Window Into Retinal Studies

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Abstract
Professor Gustavo Aguirre discusses the context of his research studies at the University of Pennsylvania, which are currently concentrating on the degenerative disease, retinitis pigmentosa.

Keywords
blindness, dogs, animals, Retinitis Pigmentosa GTPase Regulator, X-linked RP

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Window into retinal studies

Firstly, can you outline the broader aims and objectives of your research?

There are many inherited diseases that have been found to affect the retina and cause blindness. Beyond finding the disease-causing mutations, the aim of the research is to understand how these diseases develop, and then investigate the pathways that link the mutation to the death of the visual cells, and determine if these are specific or common to one or more diseases. From this, we seek to develop intelligent therapies that can be used in treating the retinal diseases. Most of our work uses dog models; in the past we have used cats, monkeys and humans, but the work we do to develop translational studies primarily involves dogs.

Can you provide an overview of the discovery of X-linked retinitis pigmentosa (XLRP) diseases?

There are several retinal diseases in humans that are known to have X-linked inheritance. There are only two where the gene has been found: RP-3 and RP-2. The pioneering work on the mapping of these diseases was done in the UK by Professors Alan Wright (University of Edinburgh) and Shomi Bhattacharyya (Institute of Ophthalmology) beginning in the 1980s. It took a long time to refine the disease intervals, and many groups were working on this difficult area. Eventually, the Wright and Meitinger groups published a seminal paper identifying a very complex gene called RPGR (Retinitis Pigmentosa GTPase Regulator). They also found part of the gene which had previously been very difficult to sequence. This DNA region – ORF-15 – accounted for about 70 per cent of mutations in patients with RPGR. After this finding, my team collaborated intensely with Wright and found that dogs had the mutation in the same ORF15 region as patients. When you take most populations of patients with X-linked RP, up to 70 or 80 per cent have mutations in RPGR. Thus it is recognised as the most common cause of X-linked RP. It is a very severe problem, not least because the regions in questions are very difficult to clone and sequence.

The first gene therapy clinical trials in human patients affected with a severe form of childhood blindness were reported 2008. Can you highlight the developments which have been made in this regard, and any further challenges clinical trials have thrown up?

The Phase 1 studies in human patients have confirmed that treatment of the study population is safe which is the primary goal of any Phase 1 clinical trial. In addition, the studies have shown efficacy in some patients. What we now need is to determine the patient population that is amenable to treatment, and the long-term safety and efficacy in treated patients.

In the studies with LCA-affected dogs over the past 10 years, we have shown stable correction and safety over this period of time. What we still need to know are the structural correlates of the restored function. In other words, does the treated retina remain normal and, if so, can it restore the chemical connections with other cells that ultimately are responsible for transmitting the visual information to the brain.

The new studies under the direction of Dr András Komáromy use a different model of severe visual impairment – achromatopsia – that occurs at birth. Patients with this severe disease have an aversion to light; they are totally blind in bright light; their fovea and macula — which contain the cone cells that enable colour vision and high visual acuity — do not function normally. We have published a paper which details that this disease can be cured and, with collaborators at the University of Florida, are planning to develop clinical trials for this disease. In this disease, the studies are directed at treating cones — the main class of cells involved in macular degeneration. If we can develop a treatment targeting cones, there is the exciting possibility that we can treat other diseases also.

What major, long-standing challenges does your field of research present?

From a scientific point of view, a main challenge now is in eliminating proteins that have an adverse effect on tissue, not only in relation to X-linked RP, but other dominant diseases in which the actual genetic defect produced a protein that has a deleterious affect on the retinal photoreceptors. One such disease is caused by mutations in the rhodopsin gene which is an extremely common cause of autosomal dominant RP. Many disease-causing mutations that are inherited as dominant or X-linked result in what is known as gain-of-function, where the abnormal proteins produced cause disease. For therapy, you first must prevent production of the abnormal protein, and then replace it with the normal version. In the X-linked RP model, which is applicable to a large number of dominant diseases, the need to eliminate the undesirable mutant protein is a challenge of utmost importance.

