2020 European Society of Human Genetics, June 6–June 9th: Live in your living room!

Hannah K. Ralph

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In early June, researchers should have been meeting in Berlin for the annual European Society of Human Genetics (ESHG) conference. Although this was no longer possible after the global outbreak of COVID-19, the conference instead came to the community and was broadcast on a virtual platform. Across Twitter, researchers shared how they were experiencing the very first virtual ESHG conference – some on the beach, others in their gardens and many juggling their conference experience with childcare responsibilities. Below are some examples of the innovative and exciting research I had the opportunity to listen to (live in my living room!).

1. Novel causative genes in male infertility

Margot Wyrwoll of the Institute of Human Genetics (University of Münster, Germany) spoke about the identification of novel causes of male infertility. Around 7% of all men are infertile and, of these, 11% have azoospermia, meaning that their semen does not contain any sperm at all. Although some male infertility associated genes are known (e.g. *TEX11*, *NR5A1* and *DMRT1*) and form part of currently trialed diagnostic panels, ~75% of patients remain without a causal diagnosis.

Margot’s work focused on the men with non-obstructive azoospermia, confirmed through testicular biopsies that showed no cells beyond the spermatocyte stage in the testis. This indicated that the lack of sperm was due to meiotic arrest. During meiosis, the proteins MSH4 and MSH5 form a heterodimer that facilitates the resolution of double Holliday junctions during crossover of the homologous chromosomes. In model organisms, biallelic loss of function of MSH4/MSH5 has been linked to infertility. In women, mutations have been associated with infertility and premature ovarian insufficiency (POI). However, no pathogenic variants in MSH4/MSH5 have so far been described in men.

In their cohort of men with complete bilateral meiotic arrest, the researchers had 54 unresolved cases with a confirmed testicular biopsy. A further 517 men had unexplained non-obstructive azoospermia but no available biopsy. Screening of whole exome sequencing (WES) data identified variants in MSH4 in two men and in MSH5 in three men. Of these, four variants were nonsense and one was a homozygous missense of uncertain significance according to clinical guidelines but considered by the researchers to be likely causative. This work demonstrates for the first-time pathogenic variants in MSH4/MSH5 in male infertility and reveals an overlap between male infertility caused by meiotic arrest and POI.

2. Methylation patterns in liquid biopsies

Professor Yuval Dor of the Hebrew University of Jerusalem (Israel) opened the session on Liquid Biopsy for Early Detection of Cancer with a presentation on the use of methylation patterns to infer tissue of origin of cell free DNA (cfDNA).

The presence of short DNA fragments circulating in the blood has become an emerging and promising biomarker in recent years, and there has been an active interest in their use for clinical diagnostics, such as in non-invasive prenatal testing (NIPT) or cancer detection and monitoring. This use is underpinned by genetic differences between the cfDNA and the ‘host’ which allow them to be distinguished from each other. Yuval Dor’s group is interested in detecting the tissue of origin of the cfDNA; a clear challenge when the genomes of the host and the tissue of interest are identical. To answer this question, the group relies on the fact that each cell type has a unique methylation pattern, which is stable and conserved across health and disease and between individuals. As a basis they use a methylation matrix of all human cell types, assembled from both publicly available datasets and their own work. These are used as markers to infer the cell type from which the cfDNA was released.

Demonstrating the clinical relevance of detecting cell death through cfDNA release was a collaboration with David Planer (Hadasah Medical Center, Israel) in which cardiac derived cfDNA was found to be significantly elevated in patients shortly after a myocardial infarction and, importantly, correlated with the conventional measurement of troponin levels.

In another recently published study, this time focused on breast cancer, the researchers demonstrated the detection of a breast cfDNA signal in women with localized, operable breast cancer. This was most strongly observed in the patients with HR-/HER2+ tumours.
Clinical follow-up of an individual patient demonstrated the potential of this approach – initially a reduction in breast cfDNA was observed with chemotherapy, followed by a rise that correlated with clinical progression and a subsequent reduction to zero following surgery. A later cancer recurrence was again detected through the elevated levels of cfDNA in the plasma. Investigations into the use of cell type specific cfDNA in therapeutic settings are continuing.

3. Cell-specific pathways associated with risk of Parkinson’s disease

In a session on Neuropsychiatry, Professor Caleb Webber of the UK Dementia Research Institute (Cardiff, Wales) described the generation of the first human single-nuclei transcriptome atlas of the Substantia Nigra (SN). The SN is a region of the midbrain which plays a role in movement. Parkinson’s disease (PD), the most common neurodegenerative movement disorder, is characterized by the selective loss of dopaminergic neurons (DaNs) in the substantia nigra pars compacta (SNpc). However, it is unknown which cell types specifically are contributing to the loss of DaNs.

