COMMENTARY

Understanding the genetic architecture of human retinal degenerations

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Overview of Inherited Retinal Degenerations

Inherited retinal degenerations are a clinically and genetically heterogeneous group of blinding diseases characterized by progressive degeneration of the neuroretina and/or the retinal pigment epithelium. Currently, over 300 genes have been implicated in retinal degenerations (RetNet: https://sph.uth.edu/RetNet/). While mutations, or causative variants, in each of these genes are relatively rare, taken together they are a significant cause of blindness, especially in working-age individuals, which increases their economic and societal impact. Clinically, inherited retinal degenerations vary from retinitis pigmentosa and Leber congenital amaurosis, which are often manifest at birth and initially involve rod photoreceptors in the retinal periphery, to early-onset macular degeneration, which is a progressive degeneration affecting central vision (1). Inherited retinal degenerations can show autosomal-dominant, autosomal-recessive, and X-linked inheritance, as well as more complex or multifactorial inheritance patterns, especially for some of the later-onset progressive diseases. While mutations in the same gene usually show the same inheritance pattern, it is not uncommon for mutations in the same gene to cause two or more forms of retinal disease, complicating the nosology of this group of diseases (2). In addition, the presence of disease-associated alleles at one gene can affect the penetrance or expressivity of mutations at a second, resulting in complicated family histories and in some cases even digenic diallelic (3) or digenic triallelic (4) inheritance. In PNAS, Hanany et al. (5) address this intimidating set of related diseases by developing a system to identify disease alleles at each of the associated genes and, beyond that, to calculate the gene-specific carrier frequency and the prevalence of homozygosity for variants causing autosomal recessive retinal degenerations. While appearing somewhat mundane on the surface, this work actually has far-reaching implications for both the basic science of the retina and the clinical practice of ophthalmology.

Genetic Architecture of Inherited Retinal Degenerations

The genes associated with retinal degeneration and the changes in them define the genetic architecture of that disease. Identification and characterization of DNA variants associated with disease is a major goal of human genetics, and it has become both clinically more important as DNA-based diagnosis has moved from the realm of research to a component of mainstream medical practice and more feasible as whole-genome and whole-exome DNA sequencing has decreased in cost and become generally available. However, estimating, and especially using, this basic information is not straightforward. As emphasized by Hanany et al. (5), there is a great difference between the summed carrier rate for retinal degenerations overall, a value often currently used in genetic counseling, and the carrier rates for specific genes, which are more useful when estimating genetic risk. Interestingly, the carrier frequency for deleterious mutations at each locus is extremely low, which is consistent with the rare frequency of recessive disease associated with each gene. However, the carrier frequency in aggregate across all inherited retinal degeneration genes is extremely high, as high as one in every second to fourth individual depending on the population. This is a significant problem for genetic diagnosis in patients in whom only one mutation has been detected.

As mentioned above, the genetic architecture of the retinal degenerations varies among populations, not only among the broad groups analyzed by Hanany et al. (5) but also in smaller subpopulations, sometimes called "special populations," which by virtue of their relatively small size and genetic isolation can be more strongly affected by founder effects, genetic drift, and as in all population groups social traditions affecting marriage patterns that lead to assortative mating, thereby elevating the consanguinity rate (6). Founder effects and genetic drift can have a major effect on allele frequencies and linkage disequilibrium, while marriage traditions can increase the frequency of

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homozygosity and hence autosomal recessive traits above that predicted by assuming Hardy–Weinberg population equilibrium. Although these characteristics of special populations can be quite useful in identifying gene mutations that can cause autosomal-recessive diseases, they also complicate genetic risk prediction in a clinical setting. However, in any population, a solid estimate of the carrier frequency for mutations in the gene of interest provides much more accurate estimates of risk than those based on a summed or average carrier frequency.

The genes causing between 50% and 70% of retinal degeneration cases have been identified (7), depending on the population and approach of various studies. This is a major complication in attempts to estimate the carrier frequencies for the various causative genes, especially since it is unclear whether the unidentified mutations result from a failure to identify causative mutations existing in known genes or from changes in genes yet to be identified. That the missing causative changes in known genes have proved more resistant to identification is not surprising since they are likely to be subtle regulatory or splicing changes or perhaps large deletions, difficult to analyze and often distant from the coding regions so that they would not show up in whole-exome sequencing. Non-Mendelian inheritance patterns, which may be apparent if large families are available, and runs of homozygosity on whole-genome sequencing are suggestive of large deletions, and some types of deep or long-run sequencing can identify them. One clue of a regulatory or subtle splicing mutation in a recessive disease is the identification of a single copy of a severe or proved mutation in an affected individual, which raises strong suspicion that it might be accompanied by an unidentified second change. However, elucidation of these changes with certainty will probably require more laborious functional studies to demonstrate pathogenicity rather than clever bioinformatic approaches.

