RP Fighting Blindness
Research Reports May 2010 – April 2011

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## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>4</td>
</tr>
<tr>
<td>PHOTORECEPTOR REPLACEMENT THERAPY AND GLIOSIS: DEVELOPMENT OF STRATEGIES TO IMPROVE CELL INTEGRATION</td>
<td>5</td>
</tr>
<tr>
<td>IDENTIFICATION AND CHARACTERISATION OF A NOVEL X-LINKED RETINITIS PIGMENTOSA GENE</td>
<td>6</td>
</tr>
<tr>
<td>MOLECULAR GENETIC STUDIES OF SPACEMAKER, A PROTEIN IMPLICATED IN AUTOSOMAL RECESSIVE (RP25) RP &amp; UNRAVELLING THE DISEASE MECHANISM FOR PRPF31 RP</td>
<td>7</td>
</tr>
<tr>
<td>INVESTIGATION OF THE INTEGRATION OF GENETICALLY MODIFIED IRIS PIGMENT EPITHELIUM DERIVED PROGENITOR CELLS INTO AN EX VIVO MODEL OF RETINITIS PIGMENTOSA</td>
<td>10</td>
</tr>
<tr>
<td>CHARACTERISING THE X-LINKED RETINITIS PIGMENTOSA PROTEIN RP2</td>
<td>12</td>
</tr>
<tr>
<td>DEVELOPING THERAPIES FOR RHODOPSIN RP</td>
<td>13</td>
</tr>
<tr>
<td>GENE THERAPY FOR RPE65: SUPPORT FOR ASSESSMENT OF SUBJECTS AND OPTIMISATION OF CLINICAL TRIAL PROTOCOL</td>
<td>14</td>
</tr>
<tr>
<td>CLINICAL TRIAL OF GENE THERAPY FOR CHILDREN WITH LEBER’S CONGENITAL AMAUROSIS</td>
<td>15</td>
</tr>
</tbody>
</table>
IDENTIFICATION OF GENES CAUSING AUTOSOMAL RECESSIVE RETINAL DISEASE EXPLOITING NOVEL LOCI DERIVED FROM AUTOZYGOSITY MAPPING OF CONSANGUINEOUS PEDIGREES  
Mr A Webster, Institute of Ophthalmology, University College London  ..  17

WIDENING ACCESS TO GENETIC TESTING SERVICES FOR HEREDITARY EYE CONDITIONS  
Professor G Black, University of Manchester and Central Manchester Healthcare Trust  ..  18

FUNCTIONAL ANALYSIS OF RPGR  
Prof A F Wright, MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, Edinburgh, UK  ..  19
INTRODUCTION FROM THE CHAIRMAN OF
THE RP FIGHTING BLINDNESS MEDICAL ADVISORY BOARD
(Extracted from the charity’s Annual Report 2010)

I have been involved in research into retinal dystrophies since the 1960s and I have never witnessed such a dramatic change as that over the past 3 years ... you can therefore imagine my excitement, as one who has observed developments over some 45 years, that we have now finally reached an era of treatment.

The first scientifically-based treatment for retinitis pigmentosa was the RPE65 gene therapy trial, in part funded by us, carried out by Prof. Robin Ali at the Institute of Ophthalmology. This groundbreaking development, initially designed to demonstrate safety of the procedures, led to one of the patients showing a significant improvement in night vision. Further funds (have now) been committed needed to sustain the infrastructure established by Prof. Ali’s group and to carry out further trials on other types of RP.

Another prospect for treatment of RP is tissue transplants, again an area that the charity has funded in the past and will certainly be asked to fund in the future. Early projects resulted in our tissue culture facility at the University of Westminster and the groundwork there, handling retinal pigment epithelial (RPE) cells, has now led us to the point where such cells can be transplanted into a diseased retina. Transplanting RPE cells may have a greater role in treating macular degeneration but the technologies are certainly going to be helpful in the future treatment of RP too, and in fact several research groups are now trying to transplant light-sensitive cells and manipulate the environment so that they can connect to other cells in the retina and provide a meaningful pathway back to the brain.

Related to this, the huge investment in stem cell research offers the prospect of providing photoreceptor cells with the potential to make connections as they would in the developing eye ... and the UK is still a leading player in this field. Although the ethical problems for some over the use of embryonic material must be recognised this is certainly a resource that is going to be key in helping us restore vision to individuals who have completely lost their sight as a result of retinitis pigmentosa.

We must also maintain our underpinning work seeking to identify all the genes responsible for RP, because only by doing this can we determine the biochemical problems that result in cell death. Understanding of such basic science is fundamental to exploring new forms of treatment.

