Molecular and cellular approaches to understand and treat some diseases of the eye*

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About 1.8% of the population of India, amounting to over 18 million people, is blind. Of these, almost 80% suffer from avoidable and treatable forms of blindness, such as cataract, refractive errors and infection of the eye. India has joined in the global effort called Vision 2020 – The Right to Sight, which sets out to prevent and treat this 80% of the blindness burden by the year 2020. Some strategies that are implementable in order to achieve this laudable goal are outlined. In addition, we also summarize some current research work in the areas of molecular genetics of chosen eye diseases, pharmacology of cataractostasis, and adult stem cell-culture and transplantation in the reconstruction of damaged ocular outer surface.

The magnitude of the problem

The Andhra Pradesh Eye Diseases Study (APEDS), conducted by colleagues at our Institute, has estimated that between 1.66 and 1.84% of the population of Andhra Pradesh (AP) is blind, as judged by a visual acuity of <6/60 or central visual field <20° in the better eye1,2. Since the 1986-89 National Survey of Blindness3 reported the prevalence and causes of blindness in AP to be similar to the average in India, and since the recently reported health indicators from the National Family Health Survey4 have suggested that the health situation in AP may be considered as being somewhat close to the average for India, it appears justifiable to use the APEDS results to estimate the prevalence of blindness in the whole of India. With this extrapolation in mind, we estimate 1.84% of Indians to be blind, which translates to about 18.66 million people. Table 1 lists the prevalence and causes of blindness in India in the year 2000. An estimated 9.5 million have impaired vision due to cataract or clouding of the eye lens, and another 3 million cannot see because they have not corrected for their ‘power’ or ‘number’. They are blind just because their myopia, hyperopia or amblyopia has not been corrected through the use of spectacles or contact lenses. Just these two causes – cataracts and uncorrected refractive errors – account for over 67% of the blindness burden in the country (Table 2), a fraction that is projected to persist at about the same level for the next two decades. The actual number of people affected is estimated to rise from 12.5 million in 2000 to about 18.8 million in the year 2020. The recent review by Dandona et al.5 summarizes and analyses these figures, and discusses their implications for a national policy of blindness control.

Vision 2020 – The Right to Sight initiative

While these numbers appear daunting and the problem before the nation appears large, it has been realized that the task of restoring normal vision to these unfortunate individuals is achievable. The technology needed for cataract surgery is available and well within the hands of the 10,000 ophthalmic surgeons practising across the nation. A large number of them have already adopted the technique of intraocular lens (IOL) implant, which involves the removal of the diseased lens and implanting in its place an artificial lens made of acrylic polymer. The surgery is done under local anaesthesia, takes less than 30 min for a practised hand and requires no hospitalization after surgery. The IOL method has increasingly replaced the earlier practice of excising the whole intact lens (intracapsular cataract extraction or ICCE) which left the patient aphakic (lensless) and needing thick corrective eyeglasses. The ICCE method, which was widely used in India to treat cataract cases in

<table>
<thead>
<tr>
<th>Cause of blindness</th>
<th>Prevalence* (as %)</th>
<th>Number of blind persons (in millions)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cataract-related</td>
<td>0.94</td>
<td>9.53</td>
</tr>
<tr>
<td>Refractive error-related</td>
<td>0.30</td>
<td>3.04</td>
</tr>
<tr>
<td>Retinal diseases</td>
<td>0.19</td>
<td>1.92</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>0.15</td>
<td>1.52</td>
</tr>
<tr>
<td>Corneal diseases</td>
<td>0.12</td>
<td>1.22</td>
</tr>
<tr>
<td>Other causes</td>
<td>0.14</td>
<td>1.42</td>
</tr>
<tr>
<td>Total</td>
<td>1.84</td>
<td>18.65</td>
</tr>
</tbody>
</table>

*Prevalence taken from the data of the APEDS study (refs 1, 2 and 5); **Calculated based on a population of 1014 million people in 2000.

*Based on the Eleventh C. V. Raman Lecture of the Indian Institute of Science, Bangalore, delivered on 4 March 2002.

