Ophthalmic Gene Testing – Ready for Prime Time?

Nick Lane PhD

The debut of genetic tests for glaucoma in the marketplace belie the fact that it will be a long time yet before sensitive and specific screening tools can deliver useful clinical information, reports Nick Lane PhD, in an in depth report.

The first commercial gene test for glaucoma (OcuGene) was launched to a TV fanfare nearly three years ago, and the developers,InSite Vision,claim their assay has "greater than 99% sensitivity and selectivity".So how long before gene testing becomes routine for the early diagnosis of complex ophthalmic disorders?

"I don't want to be a nay-sayer" Richard Parrish II MD, at the University of Miami,told *EuroTimes*. "All this genetic information is incredibly interesting and valuable, but as far as genetic screening is concerned, we're still a long way from prime time."

EuroTimes looked into why the delay. The mutations in the two genes linked with POAG tell a story of high hopes,dwindling returns and a certain amount of misinformation.

Take 1: Light at the End of the Tunnel

The first gene linked with POAG, known as MYOC (originally designated TIGR),was tracked down in 1997 by Edwin Stone MD PhD, and colleagues at the University of Iowa.It codes for a trabecular meshwork protein called myocilin, whose function is still largely unknown.

The original paper was published in Science in 1997,and an accompanying editorial by Gretchen Vogel hailed the breakthrough as a light at the end of the tunnel for patients with glaucoma.

Numerous alterations have since been detected in the MYOC gene in different populations,and more than 50 are claimed as plausible 'disease-causing variations' (DCVs).A handful of these DCVs are associated with a severe, autosomal-dominant,early-onset form of glaucoma.More generally, DCVs in the MYOC gene account for 2% to 4% of adult-onset POAG.Not only the coding regions of the gene, but also the upstream promoter region, referred to as mt.1,have been associated with POAG.Although the prevalence of the mt.1 variant is similar in patients with POAG and controls,at around 15 to 20%,Henri-Jean Garchon MD PhD and his colleagues at INSERM U25,Paris,have claimed that it influences the course and severity of disease.

Garchon and his associates argue that mt.1 "appears to be an indicator of poor IOP control and greater visual field damage in diagnosed POAG patients,potentially due to a lack of response to therapeutic intervention.Its typing may help in the selection of treatment paradigms for the management of POAG patients."

The OcuGene test,launched in October 2001,detects three of the more common DCVs in the MYOC gene itself,along with the variant mt.1 promoter region.

At its launch,InSite Vision CEO Kumar Chandrasekaran PhD made the modest claim that "The information provided by OcuGene may help physicians to individualise treatment according to the patient's genetic make-up and perhaps to initiate treatment for those high-risk individuals who may otherwise be unidentified."

At about the same time, a second gene that segregates with POAG was pinpointed by Mansoor Sarfarazi MD and his associates at the University of Connecticut.

Sarfarazi's group found that 16.7% of families with hereditary POAG had alterations in the OPTN gene, coding for a 66 KD protein that they re-named optineurin.The function of this protein is still unknown,but it is expressed in the trabecular meshwork,non-pigmented ciliary epithelium, retina and brain,and is thought to interact with the apoptosis pathway signalled by tumour necrosis factor-a.

Sarfarazi speculated that optineurin "plays a neuroprotective role in the eye and optic nerve, but when defective, it produces visual loss and optic neuropathy as typically seen in normal and high-pressure glaucoma."

The optineurin results were published in Science in February 2002.The findings were highlighted in an editorial entitled Under Pressure by James Friedman MD and Michael Walter MD, at the University of Alberta,Canada. They noted that 66 million people world-wide have glaucoma,some six million of whom are bilaterally blind,and that mutations in the optineurin gene are "responsible for a significant proportion of cases of primary open-angle glaucoma,the most common form of this disease."

Given that POAG accounts for about 60 – 70% of all the glaucoma,the implication is that perhaps six or seven million people world-wide are at an elevated risk of developing glaucoma as a result of DCVs in the optineurin gene, which could be detected before the onset of symptoms by genetic screening.

InSite Vision was quick off the marks to meet the challenge:that same February, they announced the development of OcuGene II, which would test for DCVs in the OPTN gene. So a comprehensive gene test for several different forms of glaucoma seemed to be on the horizon.

Take 2: Research Interest Only

The first serious question was raised by Wallace Alward MD, and colleagues at the University of Iowa,in the Archives of Ophthalmology in September 2002.They examined DCVs in the MYOC gene and the mt.1 promoter region in a consecutive series of 779 patients with confirmed (652 patients) or suspected (127 patients) open-angle glaucoma.They found plausible MYOC DCVs in a total of 13 patients,3.3% of this population,close to the original 2.9% reported by Stone et al.

