The Cell and Molecular Biology of Complex Forms of Glaucoma: Updates on Genetic, Environmental, and Epigenetic Risk Factors

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Glaucoma is a clinically and genetically heterogeneous disease. First-degree relatives of primary open-angle glaucoma (POAG) patients have a disease prevalence that is between 4 and 10 times higher than that of the general population, and there is a higher disease concordance in monozygotic twins than in dizygotic twins. These studies indicate the significant heritability of POAG; however, a simple mode of inheritance is not likely and cannot be assumed in genetic studies designed to identify POAG susceptibility genes. POAG is also complex clinically. The relationship between intraocular pressure (IOP) elevation and retinal ganglion cell degeneration is not simple, as many individuals have IOP elevation without optic nerve damage, and in some individuals, optic nerve degeneration develops without elevated IOP. Recent studies have shown that POAG-related clinical features, including optic nerve parameters, central corneal thickness, and IOP, are influenced by different sets of genes. Genetic and environmental risk factors and epigenetics are thought to influence complex traits such as POAG, normal tension glaucoma (NTG) and exfoliation syndrome-related glaucoma (Fig. 1).

GENETIC RISK FACTORS FOR POAG

The identification and characterization of POAG susceptibility genes would elucidate the molecular pathogenesis and could suggest new methods of diagnosis and treatment. The demonstration that the function of a particular enzyme or structural protein is impaired in POAG patients may lead to the development of novel drug therapies. The identification of DNA sequence changes associated with the disease could form the basis of diagnostic tests that are useful in identifying individuals at risk. The availability of such tests would provide a mechanism for early detection and timely treatment. Those individuals at risk who are identified early in the course of the disease and who begin therapy before significant damage to the optic nerve have the best chance of maintaining useful sight.

Although POAG has a significant heritability, family-based linkage studies have not revealed POAG genes with significant population effect. The lack of such findings suggests that novel POAG genes have modest effect sizes and that large data sets with well-defined phenotypes are necessary for discovery. The formation of multiple consortia and collaborations has been crucial in the success of the genome-wide association studies approach by increasing sample sizes, thereby increasing statistical power, enabling replication of findings from individual studies, and establishing common methods of analysis. Common complex diseases often require more than 10,000 cases and controls for successful identification of the predisposing genes.

Genome-wide association studies have recently shown promising results for POAG gene discovery. Using an Icelandic population of 1,263 cases and 34,877 population controls, two single-nucleotide polymorphisms (SNPs) in an intergenic region between the CAD1 and CAD2 genes were found to confer modest risk for POAG (odds ratio [OR] = 1.3). This finding was replicated in Caucasians of European ancestry and in a Chinese sample and, more recently, in a Caucasian case-control study from the United States. A second study from Australia of 590 cases with severe POAG and 3956 controls found significant association with SNPs located near the TMC1 gene (P = 1.7 × 10⁻¹⁰), OR = 1.68) and CDKN2BAS (P = 4.7 × 10⁻⁹; OR = 1.5). SNPs in the CDKN2BAS region were also found to be risk factors that influence an important quantitative optic nerve parameter, the cup-to-disc ratio (CDR), suggesting that quantitative trait analysis for POAG-related endophenotypes can be a successful approach in dissecting POAG’s genetic architecture. In a large case-control study of 3146 POAG cases and 3487 controls from the United States, significant associations were also observed for SNPs located in the CDKN2BAS region (rs2157719 [G]: OR = 0.69; 95% CI, 0.63–0.75; P = 1.86 × 10⁻¹⁰), as well as the SIX1/SIX6 region on chromosome 14, region q23 (rs10483727 [A]: OR = 1.32; 95% CI, 1.21–1.43; P = 3.87 × 10⁻¹¹), also previously associated with CDR. Further analysis of the normal-tension glaucoma (NTG) subgroup for the U.S. sample (720 NTG cases, defined as IOP < 22 mm Hg without treatment) identified a novel region on chromosome 8, region q22 (rs284489 [G]: OR = 0.62; 95% CI, 0.53–0.72; P = 8.88 × 10⁻¹⁰) with probable regulatory function in several cell types relevant to glaucoma, including those in the ciliary body and choroid plexus. The CDKN2BAS region was also statistically significant in the NTG subgroup analysis (rs2157719 [G]: OR = 0.58; 95% CI, 0.50–0.67; P = 1.17 × 10⁻¹⁵), suggesting that the gene contributes primarily to optic nerve disease in glaucoma. Genome-wide association studies have also identified CDKN2BAS as a genetic susceptibility locus for several other age-related conditions, including coronary artery disease, intracranial aneurysm, and type 2 diabetes. CDKN2BAS codes for a long, noncoding RNA (also known as ANRIL) that contributes to the regulation of expression of CDKN2B, a component of the transforming growth factor (TGFβ) signaling pathway. Previous studies have suggested a role for TGFβ signaling in glaucoma, both in the optic nerve and the trabecular outflow pathways. In addition, components of the tumor necrosis factor (TNF)-α pathway have recently been implicated in optic nerve disease. Collectively, these results suggest that further research is necessary.
Glaucma Pathogenesis

