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

Bigger picture

Reinterpretation and return of revised genomic results

April's issue of Genetics in Medicine features an article published by Clayton and colleagues (first published online January 2021), which lays out some of the concerns we all share regarding reinterpretation and reclassification of variants – namely when reinterpretation should occur, and by whom should the responsibility of triggering reclassification be borne- by the clinician, the scientist or the patient? The legal situation laid out by the authors is predominantly focused on the situation as it stands in the USA, but the basic principles hold true. In their concluding remarks, the authors comment that we, as a community, need to "think seriously" about out practice.

Management of individuals with germline variants in *PALB2*

Published online this week in Genetics in Medicine is the very useful Practice Resource regarding Management of patients with pathogenic germline *PALB2* variants, from Professor Marc Tischkowitz and colleagues. The authors again highlight that the risks associated with such variants depend on the family history and other modifiers. The authors also summarise current evidence regarding the biology of *PALB2* associated cancers – with enrichment among triple negative cases, demonstration of Homologous Recombination Repair deficiency mutational signatures, and more similar to that of *BRCA2* than *BRCA1*. This may have implications for treatment. There is some evidence to suggest inferior outcomes among *PALB2* carriers affected by breast cancer compared to non-carriers, but further data is required. Regarding risk-reducing strategies, carriers of *PALB2* variants should be offered *BRCA*-equivalent breast screening, and the option of risk-reducing bilateral mastectomy, although the uncertainty of survival benefit of this approach after a diagnosis of breast cancer, particularly given paucity of data regarding contralateral breast cancer risk, should be considered in women with a preceding diagnosis. Pancreatic cancer screening is not recommended outside of a research study at present. Ovarian risk management is less clear cut – the risk appears to be moderately increased (2-10%), a risk level at which, risk-reducing bilateral salpingo-oophorectomy would not ordinarily be considered. The authors suggest that risk-reducing surgery may be considered after non-directive counselling, bearing in mind reproductive and lifestyle modifiers of risk. The role of chemoprevention is not yet established.

Translational science

Using antisense oligonucleotides for the physiological modulation of the alternative splicing of NF1 exon 23a during PC12 neuronal differentiation. Biayna *et al.* (2021). *Scientific Reports*; 11: 3661. https://doi.org/10.1038/s41598-021-83152-w

- In this work the authors designed and used Antisense oligonucleotides (ASOs) known as Phosphorodiamidate Morpholino Oligomers (PMOs) to modulate the alternative splicing of E23a of the *NF1* gene in the PC12 neuronal differentiation system.
- As known, the *NF1* gene is located in the pericentromeric region of chromosome 17, at 17q11.2, and spans about 280 Kb of genomic DNA2. The gene is composed of 60 exons, 57 are constitutive exons and 3 are tissue-specific alternatively spliced exons (9a, 23a and 48a)
- Alternative splicing is a major source of proteome diversity and a key regulator of physiological processes, like neuronal branching and differentiation, brain development, etc. However, the physiological relevance of alternative splicing is still largely unknown.
- Initially the authors created Cell-based systems to study the alternative splicing of exon 23a (E23a) of the *NF1* gene. They used 3 different models and ended up with a model using pheochromocytoma derived PC12 cells. PC12 cells could be stimulated to differentiate into sympathetic-like neurons in the presence of Nerve Growth Factor (NGF). The use of NGF triggers cell division arrest, neurite extension and increases synthesis of several neurotransmitters. Then they observed different PC12 differentiation outputs, regarding cell morphology and degree of *NF1* alternative splicing, in independent experiments.
- After that the authors designed and used PMOs to induce the skipping or inclusion of E23a while maintaining physiological NF1 levels and showed that that PMO-SkpE23a treatment was able to induce E23a skipping in a specific manner even at higher levels than NGF alone, while maintaining the endogenous expression levels of Nf1.
- Following this, the authors assessed the impact of E23a alternative splicing modulation by PMOs on NGF-mediated PC12 neuronal differentiation and to further explore the molecular consequences of *NF1* alternative splicing modification by PMOs on PC12 neuronal differentiation, they analyzed the status of the MAPK/ERK and cAMP/PKA signaling pathways. From this set of experiments the authors concluded that any alteration of the natural *NF1* isoform switch produced by NGF in PC12 cells altered their neuronal differentiation phenotype morphologically, at the level of neuronal marker expression and on the regulation of NF1-related signaling pathways.
- Lastly, they attempted to dynamically modify the E23a alternative splicing by PMOs in PC12 neuronal differentiation.
- Overall, this study shows that the role of *NF1* E23a alterative splicing in PC12 neuronal differentiation, PMOs allowed to clarify the precise quantitatively and time-dependent regulation of E23a splicing along the neuronal differentiation, suggested a potential feed-back loop regulation in the NGF-triggered neurofibromin-dependent signaling, and showed the coordinated and opposite regulation of the MAPK/ERK and cAMP/PKA signaling pathways by neurofibromin.
- To conclude, this work highlights the importance of a precise quantitatively and time-dependent regulation of E23a splicing along the neuronal differentiation process. It constitutes an interesting example of how the alternative splicing of a single gene can influence cell fate by the fine, coordinated, and time-dependent regulation of different signaling pathways. Lastly, it underlies the potential of using PMOs to study alternative

