RP Fighting Blindness Research Reports

May 2012 – April 2013

Image: Typical RP Fundus

An omnibus of research reports produced by researchers funded by RP Fighting Blindness during the period.
CONTENTS

Page 4  INTRODUCTION

Page 5  1. CHARACTERISING THE X-LINKED RETINITIS PIGMENTOSA PROTEIN RP2  
Prof Alison Hardcastle and Prof Mike Cheetham  
Postdoctoral fellow Dr Nele Schwarz  
UCL Institute of Ophthalmology

Page 7  2. PHOTORECEPTOR REPLACEMENT THERAPY AND GLIOSIS: DEVELOPMENT OF STRATEGIES TO IMPROVE CELL INTEGRATION  
Dr Rachael Pearson and Prof Robin Ali  
UCL Institute of Ophthalmology

Page 9  3. INVESTIGATION OF THE INTEGRATION OF GENETICALLY MODIFIED IRIS PIGMENT EPITHELium DERIVED PROGENITOR CELLS INTO AN EX VIVO MODEL OF RETINITIS PIGMENTOSA  
Prof Andrew Lotery and Dr Heather Thomson  
Southampton General Hospital

Page 10  4. WIDENING ACCESS TO GENETIC TESTING SERVICES FOR HEREDITARY EYE CONDITIONS  
Prof Graeme Black  
St Mary’s Hospital, Manchester

Page 11  5. IDENTIFYING NOVEL GENE MUTATIONS IN INHERITED RETINAL DEGENERATIONS  
Dr Stephanie Halford  
University of Oxford

Page 12  6. STRUCTURAL RESTORATION OF RHODOPSIN ADRP MUTANTS: SUB-CELLULAR LOCALISATION AND CO-FACTOR INTERACTIONS  
Dr Philip Reeves  
University of Essex

Page 13  7. PATHOLOGY AND TREATMENT OF PROMININ1 (PROM1)-MEDIATED RETINITIS PIGMENTOSA  
Prof Shin-ichi Ohnuma  
UCL Institute of Ophthalmology

Page 14  8. MOLECULAR GENETIC STUDIES OF SPACEMAKER, A PROTEIN IMPLICATED IN AUTOSOMAL RECESSIVE (RP25) RETINITIS PIGMENTOSA  
Wendy Mustill & Shomi S Bhattacharya  
UCL Institute of Ophthalmology
9. MODIFICATION OF MUTANT BESTROPHIN-1 TO PREVENT RETINAL DEGENERATION
Dr Forbes Manson, Dr Lisa Swanton
University of Manchester

10. RP FIGHTING BLINDNESS CENTRE FOR THE DEVELOPMENT OF GENE THERAPY FOR INHERITED RETINAL DYSTROPHIES
Prof R Ali
UCL Institute of Ophthalmology

11. MECHANISMS OF PHOTORECEPTOR DEGENERATION IN CHOROIDEREMIA
Prof Clare Futter
UCL Institute of Ophthalmology

12. CHARACTERISING A NOVEL GENE FOR X-LINKED CONE-ROD DYSTROPHY AND X-LINKED RP
Prof Alison Hardcastle, Dr Michel Michaelides, Prof Mike Cheetham
Postdoctoral fellow Dr Jessica Gardner
UCL Institute of Ophthalmology
INTRODUCTION

(Extracted from the charity’s Annual Report for 2012)

In 2012 two exciting new projects were funded. Professor Clare Futter at the Institute of Ophthalmology was funded to investigate mechanisms of photoreceptor degeneration in choroideremia, and Prof Alison Hardcastle, again at the Institute of Ophthalmology, was funded to complete the identification of a novel gene causing X-linked Cone-Rod Dystrophy and X-linked RP.

I noted last year that several threads of work have entered, or are entering clinical trials phases and this has continued in 2012. Indeed, I would anticipate similar news from around the world in 2013 and 2014.

As a practising clinician, as well as a research scientist, I wholeheartedly endorse the charity’s emphasis on translating exciting science into therapies. When the Medical Advisory Board prioritises projects for recommendation for funding, this will always be a significant factor in our minds. At the same time, however, it is important to fund high quality fundamental science that has potential to be developed into new treatments.