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<table>
<thead>
<tr>
<th>Cause</th>
<th>Year 2000</th>
<th>Year 2010</th>
<th>Year 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percentage blind</td>
<td>People affected (in million)</td>
<td>Percentage blind</td>
</tr>
<tr>
<td>Cataract-related</td>
<td>51.1</td>
<td>9.53</td>
<td>51.4</td>
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<tr>
<td>Refractive error-related</td>
<td>16.3</td>
<td>3.04</td>
<td>15.5</td>
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<tr>
<td>Retinal diseases</td>
<td>10.3</td>
<td>1.92</td>
<td>10.2</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>8.1</td>
<td>1.52</td>
<td>8.3</td>
</tr>
<tr>
<td>Corneal diseases</td>
<td>6.5</td>
<td>1.22</td>
<td>6.8</td>
</tr>
<tr>
<td>Other causes</td>
<td>7.6</td>
<td>1.42</td>
<td>7.8</td>
</tr>
</tbody>
</table>

*Based on population estimates of 1014, 1168 and 1312 million people in the years 2000, 2010 and 2020, respectively.

Reducing the cataract burden

A major focus in this endeavour is on reducing the cataract burden. Methods are being worked out to make available IOLs, train and retrain eye-care personnel, modify and replace the earlier mode of rural eye camps so as to provide post-operative and year-round access to eye care. If each of the 10,000 ophthalmologists were to do but ten cataract surgeries per day, the estimated 10–14 million cataract patients per year can be easily restored normal vision. The technology, the trained personnel, the time and the wherewithal are all available. What is needed is a co-ordinated systems approach so that we achieve a ‘sight revolution’ in much the same manner as we made the white revolution and the green revolution happen.

Refractive error correction

Blindness due to the non-correction of refractive errors is both pathetic and inexcusable. Yet, it accounts for 3 million cases of blindness in India today, a number that will rise to 4.5 million if left unattended. Dandona et al. have estimated the number of blind person-years suffered over the lifetime of those who are blind from treatable or preventable causes today, and in the years 2010 and 2020, if the current trend continues. Typically, the onset of refractive error blindness is usually at a younger age (while that of cataract is at an older age) leading to a greater number of years lived by each person blinded by the former condition than the latter. They conclude that effective control of refractive error-related blindness can prevent 82 million blind person-years, slightly higher than with the control of cataract blindness, which accounts for about 78 million blind person-years. Treatment of refractive errors is indeed simple—training of adequate number of optometrists, screening the population for these errors, and establishing mechanisms for the provision of spectacles at affordable prices. Optometry is turning out to be an increasingly attractive career option, since it offers self-employment as well as hospital attachment. A four-year degree programme costs but a few lakhs of rupees. There is but a handful of institutions in India offering optometry training and degree programmes, and this aspect needs to be looked into and expanded. Addressing these two causes of treatable and avoidable blindness, and helping solve them is the main charter of Vision 2020 – The Right to Sight. The recently introduced pilot project in AP, called Village Vision Programme, has offered soft loans to about 15 village women through which each of them has been provided.
50 quality spectacles, which she can sell at twice the price, helping the buyer and at the same time make some money herself to pay back the loan and earn profit. This is a global collaborative project between L. V. Prasad Eye Research Institute and a group called the Society for Helping and Awakening Rural Women through Education (SHARE), a take-off from the successful microcredit programme initiated by Mohammed Yunus of Bangladesh. Another programme, initiated by us, called CAFÉ (Community Assisted and Financed Eye Care) has selected a rural population clutch of 50,000 people, each of whom will pay 1 rupee per person per month, and in turn will be provided comprehensive eye care at one of the LVPEI branches and affiliates. These are two examples of involving the community in the mission of Vision 2020 – The Right to Sight.

Infection of the eye

The other form of avoidable/treatable blindness is due to infection of the eye, largely in parts of its outer surface such as the cornea and sclera, and the conjunctival membrane that lines the inner surface of the eyelids. Incidence of ocular infection is high in India, particularly in rural and urban slum areas, and accounts for close to 10% of blindness. If detected early enough, it is easily taken care of, but if the treatment is delayed, vision can be lost. Effective medication is available for bacterial and parasitic infection, while antifungals and antivirals are both limited in number and are more expensive. Early detection and management thus become crucial in these situations.

Glaucoma

Glaucoma or the development of abnormal intraocular pressure is a condition, which, if left untreated, can affect the optic nerves and cause loss of vision. Two forms of this disease, namely primary angle closure glaucoma (PACG) and primary open-angle glaucoma (POAG) are known; which of the two is more prevalent in India is not yet certain. There is a genetic basis for glaucoma, and at least two genes (CYP1B1 and MYOC) are implicated in two forms of this disease. Of the two types, PACG is relatively easier to treat and prevent than POAG, which needs greater care and management. It is important to detect and diagnose PACG and POAG in their early stages, an area where Indian ophthalmic practice needs strengthening.

