IRD panels-caveat emptor-truly know your IRD panel

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Less than a decade ago, genetic testing in inherited retinal disease (IRD) patients was reliant on coverage by payors, which resulted in a very low rate of genotyping. Some tertiary centers offered options for non-CLIA certified genetic testing on a research basis, but this was not widely available to community practitioners, and research results could not be disclosed clinically. Fortunately, the cost of genetic testing has decreased markedly and the bioinformatics has improved as well. The number of genes covered in a typical next generation sequencing (NGS) panel has more than tripled over the past decade to more than 300 genes. Currently, there are multiple labs offering these large panels, but with variations in the number of genes covered and in specific cases, also the coverage of key genes. Figure 1 demonstrates the overlap between five major panels as of June 1st 2021. Now some companies are offering the panel testing "for free". While the ability to solve the genetic cause of disease in patients has been a valuable addition, we would like to point out some potential pitfalls in their use with the hope of raising awareness with providers who order these tests.

Stone et al. have extensively discussed the problem of false discovery rate.[1] With the number of tests that are involved in a panel and the number of possible variants per test, the chances of finding a positive test that is unrelated to the pathology is high. Stone et al. calculated that if 301 non-mitochondrial genes are tested, the false discovery rate would be 128%, or 1.28 plausible disease-causing mutations per person.[1] Therefore, careful pre-, and in some cases, post-test phenotyping is crucial to interpreting genetic test results, especially when large panels are utilized. A comprehensive, multimodal characterization that includes a detailed clinical history, and, depending on the case, fundus photos, autofluorescence imaging, visual fields, full-field psychophysics and electroretinograms will help avoid spurious conclusions and increase the chances that a "positive" genetic test really is the underlying cause of the pathology. Obtaining a

pedigree and evaluating family members helps to determine if the mode of inheritance is autosomal recessive, autosomal dominant, X-linked recessive, X-linked dominant, or mitochondrial. In cases where the proband has more than one presumed pathogenic variant, analyzing clinical data and family members—both affected and unaffected—can help determine the implications of these variants.

Physicians should be wary when genetic testing results do not comport with the clinical findings. For example, the finding of variants in an autosomal recessive gene should arouse suspicion when the patient has a strong family history of X-linked disease, or conversely, physicians should keep an open mind for the possibility of an X-linked or mitochondrial disease in families with a family history suggestive of autosomal dominant (or even recessive) inheritance. It is also important to consider the possibility of an autosomal dominant disorder with incomplete penetrance in a patient with no apparently affected family members, in whom autosomal recessive inheritance has been presumed. (For example, variants in *PRPF31* are a frequent cause of autosomal dominant RP, and incomplete penetrance is frequently encountered.) Finally, the finding of a non-syndromic gene should be viewed cautiously in the context of a patient with multisystem features. However, it is also crucial to maintain an open mind and realize that common genes might sometimes present in uncommon or previously unrecognized manners, although often reflecting common phenotypes modulated by disease severity, and thus, by the window in time within the natural course of the disease when patients are examined. There are a growing number of genes previously reported only in syndromic retinal degenerations that are now being associated with non-syndromic disease (e.g. *BBS*, *CLN*, etc.)[2,3]

Another important aspect that has not been discussed in depth in the literature is the range of the panel genes tested. Looking at the list from each company is initially daunting since they

appear to be so overwhelming and complete. However, ocular disease panels can vary widely in testing strategy, with some including mitochondrial genes, copy number variant detection, deep intronic sequencing, or testing for large insertions or deletions. As important as not making a false discovery is not obtaining a false negative. False negatives can occur from inadequate knowledge of all of the variants associated with a disease type. An important example are the hypomorphic variants of *ABCA4* that were initially disregarded because they are relatively common in the Northern European population.[4] Recently these variants have been deemed to confer a milder form of the disease.[4] On the other hand, *ABCA4*-associated IRDs are also an example of the possibility for false positives calls. With a relatively high prevalence of disease-causing and hypomorphic variants in the general population, there is a risk for attributing disease to mono-allelic changes, whilst these may be incidental; again, the specificity of the phenotype is important.

False negatives can also occur if a specific panel does not test for a gene of interest. An interesting illustration of this situation is testing for the *RPGR* gene, which is well known to the field and for which there are four ongoing gene therapy clinical trials. This gene causes 40-70% of all cases of X-linked RP.[1,5–8] Additionally, in a series from the USA and from the UK, *RPGR* was the third most common cause of inherited retinal disease behind *ABCA4* and *RHO*,[1,9] accounting for 6% of all IRD cases.[1] The issue with *RPGR* is that exon 15 is difficult to sequence with present techniques.[10,11] At least one "free" panel does not include it in its large list of tested genes. Imagine therefore, a male patient with severe RP in his early 30s without a notable family history who undergoes testing to determine if there is a chance that his children will have the disease. If the results of genetic testing are inconclusive, he will be told that the chances that the children will be affected is low because it probably is an autosomal recessive disease. However,

an isolated case in a man is an AD or an X-linked disorder in 5 to 10% of cases. In this case, if his disease is from a mutation in *RPGR* and it was not in the panel, he will not undergo appropriate counseling regarding the implications for his offspring and siblings. Although a male with *RPGR*-associated disease cannot pass on the variant to a son, his daughters will be obligate carriers, and might be variably affected. He may also have female relatives of child-bearing age for whom establishing carrier status might have implications in terms of consideration of prenatal or preimplantation genetic diagnosis.

Identifying the causative mutation is often viewed as the last step when in reality it is only the first, after which counseling the patient and family regarding rehabilitation, progression and treatment possibilities is a highly individualized science. It is also beneficial for the family to understand the limitations of the test, i.e. a panel test does not test every gene. One of the most challenging aspects in this field is to explain, after-the-fact, to the patient, often an entire family, the meaning of variants of unknown significance. The possibility should be given careful consideration, anticipated, and explained beforehand as part of the process of ordering 'all-inclusive' panels. It can be helpful for the ordering physician to work with a genetic counselor who can provide additional support in ensuring that the appropriate panel is chosen and the patient understands the results in context. In fact, counseling is included with one of the "free" testing programs.

In summary, although the cost of testing is decreasing and the ability to determine a genetic cause is increasing, it is paramount that the ordering physician be knowledgeable of the limitations of the positive as well as the supposedly negative results. Both the genes and variants that are known to be associated with disease are rapidly changing, and the panels are constantly

undergoing changes themselves. It is therefore incumbent on the doctor who orders the test to understand the limitations of whichever panel he or she chooses.

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Figure 1 Venn diagram showing the genes evaluated by 5 separate companies in their IRD panels represented by size of the circle[12]