Preventing sight loss using gene therapy to replace faulty or missing genes in the retinal cells, or by intervening with the use of drugs, is the objective of several projects in the UK, and indeed around the world. Our own support for Professors Robin Ali and Michael Cheetham at The Institute of Ophthalmology are good examples. It is also exciting that other groups, such as Professor Robert MacLaren’s at Oxford University Eye Hospital, are taking on gene therapy trials. We also watch with interest commercial companies such as Neurotech (USA) that are developing and testing in clinical trials technologies designed to release compounds into the eye over long periods of time to treat RP.

RP Fighting Blindness’ contribution to high quality retinitis pigmentosa research is important and can be influential … I remain confident that we have an important role to play and that we are making good use of our available medical research funds.

Prof Paul Bishop
Chair of RPFB Medical Advisory Board
April 2012.
1. CHARACTERISING THE X-LINKED RETINITIS PIGMENTOSA PROTEIN RP2

Prof Alison Hardcastle and Prof Mike Cheetham
Postdoctoral fellow Dr Nele Schwarz
UCL Institute of Ophthalmology

Background
X-linked retinitis pigmentosa is a severe form of RP, which primarily affects males in the family. One of the genes causing this condition is called RP2. Curiously, disease is restricted to the eyes, but the RP2 protein is found in every part of the body. We are therefore conducting research to understand the special function of RP2 in the eye and determine why RP2 causes a retinal disease. We have addressed this important question by identifying partner proteins that work in conjunction with RP2 in the retina, and by evaluating how these important partnerships and molecular pathways are disrupted by the RP2 sequence mistakes we find in patients. We have previously shown that RP2 is located in very specialised parts of the photoreceptors, those parts that are important for delivering proteins to the light sensing part of the cell (Evans et al 2010). We have recently discovered a direct link between vision and RP2, by identifying a cargo protein that plays an important role in light detection. We think RP2 is required to transport this protein to the correct part of the photoreceptor so that it can perform its function (Schwarz et al. 2012b, Schwarz et al. 2012c). This supports our hypothesis that RP2 has a special function in the retina.

Progress
We are continuing to make considerable progress with our research. We have now identified two new RP2 partner proteins and we are investigating how RP2 might regulate the transport and function of these proteins in the retina. In addition, we have recently established a model of how RP2 cooperates with several other proteins to traffic important visual proteins to their correct location (Schwarz et al. 2012a). Importantly, in collaboration with other groups, we have discovered a new gene for RP that is involved in a similar molecular pathway to the one we have established for RP2. We were able to experimentally test and unravel the function of this new RP gene because of our current research and working hypothesis on RP2 function (Davidson, Schwarz et al. under review).

Future work
We are currently applying for funding to continue our successful research to unravel the special function of RP2 in the retina. We also want to derive retinal cells from patient stem cells so that we can test some new drugs that may be able to bypass the RP2 mutation and restore RP2 function.

Recent Publications
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Previous Publications
2. PHOTORECEPTOR REPLACEMENT THERAPY AND GLIOSIS: DEVELOPMENT OF STRATEGIES TO IMPROVE CELL INTEGRATION

Dr Rachael Pearson and Prof Robin Ali
UCL Institute of Ophthalmology

Photoreceptor replacement therapy is an exciting potential strategy for the treatment of blindness caused by the loss of the light-detecting 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. In other projects, we have provided the first conclusive evidence 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 ‘baby photoreceptors’ but not yet fully mature. When transplanted into a model of retinal disease, these precursor cells migrate into and become integrated within the recipient retina and develop into mature photoreceptors (MacLaren & Pearson et al., Nature, 2006; Lakowski et al., HMG, 2010). Moreover, we were then able to demonstrate that these cells not only look like normal photoreceptors, but are also capable of restoring vision in an animal model of stationary night blindness (Pearson et al., Nature, 2012). We have recently developed protocols that now enable us to generate rod photoreceptor precursor cells from mouse embryonic stem cells, thus providing a renewable source of transplantable donor cells (Gonzalez-Cordero, West et al., Nat. Biotech. in revision).