Corneal blindness

Corneal diseases can occur due to genetic as well as environmental factors. Infection and injury due to chemical (acid or alkali) and fire burns are the major causes of the latter form of corneal disorder. Of all parts of the eye, cornea is the one that is transplantable from one person to another – a fact that is the basis of eye-banking. The demand for transplantation-worthy corneas far exceeds (more than ten-fold) the supply. There is thus a crying need for basic research and innovative technology to address this problem. Besides corneal infection, which we alluded to briefly above, a significant component of corneal blindness in India, particularly in children, is due to vitamin A deficiency. This deficiency occurs due to a variety of factors such as inadequate nutrition, diseases such as measles, or debilitation. Primary-health care measures such as immunization, vitamin supplementation (through programmes such as megadoses of vitamin A in schools and slums) and improvement in sanitation will go a long way to combat corneal disorders due to non-genetic factors. Dandona et al.5 remind us that if these measures are strengthened now, they can potentially result in preventing 20 million blind person-years in those who would otherwise lose vision by 2020.

Retinal diseases

Perhaps the most difficult of ocular conditions to treat are retinal disorders. Besides genetic factors, senescence- or age-related degeneration of the retina and its macula occur, and there is no chemical or pharmacological therapy available to counter them effectively. Much of the treatment is focused on managing the disease and stopping its deterioration. Retinal blindness is an area where one needs to turn to basic biology for solution. Genetics has identified some candidate genes responsible for some forms of this blindness. In the case of monogenic retinal blindness such as Leber’s syndrome, where the faulty or missing gene is identified (RPE 65), gene therapy has been attempted in dogs with success7. The long-term results of this experiment are awaited with great excitement so that it can be attempted on humans. Cell biology too holds great promise, since some early experiments involving the isolation of foetal proto-retinal cells and layering them on the diseased retina have shown promise in experimental animals. T. P. Das at our institute has attempted a similar delivery and layering in human volunteers with mixed results.

Basic biology – genetics

J. B. S. Haldane aptly described India as a living genetics laboratory. The genetic diversity of the thousands of communities and tribes that constitute the peoples of India is only too well known. The other special feature is the practice of consanguineous marriages, and marry-
ing within one’s own subcommunity, which is thought to restrict the gene pool\(^5\). In the field of eye diseases, this affords us a great opportunity to look at the genetic bases of cataract, corneal dystrophy, glaucoma, retinal disorders, and also some less common conditions such as aniridia (iris disorders), microphthalmia and nanophthalmos (very small eye balls) and some other inherited disorders. Being situated in the south-central part of India, where intra-community and consanguineous marriages are significantly prevalent, has given us a unique opportunity to study the molecular genetics of some eye diseases. We have therefore embarked on a comprehensive and active research programme in this area at our institute, and report the results of such a study here.

**Molecular genetic analysis of primary congenital glaucoma and cataract**

Primary congenital glaucoma (PCG) is a childhood condition characterized by bulging eyeballs, high intraocular pressure, enlarged cornea, oedema and other tell-tale features (Figure 1). If not treated early enough, it results in blindness within a few years. Epidemiology suggests its prevalence to be much higher in communities with restricted gene pools (1:1250 among the Slovak gypsies and 1:2500 among the Saudis) than, say, in the West (1:10,000). The APEDS study on childhood blindness in AP offers an estimated prevalence of 1:3300 in this region\(^6\). Analysis has suggested two loci for human PCG, one in chromosome 2 (GLC3A at 2p21) and the other in chromosome 1 (GLC3B at 1p36); while the gene in locus 2 is yet to be identified, that in locus 1 is known to belong to the cytochrome P450 family, called CYP1B1 (ref. 9). We have performed genetic analysis of over 150 cases of PCG presenting at our hospital and the members of the families of the affected, and specifically looked at the status of the candidate gene CYP1B1; a detailed report appears elsewhere\(^10\). A total of five mutations have been identified as shown in Table 3. Of these, the most dramatic is a frameshift mutation (376 insA) which leads to a null functional phenotype, in effect a human knockout (Figure 2). More usefully, such analysis has also provided us diagnostic leads (see Table 3). The direct benefit from such a molecular genetic analysis has been to the pediatric ophthalmologist, who can now intervene well on time, relieve the condition and offer some comfort to the child and the family. Experience at our institute shows that surgical relief at the earliest (the very first weeks after birth) gives good prognosis.