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In terms of screening,these data mean that if a gene test were able to detect all of the DCVs in the MYOC gene, and if all of these patients went on to develop glaucoma,then the best case scenario would be a 100% specificity and a 3.3% sensitivity.

This conflicts with the figures claimed by OcuGene, which are not given any explanation. I assume their claim of nearly 100% sensitivity and specificity means that their test detects nearly 100% of the people with the DCVs tested for, and that nearly 100% of these patients go on to develop glaucoma.This may be true, but is misleading,as it is not the usual meaning of the term.It ignores the fact that 97% of people who develop glaucoma do not have DCVs in the MYOC gene, and that there are other DCVs in the same gene that are not detected by OcuGene.

In fact,many of the DCVs that were detected by Alward et al.do not correspond to those tested by the OcuGene test.The test assays just three out of the dozens of known DCVs in the MYOC gene.As Richard Parrish told *EuroTimes*: "If the OcuGene test had been used to screen the patient population in Lee Alward's study, not a single one of the patients with adult-onset POAG with DCVs would have been detected.The sensitivity of this test is nearly zero."

Despite the poor sensitivity of the OcuGene test,Alward et al. calculated that the cost of screening their patient population would have been at least $78,600;and the test would have found no differences between the two groups.

But Alward's study bore yet worse tidings:they also examined the hypothesis of Garchon's group in Paris,that the mt.1 variant is associated with a more serious, refractory form of the disease.

Alward's group confirmed that the prevalence of the mt.1 polymorphism is broadly similar in patients (15.5%) and controls (23.9%) but they could find no association whatsoever between the variant and the severity of disease. In particular, they studied 208 women with the mt.1 variant (encoding an oestrogen response element) and found no differences between those with or without the allele in any measure of disease severity.

How do we account for the discrepancy? Alward et al.studied three times as many patients and used more common measures of glaucoma severity, including age at diagnosis and first treatment,highest IOP, cup-disc ratio, Goldmann visual field loss,surgical procedures,and trabeculoplasty.They were very critical of the method used by Garchon's group (the difference between IOP at diagnosis and at study entry),saying,"We feel this measure of disease severity is so flawed that it is meaningless".

A more recent study, published in 2003 by Jon Polansky MD, and colleagues at the University of California,San Francisco, re-awakened the controversy.

Polansky had also been a contributor on the Garchon paper, and now reiterated that there was after all an association between the mt.1 variant and the severity of disease over 15 years, in a retrospective study of 147 patients. Whether this finding is meaningful remains to be seen; but at best, the association between the mt.1 promoter and severity of disease is controversial.

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Alward, though, is in no doubt: "The bottom line is this. Our laboratory can genotype 2500 patients per day. I have the ability to screen for the mt.1 promoter polymorphism at no cost to my patients. I do not test for this polymorphism."

The optineurin gene has suffered a similar fall from grace. Again, the problem is not with science, but spin. The original Science paper, published in 2002 by Sarfarazi's group, reported that 16.7% of families with hereditary POAG had sequence alterations in the OPTN gene "including individuals with normal intraocular pressure." The accompanying editorial was entitled High Pressure, and claimed the gene caused the disease in a "significant proportion" of patients with POAG.

POAG, of course, encompasses the spectrum of open angle glaucoma from raised IOP with relatively little degeneration of the optic nerve, to normal or low-tension glaucoma with extensive optic nerve degeneration.

Casual readers of the Science articles might be forgiven for thinking that alterations in the OPTN gene were responsible for a broad spectrum of such cases, including patients with raised IOP. Not a bit of it. A more detailed scrutiny of the paper reveals that most of the patients were drawn from families with normal tension glaucoma, and only 13% had IOP values above normal.

In an extension of the large-scale Iowa study, of 1,048 glaucoma patients and 251 controls, Alward and colleagues found that the OPTN sequence variations were not significantly associated with any form of high-tension open-angle glaucoma. They confirmed an association between one particular sequence variation in patients with normal tension glaucoma but the alteration was responsible for less than 0.1% of all cases of open-angle glaucoma (and just 1 out of 352 unrelated NTG patients).

A second study, by Janey Wiggs MD PhD and colleagues at Harvard, also published in 2003, confirmed the lack of association between the DCVs in the OPTN gene and disease in patients with adult-onset POAG. Wiggs et al. concluded that normal tension glaucoma is not necessarily part of the phenotypic spectrum of adult open-angle glaucoma.