To what extent has genome sequencing been integral in enabling the team to identify likely targets for intervention, and can you outline the support offered by the Penn Genome Frontiers Institute (PGFI)?

The progress made in this regard is remarkable. When I first started work in this field in 1992, there were absolutely no genome resources available for the dog. So we had to carry out cloning for ourselves, taking information gained from the cloning of genes in mice, cows and humans. When I look over the lab books, I recall that people would spend several years cloning genes; today you simply access the information through a web browser. We can use SNP-chips to track diseases or identify new diseases, and arrays to look at gene expression. The resources available at the PGFI have been critical in providing the infrastructure and resources needed to do this work.
Seeing is believing

A research team based at the University of Pennsylvania are conducting essential research which could lead to promising therapies for X-linked retinitis pigmentosa, an hereditary disease that leads to blindness.

FOR THE BEST part of two decades, Dr Gustavo Aguirre of the School of Veterinary Medicine at the University of Pennsylvania, Philadelphia, has dedicated his research efforts to the long-term development of therapies for degenerative retinal diseases. Presently, Aguirre and his collaborators are conducting studies in relation to retinitis pigmentosa (RP) linked to the X-chromosome. RP represents a group of hereditary and degenerative eye conditions that invariably induce total blindness in patients. In general terms, this group of diseases is associated with dystrophies of the photoreceptors of the retina and/or the pigment epithelium.

Aguirre and his associate, Dr William Beltran, are employing dog models in identifying the genes and pathways associated with a form of RP known as X-linked PRA2 (XLPRA2). This naturally occurring canine disease is a close correlate of early-onset XLRP in humans. This work has great potential for translational studies to harness a novel therapy for XLRP caused by mutations in the ORF-15 region of the RPGR gene. Significantly for XLRP, mutations in this gene account for 8-10 and 15-20 per cent of early onset retinal diseases in the U.S. and Europe respectively. With this in mind, it is clearly manifest that Aguirre’s studies bear enormous potential and relevance in clinical terms; however, the industry and dedication shown by Aguirre and his team up to this point also warrant attention.

RP STUDIES IN FOCUS
Restoring vision in patients is the overall goal of basic and applied research in this field. However, in past decades, a significant proportion of studies and investigations have focused on slowing the rate of vision loss in patients with RP through nutritional supplementation, because of the limited availability of therapeutic alternatives. Another approach to combating the disease has seen neuroprotective agents, such as CNTF, being transported to the retina through ECT technology, which utilises genetically engineered cells that are encapsulated and placed in the eye. Whilst studies employing these techniques have delivered important findings, there is consensus that advancements in molecular science are integral to understanding the causes of RP. This, in turn, has engendered further hope for the development of effective treatment options. Molecular science has enabled significant progress to be made in identifying loci and genes linked to retinal dysfunction; unfortunately, this progress has not been mirrored to the same extent where the development of treatments is concerned. Indeed, whilst encouraging findings have emerged from a large number of experimental studies in animals, there is still a paucity of approved treatments for patients diagnosed with RP.

However, work recently undertaken by Aguirre’s group and associates has garnered extremely encouraging results that have unlocked potential human therapies, currently in Phase I trials by various groups of investigators in Europe and the U.S. The study relates to a mutation in the RPE65 gene in patients with a form of RP known as Leber congenital amaurosis (LCA).

In light of this notable achievement, Aguirre’s team remain focused and determined to continue to deliver important contributions in the development of therapies for RP; they envisage each investigation as part of an incremental process where knowledge relating to the mechanisms which lead to RP is steadily enhanced and used in formulating new treatments. As such, the success registered by the team in regard to RPE65-LCA has provided a basis from which they can explore more complex disorders. In this sense, the team’s latest proposal regarding RPCR-XLRP marks their next developmental stage in addressing forms of RP.