In order to reveal the specific cell types involved, the group began by creating an atlas of gene expression from cells in the SN and the cortex. Single nuclei were dissociated from these regions in five healthy human brains and the transcripts analysed by single cell sequencing using the 10X Chromium platform. In total, approximately 16,000 nuclei were sequenced (5943 from the SN and 10,706 from the cortex) with an average of 2000 genes detected per nucleus.

Having established a cell type specific expression atlas, Caleb’s group used this to associate genetic risk with individual cell types by annotating genes in established risk loci from GWAS to the cell type they are expressed in. Two established statistical association methods were applied to address whether these risk loci are enriched in genes from specific cell types. In the cortex, this replicated previously known associations, for example between several psychiatric disorders and excitatory neurons and between Alzheimer disease risk and microglia. As expected in the SN, an association was observed between cell types such as a gradient of Pax3 positive cells, representative of migrating myoblasts. Furthermore, increasing clear clusters of cells positive for Fgfr8, representative of the apical ectodermal ridge. Similar results were also observed for PAX3 positive cells, representative of migrating myoblasts. Furthermore, the approach was sensitive enough to detect subtle differences between cell types such as a gradient of Shh positive cells in the zone of polarizing activity.

Next, they investigated a set of 1400 genes from the Mouse Genome Informatics database that have been previously associated with limb malformation phenotypes. In their single cell atlas, they were able to detect nearly all these genes (n = 1308) and a quarter were ranked among the highest for informative gene expression variance. Going back to their trios, 323 of the 374 genes were detected in the dataset but only 44 intersected with the curated genes associated with limb phenotypes. From here, they are continuing to gather further functional evidence for the novel candidates they have prioritized. They also plan to release their resource as an interactive tool to allow other researchers to use the atlas to prioritize candidate variants.

4. New (old) treatments for genetic disorders

Elisa Giorgio of the University of Turin (Italy) spoke about her work in developing a screening strategy to support drug repositioning for rare genetic diseases. Developing and translating therapeutic options for rare diseases is a significant challenge and only 5% of diseases have an approved therapeutic solution. Although rare diseases are heterogeneous, on a molecular level they can be grouped together into a handful of pathogenic mechanisms. Gene dosage alterations are very common. Therefore, the group hypothesized that diseases with a common molecular mechanism could benefit from the same screening strategy.

To this end, Elisa described their work to develop and validate a high-throughput assay that can be used to identify compounds that modulate the level of a given protein of interest. The assay is based on the use of a stable dual-reporter cell line - the protein of interest is fused to GFP and destabilized RFP is also expressed to allow normalization and identify negative hits. This system allows the rapid identification of compounds which affect expression of the target protein at the translational level and facilitates the prioritization of compounds that either reduce or increase the protein level, depending on the respective alteration mechanism seen in the disease.

As a proof-of-principle, she discussed the rare neurodegenerative genetic disease Autosomal Dominant Leukodystrophy (ADLD). ADLD is associated with overexpression of LMNB1 and so a candidate drug was sought that would reduce the protein levels. After establishing their stable cell line, they screened a library of 717 FDA-approved molecules. They found one positive candidate (“Drug G”) and further validated it through in vitro analysis in multiple cell lines. Their findings promote the use of this candidate in clinical trials based on the practice of ‘compassionate use’. Importantly, this strategy can be used to identify existing approved drugs that may be effective in the treatment of other rare diseases.

5. Atlas of human limb malformation candidate genes

Cesar Prada-Medina from Malte Spielmann’s group at the Max Planck Institute for Molecular Genetics (Berlin, Germany) spoke about efforts to prioritize limb malformation candidate genes. Limb malformations are clinically and genetically heterogenous. From 50 trios with limb malformations, the group identified 374 genes with candidate de novo mutations. To gather causative functional evidence, Cesar described their work setting up a high-throughput, single cell transcriptomic profiling assay using mice.

Starting from the first stages of limb bud development (E9.5, E10.5, E11.5, E12.5), single cell suspensions were prepared and sequenced using the 10X and Illumina platforms. They first checked whether the single cell results recapitulated known features of limb development. At each developmental time point they observed increasingly clear clusters of cells positive for Fgfr8, representative of the apical ectodermal ridge. Similar results were also observed for Pax3 positive cells, representative of migrating myoblasts. Furthermore, the approach was sensitive enough to detect subtle differences between cell types such as a gradient of Shh positive cells in the zone of polarizing activity.

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