

# TAKING CARE OF EYES AND EARS

MORE THAN 60 SEMINARS TOOK PLACE DURING TWAS'S 23<sup>RD</sup> GENERAL MEETING IN TIANJIN, WHERE SCIENTISTS PRESENTED A BROAD SPECTRUM OF INVESTIGATIONS, FROM DEFENSIVE STRATEGIES USED BY PLANTS AGAINST PARASITES, TO INNOVATIVE MATERIALS, TO FUTURE PLANS TO EXPLOIT SOLAR ENERGY.



*A selection of these presentations focused on pathologies affecting eyes and ears, which are subject to a much higher percentage of serious disease in developing countries than in developed countries. Promising results for eyes were presented by Sheikh Riazuddin (TWAS Fellow 1989) from the Centre of Excellence in Molecular Biology at the University of Health Sciences, Lahore, Pakistan and by Dorairajan Balasubramanian (TWAS Fellow 1997), Director of Research at the L.V. Prasad Eye Institute in Hyderabad, India. Research on hearing loss was presented by Ana Belen Elgoyhen from the Instituto de Investigaciones en Ingeniería Genética y Biología Molecular (INGEBI-CONICET) in Argentina. An overview of their research follows.*

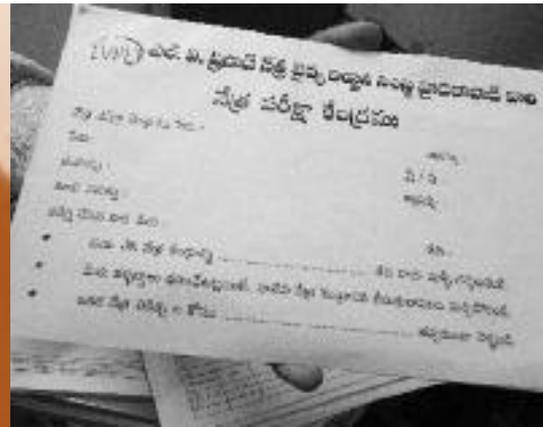
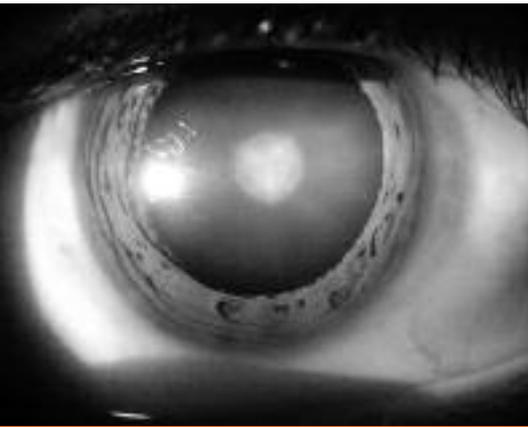
**M**any of the pathologies that affect sight and hearing are chronic and defined as non-communicable diseases. While industrialized nations can sustain the eco-

nomic costs of providing adequate healthcare programmes to keep these pathologies at bay, developing countries cannot.

According to World Health Organization (WHO) estimates, 285 million people are visually impaired

worldwide: 39 million are blind and 246 million have reduced vision. Of this latter group, about 90% live in low-income countries, where healthcare systems are often inadequate and the medical assistance provided is not free of charge.

It should not be surprising then, to learn that up to 80% of the cases of visual impairment – including uncorrected refractive errors (myopia, hyperopia and astigmatism), cataracts and glaucoma – could be avoided, either by early treatment or prevention. Given the social and economic burden that eye disease causes, WHO and the International Agency for the Prevention of Blindness (IAPB) have launched a global



initiative, ‘Vision 2020: The right to sight’, which aims to eliminate *avoidable blindness*.

### THE VIEW FROM PAKISTAN

Cataracts, glaucoma, retinitis pigmentosa (RP) and macular degeneration are some of the most common conditions that impair vision, causing blurred or spotty images and creating blind spots. If not treated properly and at an early stage they can lead to blindness.

Although they occur worldwide, these conditions are more frequent in developing countries. Indeed, Pakistan has an unusually high prevalence of the first three conditions (cataract, glaucoma and RP). According to scientists, this could be due to a combination of historical factors that have influenced the local gene pool.

Marriages between first or second cousins have long been a common practice in Pakistan. This, in time, has led to a high degree of consanguinity within families and, as a consequence, to lower genetic variation. There is a high risk that existing mutations will be transmitted from one generation to the next, increasing the likelihood of disease.