These exciting results raise fundamental questions about whether photoreceptor transplantation will be equally able to treat the very heterogeneous degenerations encompassed within AMD and Retinitis Pigmentosa (RP). In our most recent paper (Pearson et al., Nature, 2012), we used a model of stationary night blindness, which undergoes a relatively mild form of degeneration. However, we will need to be able to treat much more severe forms of degeneration. In this RP Fighting Blindness-funded project we performed the first comprehensive comparison of photoreceptor transplantation outcome in animal models of slow, moderate and fast photoreceptor degeneration caused by RP. We found that disease type has a significant bearing on transplantation success; it is possible to treat even the severely degenerate retina however, the number of transplanted donor photoreceptors that are able to correctly integrate within the recipient can increase, decrease or remain constant with disease progression, depending upon the initial disease-causing genetic (Barber et al., PNAS, 2013). Importantly, we have identified a number of key features of the degenerating retina that have a significant impact upon transplantation success. These include a process called glial scarring, much like the scarring you might see in the skin following a cut, and changes in the integrity of naturally occurring barriers within the retina. We are now performing a comprehensive study of gliosis in order to understand this complex process and to find targeted ways of manipulating it (Hippert et al., manuscript in preparation). In support of this strategy, we have already identified two ways of manipulating these barriers, each of which significantly improve transplantation outcome even in cases that would otherwise perform badly (Barber et al., PNAS, 2013; Hippert et al., manuscript in preparation).
Publications supported by this grant:

Manuscripts in revision/in preparation
3. INVESTIGATION OF THE INTEGRATION OF GENETICALLY MODIFIED IRIS PIGMENT EPITHELIUM DERIVED PROGENITOR CELLS INTO AN EX VIVO MODEL OF RETINITIS PIGMENTOSA

Prof Andrew Lotery and Dr Heather Thomson
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.

We receive iris tissue as a by-product of glaucoma surgery and from donor eyes which have been consented for research. We have used this tissue to derive immature (progenitor) cells. Unlike other types of progenitor cells, iris derived cells cannot be grown in the laboratory indefinitely therefore we have continually grown iris derived progenitor cells throughout the duration of the project. In an effort to manipulate these cells into becoming like photoreceptor (light-sensitive) cells, we have genetically modified the cells with a transcription factor known as cone rod homeobox (Crx). This small piece of genetic material acts as a master switch during normal eye formation to turn on development of these highly specialised light sensitive (photoreceptor) cells. We have used Crx in the hope that it will be sufficient to drive iris cells to look and function like photoreceptors. The cells which we have produced resemble photoreceptors, however we have found that Crx alone is insufficient to induce light sensitivity in our cell population.

Using healthy pieces of retina we have produced a model of retinal damage, which we have used to study progenitor cell integration and movement. Using a drug, we have produced cell death which is specific to the light sensitive photoreceptor cells. This pattern of cell death means that the rest of the retina remains functional and mimics that seen in retinitis pigmentosa. We have grown iris progenitor cells with this damaged retinal tissue. We have seen that the cells can become part of the retina.

We have carried out initial experiments to deliver iris cells which have been tagged with a green fluorescence, so that we can track where they go in the eye. It has been documented that transplantation of cells into the eye attached to scaffolds reduces cell death and improves survival. We have produced artificial cell scaffolds using biodegradable plastic (small solid spheres) and fibre glass like material which does not degradable (fibrous mats). To maximise cell attachment we have modified the surface of the scaffolds with whole proteins and small active pieces of protein. In our experiments we found that changing the surface by simple chemical modification can increase both cell attachment and viability. We have been carrying out experiments to deliver the small solid sphere (microspheres) into the eye. To allow better visualisation of the spheres we have coated them with green fluorescence. We are currently analysing if these microspheres are tolerated within the eye.