Prof John Marshall, Chair of Medical Advisory Board, April 2011.
PHOTORECEPTOR REPLACEMENT THERAPY AND GLIOSIS: DEVELOPMENT OF STRATEGIES TO IMPROVE CELL INTEGRATION

Dr R Pearson & Prof R Ali, UCL Institute of Ophthalmology

Cell replacement therapy is an exciting potential strategy for the treatment of blindness caused by the loss of the light-detecting cells of the eye, the photoreceptors. For photoreceptor transplantation to be successful, the donor cell must be transplanted into the eye, whereupon it must migrate into the recipient retina and then mature into a functional photoreceptor that is correctly ‘wired up’ to the rest of the visual pathway. There have been many attempts to transplant different stem cell populations but none had been able to demonstrate both appropriate migration and correct maturation. Broadly, neural and embryonic stem cells can migrate into the retina, but fail to generate retinal cell types, while retinal progenitor cells form the appropriate cell types but do not migrate into the recipient retina. In 2006, we demonstrated that rod photoreceptor transplantation is possible, provided the donor cells are at a critical developmental stage. They must be photoreceptor precursor cells, that is to say committed to becoming photoreceptors but not yet fully mature. When transplanted into a model of retinal disease, these precursor cells migrated into the recipient retina, made functional connections and improved a basic visual function, the pupil reflex (MacLaren & Pearson et al., Nature, 2006). We have recently demonstrated that similar principles apply for the transplantation of cone photoreceptors (Lakowski & Baron et al., HMG, 2010).

These established important proofs of concept, but photoreceptor cell transplantation is a long way from clinical application. A major hurdle is getting sufficient cells transplanted. In our original study, we could transplant ~1000 rods per eye, but a patient is likely to require many more to restore significant levels of vision. We therefore need to improve transplantation efficiency. In another project we have spent much time trying to improve the transplantation procedure itself and this has led to improvements sufficient to permit restoration of scotopic vision in animal models. A fundamental question remains, however, as to how broad an application photoreceptor transplantation will have for the very heterogeneous degenerations encompassed within AMD and Retinitis Pigmentosa (RP). Moreover, it is not yet known whether it is possible to treat end stage disease by this strategy and what role the specific type of degeneration may have on transplantation success. In this BRPS-funded project we have performed the first comprehensive comparison of photoreceptor transplantation outcome in animal models of slow, moderate and fast photoreceptor degeneration caused by RP. Our findings demonstrate that disease type has a significant bearing on transplantation success with integration increasing, decreasing or remaining constant with disease progression, depending upon the initial genetic defect. We have identified a number of features of the degenerating retina that significantly impact upon transplantation success, namely scarring, deposition of inhibitory proteins and changes in the integrity of naturally occurring barriers within the retina. Importantly, we have found that the breakdown of these barriers permits significantly increased levels of transplanted photoreceptor integration even in cases that would otherwise perform badly. Thus the degenerating recipient retinal environment is important in determining photoreceptor transplantation success. However, it would appear that it is possible to repair even end stage degeneration by photoreceptor transplantation.
IDENTIFICATION AND CHARACTERISATION OF A NOVEL X-LINKED RETINITIS PIGMENTOSA GENE

Prof A Hardcastle, Dr T Webb, UCL Institute of Ophthalmology

Background
Retinal degenerations that are carried on the X-chromosome result in symptoms that are severe in young boys. There are two known genes on the X-chromosome that cause retinitis pigmentosa. We have previously identified the approximate location of a new X-chromosome retinitis pigmentosa gene, called RP23, but we do not know what the gene is. Our aim is to identify genes on the X-chromosome causing retinal degenerations, and specifically to find the RP23 gene. This will allow us, not only to provide a precise molecular diagnosis for the patients and families, but also to assess the similarities and differences in visual loss in different families and to explore why the faulty gene results in photoreceptor cell death and blindness.

Progress
We have used new technology, called ‘Next Generation Sequencing’, that has enabled us to examine the X-chromosome in detail. As a result, we have now identified a unique DNA sequence variant in an RP23 patient, which we believe may be the cause RP. Further experimentation is underway to test how this altered DNA sequence is affecting normal function of the gene in the retina, and how many people have RP as a result of DNA sequence changes in the RP23 gene.