### IN THE GENES

“The history of the Pakistani population is written in its genes, which give us information not only about past

migrations and historical events but also about social habits,” explained Sheikh Riazuddin (TWAS Fellow 1989) during his presentation at the TWAS General Meeting. Riazuddin is professor and director of the National Centre of Excellence in Molecular Biology (CEMB) at the University of the Punjab in Lahore. “If we can identify the genes responsible for



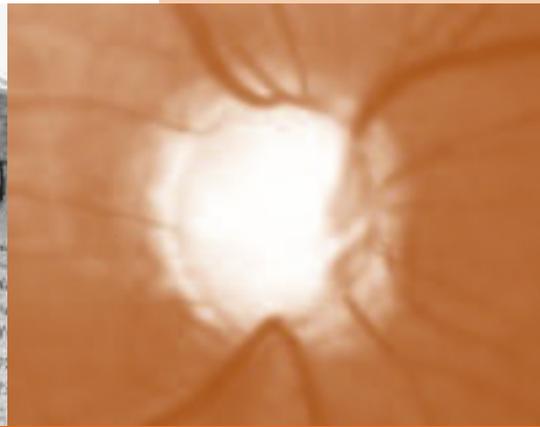
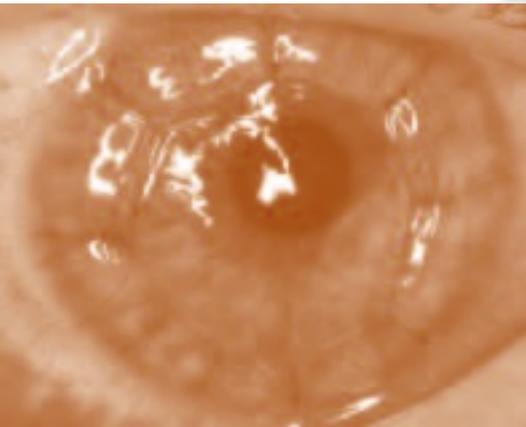
**Sheikh Riazuddin**

***If we can identify the genes responsible for eye diseases, we can observe DNA mutations and their effects in modulating the onset of the hereditary conditions that damage vision.***

eye diseases, we can observe DNA mutations and their effects in modulating the onset of the hereditary conditions that damage vision. Genetics data”, he added, “can be used to orient future healthcare policies in a better way.” The results he showed sum up several years of investigations carried out by his team on the most common eye disorders.

RP is an inherited, degenerative eye condition in which the retina – the sensory membrane lining the back of our eyes – slowly degenerates leading to severe vision impairment and blindness. “Despite the early appearance of predictive signs,” explains Riazuddin, “it is difficult to quantify the extent of future vision loss. And once the degenerative process is complete there is no treatment to reverse it.” Even during the disease progression, doctors are powerless to intervene since

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there are no medicines to slow its course. The only way to prevent the occurrence of RP is through knowledge of the genetic pattern of the two partners (with a family history of RP) who are planning to raise children.

“The identification of healthy carriers is particularly important in families where consanguineous marriages are a common practice”, continued Riazuddin. Consanguinity, in fact, increases the risk of inheriting not only an eye disease, but any one of the 5,000 or so recessive genetic diseases that can affect any part of the body.

Five consanguineous Pakistani families with early onset RP identified by Riazuddin’s team gave their consent to participate in a collaborative investigation, carried out by the National Center of Excellence in Molecular Biology in Lahore and the National Eye Institute in Maryland, USA. The study aimed to identify specific pathogenic mutations which trigger the recessive form of the disease. A recessive disease develops when both the parents host a defective gene – with a “spelling mistake” or mutation – and transmit it to their offsprings.

“We found seven new genes involved in the onset of familial RP”, explained Riazuddin, “a discovery which will greatly contribute to our understanding of the molecular mechanisms underlying this condition.”

Working with another group of 12 consanguineous Pakistani families and one Arab-Israeli family (a total

of 44 individuals), two brand new genes and nine mutations in a gene called *FYCO1* were identified. The latter accounts for the recessive form of congenital cataract, a clinically diverse and genetically heterogeneous group of disorders which trigger the degeneration of the crystalline lens (the eye structure that, along with the cornea, helps to refract light and focus it on the retina). Cataracts are responsible for one third of the cases of infant blindness, and are prevalent in the Pakistan population.

“We also obtained important evidence on the genes and mutations involved in congenital glaucoma and night blindness,” added Riazuddin.

***If current trends are not halted, by 2020 we could face an emergency of up to 80 million blind individuals.***

Congenital glaucoma is a neurodegenerative process of the optic nerve and the second leading cause of visual loss. Almost 60 million people are affected today, but future predictions are grim: if current trends are not halted, by 2020 we could face an emergency of up to 80 million blind individuals.