Once again we thank RP Fighting Blindness for their invaluable support of this work.
4. WIDENING ACCESS TO GENETIC TESTING SERVICES FOR HEREDITARY EYE CONDITIONS
Prof Graeme Black
St Mary’s Hospital, Manchester

There has been exciting progress over the year 2012-2013.

Thanks to funding from RP fighting blindness, in conjunction with Fight for Sight, it has been possible to develop and launch a novel method of genetic testing for patients with retinitis pigmentosa. Based around a new technology – so called ‘next generation sequencing’ – this allows the simultaneous testing of over 100 of the genes that are known to be faulty in patients with RP. Having published results in the Journal of Medical Genetics in early 2012, the test was launched by the NHS in April 2012.

This is extremely exciting. The test now means that NHS testing is relevant to virtually all patients with RP. Furthermore our evidence that it is successfully finding the gene faults in around two thirds of all patients with classical RP. Not everyone needs a test, but gene testing can help patients and their doctors to hone the understanding of an individual’s form of RP. In turn this can help patients (and their families) ability to plan the future, and with their decision making This represents a huge step towards making a worthwhile test available to as many patients as possible. Early evidence suggests that uptake of the test by the NHS is encouraging! Delivering the test is by no means the end of the story – it is important at the same time to take care of ‘simple’ practical matters such as how patients consent for testing, how the cost of the test will affect access to it and how to deliver results back to patients and their doctors in a way that is clear and concise. This has taken a great deal of care as the test is perhaps as complex as any delivered before in the UK.

Around one third of all patients remain to have the form of RP defined, so this also represents an opportunity to begin the process of trying to find new forms of retinitis pigmentosa and to identify the genes that remain to be discovered. Therefore the testing programme should fit nicely to the ongoing work of basic science and help to make that more efficient. Furthermore the results of this work will be publically available to ensure that – should it be appropriate and with patients’ consent - access to clinical trials is enhanced by the testing programme.
5. IDENTIFYING NOVEL GENE MUTATIONS IN INHERITED RETINAL DEGENERATIONS

Dr Stephanie Halford
University of Oxford

Degeneration of the retina due to the inheritance of faulty copies of genes affects approximately 1 in 2500-3500 people and causes either partial or total blindness. Currently, over 220 regions of DNA have been associated with causing blindness, each of which may contain from a few to hundreds of different genes. In about 80% of cases the specific gene and mutation causing blindness has been found. To help identify which genes and mutations are involved in the remaining 20% of cases we have performed experiments to determine which genes are specifically used within the light detecting cells of the retina.

The retina is a complex structure of many layers of several types of cells but it is the rods and cones that are responsible for vision. The rods allow black and white vision in dim light conditions, whereas the cones provide colour vision. We have examined the extent to which more than 28,000 genes are turned on in normal retina compared to retina missing either the rods, cones, or both. This has allowed us to identify those genes which are switched on in rods and cones and are therefore likely to be vital to how they function. Comparing this subset of genes to those already known to cause inherited forms of blindness showed considerable overlap. This indicates that our approach is successfully identifying genes that are important in rod and cone function. Most importantly, many additional genes were also found to be turned on at high levels in the rods and cones, indicating that they are important for these cells to function properly. These new genes are important novel candidates and may cause blindness in some families.

We are continuing to utilise these data in two important ways in order to help find mutations which cause retinal degeneration. First, we are collaborating with partners both within our department and in other institutions to identify patients with inherited forms of blindness for which the causal gene is currently unidentified but has been localised to a region of DNA that contains many genes. We are then using our data to examine the genes in these regions to find which have a role in the photoreceptors. These can then be checked in the patients to determine if there are any mutations which affect how the gene works.