Cataract, or the progressive opacification of the eye lens, is a multifactorial disease. Diverse etiological factors such as toxins from smoke, diabetes, steroid drugs, dehydrating episodes of diarrhoea, impaired nutrition and excessive radiation are known to cause cataract. Age-related or senile cataract, which is most common and affects people in their 50s and 60s, is thought to arise due to cumulative oxidative stress that damages lenticular components\(^11\). Genetics is also known to be implicated in causing certain forms of cataract\(^12\). We shall highlight the results of our studies on two of these cataractogenic factors, namely genetics and oxidative stress.

![Figure 1. Clinical presentation of cases of primary congenital glaucoma. Photographs courtesy Anil K. Mandal.](image-url)
Table 3. Mutations causing PCG phenotype

<table>
<thead>
<tr>
<th>Pedigree Exon</th>
<th>Novel*</th>
<th>Mutation</th>
<th>Heterozygous/ homozygous</th>
<th>Codon change</th>
<th>Mutation type</th>
<th>Restriction site change</th>
<th>Diagnostic method developed</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCG 4</td>
<td>Novel*</td>
<td>II</td>
<td>376insA</td>
<td>Homozygous</td>
<td>Ter@223</td>
<td>Frameshift</td>
<td>'Eco130I PCR followed by Eco130I digestion</td>
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<tr>
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<td>Reported</td>
<td>II</td>
<td>528G → A</td>
<td>Homozygous</td>
<td>G61E</td>
<td>Missense</td>
<td>'TaqI PCR followed by TaqI digestion</td>
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<td>P193L</td>
<td>Missense</td>
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<td>PCG 2 and 6</td>
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<td>II</td>
<td>959G → A</td>
<td>Heterozygous</td>
<td>E229K</td>
<td>Missense</td>
<td>'Eam1104I PCR followed by Eam1104I digestion</td>
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<tr>
<td></td>
<td></td>
<td>III</td>
<td>1449G → A</td>
<td>Homozygous</td>
<td>R368H</td>
<td>Missense</td>
<td>'TaqI PCR followed by TaqI digestion</td>
</tr>
</tbody>
</table>

*Data courtesy Panicker et al. Gain and loss of restriction sites are indicated by + or − sign, respectively.

Figure 2. Example of a frameshift mutation (labelled as PCG 4) in the gene CYP1B1 resulting in a null phenotype, or a ‘human knockout’ case of primary congenital glaucoma. The parents (consanguineously married) are carriers, as also one daughter; but the other daughter and her brother are affected.

One form of cataract, known as autosomal dominant zonular cataract, is characterized by prominent opacities of both the anterior and posterior Y-sutures in the area enclosed by the zonular component (Figure 3). Linkage analysis localized the gene causing this form of cataract to a region in chromosome 17q11-q12, including the gene for the lens structural protein βA3/α1 crystallin, with a lod score of 3.91 (Figure 4). Molecular genetic analysis of the affected members confirmed the involvement of the βA3/α1 gene, and revealed a splice-site mutation at the end of the third exon of this gene (Figure 5). One possible consequence of this is that the protein would be terminated near the end of a Greek key motif, disrupting the latter and leading to misfolding of the chain. Current efforts in the laboratory are to heterologously express the proteins from the normal and the splice-site mutated genes in sufficient amounts and compare the changes in the protein structures, which might give us an insight into the function (e.g. changed solubility, nidus for the precipitation of other damaged proteins, inter-protein interactions).

Pharmacology of cataract

We now move from genetic analysis to biochemistry and pharmacology. First we look at cataract. While the
problem of cataract is quite easily solved through surgery, the sheer volume of cases calls for non-surgical modes of delaying the onset or retarding the progression of cataract, if not its cure. It has, in fact, been estimated\textsuperscript{15} that postponement of cataract surgeries by five years, would lead to significant saving in effort, resources and money. Since the most common form of cataract is age-related, which is thought to arise due to oxidative stress, one mode of delaying its onset or progression would seem to be through antioxidant administration.

In a long-duration study called the REACT survey, conducted in the US and Western Europe, it was found that cataract progression was delayed in volunteers who were given regular doses of an antioxidant formulation of vitamins, compared to the control group\textsuperscript{16}. Though doubts have been raised about whether such supplementation provides any benefit in this context to people who are already well nourished\textsuperscript{17}, it is more likely that such antioxidant intake will impart greater benefit to populations in the developing world, such as in India. It is worth noting that items of traditional diet, native herbs, potions and medications that are naturally rich in antioxidants and cytoprotectants (e.g. flavonoids) are more easily available, affordable, and culturally acceptable than vitamins and supplement pills and capsules. To this end, we have looked at natural products such as tea (black and green), ginkgo biloba and ashwagandha for their potential as cataractostatic agents.