Night blindness (or nyctalopia), another condition that Riazuddin examined, is not a severe condition *per se*, but makes it difficult or impossible for sufferers to see in relatively low light. Importantly, it is often associated with more severe degenerative conditions.

“Even though we have carried out extensive investigations, our results are far from being conclusive,” Riazuddin admitted. “We still don’t have a treatment for these conditions. However, we have paved the way



### THE COSTS OF CLEAR VISION

*Eye disease is not just a health issue: it has dramatic consequences for society and the economy. In 2010, nearly three trillion dollars were spent on providing healthcare and specific assistance to people living with low vision and blindness worldwide. This amount – which includes direct healthcare expenditure, loss of productivity at work, and caregiver time – is set to rise through 2020, unless measures to prevent and treat the problem are adopted soon. But eye disease is not just a financial issue. According to WHO’s measurements of the overall burden of disease calculated using so-called DALYs (disability-adjusted life-years), people with visual impairment have been deprived of the equivalent of 118 million years of healthy life due to disability and premature death. This value will peak to 150 million DALYs by 2020 if current trends are not checked soon.*

to a better understanding of the molecular mechanisms underlying these disorders and, far more importantly, we have raised awareness – both at the individual level and at a general level – of the importance of offering preventive screening to avoid undesired genetic combinations that affect not only the patient, but their families, and, ultimately, society at large.”

### RECONSTRUCTING THE EYE

Any damage to the outer surface of the eye, or cornea, (caused by thermal or chemical burns, for example) will affect sight. Corneal injuries are not uncommon: both the workplace and the home can be dangerous environments for our fragile ocular organs. The resulting corneal scars can severely impair vision, sometimes even causing blindness.

Dorairajan Balasubramanian, director of research at the L.V. Prasad Eye Institute (LVPEI) in Hyderabad, India, has been addressing this specific problem for more than ten years and is an internationally recognized specialist in eye-related research. The institute, in addition, is a WHO Collaborating Centre for the Prevention of Blindness and a Global Resource Centre for

VISION 2020: The Right to Sight initiative.

“Networking is essential if we are to provide equitable and efficient eye care to all sections of society,” underlined Balasubramanian at the Tianjin meeting.

“The LVPEI network, in fact, includes a centre of excellence in Hyderabad, three tertiary centres in Bhubaneswar, Visakhapatnam and Vijayawada, ten more secondary centres and 89 primary care centres that cover the remotest rural areas in the state of Andhra Pradesh, India, as well as several city centres.”

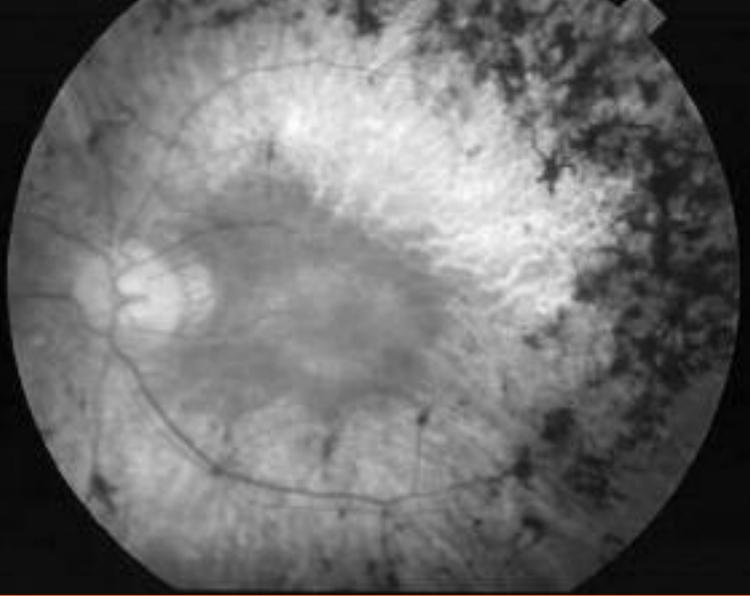
Corneal transplants, which might seem an option for replacing diseased or damaged corneas, in fact have very poor outcomes: graft survival at one year is disappointing (33-46%) but at three years it is disastrous (0%). New avenues must be explored to ensure, in the words of the LVPEI logo “That all may see.”

Balasubramanian described the alternative and highly successful treatment his team has been working on, taking the audience through ten years of pioneering



**D. Balasubramanian**

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applications of limbal stem cells, which are now widely used to reconstruct the damaged corneal epithelium. “Limbal stem cells”, he explained, “are a versatile tool for doctors. These cells sit in the anular ring surrounding the cornea and not only self-renew, but also differentiate. This means that, under proper cultural conditions, they can be induced or taught to become the cells we need and used to patch damaged tissue, which is done with the patient’s own cells.”