In a complimentary study, we have investigated a new form of X-linked cone dystrophy. This is a progressive degeneration in which the cone photoreceptors are lost before the rod photoreceptors; the opposite of retinitis pigmentosa. We have shown, for the first time, that sequence variants in the red and green cone opsin genes can cause retinal degeneration. Usually sequence variants in the red and green cone opsin genes cause various forms of stationary colour blindness, but we now know that certain sequence variants in these genes cause progressive degeneration, in a similar manner to mutations in the rod opsin gene causing retinitis pigmentosa. We went on to determine how the mutation affects the cone opsin proteins, and demonstrated that the protein fails to form the correct shape. Next we tested a pharmacological agent known to help with rod opsin shape formation in models of retinitis pigmentosa, however this agent did not help the cone opsin protein form the correct shape. This research has provided a molecular diagnosis and an explanation for cone dystrophy in some patients, and through publication of our work, we hope that many more patients will benefit from these findings and that a treatment may be developed.

Publication
MOLECULAR GENETIC STUDIES OF SPACEMAKER, A PROTEIN IMPLICATED IN AUTOSOMAL RECESSIVE (RP25) RETINITIS PIGMENTOSA & UNRAVELLING THE DISEASE MECHANISM FOR PRPF31 RP

Dr W Mustill, Dr G Alfano, Prof S Bhattacharya, UCL Institute of Ophthalmology

Retinitis pigmentosa (RP) is a disabling disease of the retina of the eye that starts with difficulty seeing in the dark and can eventually lead to complete blindness over time. RP is a hereditary disease, and can be passed from parent to child in several different genetic processes. RP can be passed on by a parent who is affected by RP themselves (autosomal dominant or adRP), or by one or both parents who have a genetic mutation but no symptoms (autosomal recessive or arRP). Our research is supported by BRPS and we are working on two related projects. In the first one we are characterising a protein implicated in the RP25 form of arRP. In the second project we are trying to understand the molecular mechanisms associated with the PRPF31 (RP11) form of adRP.

RP25 is known to be mutated in arRP patients from countries including France, Spain, China and Israel. arRP is the most common and most severe form of RP and accounts for as much as 50% of all cases. We started by cloning the RP25 gene, and found that it had significant similarity to another gene found in flies which was already being studied by another group of scientists. In flies, mutating this gene leads to a changed structure of the insect compound eye, and is known as Eyes Shut (EYS). It appears that the RP25 gene is in fact the human version of EYS. This suggests that in humans, mutations in RP25 could lead to RP because the retina is not formed properly. Genes provide the information the body needs in order to make proteins, allowing normal body functions, and essentially keeping a person well. The protein encoded by EYS is a large protein called Spacemaker (SPAM). Our work over the past year has been on SPAM.

We have found that SPAM is expressed in a wide range of animals, not just humans and flies. It is present in the retina of mammals, birds and amphibians. Interestingly, they express SPAM in different locations in the retina. This appears to illustrate that as animals have evolved in different ways over millions of years, SPAM has also evolved. The evolution of the eye has been a subject of significant study, as a distinctive example of an organ that is present in a wide variety of animals, indicating that all animals with eyes had a common ancestor millions of years ago. However, although eyes have evolved differently in different branches of the evolutionary tree, key genes and proteins often remain the same. This appears to be the case with SPAM. As SPAM is present in different locations in the different animals, this suggests that it has different key functions. This may help to provide suggestions on exactly what function SPAM has in the human retina, and what other proteins it may interact with. Together, this could illustrate how mutations in EYS can cause RP. We are not only interested in where in the retina SPAM is expressed, but also the location within the retina cells. For this reason we are currently working on several experiments using cells grown in flasks in the lab. We already know that under
normal conditions SPAM is present in the cytoplasm of cells. In the experiments, cells derived from human retina will be cultured under conditions that will make them express mutant SPAM, like the SPAM in arRP patients. We want to see if this will change the location in the cell that SPAM is present compared to normal SPAM. We may also see a change in the distribution, such as large masses of mutant protein. This may be seen by looking at the cells under a microscope.

Identifying the genetic mutations in \textit{EYS} and characterising the effects they have in the eye could lead to new diagnostic tests and therapies. These tests would allow faster identification of children who have inherited \textit{EYS} mutations from their parents, and are therefore likely to develop RP. This may allow them to be treated faster and therefore save their sight from degenerating with age.

The aim of our second project is to investigate the molecular mechanism of retinal degeneration caused by mutations in the \textit{PRPF31 (RP11)} gene. \textit{RP11} is ubiquitously expressed and is commonly mutated in adRP patients. The majority of our genes contain areas of coding and also non-coding DNA (exons and introns, respectively). A splicing factor is a protein involved in the removal of exons and introns. A splicing factor may act by removing all the introns thus allowing the exons to join together in a single RNA molecule leading to the eventual synthesis of the gene specific protein. The \textit{RP11} gene encodes a pre-mRNA splicing factor.