splicing, since they preserve physiological gene expression conditions and can be used in a quite flexible way in contrast to static genetic methods.

Distinct CDK6 complexes determine tumor cell response to CDK4/6 inhibitors and degraders. Wu *et al.* (2021). *Nature Cancer*; 2: 429-443. https://doi.org/10.1038/s43018-021-00174-z

- Cyclin-dependent kinases (CDKs) 4 and 6 inhibitors (CDK4/6is) are effective in metastatic breast cancer, but they have been only modestly effective in most other tumor types. Recently, CDK4/6is in combination with hormonal therapy showed significant clinical activity in RB-proficient metastatic ER+ breast cancers and three CDK4/6is, palbociclib, abemaciclib and ribociclib, are now US Food and Drug Administration (FDA)-approved for this indication. As the activity of CDK4/6 requires a functional RB protein, tumors that do not express functional RB are resistant to these drugs. However, in many tumor types predominantly expressing wild-type (WT) RB1 (lung adenocarcinomas, melanomas, colon cancers and others) preclinical and clinical studies have shown only modest effectiveness of CDK4/6is, suggesting that other mechanisms limit their efficacy in these tumor types.
- The authors here used non-small cell lung carcinoma (NSCLC), melanoma and colorectal carcinoma and showed that tumors expressing low CDK6 rely on CDK4 function and are exquisitely sensitive to CDK4/6is.
- They also showed that tumor cells expressing both CDK4 and CDK6 have increased reliance on CDK6 to ensure cell cycle progression.
- The authors also identified that CDK4/6is and CDK4/6 degraders potently bind and inhibit CDK6 selectively in tumors in which CDK6 is highly thermo-unstable and strongly associated with the HSP90–CDC37 complex. In contrast, CDK4/6is and CDK4/6 degraders were shown to be ineffective in antagonizing tumor cells expressing thermostable CDK6, due to their weaker binding to CDK6 in these cells.
- All these observations led to the conclusion that a new general mechanism of intrinsic resistance to CDK4/6is and CDK4/6i-derived degraders has been identified. This work also suggests the urgent need for new inhibitors targeting the CDK4/6i-resistant, thermostable form of CDK6 for application as cancer therapeutics.

In the clinic

The clinical features of polymerase proof-reading associated polyposis (PPAP) and recommendations for patient management. Palles *et al.* (2021). *Familial Cancer*; https://doi.org/10.1007/s10689-021-00256-y

- The clinical features and management of patients with Polymerase Proofreading Associated Polyposis has been outlined in a new paper by Claire Palles and colleagues in Familial Cancer, building on their earlier paper in 2013.
- In this paper, the authors delineate the phenotype of 105 carriers of exonuclease domain (ED) variants in POLE, 27 carriers of such variants in POLD1. Carriers were ascertained from testing via the CORGI and QUASAR2 studies.
- The phenotypic spectrum, the authors conclude, is a hybrid of adenomatous polyposis and Lynch Syndrome, and includes polyposis; colorectal, endometrial, ovarian, duodenal and brain tumours, and possibly other tumours.