Solving the problem of mutations in as-yet-unidentified genes might be more amenable to a combination of high-throughput sequencing and bioinformatics. One example of this is seen in the report by Carss et al. (7), in which whole-exome and whole-genome sequencing of a large group of individuals with inherited retinal degenerations identified putatively damaging biallelic sequence changes in genes not previously associated with retinal degenerations. While these results are highly suggestive, especially if they are seen in more than one unrelated individual, they should probably also be confirmed by functional studies, depending on the level of certainty required, which would be higher for use in clinical diagnosis than in a research setting. Finally, if families with a sufficient number of affected individuals can be identified, linkage analysis showing cosegregation of a variant allele with the disease can provide strong support for causality, often limiting candidate genes to the 50 to 100 that lie in a 5- to 10-cM linked region. This approach is somewhat laborious and, more importantly, requires the availability of large families segregating the disease, more common in the "special populations" discussed above who may not have the same distributions of mutations seen in the larger population groups, at least at high frequencies. The approach taken by Hanany et al. (5) combines the use of large population-specific databases containing all sequence changes as well as more specific databases containing putative causative changes. Because the latter are known to misclassify some variants (especially rare ones) (8) they used a manual filtering process to examine literature reports, which should make the process significantly more robust but does not circumvent the problems of undetected variants. However, as an increasing fraction of causative mutations and genes are identified, discovery of the remainder might be simplified as subtle changes become paired with obvious or known causative ones more frequently and multiple individuals and/or families with mutations in novel genes are identified.

Importance for Basic Science and Clinical Practice

As human genetics comprises study of the sum of the genetically determined similarities and differences between individuals and populations, understanding the genotype–phenotype correlations of those differences provides perhaps the most powerful tool available to characterize the function of the included genes and their products. In terms first popularized by Victor McKusick, who had perhaps the clearest early vision of this undertaking, the comparative anatomy, functional anatomy, developmental anatomy, and particularly the applied anatomy of the human genome are all interrelated with and critically dependent on knowing its morbid anatomy (9). This is also true for understanding the function and physiology of the corresponding genes and even appreciating that they exist, as emphasized earlier by Garrod (10) and recapitulated many times since by elucidation of the roles of many underappreciated or even completely unknown biochemical and developmental pathways in the retina and other tissues as a result of genetic studies identifying mutations in a causative gene. The basic biochemistry of proteins can, and perhaps must, be understood through in vitro studies, but their true relevance in humans has to be examined in the actual tissue and organism. Cell culture and animal models are useful in this respect, but neither they nor even induced pluripotent stem cell-derived retinal organoids (11) provide a completely accurate model for the human retina. The choice of autosomal recessive diseases for the study by Hanany et al. (5) provides not only an analytical advantage in identifying causative mutations but also a look at the effects of the absence of a specific gene or process on the retina and its cells without the potentially complicating gain of a deleterious function frequently seen in dominant mutations. In addition, the specific amino acids mutated in target genes allow one to identify critical parts of the protein encoded by that gene and their effect on the enzymatic or structural properties of that protein. The study by Hanany et al. (5) represents a timely advance along the pathway from having studies of individual genes elucidate functional aspects of the retina to synthesis of a coherent view of the retina from the sum of currently known mutations and the genes in which they occur.

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In addition to expanding understanding of the retina and its biology, an exhaustive knowledge of the mutations and genes contributing to retinal degenerations will be a significant benefit to clinical diagnosis and perhaps treatment of retinal diseases. While molecular diagnosis using direct detection of a known mutation is exquisitely accurate and technically straightforward, in other situations diagnostic ability can suffer due to both uncertainties regarding the genes that can cause disease when mutated

and difficulties in distinguishing benign variants from causative mutations. As the morbid anatomy of the genome is increasingly well understood these difficulties will decrease correspondingly. Similarly, expanding the knowledge of genes causing retinal degenerations allows gene therapy to be performed for a wider spectrum of retinal diseases, including replacement of specific gene products and ameliorative therapy modifying retinal defensive processes, with diseases sharing common pathological mechanisms potentially responding to similar supportive therapies (12). The latter benefit would require more sophisticated understanding of retinal biology and pathophysiology.

The retina provides an excellent system in which to carry out precise disease diagnosis and phenotype–genotype correlations. It has a precisely organized structure arranged to carry out the specific functions of phototransduction, immediate processing of signals, and transmission of the resulting signals to the brain. Further, because of this it is amenable to direct visualization, precise imaging techniques, and elegant functional tests. All of these allow differentiation of the retinal degenerations into a multitude of precise clinical diagnoses so that the genes and mutations responsible for each can be identified and characterized. However, this ability is not unique to the retina, or retinal degenerations, and the difficult process of exhaustively delineating and correlating mutations with the diseases they cause is ongoing in essentially all organs and tissues. These relatively small individual steps will eventually sum to provide an exhaustive knowledge of the genes and mutations responsible for human inherited disease and from there human pathophysiology and biology.

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