It has been found that \textit{RP11} is commonly affected by truncation mutations. These cause the gene to be shorter than normal and this affects how it works. The preponderance of protein truncation mutations in \textit{RP11} suggests that adRP patients affected by mutations at the \textit{RP11} locus have loss of one copy of the gene, resulting in decreased amount of protein synthesis. In addition we are also trying to identify regulatory elements leading to \textit{RP11} expression level variation, as we suspect these are also affected in adRP patients.

An interesting feature seen in almost all \textit{RP11} families worldwide is the presence of symptomatic and asymptomatic individuals even though both carry the mutant copy of the gene. This is due to the different levels of expression of the wild type copy of \textit{PRPF31} in the general population. In addition, it was found that people who may otherwise have RP but expressed the wild type \textit{PRPF31} allele of \textit{RP11} at a high level did not have RP. This insight revealed a potential avenue for future therapy for the adRP locus, as increased expression of wild type \textit{PRPF31} may prevent clinical manifestation of the disease in RP patients with normal expression.

One of the questions regarding \textit{RP11} is why mutations are only able to cause photoreceptor cell degeneration when \textit{RP11} is expressed throughout the body. Why are other cells unaffected? We think this may be due to the splicing factor normally encoded by \textit{RP11}. The rod photoreceptor cells are very specialized cells likely to require a very high level of splicing both for structural and functional proteins. It is possible that any defect in mRNA splicing may severely compromise the cells. Therefore, when \textit{RP11} is mutated, the splicing factor protein normally encoded by \textit{RP11} cannot be formed properly and improperly spliced proteins may cause damage to the photoreceptor cells.

To try to shed light on this potential disease pathology we are currently investigating \textit{PRPF31} expression within retina cells using the mouse as an animal model. From previous experience in our laboratory and also those of other scientists, splicing factors are normally found in the nucleus of the cell. Surprisingly, instead of a nuclear localization, our preliminary results show stronger expression of \textit{PRPF31} in the photoreceptor cell cytoplasm. A cytoplasm expression pattern is very challenging and seems to suggest a new role for \textit{RP11} in adult photoreceptor cells. In other
words this protein may act as a splicing factor and could also be involved in several other molecular pathways that are implicated in RP. To explore these pathways further, we aim to induce retinal degeneration in mice and then apply a gene replacement therapy introducing wild-type (normal) \( PRPF31 \). Retinal degeneration will be induced by shRNA injections and light damage, which has been characterized from previous research projects. The effects of \( PRPF31 \) treatment will be evaluated in wild-type (normal) mice and then also in \( PRPF31 \) knock-in and \( PRPF31 \) knockout (test) mice. We hope that this may lead to gene therapy for human adRP patients. In summary, this has been an exciting year for RP research in our laboratory. We are grateful to the British Retinitis Pigmentosa Society for continued funding of our research project.

**Publications:**


INVESTIGATION OF THE INTEGRATION OF GENETICALLY MODIFIED IRIS PIGMENT EPITHELIUM DERIVED PROGENITOR CELLS INTO AN EX VIVO MODEL OF RETINITIS PIGMENTOSA

Prof A Lotery, Dr H Thomson, Division of Clinical Neurosciences Faculty of Medicine, Southampton General Hospital

Aims of the project: This project aims to produce light sensitive retinal cells from human iris tissue. We aim to produce a model which mimics the retinal damage which is observed in Retinitis Pigmentosa. Finally we aim to examine if the cells we produce can become part of a diseased retina in the laboratory.

Progress to date:
This project began in January 2010 and we are currently working on all three of the aims described above. Our work remains focused on acquisition of human iris tissue samples, which we receive as a by-product of glaucoma surgery (Trabeculectomy). This tissue is used for isolation and growth of stem/progenitor cells. The tissue samples which we receive are extremely small (size of a pin-head) and the quality varies widely between patients. Therefore it is important to continue acquiring as many samples as possible throughout the project. Also unlike other types of cells, iris cells can not be grown in the laboratory for extended periods of time, thus it is not possible to produce cells lines which can be used continually. To overcome these issues with quantity and quality of tissue we are also utilizing tissue from donor eyes which have been consented for use in research.

We are continuing to optimise the model of retinal damage, which we propose to utilise in order to study cell integration and migration. This model involves maintaining small pieces of retina in the laboratory. We then use a drug to produce cell death which is specific to the light sensitive retinal cells (rod-photoreceptors). This pattern of cell death mimics that seen in retinitis pigmentosa. We have now found the appropriate concentration and exposure time to produce photoreceptor cell death while preserving the viability of the other cells in the retina.

