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Perspective

Molecular Diagnosis of Inherited Retinal Dystrophy

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In the last few years, we have witnessed a revolution in molecular diagnosis of inherited retinal dystrophy. The progress has been extensively reviewed recently [1-3]. The purpose of this article is to provide personal insight and perspective on the progress and challenges. There is no doubt the views presented here may be seen as unconventional by many. However, I have been in a unique position by running a molecular diagnostic operation for many years. The operation is a combination of clinical service, business and research. Therefore, the views shared here are products of the unique environment. My mission has always been providing the best service with the lowest cost possible. At the same time, I also want to improve mutation detection rate. I have outlined several important issues we are facing in the molecular diagnosis of IRD and I will attempt to share my perspective on these issues.

Single gene vs. Multi gene panel testing:

The lack of precise clinical diagnosis, phenotype/genotype correlation and the large number of genes involved have long been the barriers facing molecular diagnosis of inherited retinal dystrophy (IRD). The arrival of Next-Generation Sequencing (NGS) has liberated the field allowing that any number and any combination of genes can be sequenced in one reaction now. The problem with single gene sequencing is not just limited to the likelihood of missing the causative genes involved in disease. Single gene sequencing can only provide a very limited view of mutation spectrum while missing additional information from entire pathways/genes involved with IRD. The presence of modifiers, digenic inheritance, multi-allelic inheritance and the co-incidental presence of mutations in multiple genes in the same patient will all be missed.

At this time, the argument against single gene testing is well accepted in the community. However, a different argument against single gene testing has rarely been mentioned. Single gene testing mostly relies on PCR and Sanger sequencing. From the operational point of view, PCR and Sanger sequencing are harder to streamline or scale up. It is very labor-intensive; mostly done manually and prone to human errors. The arrival of NGS thus provides an opportunity to automate the entire workflow from sample preparation, sequencing, to data analysis and reporting. Standardization of the entire procedure can increase quality, prevent human error while eat the same time reducing costs. Therefore, a multigene panel should really be the test of choice.

Targeted panel vs. whole exome sequencing (WES) or whole genome sequencing (WGS)

At this time, each platform has unique advantages and disadvantages. For testing patients with IRD, the consensus at this time is to order a comprehensive multigene panel with array comparative genomic hybridization (CGH) as a tier II test. It is a well-known fact that WES does not cover entire targeted regions and coverage is uneven. Because WES is designed to be a generic test aiming to identify mutations from most known disease genes, gene feature and mutation spectrum unique to IRD cannot be completely covered. For example, RPGR ORF15 is extremely difficult to sequence, which usually requires a separate ORF15 specific sequencing (Chiang, et., manuscript in preparation). Additionally, there were many reported deep intronic mutations identified recently in the IRD genes and these mutations cannot be identified by the generic WES testing. Also very importantly, the emergence of WES as an all-in-one test was mostly due to the low mutation detection rate for genetic conditions unrelated to IRD. When mutation detection rate is very low, due to many different reasons, the ~25-30% mutation detection rate of WES is justifiable to be the platform of choice. However, for genetic conditions with very specific clinical presentations such as retinitis pigmentosa (RP), USHER syndrome and pigmentation disease (such as Oculocutaneous albinism), using the generic approach of WES does not make much sense. When compared side by side, multigene panel outperformed WES [4]. Also many testing laboratories run trio WES testing (sequencing patient and their parents). Therefore, cost-wise, WES testing is more expensive than multigene panel testing.

The most exciting development in the molecular diagnosis of IRD may be the coming of $1,000 whole genome sequencing.

The initial announcement of $1,000 genome by Illumina HiSeqX Ten was criticized to be overly exaggerated by merely counting sequencing cost but not including interpretation or other additional costs. The latest claim of $1,000 genome was from Veritas Genetics. Veritas Genetics offers WGS, data interpretation, reporting and genetic counseling for the price of $1,000. Clearly the $1,000 benchmark is the Holy Grail in precision medicine. However, put aside marketing gimmick and/or unsustainable business practice, the total cost of molecular diagnosis of IRD by any platform today is still above $1,000. We are just not there yet.