CURRENT INVESTIGATIONS
While a considerable proportion of patients with retinal diseases carry mutations in the RPGR gene, significant gaps persist in our understanding of the function of the protein, as well as the molecular mechanisms that lead to photoreceptor degeneration.

In addressing this issue, part of Aguirre and his investigators’ latest research involves the identification of genes and pathways associated with Retinal Dysfunction (RD) in dogs affected with XLPRA2, also known as RPCR2. This is a canine model of early-onset XLRP caused by an RPCR exon ORF15 microdeletion. The connection between RPCR2 in dogs and RPCR in humans is extremely useful: should the team determine that the molecular mechanisms underlying XLPRA2 in dogs are the same as those of XLRP in humans, it is anticipated that therapies proven effective in canine models will be likely to induce comparable effects in patients.

The team’s investigations in canine models will run in parallel with a comprehensive study in patients seeking to enhance knowledge regarding the range and history of XLRP. This corresponding study, carried out by Drs Samuel Jacobson and Artur Cideciyan at the University of Pennsylvania,
INTELLIGENCE
MODELS OF X-LINKED RETINITIS PIGMENTOSA

OBJECTIVES

• To develop specific gene-based therapies for RPGR-XLRP past the proof-of-principle stage so that clinical trials are then generated

• To assist collaborators, Drs S Jacobson and A Cideciyan, to conduct prospective studies in RPGR-XLRP patients to assess the feasibility or facility of translating pre-clinical therapy to the clinic

• To carry out complementary in vivo studies in the canine model to quantify photoreceptor laminar structure and function, with emphasis on the central retina, at comparable disease stages as the patients, to establish the morphological/immunocytochemical correlates to the observations made in dogs that can be extrapolated to patients

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will aim towards the development of a set of outcome measures tailored to pre-clinical treatment strategies. The concept of running concurrent studies is ambitious; yet, given the capabilities of the investigators, it would seem that their bold objective is well-placed and feasible.

A MULTIDISCIPLINARY APPROACH

The complexity and breadth of this research programme requires the expertise of a team with proven experience in translational research. The group members’ collective ability to engage in thorough investigations – dealing with science-based ideas to clinical application of therapies – is perhaps epitomised in their harnessing of an effective treatment for LCA. The antecedent study, in which the team translated dog and murine results to human RPE65-LCA, has cultivated a greater appreciation that proof-of-principle treatments in animal models generate a multitude of questions regarding exact human application.

Crucially, over the years Aguirre has forged and enjoyed fruitful partnerships with other leading researchers and clinicians across the U.S. and beyond. In regard to interaction and studies in patient populations, the research benefits from cooperative efforts, as Aguirre highlights: “Drs Samuel Jacobson and Artur Cideciyan, collaborators at the Scheie Institute who also run the Center for Retinal Degenerations here at the University of Pennsylvania, are really crucial in this respect because they see the actual patient population”. He continues: “They have a great interest in the diseases in dogs, enabling them to better delineate these diseases in humans”.

Furthermore, the development of virus vectors – which are required to transport genes to the retina – is conducted by external partners, Drs William Hauswirth and Alfred Lewin, at the Departments of Ophthalmology, Molecular Genetics and Microbiology, and the Powell Gene Therapy Center, University of Florida. This valuable collaboration is pivotal to the advancement of studies which require specific targeting of the retinal cells, elimination of the mutant gene product and replacement with a normal copy of the gene.

Looking beyond the current research programme, Aguirre is excited by the prospects and promise held in the laboratories at Penn. His praise is unreserved: “It is a joy to have here at Penn a group of scientists who are young, talented, productive and enthusiastic, and it is reassuring to know that they will be here to continue the work in the long term,” he comments.

The tools, it would appear, are in place; it will only be a matter of time before Aguirre and his wider team make further valued contributions to this field of enduring and essential research.