Transplantation of cells into the eye attached to plastic scaffolds has previously been shown to reduce cell death and improve survival. We are concentrating on production of artificial cell scaffolds using biodegradable plastic and fibre glass like material which in non-degradable. For the experiments we have carried out to date, we have used cells which support photoreceptors known as retinal pigment epithelium cells (RPE). We have produced different conformations of scaffold including small beads (microspheres) and fibrous scaffolds.

Our preliminary experiments with RPE cells grown on these biodegradable microspheres were published last year. In this study we explored for the first time the attachment of RPE cells to microsphere for the purpose of ocular transplantation.

In order to provide a more permanent support for transplanted cells, we have produced fibrous scaffolds which do not degrade in the body. To maximise cell attachment on the fibrous mats we are investigating the effect of coating the surface with different types of proteins. These proteins normally help cells to adhere to
membranes within the body. In our experiments we found that coating our scaffolds with these proteins did increase both cell attachment and viability.

Overall we are satisfied with the progress of this work which ultimately we hope will improve the outcomes of patients with retinitis pigmentosa.

Once again we thank RP Fighting Blindness for their invaluable support of this work.
CHARACTERISING THE X-LINKED RETINITIS PIGMENTOSA PROTEIN RP2

Prof A Hardcastle, Prof M Cheetham, Dr N Schwarz. UCL Institute of Ophthalmology

Background
Retinal degenerations that are carried on the X-chromosome result in symptoms that are severe in young boys. Mutations in the RP2 gene on the X-chromosome cause retinitis pigmentosa. We have previously characterised the RP2 protein in the eye and shown that the patient mutations lead to disease either through the protein failing to reach to the correct part of the photoreceptor cell or because the mutant proteins are recognised as faulty and are degraded. However, RP2 is found in every tissue in the body, not just the eye, and we believe that unlocking the seemingly special function of this protein in the eye is key to understanding why patients suffer retinal degeneration. We have made considerable progress towards achieving our aims by locating RP2 in very specialised structures in the photoreceptors in the retina, which are crucial for the transport of proteins to the correct place. This is important for cell survival and lack of RP2 can lead to misrouting of proteins in the retina. This research has led to defining an important role for RP2 in photoreceptor function (Evans et al. 2010). We are now focused on the identification and characterisation of retina specific RP2 partner proteins, and we are pleased to report we have made significant progress towards achieving this aim.

Progress
We have applied a different technique (called ‘proteomics’) to look for important interactions with RP2 in the retina. Using this method, we have identified a retina specific protein as a partner of RP2. This novel partner protein plays an important role in turning light detected by the retina into a signal that is passed to the brain, and as such we have now made a direct link between vision and RP2. We have shown that RP2 is required to take this new light regulated protein partner to the membrane of the photoreceptor cell so that it can help transmit the light signal. This finding supports our hypothesis that RP2 has a very special function in the eye, by taking important proteins to the correct structures in the retina so they can perform their function. This finding greatly enhances our understanding of RP2 function in the eye. We plan to submit this exciting research for publication within the next few weeks so that other vision researchers can benefit from this research.

Please note this grant is currently in abeyance while Dr Schwarz is on maternity leave.
DEVELOPING THERAPIES FOR RHODOPSIN RP

Professor Mike Cheetham, UCL Institute of Ophthalmology

Retinitis Pigmentosa (RP) is a group of inherited diseases that cause blindness due to the progressive death of rod and cone photoreceptors in the retina. Symptoms start with decreased night vision and later progress to tunnel vision and ultimately blindness. There are currently no effective treatments for RP. Inherited mutations in rhodopsin, the light sensing protein of rods cells, are the single most common cause of autosomal dominant RP and the majority of mutations cause protein to not take up the right shape or 'misfold' this means it will not work properly. The misfolded rod opsin is normally removed by the cell but if it builds up then it will form clumps of aggregated material, similar to those found in many other neurodegenerative diseases.

Through the previous support of BRPS (grant GR544) we developed a cell based laboratory model to test drugs to combat the adverse consequences of this rhodopsin misfolding. Importantly, we showed that several classes of drugs could reduce the toxic effects of the mutant rod opsin protein. These drugs did not have to improve the folding of the mutant protein to have beneficial effects; it appears that reducing protein aggregation was sufficient to improve cell health. Therefore, we believe that some of these drugs could be developed as potential treatments to delay or prevent blindness in rhodopsin retinitis pigmentosa patients.

