Next Generation & Beyond...

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ExACtly! [Lek et al., 2016]

The Exome Aggregation Consortium or the ExAC is an effort to aggregate and harmonise exome sequencing data from various sequencing projects around the world. The group in its first publication has published the analysis of genetic variation in protein coding regions of the human genome in about 60,706 individuals. This is by far the largest dataset of variations in the human exome. A staggering 74, 04, 909 high quality variants were identified averaging to one variant for every 8 base pairs. Such a large data set helps vastly to improve the human reference genome and provides for better variant calling. About 163 of 192 variants which were classified as pathogenic have now met the criteria to be called as benign or possibly benign variants raising serious concerns about how to interpret variants in rare diseases. Many protein-truncating variants with no established human disease phenotype have also been identified. This article is thus a must read in this era of Next Generation Sequencing.

Inborn Exome of Metabolism

[Tarailo-Graovac et al., 2016]

In the past few years, whole-exome sequencing has become a well-established modality for the discovery of new genes and the diagnosis of rare genetic disorders. It is now being taken to the next level – that of translational medicine. Tarailo-Graovac et al. have reported a study wherein they have combined whole exome sequencing analysis with deep clinical phenotyping (i.e. a detailed characterization of the patients' clinical and biochemical phenotype) through a semi-automated bioinformatics pipeline in individuals with intellectual developmental disorder and unexplained metabolic phenotypes. Through the application of this combined modality in 41 such probands without an identifiable etiology, they were able to clinch the diagnosis in 28 patients (68%) and identify 11 new candidate genes for neurometabolic disease phenotypes. Most importantly, with the identification of the diagnosis they were able to offer beneficial targeted intervention in 18 (44%) patients. This is indeed an example of bench-to-bedside application of technology!

CRISPieR [Arbab et al., 2016]

CRISPR/CAS9 has been a big bang in the world of genome editing. The relative ease, efficacy and specificity have made this the preferred methodology in gene editing. A slew of discoveries have helped to better the CRISPR-based technology, the latest being the self cloning CRISPR reported by Arbab et al. Self-cloning CRISPR/Cas9 (scCRISPR) makes use of a self-cleaving palindromic sgRNA plasmid (sgPal) that recombines with short PCR-amplified site-specific sgRNA sequences within the target cell by homologous recombination and circumvents the process of sgRNA plasmid construction. scCRISPR enables gene editing within 2 hours once sgRNA oligos are available. The efficiency (90% knock out rates) is high and equivalent to conventional sgRNA targeting. PCR-based addition of short homology arms leads to efficient site-specific knock-in of transgenes (2% to 4% knock-in rate). This paper describes the most rapid and efficient means of CRISPR gene editing to date.

Linking the dots..... [Perla-Kajan et al., 2016]

Hyperhomocysteinemia caused by Cystathionine β-synthase (CBS) deficiency is characterised by elevated levels of plasma homocysteine with resul-

tant neurological, vascular and connective tissue abnormalities. The underlying mechanisms, however, are poorly understood. Elevated plasma homocysteine- thiolactone is believed to cause the connective tissue abnormalities. Perla-Kajan et al. have studied CBS knockout mice which replicate the connective tissue abnormalities in humans with CBS deficiency. Their hypothesis that homocysteine- thiolactone residues form isopeptide bonds with lysine residues and impair cross linkage was affirmed when N- homocysteinylated collagen was found elevated in the bone, tail and heart of the CBS knockout mice. Further, in vitro and in vivo studies have demonstrated N-homocysteinylation of collagen type 1 α1 chain explaining, at least in part, the cause for connective tissue abnormalities in Hyperhomocysteinemia.

References

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3. Perla-Kajan J, et al. N-Homocysteinylation impairs collagen cross-linking in cystathionine β-synthase-deficient mice: a novel mechanism of connective tissue abnormalities. FASEB J 2016 Aug 16; DOI: 10.1096/fj.201600539 [Epub ahead of print]
4. Tarailo-Graovac M et al. Exome Sequencing and the Management of Neurometabolic Disorders. N Engl J Med 2016; 374: 2246-2255.