Commercial/clinical vs. research testing

With the arrival of NGS, the boundary between commercial testing and research testing is disappearing. Ideally, clinical testing should really be the choice. The reasons are as follows: (1) The era of "new" gene discovery is coming to an end. Many of the newly identified "IRD" genes are syndromic genes. The research value of sequencing patients with IRD is gradually becoming less significant. (2) Sequencing cost is coming down and economy of scale further brings down cost. Dedicated commercial laboratories are more cost-effective and they are designed to run large scale and repetitive testing. Funding agencies may want to take a different approach by consolidating sequencing in order to standardize genetic testing and data collection. (3) Clinical testing must be performed with higher standards and clinical laboratories are regularly inspected by various agencies. (4) Patients should not be treated merely as research subjects. De-identification of patients does not serve these patients' interests. Returning clinical reports to the patients can be used as incentive in enrolling patients to "research" studies.

In fact, a better strategy for researchers is to efficiently and accurately identify all of the patients with known mutations first by clinical laboratories. The remaining patients are valuable research subjects for the study of new disease genes, novel genetic mechanisms and different differential diagnosis.

DTC (direct to consumer) testing - a force of disruption?

Genetic testing of rare conditions including IRD is usually ordered by clinical geneticists or in the case of IRD by ophthalmologists specialized in retinal degeneration. This setup is preferable because clinical diagnosis and genetic counseling require expertise. However, this traditional practice does have some drawbacks. Fundamentally, the ownership issue could be the biggest problem. In some research studies, patients are de-identified, so they have no easy way to receive testing results back from the research studies (even when research studies are done by clinical laboratories with CLIA license). Even though many of this type of research is supported by federal funding (taxpayers' money), patients do not directly benefit from the research results. In fact, many researchers treat patient clinical information and genetic data as their private properties. This fragmented practice and ownership issue hinder the development of public database and also disfranchised some patients from participating clinical trials run by other centers. With the arrival of NGS, especially the price of WGS is coming down very significantly and also because of the democratization of medical knowledge through internet and by various disease specific social groups, more and more patient families pursue genetic testing with or without the involvements of medical professionals. One could argue that this liberation can have some positive outcomes with proper regulations. In fact, patients are the true owners of their genetic maternal and medical information. One way to circumvent the private ownership of database is to incentivize direct submission of medical information and genetic information from patients to public database. This DTC liberation may also bring down the cost of genetic testing by offering more choices to consumers/patients. Ultimately, the knowing of one's own genetic makeup should be a right to each individual. Finally, the emergence of private independent genetic counseling services may also contribute to this democratization process. Patients now have the option to use private genetic counseling service through phone especially for those patients with clinical diagnosis already.

$1,000 genome sequencing including data interpretation has arrived through "mass production"

With the fast development of NGS technologies, sequencing is gradually becoming a commodity. In fact, for some generic sequencing such as WES, outsourcing sequencing to CLIA labs at big genome centers will probably make more economic sense. Even for sequence alignment, data analysis and variation calling, various commercial pipelines are available. The entry barrier of "clinical sequencing" is virtually non-existent. Also database such as Human Gene Mutation Database (HGMD), ClinVar and ClinGen are being widely used. Building an automatic pipeline by reporting mutations as defined by HGMD and/or ClinVar can make data analysis very "straight forward". Indeed, the $1,000 genome including "data analysis and interpretation" has already arrived through this type of mass production! However, similar to all of the mass produced products, disease and sequence specific and unique information are lost in the process. It is also true that HGMD and ClinVar are far from accurate. Taken together, true clinical utility of the $1,000 genome including interpretation is still in question at this time. Specialty/boutique molecular diagnostic laboratories still have some advantages.

