qualitative study looking at decision-making process among *BRCA* carriers considering prophylactic surgeries in the PROTECTOR trial
- 24 in-depth semistructured 1:1 telephone interviews
- 7 themes integral to surgical decision making were identified: fertility, menopause, cancer risk reduction: surgical choices, surgical complications, sequence of ovarian and breast prophylactic surgeries, support with decision making, satisfaction with treatment choices
- RRSO preferred by women who felt maximising ovarian cancer risk reduction was relatively more important than early menopause/quality-of-life, and also by women who were nearer the age of natural menopause or who had a strong FH of OC
- RRESDO preferred by women who were more concerned about detrimental impact of menopause, and by women with a strong FH of BC (as opposed to OC)
- RRESDO offers an alternative surgical prevention strategy for premenopausal women who have completed their family but decline/wish to delay RRSO
- Women managed in specialist familial cancer clinic settings compared with non-specialist settings felt they received better quality care, improved HRT access and were more satisfied
    - Care of high-risk women should be centralised to such specialist centres

Uptake and efficacy of bilateral risk reducing surgery in unaffected female *BRCA1* and *BRCA2* carriers. Marcinkute *et al*. (2021). *Journal of Medical Genetics*. doi: 10.1136/jmedgenet-2020-107356

- Prospective follow up of women with positive genetic test (GT) results (between November 1994-March 2019) to evaluate long-term uptake, timing and effectiveness of RRM and bilateral salpingo-oophorectomy (RRSO) in healthy *BRCA1/2* carriers (n=887)
- RR-surgery was performed in 512 women, 73 before genetic testing
- 34.5% of those with *BRCA1/2* pathogenic variant underwent RRM (mean age at GT = 37.9 years, mean age at RRM = 39.2 years)
    - Median time from GT to RRM was 18.4 months, mean time was 28.4 months
- 46.7% of those with *BRCA1/2* pathogenic variant underwent RRSO (mean age at GT = 43.8 years, mean age at RRSO = 44.6 years)
    - Median time from GT to RRSO was 10.0 months, mean time was 29.5 months
- 23.5% of women underwent both RR-surgeries – of these, 51% underwent RRSO before RRM, 47% underwent RRSO after RRM, and 2% underwent RRM and RRSO simultaneously
- For every year increase in age, women were more likely to undergo RRSO but slightly less likely to undergo RRM
- Annual BC incidence in the study population was 1.28%
- Relative BC risk reduction (RRM vs non-RRM) was 94%
- Risk-reduction of OC (RRSO vs non-RRSO) was 100%, but OC incidence was not statistically significantly decreased in those undergoing RRSO (possible due to very small incidence rate which is likely due to young study population)
- Observed increasing number of women opting for RR-surgery over a 24-year period

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