Tin Aung, MD PhD and colleagues at University College in London came to a similar conclusion from a study of 315 unrelated British patients. They found that two of the mutations in the OPTN gene reported by Sarfarazi's group were indeed associated with sporadic normal-tension glaucoma in the UK, but not with high-tension glaucoma.

They concluded that the association of OPTN mutations with normal-tension but not high-tension glaucoma "suggests genetic or allelic heterogeneity, implying different mechanisms of optic nerve damage". In other words, the two forms are probably best seen as two different diseases, and we shouldn't make inferences about one based on the other.

So although the OPTN gene variants are indeed associated with some forms of NTG, their utility in gene testing for POAG in general is worse than that for the MYOC gene. Screening for DCVs in the OPTN gene would detect less than 0.1% of patients with POAG.

Take 3: Limited Utility at Present

"It's important not to be too negative" Janey Wiggs told EuroTimes. "We need to make a distinction between the relatively few cases where gene testing is useful, and screening more generally. In some cases, particularly familial cases, gene testing can be very useful."

For example, Dr Wiggs believes it is already worth testing other family members in severe early-onset phenotypes, such as the Pro370Leu mutation (i.e. leucine is substituted for proline at position 370) in the MYOC gene that Sarfarazi's group reported in a French Canadian family.

"If someone develops glaucoma before the age of 45, it would certainly affect our management. If they tested positive for the Pro370Leu mutation, we might want to screen other family members to check their risk, and if necessary start treatment before the onset of symptoms. In this case, it would be similar to treating congenital single-gene conditions like retinoblastoma."

The picture is less clear with familial optineurin mutations, where treatment options are less certain. For example, the E50K mutation correlates with normal tension glaucoma, so if a family has a history of normal tension glaucoma it may be worth testing for this mutation. But if positive, would it change management?

Dr Wiggs again: "All we could do would be to try and lower IOP as much as possible, because we don't have a specific treatment for the disease. As yet we aren't able to treat optic neuropathy, so all we can do is follow it. That is worth doing in itself. We still don't know very much about penetrance in the case of the optineurin mutations."

"We need to know which genes modify the action of others, and about the clinical outcomes in each case." **Dr Wiggs**

So how long will it be before it's useful to screen whole populations for glaucoma?

"I think it will take 10 years and a panel of 20 genes," said Dr Wiggs. "If each gene adds 5% to the risk of glaucoma, and we test for all the known DCVs, then we would have a test worth doing. That's very possible to do with a DNA chip, but before we can build such a chip we need to know far more about clinical correlates.

We need to know about the penetrance of different DCVs, we need to know which genes modify the action of others, and about the clinical outcomes in each case. And of course the treatment possibilities.

There's a lot of basic research in pharmacogenomics that needs doing."

Any population screening programme at present puts the cart before the horse.

Glossary

Polymorphism:
Any genetic variation that occurs at a relatively high frequency in a population, usually greater than 1%. The most common form is the single nucleotide polymorphism (SNP). Polymorphisms are usually considered neutral variations, but this is not necessarily true: polymorphic alleles such as ApoE4 are associated with degenerative diseases such as Alzheimer's disease.

Mutation:
Any relatively rare genetic variation, usually present in a population at a frequency of less than 0.1%. Mutations may be to single nucleotides, or larger deletions, additions or other modifications to chromosomes. Mutations tend to be thought of as negative, but many are neutral and some are beneficial.

Disease-Causing Variant (DCV):
A more recent term that replaces polymorphisms and mutations, denoting specifically a negative effect. The problem is that some common polymorphisms, such as ApoE4, cause disease, whereas many mutations are neutral (or beneficial). DCVs refer to both rare mutations and common polymorphisms that segregate with a disease.

Sensitivity:
The ratio of true positive tests to the total number of patients tested, as a percentage. A test with high sensitivity has few false negatives. In the case of OcuGene, the test has a 99% sensitivity for 3 out of more than 50 DCVs on the MYOC gene, and a 0% sensitivity for all the rest. Because the MYOC gene DCVs are present in just 3% of people with glaucoma, 97% of people with glaucoma would test false negative, even if the gene test examined all of the DCVs. In the case of OcuGene, the sensitivity is nearly zero.

Specificity:
The ratio of true negative tests to the total number of people tested, as a percentage. A test with high specificity has few false positives. In the case of OcuGene, most people with DCVs in the MYOC gene do go on to develop glaucoma (if the test is positive it is likely that the patient will indeed develop glaucoma). However, the specificity of the mt.1 variant is zero: people who test positive for the variant are no more likely to develop the disease than people who test negative.

Penetrance (predictive power):
The probability of developing a disease when a DCV is present. If a DCV has incomplete penetrance, individuals with the DCV may never get the disease; the predictive power is low.