- The vast majority of carriers of pathogenic variants in POLE (100/105) or POLD1 (23/27) had a colorectal phenotype, with adenomas and/or cancer. Fifteen patients were diagnosed with colorectal cancer before age 30. Affected individuals mostly demonstrated a phenotype of oligopolyposis (median 12), ranging from 1-100 in those carriers for whom polyp burden was available. Colorectal cancers were also reported in the absence of polyposis.
- Data from Mismatch repair immunohistochemistry and or microsatellite instability was available for a minority of tumours – of which a minority (14%) demonstrated MMR deficiency.
- The risk of colorectal cancer by age 70 is estimated to be 90% for POLE carriers and 50% for POLD1 carriers.
- Duodenal tumours were not infrequently reported in carriers of POLE variants, with 10/105 developing duodenal cancer, and 16/105 developing duodenal adenomas – including two before age 18. No duodenal disease was reported in the POLD1 carriers included in this cohort.
- Endometrial cancers are also a part of the phenotypic spectrum of PPAP, particularly among female POLD1 carriers (9/17), and less frequently among POLE variant carriers (5/43). The risk of endometrial cancer by age 70 is estimated to be 25% for POLE carriers and 75% for POLD1 carriers.
- Ovarian cancer cancers were reported in female POLE variant carriers (5/43), with diagnosis between ages 33-40, but no cases were reported among POLD1 carriers.
- Brain tumours were reported in 9/105 POLE carriers.
- Breast cancer was also reported in female carries in this series; in 6/43 POLE carriers and 4/17 POLD1 carriers.
- Prostate cancer was reported in only one case in this series.
- Recommended screening from this study includes biennial colonoscopy and FAP-equivalent upper GI surveillance starting from 25. In the absence of proven screening for endometrial or ovarian cancers, screening is not recommended in female carries, but the authors suggest that consideration should be given to risk reducing hysterectomy for POLE and POLD1 carriers, with due consideration for risk-reducing salpingo-oophorectomy in POLE carriers. Breast cancer risk management should be guided by the family history.
- The authors also highlight challenges in variant interpretation, and in standardisation of criteria for testing.

Naproxen chemoprevention promotes immune activation in Lynch syndrome colorectal mucosa. Reyes-Uribe *et al.* (2021). *Gut*; doi:10.1136/gutjnl-2020-320946

- Immune system increasingly recognised to play an essential role in LS tumour development, making it an ideal target for cancer prevention
- Phase 1b, placebo-controlled, randomised clinical trial of two dose levels of naproxen sodium (440 and 220 mg) administered daily for 6 months to 80 participants with LS, and a co-clinical trial using a genetically engineered mouse model of LS and patient-derived organoids (PDOs)
- Aim was to evaluate safety, assess activity, and discover novel molecular pathways involved in the activity of naproxen as primary and secondary chemoprevention in patients with LS
- The trial showed that naproxen is a safe primary and secondary chemopreventive intervention
- Both high and low dose levels of naproxen promoted immune activation of different resident immune cell types

- The level of prostaglandin E2 (which promotes cancer progression) in the colorectal mucosa was significantly decreased after treatment with high and low dose naproxen when compared with placebo
- Naproxen was effective as chemoprevention by modulating tumour growth and prolonging survival in the co-clinical trial using a tissue-specific mouse model of LS
- Gene expression profiles induced by naproxen in humans showed perfect discrimination of mice with LS and PDOs treated with naproxen and control, thus providing novel predictive biomarkers of naproxen activity
- Naproxen is a promising potential strategy for immune interception in LS

Universal newborn genetic screening for pediatric cancer predisposition syndromes: model-based insights. Yeh *et al*. (2021). *Genetics in Medicine.* doi: 10.1038/s41436-021-01124-x.