Secondly, for some of the genes we have identified in the rods and cones very little is currently known about their role in the retina. Therefore, we are performing additional experiments to determine more information about these genes such as what their possible function is, where else in the retina they are used and whether they are only functional in the retina or in other tissues too. By gathering such information we will be better able to identify genes that are strong candidates for causing retinal degeneration in patients with very little or no genetic information available.
Rhodopsin is a major constituent of the rod photoreceptor cell where it is responsible for detecting vision under dim-light conditions. Faults in the rhodopsin gene are the most common cause of Autosomal Dominant Retinitis Pigmentosa (ADRP). These faults cause rhodopsin to misfold and become toxic which results in death of rod photoreceptor cells. Our goal is to understand why (and how) these defective rhodopsin ADRP proteins misfold, and then use this information to identify new ways to stabilize the faulty proteins or prevent them from becoming toxic. We are also undertaking studies to determine how known proteins (gatekeepers) interact with these misfolded rhodopsin proteins and we are also trying to identify new interacting partners that may be future drug targets. This grant has allowed us to develop methods and characterise rhodopsin ADRP proteins as follows:

1) We have determined that certain ADRP faults that lead to alterations at the beginning of the rhodopsin protein can form the correct light sensitive ‘pigment’ under suitable conditions. However, these pigments are very unstable and do not function properly. This work has led to identification of a key part of rhodopsin that plays a vital role in stability and function. We are expanding our work on this section of the protein in order to understand why it is so important for correct folding.

2) We have used cell imaging to identify the location of rhodopsin ADRP proteins in cells. The misfolded proteins have been observed to aggregate. The location of these mutants in the cell is the same as that for proteins known to be involved in assisting folding or degrading misfolded proteins.

3) The full range of proteins that interact with normal and faulty rhodopsins is not known. We are developing a ‘proteomics’ approach to enable us to identify the full range of interactions that take place in model cell systems and in the retinas of transgenic mice carrying rhodopsin ADRP genes. These techniques offer unprecedented sensitivity and will allow us to identify even weak or temporary interactions. Using this approach we have already identified one new protein that interacts with a faulty version rhodopsin. This protein is thought to perform a key role in photoreceptor biology and this interaction may help to explain why rhodopsin ADRP faults have such serious affects on the rod cell.

Invited talks and publications
1. Dr Phil Reeves presented this work as an invited speaker at the 15th International Conference on Retinal Proteins, Monte Verità, Ascona, Switzerland (30th Sept - 5th Oct, 2012). ‘Restoration of folding and function in rhodopsin ADRP mutants.’ A manuscript describing this work has also been submitted for peer reviewed publication.

7. PATHOLOGY AND TREATMENT OF PROMININ1 (PROM1)-MEDIATED RETINITIS PIGMENTOSA

Prof Shin-ichi Ohnuma

UCL Institute of Ophthalmology

Background
The death of photoreceptor cells is a major cause of blindness. Recently, the genetic basis for photoreceptor cell death in many patients has been identified but treatment options are still very limited. This lack of treatment largely results from a limited understanding of the pathology of the disease. Mutations in the gene called Prominin1 (Prom1) cause retinal dystrophies including retinitis pigmentosa and this project aims to elucidate the disease mechanism through the use of a mutant mouse that does not express the Prom1 gene. This mouse provides an excellent model system for the human disease.

Progress
In the first year of the project we overcame the Prom1 mouse fertility issues we experienced and proceeded with our experiments utilising these mice. We observed rapid retinal degeneration in our mouse model (75% photoreceptor loss within one month). We next examined whether or not application of a drug prevented the progression of retinal degeneration. We found that this drug helped to partially slow the retinal degeneration. We performed 2 trials with the drug and are currently trying to optimise the dose and delivery of this drug in order to see better results. We are also trying to determine the underlying mechanisms using biochemical analyses of the treated and untreated eyes.

This drug is already widely used in clinical trials for other diseases, so if it is effective in treating the PROM1 mutant mice it should be directly applicable to human retinitis pigmentosa patients and therefore quickly lead to early phase clinical trials. This study has the potential of making a significant contribution to the understanding of retinitis pigmentosa and the development of a novel therapy.
8. MOLECULAR GENETIC STUDIES OF SPACEMAKER, A PROTEIN IMPLICATED IN AUTOSOMAL RECESSIVE (RP25) RETINITIS PIGMENTOSA

Wendy Mustill & Shomi S Bhattacharya
UCL Institute of Ophthalmology

Retinitis pigmentosa (RP) is a debilitating condition of the retina of the eye that progresses from difficulty seeing in the dark and can lead to complete blindness. RP is a hereditary disease, and can be passed from parent to child in several different genetic processes. For example, 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).