In the first year of the project, compounds with the potential to reduce protein misfolding were tested in a cell model of retinitis pigmentosa. In addition, we imported mice and rats with rhodopsin retinitis pigmentosa. During the second year, we have completed baseline studies of both mutant lines of rat, and the normal control rat, for both the functional and structural components. Baseline studies of the retinal function and baseline histology in the mutant mice and normal control mice have also been completed. As the baseline data was in line with previous studies, we have begun testing compounds that were shown to reduce protein misfolding and aggregation in our established cell model of rhodopsin retinitis pigmentosa, in our animal models.

At this stage, 2 trials have been completed. In the first trial, normal and mutant rats were treated with a ‘chemical chaperone’ drug, previously found to be effective in our cell culture studies. No difference was observed in the responses of treated rats when compared to untreated rats of the same strain, for either the functional testing or the structure of the retina. In the second trial, the mutant strain of mice and normal littermates were treated with a drug which stimulates the natural cellular machinery for dealing with faulty proteins. Preliminary results from this trial suggest a possible improvement in the function and survival of the retina in the mutant mice when compared to untreated mutant mice. Further analysis is required to confirm this promising finding.
GENE THERAPY FOR RPE65: SUPPORT FOR ASSESSMENT OF SUBJECTS AND OPTIMISATION OF CLINICAL TRIAL PROTOCOL

Professor R Ali, Institute of Ophthalmology, University College London

In April 2008 we reported the results of the first phase our clinical trial of gene therapy for Lebers congenital amaurosis (LCA), which included 3 affected subjects aged 23 yrs, 18 yrs and 17 yrs. We demonstrated that a low dose of viral titre is safe and can lead to improved vision. For this first phase I/II clinical trial we included subjects who retained only limited residual retinal function. Despite advanced retinal degeneration we found unequivocal evidence of improved vision in one subject using a variety of techniques. The difference in the performance of this subject in visual mobility in low light was also significantly greater. Even though he was not the youngest subject, it is likely that he had less advanced retinal disease at baseline and this probably explains the improvement that was not observed in the other subjects.

We chose to use a low viral dose in the original protocol because this was the minimum effective dose in preclinical studies in dogs with a similar condition. While administration of the viral vector at the current dose is very well tolerated in the human subjects, its efficacy to date appears to be relatively modest compared to its effect in dogs. We therefore decided to evaluate outcomes following the administration of a higher dose of vector.

We have now tested a new high dose preparation of vector that been manufactured to clinical grade standards according to the same protocols as before and has passed quality assurance testing. We have assessed the vector in dogs and shown that it is safe and effective. This data, along with the safety and efficacy data from the first set of patients formed part of a regulatory submission to the regulatory authorities (GTAC and MHRA) in which we sought approval to amend our clinical trial protocol to enable us to administer the high dose preparation. Following a positive response from the regulatory authorities we have administered high dose vector to two adult patients in summer 2009 and subsequently to two children aged 10. The results to date have been very encouraging as we have seen significant improvements in visual function in 3 of the 4 subjects. Furthermore, the improvements in retinal sensitivity are much greater than we observed with the lower dose and appear to reflect the dose escalation. We have also administered vector to two children aged 5 but it is too early to know the effects of treatment. The last two subjects in the trial have now been enrolled and will receive vector in July.

We have also carried out studies in dogs to assess the feasibility of re-administration of vector in the second eye. To date we have re-administered vector in twelve RPE65-deficient dogs. Our results suggest that effective re-administration is possible since we observe good rescue following re-administration and a through analysis of the immunological responses to vector and transgene following vector re-administration suggest that it may be safe to do this in human subjects.
CLINICAL TRIAL OF GENE THERAPY FOR CHILDREN WITH LEBER’S CONGENITAL AMAUROSIS

Professor R Ali, Dr M Bainbridge, Institute of Ophthalmology, University College London

In February 2007 we started the world’s first clinical trial of gene therapy for the treatment of inherited retinal degeneration. The trial involves patients with a condition called Leber’s Congenital Amaurosis (LCA), a rare form of inherited eye disease caused by an abnormality in the gene RPE65. The condition is symptomatic at birth or in the first few months of life and causes progressive photoreceptor degeneration and loss of vision. There are currently no effective treatments available and most patients are legally blind by their late teens or early twenties. The purpose of the first phase of the trial was, firstly, to find out whether gene therapy for retinal disease is safe, and, secondly, to find out if it can benefit vision in young adults who already have advanced retinal disease. The technique used in the trial involves inserting healthy copies of the missing RPE65 gene into the cells of the retina to help them to function normally. This involves an operation to deliver the normal genes underneath the retina, using a genetically modified virus to carry the gene into the cells. In May 2008 we reported our results in the three young adult patients. Importantly, we found that the experimental treatment caused no side effects in this trial. Following the treatment, the three patients involved underwent a series of tests designed to establish the effects of the therapy on visual function. They all achieved levels of vision at least equivalent to before the operation, but one patient showed significantly improved visual function. Detailed vision testing demonstrated improved retinal sensitivity in the treated eye, but not in the control eye. We were also able to demonstrate improved visual function in a real world setting. This was demonstrated by the patient’s ability to negotiate a specially constructed simulation of a night-time street scene. Before the operation, he completed the task very slowly and made several mistakes; but, following the surgery, he was able to navigate quickly and without mistakes. (http://news.bbc.co.uk/1/hi/health/7370694.stm). The results of this trial demonstrated that gene therapy can be safe and can improve visual function in adults with advanced disease due to RPE65 deficiency.