Figure 1. Complex pathogenesis of POAG. Risk factors for POAG may include genes, environmental factors, and epigenetic effects (NTG, normal tension glaucoma; ES, exfoliation syndrome).

on the contributions of the TGFβ and TNFα pathways could lead to the identification of interesting targets for neuroprotective strategies for glaucoma.

ENVIRONMENTAL RISK FACTORS FOR GLAUCOMA

Environmental risk factors may influence POAG through effects on IOP and/or the rate of retinal ganglion cell apoptosis. Currently, there are few environmental factors known to contribute to POAG. Some activities may increase IOP, such as playing high-resistant wind instruments, wearing tight neckties, and gazing in certain yoga positions. Others may lower IOP, such as general physical exercise. Nutritional factors, such as dietary fat and antioxidant intake, and other lifestyle factors, including smoking and postmenopausal hormone use, may influence the development of POAG. Further research is necessary to define the role of these factors in glaucoma’s pathogenesis. A gene-environment interaction involving hormone replacement therapy and NOS3 (the gene coding for nitric oxide synthase 3) has been identified as a risk factor for POAG.29

Residence in northern latitudes is a significant risk factor for exfoliation syndrome (ES) and the related exfoliation glaucoma. Exfoliation syndrome (ES) is an extracellular deposit disorder that is the most common cause of secondary open-angle glaucoma. In a retrospective observational study of 3367 incident ES cases in patients residing in the northern tier of the United States (above 42°N), there was an association with an increased hazard of developing ES (adjusted hazard ratio [HR] = 2.14; 95% CI, 1.94–2.35). Living in the southern geographic tier (below 37°N) was associated with a reduced hazard of ES (HR = 0.83; 95% CI, 0.75–0.93).29,30 After adjustment for joint environmental effects, for every 1° increase in July high temperature, the hazard of ES decreased by 9% (HR = 0.91; 95% CI, 0.89–0.93). For every 1° increase in January low temperature, the hazard decreased 3% (HR = 0.97; 95% CI, 0.96–0.98). For each additional day of sunshine exposure annually, the hazard of ES increased by 1.5% (HR = 1.02; 95% CI, 1.01–1.02) for persons living with average levels of other climatic factors. Overall, these results suggest that ambient temperature and sun exposure are environmental triggers of ES, although other features of northern latitude exposure, such as reduced vitamin D metabolism, could contribute as well.

ROLE OF EPGENETICS IN GLAUCOMA

Epigenetic effects regulate gene expression without changing the primary DNA sequence. Recognized epigenetic modifications include methylation of CpG dinucleotides and covalent modification of histones, which form the protein cores of nucleosomes, the basic unit of DNA packaging in eukaryotic cells. Normal development and aging are influenced by epigenetic processes, and a role for epigenetics in the etiology and progression of age-related diseases is likely. Numerous studies support a role for epigenetics in cancer development,31 and emerging evidence suggests that epigenetic modification can also influence chronic neurodegenerative disorders such as Alzheimer’s disease.32 Epigenetic regulation of gene expression may be influenced by environmental exposures such as diet, smoking, and pollution. Epigenetic effects may be reversed through small-molecule therapies. Interestingly, the CDKN2BAS locus recently associated with POAG and optic nerve CDR is a genomic region that appears to be regulated by epigenetic mechanisms.33 Investigations into epigenetic effects in glaucoma are at early stages, but they could have a significant impact on future therapeutic approaches.

SUMMARY: KEY NEEDS AND OPPORTUNITIES

Glaucma is a complex disease, and all the factors that lead to it must be integrated, starting with the gene mutations that may precipitate the disease process. In particular, more genetic data are needed that can come from whole-genome genotypes and exome and/or whole-genome sequence data. With current advances in molecular biology, we have an opportunity to do secondary analyses using methods for studying gene × gene interactions, gene × environment interaction pathway analyses, and functional mutation assessments. Eventually, the goal will be to use information from genetic studies to develop DNA-based diagnostic screening tests and gene-based therapies, including neuroprotective therapies. Pursuing these opportunities will require well-phenotyped patient cohorts and consortia of investigators and clinicians.

References