Mutations in the genetic locus RP25 have been found to cause arRP in patients around the world. The gene affected is called \textit{EYS} and is a major cause of arRP. It encodes a protein called Spacemaker (SPAM). We know that in flies SPAM is very important in organizing the compound eye, and we think that in humans and other animals it is involved in organization of the retina, which is the nervous tissue of the eye responsible for vision and is connected to the brain via the optic nerve.

We have modeled \textit{EYS} mutations identified from arRP patients using cultured cells in the lab. This has been necessary since the gene is not expressed in mice and therefore we are unable to create a mouse model of this gene defect. When we looked at the cells using a microscope, we could see that the cells were abnormal and they quickly died. This may illustrate how the retina of patients with \textit{EYS} mutations degenerates, leading to loss of sight.

The cell model gave us insight into RP, but this is a simple system compared to the human retina. We are currently trying to understand the other factors involved in retinal degeneration due to \textit{EYS} mutations. We know that in flies SPAM works in concert with another protein called Prominin, and that humans also express this protein. Computer modeling has also indicated another protein, Dystroglycan, that SPAM may interact with in humans. These potential interactions are currently being investigated using a cell model.

In addition to intensifying our cell model work, we are investigating the role of SPAM in a fish model (zebrafish, \textit{Danio rerio}). We hope that this will help us to understand the role of SPAM better in development of the eye. The zebrafish is an excellent model to use as we can manipulate eggs at a very early stage in formation when they consist of only one cell. At this stage we can cause the \textit{EYS} gene to be expressed at higher or lower levels than normal. By comparing resultant fish to normal fish, we hope to understand the role of \textit{EYS} in formation of the retina. Further to this, the absence of \textit{EYS} expression will help us understand RP as several \textit{EYS} mutations identified in patients resulted in expression of only a fragment of the gene (and also only a fragment of the SPAM protein).

Characterising the \textit{EYS} gene and its mutations could lead to new diagnostic tests and therapies for arRP patients. These tests would allow more accurate identification of children who have inherited \textit{EYS} mutations from their parents, and are therefore likely to develop RP.

We are grateful to RP Fighting Blindness for funding this project.
9. MODIFICATION OF MUTANT BESTROPHIN-1 TO PREVENT RETINAL DEGENERATION
Dr Forbes Manson, Dr Lisa Swanton
University of Manchester

Bestrophin-1 is a protein located in the pigmented layer of the eye’s retina, the retinal pigmented epithelium (RPE). The role of the RPE is to support the light-sensing photoreceptors of the retina and if this function is compromised the photoreceptors will die, leading to poor vision and blindness. The bestrophin-1 protein is located in membranes, either at the cell surface where it may regulate the passage of chloride ions, or within the cell where it may help regulate the internal storage of calcium ions. However the exact role of bestrophin-1 in the RPE remains unclear. Mutations in the gene for bestrophin-1, BEST1, cause a range of retinal diseases that are collectively known as bestrophinopathies. Depending on the particular disease, these can be inherited in an autosomal dominant (50% chance of a child developing the disease from one affected parent) or an autosomal recessive (25% chance of a child developing the disease from two carrier parents) manner.

In order to function correctly a protein must fold into its proper three dimensional shape and be transported to the correct cellular location. Mutations in a protein can disrupt these processes, leading to abnormal localisation and premature degradation of the mutant protein. In our previous work we have used cells growing in culture to model the RPE, and have found that mutations in bestrophin-1 negatively affect its stability, location and ability to conduct chloride ions. The aim of the current work is to identify small molecule drugs that can restore the proper localisation and function of mutant bestrophin-1.

Small molecules known as chemical chaperones have been used in the past to correct the function and cellular localisation of mutant rhodopsin, mistakes in which are a major cause of retinitis pigmentosa. It is hoped a similar strategy can restore the function of bestrophin-1 and this may present new therapeutic approaches for the treatment of bestrophinopathies.

