The overall goal of the Institute for Vision Research at the University of Iowa (IVR) is to be able to treat all patients with inherited eye disease as effectively as possible. Some of the major components of this effort are:

1. establishing every patient's molecular diagnosis as accurately, efficiently, and affordably[1] as possible;
2. establishing the molecular mechanisms of each patient's disease in sufficient detail that an effective treatment can be devised (a given patient's "gene-directed treatment" would likely include one or more of the following: counseling (e.g., prognosis and recurrence risk), customization of their diagnostic workup (e.g., ERG, MRI, etc.), pre-implantation genetic testing, gene-directed drug therapy, gene replacement therapy and genetically-corrected autologous stem cell therapy);
3. establishing the "natural history" of each disease with sufficient accuracy that the optimal timing and anatomic location of each treatment can be established;
4. demonstrating sufficient proof of principle of each new therapy in patient derived cells, and sufficient safety in small animals, that phase I-II trials can be embarked upon in humans; and,
5. conducting philanthropically supported gene therapy clinical trials for less than $20,000 per subject and clinical trials of stem cell transplantation for less than $50,000 per subject.

The genetic testing unit of the IVR is the John and Marcia Carver Nonprofit Genetic Testing Laboratory. The goals of this laboratory are to:

1. devise, implement and share technical approaches that will allow the most clinically meaningful molecular results to be obtained with a clinically acceptable turnaround time while consuming the fewest health care dollars per family; and,
2. use these approaches to identify and care for individuals who may wish to participate in the natural history, disease mechanism, and therapeutic studies of the IVR.

The following "cardinal principles" guide all genetic testing within the IVR.

1. The primary goal of genetic testing is to establish a patient's molecular diagnosis with sufficient certainty (note that it is never 100%) that the likelihood of benefit from gene-directed treatment or counseling is greater than the likelihood of harm.
2. Before using a genetic test result to guide gene therapy, stem cell therapy or pre-implantation genetic testing, one should obtain two-sample confirmation (a

second independent sample from the proband, a sample from a similarly affected family member, samples from parents, etc.)

3. One should not use research data (i.e., data that are not validated enough that a CLIA report could be written) for clinical decision making or counseling a patient. However, research data can be used to guide CLIA approved clinical testing.
4. There is a threshold probability that a specific genotype causes the patient's disease that must be exceeded before embarking upon a gene directed treatment. For gene therapy trials or pre-implantation genetic testing, this threshold is greater than 95%.
5. The probability that a given variant is contributing to a patient's disease is inversely proportional to the amount of the genome that one considers to be potentially disease-causing before the variant is identified. In other words, the probability that a given finding is correct is greatly influenced by the pre-test hypothesis. Thus, the indiscriminate use of massively parallel genetic testing has the possibility of reducing the probability of a discovered genotype below the threshold that will allow treatment. This is because there is a multiple measurements penalty associated with massively parallel genotyping. Therefore, pre-test hypotheses should be carefully and formally considered.[2]
6. The multiple measurements penalty can be minimized by using a phenotypically focused tiered testing approach that first investigates the most likely places in the genome that the patient's disease-causing variants might lie, before expanding the search with a greater and greater statistical penalty. In some cases these tiers will be analytical (e.g., considering larger portions of an NGS data set when an initial focused analysis of that set failed to reveal a plausible genotype) and in some cases the tiers will be physical (e.g., Sanger sequencing of a single gene followed by NGS sequencing of the coding sequences of a group of candidate genes when the initial sequencing is negative). Many patients can be quickly, accurately and inexpensively diagnosed by phenotypically focused allele-specific testing and/or Sanger sequencing of one or a few genes. An under-appreciated advantage of physical tiers is that some genotyping methods are significantly superior to others for certain regions of the genome and certain types of mutations. There is no single method of genotyping that is best for all patients and for all clinical diagnoses. For example, low complexity regions (like codons 796 to 1081 of ORF15 of *RPGR*) will need to be interrogated using non-NGS methods (such as Nanopore sequencing or plasmid cloning followed by Sanger sequencing) for the foreseeable future.
7. The most important probability in the context of genetic testing is not the probability that a given genetic variant alters the encoded protein enough to cause a cellular phenotype (which is the goal of many *in silico* prediction methods), but the probability that an observed genotype in an individual patient causes the disease that the patient is seeking treatment for.