Mutation detection rate and clinical utility

Mutation detection rate for molecular diagnosis of IRD is much higher than many other genetic conditions for the following reasons: (1) Clinical presentation is highly specific. (2) The number of genes involved is limited. (3) Most of the IRD disease genes have already been identified. The published and unpublished mutation detection rate for patients with IRD is ~50% - 70%. At this time, the major focus is more on finding mutations for every patient with IRD. However, in order to achieve that goal, several improvements

must happen: (A) Sequencing the entire known IRD genes should be the choice of test even for patients with more specific clinical diagnosis such as LCA, Achromatoptia and Cone Rod Dystrophy. Clinical diagnosis does have its limitation and phenocopy or overlapping clinical presentation do occur. (B) An accurate molecular diagnosis requires information sharing and collaboration between testing laboratories and clinicians. For example, in the case of retinopathy, non-genetic conditions such as autoimmune retinopathy may not follow the standard genetic mechanisms of IRD. Since most clinical laboratories do not pre-screen patients, accurate mutation detection rate is hard to establish. (C) A larger scale and systematic phenotype and genotype correlation study is necessary in order to improve mutation detection rate. The randomness and fragmentation of current sample collection and sequencing are not helping the cause. (D) The current sequencing efforts mostly focus on coding regions and exon/intron boundaries. Promoter region, 5' and 3' UTR and deep intronic regions are usually not covered. A systematic effort to screen patients through standard clinical sequencing and followed by WGS will most likely increase the yield. This approach may work well, especially for patients with only one mutation identified and array CGH analysis has already ruled out del/dup mutations.

Ultimately, molecular diagnosis of IRD has the opportunity to become the first line of diagnosis. Sequencing and data interpretation are becoming better and cheaper. For genetic conditions, finding the underlying mutations is fastest, cheapest, most objective and potentially earliest diagnosis (through new born sequencing). Also for the purpose of better managing health care resources, general practice ophthalmologists may become the ordering physicians. Patients can then be referred to specialists for follow-up. Even negative results one day will become useful information to rule out genetic conditions. However, for this to happen, cost will be a deciding factor.

It will take a village and a revolution in thinking

The promise of precision medicine is well accepted but to get there will take some extraordinary efforts. Most importantly, current medical practices and funding mechanisms are hindering the progress. Patients are often being treated as research subjects and assets. The ownership issue is a real concern. The concentrations of specialists in big medical centers tend to intensify competitions. Also the fierce competitions in publishing interesting cases make sharing less desirable. Even for our clinical molecular diagnostic service, some clients refuse to provide us any medical, gender and age information. Clearly, the current path will not lead us to the Promised Land. Sequencing without knowing specifics about the patients will not improve molecular diagnostic service. The owners (researchers) of those patients benefit from this type of practice but the gain is shortsighted and fragmented.

Unfortunately, even though the solution is obvious, in practice, it is almost mission impossible. It will need unconventional thinking and require resolute action. First and foremost, funding agencies should require sharing of relevant medical information when genetic testing is part of the funded projects. Gradually, some special interest groups are realizing the problem and they may take the lead of funding sequencing projects with the goal to collect data in order to better understand phenotype and genotype correlation. A sequencing project of 3,000 unrelated patients with clinical diagnosis will probably be enough to lay the foundation of a good database. Once the database is built, it can be opened to researchers and clinicians willing to contribute their private data to the common database. There are many advantages of this approach including (1) testing methods can be standardized; (2) variation calling and interpretation can be standardized; (3) data re-interpretation and comments by members can further improve data interpretation and phenotype and genotype correlation; (4) samples that test negative can become research subjects for gene discovery program; (5) similar cases with unique findings can now be bundled together for publications; (6) the database and patient cohort can be valuable to clinical trials; (7) the better coordinated effort can actually save money and improve patient care.

Finally, individual patients may want to contribute their data even when their clinicians refuse to collaborate. A different level of data sharing with individual patient may be desirable not only to broaden the data collection, it can also offer unique education opportunity to patients.

Conclusion

We are at a juncture of a revolution in precision medicine. The old structure and practice in medicine will be challenged by new thinking and business models. Democratization is happening at many different levels including sequencing, data interpretation, data ownership, etc. The market force and the force to cut medical cost/waste will likely be smashing barriers and bring in necessary changes. The awaking of patient right and ownership will undoubtedly be a driving force in making all the right changes. It is exciting to be in the business of molecular diagnosis of inherited retinal dystrophy.

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