In order to allow us to screen potential drug candidates, we have engineered a cell type that is similar to the RPE to permanently express normal bestrophin-1 or various mutant forms of bestrophin-1 that cause autosomal recessive bestrophinopathy (ARB) in humans. We have determined the optimal conditions to express bestrophin-1 and ensure that normal bestrophin-1 is correctly localised to the cell membrane. We are now in the process of testing a range of small molecules in this cell system, and for each will determine whether they increase the level of the mutant protein and promote the correct localisation of the rescued protein within the cell. Positive ‘hits’ from this screen will then be tested for their ability to restore the function of mutant bestrophin-1.
10. RP FIGHTING BLINDNESS CENTRE FOR THE DEVELOPMENT OF GENE THERAPY FOR INHERITED RETINAL DYSTROPHIES
Prof R Ali
UCL Institute of Ophthalmology

The inherited retinal dystrophies (IRDs) are a large group of sight loss conditions that are caused by mutations in over 150 genes. IRDs represent a major cause of sight loss, affecting around 1/3000 people, with no effective treatments currently available. Research over the past 25 years has enhanced our understanding of how genetic mutation leads to sight loss, leading to new potential treatments such as gene therapy and cell replacement.

We are developing clinical trials of gene therapy for many forms of IRD, having established robust proofs-of-principle in several animal models of disease. The core expertise within our expanding Centre allows us to translate laboratory research findings into clinical trials of new treatments – as shown this year by the development of gene therapy for achromatopsia caused by mutations in the \textit{CNGB3} gene. This is the commonest form of achromatopsia, a disease in which abnormal cone photoreceptor function leads to very poor central vision, including absent colour vision and extreme sensitivity to light. We have recently demonstrated that delivering a working copy of the \textit{CNGB3} gene using an engineered virus can restore sight in a mouse model of achromatopsia (Carvalho et al, Human Molecular Genetics, 2011).

We are now working towards developing a clinical trial of gene therapy for this condition. This year we have developed a clinical trial protocol and submitted it for consideration by the regulatory authorities and it has now received approval from the National Research Ethics Committee (NRES). We have also applied for further funding that will allow us to complete the necessary pre-clinical testing and to conduct the trial itself and have secured £2.1M funding from the Medical Research Council (MRC). This will enable us to produce a clinical grade adeno-associated virus (AAV) vector that carries the \textit{CNGB3} gene and assess its safety in pre-clinical laboratory tests. With ethical approval and funding now in place, we will continue the pre-clinical safety testing and will identify and assess potential participants before seeking approval from the Medicines and Healthcare products Regulatory Agency (MHRA) to begin the trial. We are continuing to conduct baseline assessments of visual function on potential trial participants, and we aim to start the trial in 2014/2015.

We have previously shown that gene therapy can be safe and effective in our clinical trial for Leber congenital amaurosis (LCA) caused by defects in \textit{RPE65}, establishing proof of principle that gene therapy can be used to treat RPE cell defects. The achromatopsia trial forms a crucial element of our research pipeline. We aim to use this trial to demonstrate proof of principle that gene therapy can be used to treat photoreceptor cell defects.

We also continue to progress towards clinical trials in other forms of inherited sight loss. We have recently completed our first clinical trial of gene therapy for LCA caused by \textit{RPE65} deficiency, and we will report on long-term follow-up of patients later this year. Although we have observed substantial improvements in retinal sensitivity and night vision in many subjects, we believe that the therapy could be
improved further. We have now developed a new optimised vector and tested it in small and large animal models and are now planning a new trial for LCA caused by RPE65 deficiency using this new optimised vector.

We also continue to work towards a trial for LCA caused by mutations in the AIPL1 gene, by identifying and assessing potential trial participants.