Showing for the first time that gene therapy can work in patients with eye disease is a very significant milestone. The trial establishes proof of principle of gene therapy for inherited retinal disease. It is very encouraging to see that this treatment can work, even in young adults who have severely advanced disease. We anticipate an even better outcome in younger patients.

We are now building on our experience from the first phase of the trial and have extended the trial by two years in order to include a group of subjects under the age of 16 years. We have obtained approval from the regulatory authorities (GTAC and MHRA) to enable us to use a higher dose of the vector and to include patients as young as 5 years old. We administered high dose vector to two adult patients in summer 2009 and subsequently to two 10 year old children. The results to date have been very encouraging as we have seen significant improvements in visual function in 3 of the 4 subjects. Furthermore, the improvements in retinal sensitivity are much greater than we observed with the lower dose and appear to reflect the dose escalation.
We have also administered high dose vector to our youngest subjects who are 5 years old, but it is too early to report on the effects of treatment. We have been developing specialist tests for assessing vision in young children and these will be used to assess whether there have been any improvements in vision following treatment. The last two subjects will receive vector in July 2011 and the trial will be closed. We will publish our findings within the next 12 months.
IDENTIFICATION OF GENES CAUSING AUTOSOMAL RECESSIVE RETINAL DISEASE EXPLOITING NOVEL LOCI DERIVED FROM AUTOZYGOSITY MAPPING OF CONSANGUINOUS PEDIGREES

Mr A Webster, Institute of Ophthalmology, University College London

GR564 and GR569 are two grants kindly funded by RP Fighting Blindness to support a scientist and PhD student respectively, together with laboratory consumable costs, for a program of work concerning retinal dystrophies. The aims of the work are two-fold: i) determine the natural history of specific forms of retinal degeneration. This is vital in order to understand which disorders are best candidates for gene-directed therapy and which metrics of retinal dysfunction and structure can be used to assess efficacy. ii) to determine new mutations in known genes and mutations in novel genes. The work utilises the large numbers of patients and families who attend Moorfields Eye Hospital (MEH) to see the clinical teams of Professor Tony Moore, Mr Andrew Webster, and Mr Michel Michaelides. Together this represents the largest single centre in the Western world managing retinitis pigmentosa and related conditions. Moreover, the technology available to investigate the patients including imaging, electrophysiology and psychophysical is routinely available for most patients either within the hospital or the adjacent Institute of Ophthalmology. This allows the accurate determination of the structure and function of the human retina in these families. Overall the MEH resource includes 14744 subjects affected with inherited eye disease from 12792 families, which includes 8046 patients currently being managed at the hospital, at the time of writing.

The study consists of both clinical data collection as well as experiments in the molecular genetic laboratory. Since the start of the first grant in February 2010, important clinical findings with families known to be mutated for genes that affect rod, cone photoreceptor structure and function as well as retinal pigment epithelium and inner retinal function have been determined and published in the scientific literature. In one recent example of this approach, a small cohort of families with mutations in a gene called LRAT, has been identified and has allowed the acquisition of vital clinical data to be used in a clinical trial of an analogue of 9-cis retinal, an approach that has shown promise in animal models. Moreover, some of the family members are participating in the clinical trial (collaboration with Dr R Koenekoop, McGill University Montreal).

The work has generated an informative set of families who do not harbour mutations in known genes, and which are invaluable for finding novel genes. Recently, a selection of such patients have been put forward for ‘full exome sequencing’ at the UCL Genomic Centre. This is a revolutionary new technique in which all the protein coding parts of the genome are ascertained in one reaction. Already, one family has shown a mutation in a novel gene. This is a gene encoding a ion-channel in the retinal pigment epithelium, and the discovery illuminates a novel physiological pathway, essential for normal retinal development and function. The discovery has been submitted to a high-impact journal. The team anticipate further clinical and genetic discoveries as the work continues.
WIDENING ACCESS TO GENETIC TESTING SERVICES FOR HEREDITARY EYE CONDITIONS

Professor G Black, University of Manchester and Central Manchester Healthcare Trust

Genetic counselling is now an essential element to the management of many families with hereditary eye conditions. A genetic test result can aid in establishing a firm diagnosis and can offer families options that were hitherto not available including prenatal diagnosis and carrier testing.