Tea leaves contain about 35% polyphenols by dry weight. Green tea is a rich source of flavonoids of the catechin class and their gallate esters, all of which are known to be excellent antioxidants\textsuperscript{18–20}. In black tea, some of these catechins are converted during the processing steps to the richly-coloured condensation products theaflavins and thearubigins, which too are antioxidants\textsuperscript{21}. In our experiments, we showed that extracts of green and black tea quench reactive oxygen species such as singlet oxygen, superoxide and hydroxyl radicals. We also found them to effectively prevent the oxidative cross-linking of proteins, and to enter cells and inhibit single-strand breaks in the DNA in their nuclei. In rats in which cataract was induced by oxidative

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Slit lamp photograph of the right eye of a patient afflicted with autosomal dominant zonular cataract. Notice the well-defined zonular (arrow) opacity at the Y-shaped sutures. Picture courtesy L. V. Prasad Eye Institute records.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Pedigree and restriction endonuclease analysis for the G to A transition of the family described by Basti et al.\textsuperscript{17}. Taken from ref. 14.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Effect of splice mutation described in the AZC family (ref. 14) on the betaA3/A1 mRNA.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Excised rat lenses. a, Typical of a control group of animals subcutaneously injected with a single dose (2.2 mg per kg body wt) of Na selenite on post-natal day-10; b and c, Rats treated daily (from day-8 through day-20) with green tea or black tea aqueous extracts, respectively, in addition to the selenite. Lenses were excised on day-20 (from ref. 22).}
\end{figure}
stress (selenite injection), administration of green or black tea extracts led to retardation in the progression of lens opacity, suggesting the potential cataractostatic ability of tea (Figure 6). While these results are tantalizing, we need to translate them into terms of daily life, such as dosage required per day, possible contraindications (iron chelation by tannins) and so forth. In current experiments in the laboratory, we find extracts of the plant Withania somnifera (ashwagandha) to show excellent antioxidant, cytoprotectant and other relevant properties, and their use as cataractostatic agents is being evaluated. It is worth noting that oxidative stress is a common feature implicated in other eye pathologies too, such as age-related macular degeneration, some forms of retinopathies and light-induced damage.

Ocular microbiology

Pathogenic infection of the eye is common in an agrarian and developing country such as India. On a typical day when about 500 outpatient visitors come to our institute, there are at least six or seven fresh cases of eye infection. The pathogens are bacterial, fungal, viral and protozoan in nature. The effective drug for bacterial infection has been ciprofloxacin, through which the condition resolves, though we have now started encountering drug-resistant strains. The basic biology of such strains, particularly fluoroquinolone-resistant and vancomycin-resistant species is important to study in order we understand them and attempt newer strategies. Such a programme on bacterial keratitis is being pursued by my colleagues Savitri Sharma and Aparna Jagannathan. Fungal infection of the cornea (fungal keratitis, keratomycosis) is another frequently encountered problem in this region, and is often refractory to most of the currently used antifungal agents. Besides eliminating the fungal agent from an infected cornea, it is also essential to regulate factors that contribute to corneal matrix degradation that alters the corneal tissue integrity resulting in visual loss. Usha Gopinathan et al. have been investigating the fungal proteases and host factors (activated matrix metalloproteinases) mediating pathogenesis of fungal keratitis in a rabbit model. The results of this investigation indicate that matrix metalloproteinases released by the activated resident corneal cells or inflammatory cells may largely contribute to the tissue damage in fungal keratitis. We have therefore proposed that a combined approach using antifungal agents and protease inhibitors may be reasonable for regulating fungal proliferation and tissue damage simultaneously.

Herpes simplex virus keratitis (HSV) is a sight-threatening ocular infection and occurs worldwide. Our own data indicate that 7–10% of all infectious keratitis is caused by herpes simplex virus. Various aspects of this disease, including novel diagnostic techniques, molecular pathogenesis and anti-viral resistance of HSV are being studied by my colleagues S. Athmanathan et al.