### IN LIMBUS: CLET AND SLET

The corneal limbus – the white part of our eye – is the perfect factory for stem cells. These are commonly dubbed baby cells for their capacity to grow and evolve into different adult types, providing they are given adequate nutritional support.

Balasubramanian and his team have been experimenting on the use of stem cells in corneal transplants for more than a decade now. First they used a procedure called CLET (corneal limbal epithelial transplantation). As he explained: “We first take a tiny patch of corneal tissue – a biopsy – from a healthy part of the injured eye and put this fragment, less than 2 mm wide, into a special tissue culture medium. Then, after 10-14 days’ growth, the fragment reaches a suitable size. The final stage is then to transplant the patch in the recipient eye.” The transplant can be obtained from the person’s unaffected eye (an autolo-

gous transplant) or, if both the eyes of the patient are injured, from an external donor (an allogenic transplant).

This three-stage technique, which has been tested on 200 patients, has many advantages: it is safely repeatable for the donor eye, allows for a rapid recovery, and after 1-2 years the overall success rate (no tissue rejection) is around 71%.

Encouraged by this success, Balasubramanian and his collaborators decided to further elaborate the technique. “We tried an exciting *in vivo* single step procedure,” he said. “We took a strip of limbal tissue from the healthy eye of a patient, chopped it into smaller pieces and distributed these fragments over

an amniotic membrane placed on the patient’s cornea.” Results were encouraging: visual acuity improved in 66.6% of patients, with no complications observed. Balasubramanian’s team called this procedure ‘simple limbal epithelial transplantation’, or SLET.

“By avoiding the *ex vivo* step,”

Balasubramanian commented, “we simplified the procedure, cut the risks for patients and reduced the costs. In addition, the procedure is so simple – and effective – that any qualified corneal surgeon, after minimum training, is able to perform it.”

Damaged eyes represent a serious health problem in developing countries, but so too do damaged ears.

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### TURN DOWN THE VOLUME

Sound-induced acoustic injury is one of the most common causes of hearing loss and tinnitus (ringing in the ear). Tinnitus does not cause hearing loss, but people with hearing loss can have tinnitus. The condition is common among rock stars and their fans who play or listen to loud music.

However, as Ana Belén Elgoyhen explained in her opening remarks to the Tianjin meeting, “It’s not just music: our ears are assaulted by daily roars, shrieks, and general blaring noises that severely jeopardize our listening ability.” Elgoyhen’s research, in fact, has been dedicated to finding ways to protect our fragile ears from acoustic trauma.

Elgoyhen is professor and principal investigator at the *Instituto de Investigaciones en Ingeniería Genética y Biología Molecular* (INGEBI-CONICET) in Buenos Aires, Argentina, and one of two recipients of the 2011 TWAS Prize in Biology, awarded during the opening ceremony of the Tianjin meeting. In her laboratory she studies the neurochemical mechanisms that regulate hearing, trying to devise a strategy to protect the ear from potentially damaging noises.

Elgoyhen summarized how the mammalian ear works, focusing in particular on the inner and outer hair cells. The inner cells, she explained, are connected to the acoustic nerve and are instrumental in sending neuronal signals to the brain. The outer cells, in contrast, have two roles: on the one hand, they increase the sensitivity of the hearing apparatus; on the other they receive messages from the brain. In response to

loud noises, the neurons in the brain release a chemical compound (acetylcholine) that binds to receptors on the outer cells. After this binding, the outer cells’ sensitivity to loud sounds diminishes.

“This chemical reaction was known long ago, but its precise function was still to be determined. To do this, we devised a mutant receptor which was able to respond for a longer time – and more strongly – to the stimulus of acetylcholine,” explained Elgoyhen.

When tested on a laboratory model, Elgoyhen and her team were able to observe that the engineered receptor reduced the amplification ability of the hair cells. “In other words,” concluded Elgoyhen, “the ear was more resistant and more protected against obnoxious sounds known to induce permanent hearing loss.”

### EYES AND EARS TOWARDS THE FUTURE

“What is important in these investigations”, commented Romain Murenzi, TWAS executive director, “is not just the results. Certainly their findings will improve our ability to treat and prevent eye and ear disorders, but the fact is that these scientists have begun with solving local problems, of relevance to their own working environments in developing countries, but they are also looking towards the application of these solutions in both national and global contexts.”



A. B. Elgoyhen