- The authors developed the Precision Medicine Policy and Treatment (PreEMPT) model to evaluate potential risks and benefits of genetic testing of a cancer predisposition syndrome (CPS) panel (*RET, RB1, TP53, DICER1, SUFU, PTCH1, SMARCB1, WT1, APC, ALK, PHOXB2*)
- Each newborn was assigned a probability of carrying a pathogenic or likely pathogenic (P/LP) variant in each of the 11 genes
- Cohorts of newborns representative of a modern US birth cohort were simulated under the scenarios of usual care and targeted NGS (t-NGS) at birth and followed throughout their lifetimes
- It was assumed that newborns with identified variants underwent guideline surveillance (also assumed 100% adherence)
- Survival benefit was modelled via reductions in advanced disease, cancer deaths, and treatment-related late mortality
- In a cohort of 3.7 million newborns, the model estimated 1,803 would develop a CPS-associated cancer before age 20, 13.3% of whom would have P/LP CPS variants
- The model estimated that compared with usual care, t-NGS would reduce cancer deaths before age 20 overall by 7.8%, and would reduce cancer deaths before age 20 in P/LP heterozygotes by 53.5%
- Given a test cost of $55, universal screening cost $244,860 per life-year gained; with a $20 test, the cost fell to $99,430 per life-year gained.
- Population-based genetic testing of newborns may reduce mortality associated with pediatric cancers and could be cost-effective as sequencing costs decline
- Would be important to consider other factors, such as incomplete penetrance of genes and parental anxiety, implications for relatives, reproductive planning

Counselling and ethics

Considerations in genetic counselling of transgender patients: Cultural competencies and altered disease profiles. Von Vaupel-Klein & Walsh. (2021). *Journal of Genetic Counselling*; 30(1): 98-109. https://doi.org/10.1002/jgc4.1372

- Trans people have same health needs as cisgender patients, and often being trans is not always relevant to clinic. There are however some aspects of genetics clinical practice where there may be more specific needs
- Paper covers several important and relevant discussion points which are summarised here

- Transgender cultural competency: trans health disparities remain understudied. Gender dysphoria can be a source of distress for trans people – the awareness of difference between their gender identity and their body or others' perceptions of their gender. Distress can be triggered by errors of cultural competency and the authors provide appropriate vocabulary and advice inclusion in clinic settings such as use of names when addressing individuals in public and using gendered words
- What is gender, really? The authors discuss ways of defining sex/gender and the authors propose that a biopsychosocial model of humans shows that gender is multifactorial and multidimensional. Therefore, assumptions cannot be made based on observable biological characteristics only.
- Medical interventions for trans people: relevance for risk assessment: Medical interventions for transition can have implications for cancer, cardiovascular and other medical conditions. The authors discuss the impact of hormone therapy and gender-affirming surgical options and advice how to ask about relevant aspects of genetics, anatomy and hormonal characteristics.
- Sociocultural risk factors: Transgender people are more likely to be poor and unemployed, meaning healthcare and healthy nutrition may not be as easily available. Job market discriminations can mean participating in "underground economies" which carry health risks. Trans people can also experience harassment, chronic stress and policy barriers
- Physical examination: Clinical genetics examinations often require explanation and heighten awareness of body parts which trans people may be uncomfortable with. Use of photography should be explained and compromise needs to be established if there are levels of discomfort.
- Pedigree charting: The authors comment on trans people's preferences for pedigree syndromes. They discuss a study where most participants prefer to use traditional symbols to indicate gender identify, and use of annotations such as AMAB (assigned male at birth).
- Culturally competent healthcare for trans people begins with creating a safe, welcoming environment where there is room for the patient's own language, gender identify and anatomy. More research is needed to better understand these communities.

Monthly Journal Round-Up brought to you by:

Izzy Turbin, Genetic Counsellor, Addenbrooke's Hospital, Cambridge
Alice Coulson, Genetic Counsellor, Guy's Hospital, London
Ouranio Anastasiou, Genturis Project Manager, ERN GENTURIS Affiliated Partner Cyprus
Terri McVeigh, Consultant Clinical Geneticist, Royal Marsden