Figure 7. UPGMA phenogram based on 18S rDNA typing showing the genetic relationships of Acanthamoeba strains isolated from keratitis patients, reference Acanthamoeba sp. and related protozoa Balamuthia and Naegleria. Note that all Indian isolates are included in the T4 cluster of reference Acanthamoeba. Data courtesy Gunisha Pesarla and Savitri Sharma.
Protozoan infection of the cornea, particularly by the organism *Acanthamoeba*, has been described as a recent epidemic. The act of not keeping the contact lenses clean has been the greatest risk factor in *Acanthamoeba keratitis*, though it has been increasingly encountered in patients with no apparent predisposing factors. Savitri Sharma et al.\(^\text{26}\) at our institute have retrospectively analysed 151 consecutive patients affected by this affliction, none of whom had been wearing contact lenses. Treatment with biguanides (± chlorhexidine digluconate) was only reasonably successful, probably because the patients presented themselves at an advanced stage. They further went ahead and did the molecular genetic typing (ribotyping of the 18S and 26S rDNAs), which
revealed the pathogen to belong to the T4-type sequence group, as determined using UPGMA phenograms (Figure 7). It has become possible now, because of this, to develop a rapid multiplex PCR diagnostic method to detect *Acanthamoeba* infection at an early stage with assurance and mount appropriate treatment.

**Stem cell technique**

We now turn to the last topic, an exciting and contemporary one involving the use of stem cell technology to reconstruct damaged ocular outer surface. Incidents such as acid, alkali or fire burns, or very severe and unresolved infection lead to a damage to the outer surface of the eye (cornea, sclera and the limbus between the two, and conjunctival membrane), threatening or depriving vision. Corneal grafting (keratoplasty) often fails in these cases because the supporting cells are lost, and/or neovascularization and other complications interface. One stop-gap treatment introduced a few years ago was to clean the damaged surface and graft it with human amniotic membrane (obtained from maternity hospitals), so as to heal the surface and prepare it for eventual keratoplasty. The discovery that the limbus has a supply of (adult) stem cells led to the possibility of harvesting and cultivating them in order to produce at least the corneal epithelium. This was attempted in the last two years by groups in Italy, US and Taiwan, using limbal stem cell-cultured epithelium obtained by this technique. Virender Sangwan and Geeta Vermuganti at our institute decided to combine the amniotic membrane method and the stem cell technique, and began to explant culture limbal stem cells (taken from a 1 mm x 1 mm region of the contralateral eye or from next of kin, or cadaver eye) on prepared samples of human amniotic membrane. The growth of the stem cells and their layering to produce the corneal epithelium was excellent, as monitored by histological, immunohistochemical and thymidine uptake assays (Figures 9 and 10). After several days (usually < 10 days) of explant culture, it produced the desired epithelium, which was grafted onto the cleaned outer surface of the patient. Trials on over 50 patients, some unilateral and some bilateral, some already gone through subsequent corneal graft and some still awaiting this treatment have proved very satisfactory. This is perhaps the first successful large-scale application of adult stem-cell technique to provide satisfactory results to patients. Current efforts in the institute are focused on co-culturing limbal stem cells and conjunctival stem cells, and also on strategies to go beyond the epithelium to the stroma and endothelium, so that the total cornea may be produced using stem cell technology. This is a very ambitious goal, but with the rapid advances being made in cell biology, the dream of generating the entire cornea may yet be realized sooner than later.


ACKNOWLEDGEMENTS. The work described here is the collective effort of a number of my colleagues, to whom I owe a debt of gratitude. Special mention must be made of Drs Gullapalli N. Rao, Lalit and Rakhi Dandona for the epidemiology work; Savitri Sharma, Sreedharan Athmanathan and Usha Gopinathan for the ocular microbiology; Virender Sangwan and Geeta Vemuganti for the stem cell work; Anil K. Mandal, Shirlly Panicker and A. B. M. Reddy for the primary congenital glaucoma work; Chitra Kannabiran for the cataract genetics work, and of our collaborators; Drs Seyed Hasnain, Niyyaz Ahmed and Nagarajaram of CDFD; Drs Ramesh Aggarwal, Shashi Singh, Ch. Mohan Rao, T. Ramakrishna and Voley Srinivas of CCMB, and T. Padma of Osmania University. Financial support from the National Eye Institute (NIH, USA) and the 2 Foundation (Texas, USA), and DBT, DST, CSIR and ICMR of India has helped build and equip the laboratories and pay for research staff and for the supplies. We are thankful to these agencies for timely support. Most of all, it has been possible for us to attempt to work on these projects with confidence because of the willing, intense and intimate interaction that we have been having with our clinical colleagues at the LVPEI. I am grateful to the IISc Alumni Association and the Indian Institute of Science for inviting me to deliver the Eleventh C. V. Raman Lecture on 4 March 2002, a portion of which is elaborated in this paper.

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