The eye genetics service based in the Department of Genetic Medicine in Saint Mary's Hospital is run as an equal collaboration between the Manchester Biomedical Research Centre and the North-West Regional Genetics Laboratory Services under the Supervision of Professor Graeme Black and Dr Simon Ramsden.

We first obtained funding from RP Fighting Blindness to develop genetic testing for disorders of retinal degeneration in 2005. GR547 was a five year grant provided in order that we could expand the repertoire of ophthalmic genetic tests available in the NHS. This grant came to an end in August 2010 and it is thanks to this generous funding that we can now offer testing for retinitis pigmentosa (various forms), Leber congenital amaurosis, late onset retinal dystrophy (LORD), Sorsby pseudoinflammatory fundus dystrophy (SFD), Doyne familial honeycombed choroiditis (autosomal dominant radial drusen) and Best1 (VMD1) mutation screening to patients across the UK. Thanks to this funding we can also provide an accredited laboratory testing environment for the delivery of testing to support two ophthalmic gene therapy trials (RPE65 and choroideremia).

Now the case for these services has been made they are no longer supported by the society, and funding is now subject to conventional NHS commissioning. We estimate that as a consequence of this programme the NHS now supports genetic testing for retinal dystrophies to the order of about £150,000 pa and this contribution is increasing year on year. These services are provided free to UK patients. We continue to monitor the uptake of tests in order to identify geographical gaps in the provision of services.

Unfortunately, however the limitations imposed by conventional genetic testing means that it is still only applicable to approximately 50% of patients with an inherited retinal degeneration. For many patients we still cannot identify individual genes of interest and as a consequence cannot provide the necessary testing.

In the Department of Genetic Medicine in Saint Mary’s Hospital we are currently trialling new genetic screening techniques that offer us the potential to screen large numbers of genes simultaneously. For the first time this offers us the potential to extend testing to all families with inherited eye conditions. This technology will require extensive testing and validation before it can be considered appropriate for delivery under NHS funding streams. Therefore we were delighted to receive funding from RP Fighting Blindness to support this important work (GR570 – funding commenced January 2011).
FUNCTIONAL ANALYSIS OF RPGR

Prof A F Wright, MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, Crewe Road, Edinburgh, UK

Genetic changes (mutations) in the retinitis pigmentosa GTPase regulator (RPGR) gene are the commonest cause of X-linked retinitis pigmentosa1. The RPGR gene produces several slightly different forms of the RPGR protein, one of which, RPGR<sub>ORF15</sub>, appears to be the main cause of XLRP. RPGR<sub>ORF15</sub> is most strongly localised to the light-sensitive photoreceptor cell, in a region called the “connecting cilium” but it is also expressed in many other cells in lower amounts. A cilium is a small cellular tail or extension which is important for recognising signals from the environment and a photoreceptor is a modified and highly specialised cilium. The connecting cilium links the body of the cell with the ciliary extension (outer segment) where light signals are received and so is important for the maintenance of photoreceptor structure and function. A malfunction in RPGR<sub>ORF15</sub> causes disorganisation of the photoreceptor outer segment, eventually leading to cell death and visual loss. There are several findings that suggest a ciliary role for RPGR<sub>ORF15</sub>, but its exact function remains unclear. We have been trying to answer three specific questions. Firstly, how does RPGR<sub>ORF15</sub> influence cilia formation? We blocked or over-expressed RPGR<sub>ORF15</sub> in a ciliated cell and found that it does play a role in the initial steps of cilia formation. Secondly, does RPGR<sub>ORF15</sub> influence the molecular motors that transport substances in and out of the outer segment? We used a zebrafish model to study this and found that lack of RPGR<sub>ORF15</sub> causes a delay in movement of substances involving the same molecular motors that are used in cilia2. Thirdly, how does the RPGR<sub>ORF15</sub> protein function? We unexpectedly found that RPGR interacts with what is called the “actin cytoskeleton” and regulates formation of cilia by means of changes in this network of fibres that are important in linking the transport of materials, carried by the molecular motors, through the connecting cilium. We are about to submit a paper on this finding3.

These experiments give us a better understanding of the cellular functions of RPGR, but also provide a more solid platform on which to base XLRP gene therapy trials.

References

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