8. The IVR's term for the probability that a given variant is causing the patient's disease is the "Estimate of Pathogenic Probability" or EPP.[3]
9. The calculation of the EPP is different for different inheritance patterns and different testing strategies.
10. When trying to establish the EPP for a given allele in a given patient, the patient's clinical findings, clinical history, gender, ethnicity, the clinical findings in their relatives, the apparent inheritance pattern of their disease, the genotypes of their relatives, and the phenotypes previously associated with their putative disease-causing variants are all of great importance. These data and samples should be sought in every case (e.g., samples should be obtained from parents and sibs at the initial visit if present) to increase the probability that the genotype that is identified is indeed the cause of the patient's disease.
11. Given the importance of clinical data to the EPP, and the distinct "personalities" of each disease-causing gene, it is very valuable (essential really) to have clinicians who care for patients with the diseases being tested involved in the design and interpretation of these tests.
12. EPPs[3], (MDPDs)[4], M numbers[5], genotype-phenotype correlations and the like all benefit from access to tens if not hundreds of patients. Therefore, if a disease is important to the mission of your organization, you should take steps to rapidly collect tens of patients and subject them to uniform objective analysis to create a tiered test that is worthy of fee-for-service deployment.[6,7]
13. Like any aspect of medicine, one can be wrong in one's interpretation. In genetics, the truth has a way of making itself known through an unexpected new symptom or an unexpected recurrence of the disease. Choosing a variant or two from a list of equally plausible variants -- and asserting them to be disease-causing -- does not make this assertion true. Physicians should treat every molecular diagnostic decision they make as though another knowledgeable person is going to re-evaluate all the data at some future date. Everyone will make misdiagnoses. The goal is to make molecular misdiagnoses as infrequently as possible, and when one makes them, to discover them oneself by continuous re-review of one's own data (issuing amended reports as needed).
14. All samples should be subjected to quality control tests using genetic markers to lessen the risk of a sample swap anywhere in the chain of sample handling.
15. All results obtained initially with an allele-specific method or next generation sequencing should be confirmed by Sanger sequencing before reporting.
16. One should vigorously attempt to obtain phase determination of all putative disease-causing recessive genotypes. If phase cannot be determined (e.g., relatives are unavailable or unwilling), one should clearly state this limitation in the report (and downgrade the probability that the genotype is disease-causing).
17. One should inform patients that they (or their child) may have an untreatable systemic degenerative disorder (and document this in writing) before attempting

to confirm this clinical suspicion with genetic testing. In cases where clinical findings and/or family history support such a diagnosis, the patient should be offered such testing in order to avoid a diagnostic odyssey.

18. One should avoid testing presymptomatic minors (when no effective treatment or active clinical trial is available for the disease).
19. When using genome-wide next generation sequencing as a means of inexpensively investigating a subset of the genome, one should a) let the patient know that the test is not designed to assess all genes, b) be prepared to counsel the patient for any plausibly disease-causing variants one observes, and c) informatically mask the unanalyzed portion of the genome so that no laboratory or healthcare personnel are aware of a potentially disease-causing variant that is not communicated to the patient. In the future, subsets of masked data may be unmasked when research studies reveal that such subsets are compatible with the patient's original "eye disease focused" consent.
20. Variants with M numbers[5] below 7 should be interpreted with great caution, especially when detected in a screen of more than a few genes.[6]
21. Heterozygous genotypes in patients with recessive diseases should be interpreted with great caution and should in most cases not be used for publication, counseling, family planning or other aspects of medical care. The clinical phenotype should drive all decision-making and publication unless and until a properly segregating complete genotype is identified.
22. Variants that are too common in the general population to cause rare high penetrance disease should be interpreted with great caution and should in most cases not be used for publication, counseling or other aspects of medical care. The clinical phenotype should drive all decision-making and publication unless and until a statistically significant relationship has been established between the disease and the low penetrance variant and/or the disease mechanism of this variant has been elucidated in *in vitro* or animal studies.
23. It is a critical component of the IVR mission to keep the costs of genetic testing low, and the efficiency of testing (the likelihood of a meaningful positive result per dollar of expense) high, so that everyone affected with one of these rare diseases will have a chance to be diagnosed and treated. Turnaround times should be short enough for optimal clinical decision making but long enough to allow the test to be performed efficiently and the data to be analyzed thoroughly.[8]
24. It is part of the IVR mission to use the findings of our laboratories and clinics to continuously contribute to the published knowledge of the natural history of inherited eye diseases and the genotype-phenotype correlations of specific genes and mutations (e.g., StoneRounds.org)
25. It is part of the IVR mission to perform philanthropically supported "research testing" on a subset of individuals with inherited eye disease who a) have a

disease that is not yet well enough understood for an efficient fee-for-service test to be deployed and/or b) have a phenotype that is of importance to other aspects of the IVR mission. Special features of research testing include: a) longer turnaround times (to maximize the value of the philanthropic dollars used to perform the tests); b) the need for informed consent; and, c) the lack of a written report for "negative" tests (or more accurately, "ongoing research studies that are not yet positive").

26. One should avoid routine genetic testing for genetically complex disorders such as age-related macular degeneration and late-onset primary open-angle glaucoma until specific treatment or surveillance strategies have been shown in one or more published prospective clinical trials to be of benefit to individuals with specific disease associated genotypes. In the meantime, one should confine the genotyping of such patients to research studies.[8,9]

References

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