GeNeXpreSS

What Diagnoses Are Missed in Next-Generation Sequencing?

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Exome sequencing is quite popular among the clinicians for diagnosis of rare Mendelian disorders but is often non-diagnostic even when we have a strong clinical suspicion of a monogenic disorder. A proportion of them are solved by whole genome sequencing. Some families still remain undiagnosed with detection of only one of the two variants necessary to confirm the diagnosis of an autosomal recessive disorder. Let us look into some of the recent publications that have solved this problem in some families by identifying the variants that usually escape detection in next-generation sequencing (NGS).

Cryptic second variants in autosomal recessive diseases with one mutation (Moore et al., 2023)

Moore and colleagues investigated definitive autosomal recessive diseases with one hit (only one mutation identified) for the second hit by whole genome sequencing. They studied 31 patients from the 100,000 Genomes Project who had only one mutation despite having a strong clinical suspicion of an autosomal recessive disease. Whole genome sequencing (short read) revealed a diagnosis in eight additional patients by finding the second mutation. These include a patient with cystic fibrosis harboring a novel exonic LINE1 insertion in *CFTR* and a patient with generalized arterial calcification of infancy with complex interlinked duplications involving exons 2–6 of *ENPP1*. They had to undertake optical genome mapping and RNA analysis for the *ENPP1* variant.

Retroelements are missed by exome sequencing and can be the second (missing) mutation in ataxia–telangiectasia (Zhao et al., 2023)

Retroelements (retrotransposons) are stretches of DNA that copy and paste themselves into different genomic locations (transposons). They do this by converting RNA back into DNA through the reverse transcription process. Examples include Long Interspersed Nuclear Elements-1 (LINE-1 or L1), SINE-VNTR-Alus (SVA) and pseudogene insertions. Retroelement insertions are already known to cause Mendelian disorders and might be amenable to antisense oligonucleotide therapy. Zhao and colleagues studied 237 patients with ataxia-telangiectasia who had whole genome sequencing data and checked for retroelement insertions. They observed 15 individuals harboring one of the five retroelements. While one was in the coding (exonic) region, the rest were integrated in the non-coding regions. RNA sequencing, RT-PCR, and/or minigene splicing assays were used to study the functional consequences of these insertions. Twelve out of 14 intronic insertions led to or contributed to loss of ATM function by exon skipping or activating cryptic splice sites. Interestingly, these were second (missing) variants in some and third variant in others! They estimate the contribution of retroelements to the genetic architecture of recessive Mendelian disorders as ~2.1%–5.5%.

Deletions and a complex insertion in hereditary hemorrhagic telangiectasia (Xiao et al., 2023)

Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome is caused by loss-of-function (LoF) heterozygous variants (and a

second hit at in the affected tissues) in *SMAD4*. Xiao and colleagues developed GROFFFY to prioritize variants in non-coding regions rich in transcribed and critical regulatory sequences. This is an analytic tool that integrates coordinates for regions with experimental evidence of functionality. They applied GROFFFY to the whole genome sequence data from solved and unsolved hereditary hemorrhagic telangiectasia recruits to the 100,000 Genomes Project. They detected three ultra-rare deletions within the 3' untranslated region (UTR) of the tumor suppressor gene *SMAD4* which disrupted the sequence context of the final cleavage and polyadenylation site necessary for protein production. In another individual, a complex insertion was identified. Four undiagnosed cases were thus solved.

DNA methylation signature for unsolved cases of Fanconi anemia (Pagliara et al., 2023)

Fanconi anemia results from inactivating biallelic (predominantly) mutations in one of about 20 genes in the DNA repair pathway. The wide spectrum of mutations and structural rearrangements make molecular diagnosis of Fanconi anemia challenging. Assessment of chromosomal fragility is often required to confirm the pathogenicity of the variants and to firmly establish the diagnosis. Pagliara and colleagues studied the peripheral blood genome-wide DNA methylation profiles in 25 subjects with molecularly confirmed Fanconi anemia and observed 82 differentially methylated CpG sites that allow distinguishing subjects with Fanconi anemia from healthy individuals and patients with other diseases. The episignature was validated using a second cohort of subjects with Fanconi anemia involving different complementation groups and documented its sensitivity and specificity. The episignature properly classified DNA samples obtained from bone marrow aspirates, demonstrating robustness. They also trained a machine learning tool for identifying DNA methylation signature for this condition.

To summarize, we need to look for complex rearrangements, retroelements, and alterations in regulatory regions whenever we do not have a diagnosis for a monogenic disease. Specific DNA methylation signatures might also be a tool for diagnosis of genetically heterogenous conditions like Fanconi anemia.

References

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