Patient and public engagement with our research programme remains key, and this year we have been very active in communicating our progress:

- Updated the content and accessibility of our online resources, including our website, a new blog and social media accounts on Twitter and Facebook.
- Developed a system for engaging with patient enquiries, through the dedicated email address eye.info@ucl.ac.uk – this is linked to a new database of patient contacts, who stay up-to-date with our research through our new newsletter
- Secured funding to undertake three Patient Awareness Days, following the success of Retina Patient Day 2012 (those interested in future events should contact us at eye.info@ucl.ac.uk)
11. MECHANISMS OF PHOTORECEPTOR DEGENERATION IN CHOROIDEREMIA
Prof Clare Futter
UCL Institute of Ophthalmology

Choroideremia (CHM) is a retinitis pigmentosa (RP)-related disease characterized by gradual retinal degeneration and vision loss. The photoreceptors and the adjacent retinal pigment epithelium (RPE) degenerate, as well as the choroid that provides the blood supply to the RPE. CHM is caused by mutation in Rep1, which is required for the function of a family of proteins called Rabs. Rabs regulate the trafficking of membrane proteins from one region of the cell to another and so are essential for cellular function. We predict that loss of Rep1 function would cause partial defects in multiple membrane protein trafficking pathways in both photoreceptors and the RPE, gradually leading to their death. We are using CHM models that we have generated to determine how photoreceptors die in this disease and other forms of RP where protein trafficking is defective.

We have established the time course of photoreceptor death in our CHM models and have now generated retinal sections from eyes at time points at which photoreceptor cell death is maximal. We are labeling these sections with established markers of cell death so that we can identify the numbers and distribution of dying cells and determine the cell death pathway that has been activated. In parallel we are using high resolution electron microscopy to i) analyse in more detail the way in which the cells die, ii) identify morphological changes that occur in the photoreceptors as a result of defects in trafficking pathways and iii) analyse the distribution of the visual pigment, rhodopsin. Rhodopsin is made in huge quantities by photoreceptors and must be correctly trafficked in order to function. Defects in rhodopsin traffic have been linked with several forms of RP and defects in rhodopsin traffic could lead to the activation of cell death pathways in CHM.

These studies should lead to a greater understanding of the regulation of rhodopsin traffic and how defects in that traffic can contribute to photoreceptor cell death in CHM and other forms of RP.
12. CHARACTERISING A NOVEL GENE FOR X-LINKED CONE-ROD DYSTROPHY AND X-LINKED RP
Prof Alison Hardcastle, Dr Michel Michaelides, Prof Mike Cheetham
Postdoctoral fellow Dr Jessica Gardner
UCL Institute of Ophthalmology

Background
Retinitis pigmentosa (RP) is a degenerative blinding disease, in the first instance affecting the rod photoreceptors. In contrast, a related form of retinal degeneration, cone-rod dystrophy, initially affects the cone photoreceptors. Genetic studies have resulted in remarkable insights into which genes and molecular pathways are essential for normal vision.

The X-linked forms of retinal degeneration are very severe, affecting males (although not exclusively) in the family and often presenting in the first decade of life. The purpose of our research is to identify a new gene for X-linked forms of retinal degeneration.

Progress
In some of our families, where X-linked inheritance is suspected, we have excluded the known X-linked RP genes, RPGR and RP2, as the cause of their condition. We have also excluded rarer causes of X-linked retinal degeneration that we recently identified, namely the cone opsin genes and the gene OFD1. Through collaboration, we have recruited new families to our study. Every new family we study will help in the search for the missing gene. Some families have been diagnosed with RP, and others with cone-rod dystrophy. We have pinpointed a region on the X-chromosome where we believe the new gene causing these conditions must lie. We have applied state-of-the-art genetic techniques to home-in on the region that contains this missing gene, and we have excluded known genes within the region as causes for these conditions. Using these techniques we have identified unique DNA changes that lie in ‘junk DNA’ in-between genes. Currently we are investigating the possibility that these regions of the chromosome may not be ‘junk DNA’, but instead contain an as yet unrecognised gene.

Future work
Identification of the missing X-linked gene is a technical challenge, and we will apply different techniques to address these challenges. If we are successful, it will allow us, not only to provide a precise molecular diagnosis for the patients and their families, but also to explore why the faulty gene results in degeneration of the rod and cone photoreceptors.