RP Fighting Blindness

Research Reports May 2011 – April 2012

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CHAIRMAN, RP FIGHTING BLINDNESS MEDICAL ADVISORY BOARD

(An extract from the charity’s Annual Report for 2011)

RP Fighting Blindness is pleased to reproduce project reports submitted during the year by the researcher we have funded.

It is an important time for the charity as it strives to increase its influence and contributions to RP research, and an important time for research with a number of exciting new avenues being explored, including several clinical trials. Clinical trials of treatments using gene and cell replacement, as well as pharmaceutical approaches, are very exciting and tantalisingly suggest that effective therapies might be generally available in the next few years.

However, there are a few things to bear in mind; clinical trials are very expensive (and can) take several years, and history tells us that promising looking treatments often fail during this process. Therefore, it is important that we continue to invest in laboratory science that improves our understanding of retinal dystrophies … Nonetheless, great strides forward are reflected in several high profile announcements in 2011 and in the work we continue to fund ourselves.

The application of stem cell based therapy to retinal dystrophies took a major step forward. Further trials using gene therapy were announced in the USA and here in the UK and we have committed further funding to this approach by approving our largest ever grant to support Professor Robin Ali’s work at the Institute of Ophthalmology. Retinal implants from Second Sight Inc. are being used in patients in France, Germany and Italy - the first commercially available treatment for endstage RP. The company QLT recently announced that an orally taken synthetic retinoid may be an effective treatment for particular forms of Leber Congenital Amaurosis, and intriguingly, a recent report suggests that diets high in omega-3 (in this study in conjunction with vitamin A) could be effective in slowing the progression of RP.

Our own programme of new grants in 2011 was more diverse and a larger investment than ever before.

Prof Paul Bishop, Chair of Medical Advisory Board, April 2012.
FUNCTIONAL ANALYSIS OF RPGR

Prof Alan Wright and Dr Xinhua Shu, MRC Human Genetics Unit, Crewe Road, Edinburgh EH4 2XU

Original Grant aims:

(1) determine the role of RPGR in cilia formation by knocking-down each isoform and analysing RPGR posttranslational modifications.

(2) establish whether RPGR has a guanine nucleotide exchange factor (GEF) role for an unknown small GTPase by screening candidate effector GTPases that are associated with basal bodies or cilia.

(3) analyse the role of RPGR in melanosome transport;

Summary:

Mutations in the RPGR gene cause one of the most common and severe forms of inherited retinal dystrophy. In spite of numerous studies, the exact physiological function of RPGR is not yet clear, nor is the disease mechanism. The aim of this project was principally to elucidate the normal physiological functions of RPGR to complement on-going studies into disease mechanisms resulting from RPGR mutations and into RPGR gene replacement therapy. We focused on the two most promising approaches: firstly, examining the cellular consequences of RPGR knockdown in a cellular model of ciliogenesis; secondly, testing the hypothesis that RPGR can function as a guanine nucleotide exchange factor (GEF) for a small GTPase.

The consequences of RNAi-mediated knock-down of RPGR in hTERT-RPE1 cells was examined in detail. RPGR-deficient cells exhibited a reduced number of cilia, slower cell cycle progression, and impaired attachment to fibronectin, but showed no evidence of migration defects in a wound-healing assay. An unexpected observation was that RPGR knock-down cells also had stronger actin filaments, a novel finding which could explain all of the above phenotypic abnormalities. We showed that this is accompanied by dysregulation of basal and fibronectin-induced signalling, together with lower β1-integrin expression. The data point to the importance of RPGR both in ciliogenesis and in the regulation of actin stress filaments. These findings are both novel and potentially relevant to disease pathogenesis, for example if RPGR affects maintenance of photoreceptor connecting cilia. The RPGRIP1 protein interacts with and localises RPGR to the photoreceptor connecting cilium[1] and is implicated in nascent disc formation, which is thought to involve long-range vesicle transport and docking within the cilium[2, 3], which recent data show can be mediated by the actin cytoskeleton[4].
Publications acknowledging BRPS (GR568) funding:


Under review:
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

Mutations in the \textit{RP2} gene on the X-chromosome cause a severe form of retinitis pigmentosa, which primarily affects young boys. In the first decade of life patients suffer from night blindness and impaired peripheral vision, caused by photoreceptor cell death. As the disease progresses, central vision becomes also restricted, leading to blindness, usually in the forth decade of life. We have previously characterised the RP2 protein in the eye and shown that the patient sequence changes lead to disease either through the protein failing to reach to the correct part of the photoreceptor cell or because the altered proteins are recognised as faulty and are degraded. However, RP2 is found in every tissue in the body, not just the eye. It is therefore vital to understand the special function of RP2 in the eye, as it is not clear why loss of RP2 function causes only retinal problems. This research project is aimed at identifying the normal function of RP2 in the retina and identifying and characterising proteins, which work in conjunction with RP2 in the retina.

Progress

We have made considerable progress towards achieving our aims. Recently, we located RP2 in very specialised structures in the light sensitive photoreceptors in the retina, which are crucial for the transport of proteins to the place for detecting light. This is important for cell survival and suggests lack of RP2 could 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).

In addition, we have identified an important retina protein as a specific cargo of RP2. This cargo protein plays an important role in turning light detected by the retina into an electrical 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 move this protein to the membrane of the photoreceptor cell and help its transport to the light detecting area of the cell. This finding supports our hypothesis that RP2 has a very special function in the eye, by ensuring important proteins reach the correct
structures in the retina so they can perform their function. This finding further enhances our understanding of RP2 function in the eye and has been recently published (Schwarz et al. 2012).

Future work

Since the start of the project in October 2006, we have been using RP2 as ‘bait’ to pull out binding proteins using a range of genetic and biochemical screens. The RP2 bait has ‘caught’ several proteins that we believe may interact with RP2 in the retina, which have not been reported to bind RP2 before. For example, one of these novel RP2 interacting proteins is important for the movement of proteins and we believe it may act with RP2 to regulate the transport of proteins in the eye. We are now experimentally testing this hypothesis using cell models and protein based assays.

Publications


DEVELOPING THERAPIES FOR RHODOPSIN RP

Professor Mike Cheetham, Postdoctoral fellow Dr David Parfitt, UCL Institute of Ophthalmology

Background

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. There are currently no effective treatments for RP. Inherited changes in rhodopsin, the light sensing protein of rods cells, are the single most common cause of autosomal dominant RP and the majority of changes cause protein to not take up the right shape or ‘misfold’ this means it will not work properly. The cell normally removes the misfolded rod opsin, but if the rod opsin 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 faulty 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. This BIG Lottery funded project aims to test some of these compounds in animal models

Progress

In the first year of the project, additional compounds were tested in our cell model. We also imported rats and mice that had been genetically altered to be models for human rhodopsin retinitis pigmentosa. During the second year, we completed baseline studies of the retinal survival and function on two types of rat model, and the normal control rat, testing both the function and structural preservation. Baseline studies of the visual function and retinal survival in the mouse model were also compared to control mice. As the baseline results were in line our expectations and
with previous studies, we started testing compounds that were shown to reduce protein misfolding and aggregation in our cell model in these animal models.

At present, two trials have been completed. In the first trial, control animals and the rat models were treated with a ‘chemical chaperone’ drug, previously found to be effective in our cell culture studies. Unfortunately, no difference was observed in the responses of treated animals when compared to untreated animals, for either the functional testing or in the survival of the retina. In the second trial, the mouse model and control animals were treated with a drug that stimulates the natural cellular machinery for dealing with faulty proteins. The preliminary results from this trial suggested an improvement in the function and survival of the retina in the mouse RP model when compared to untreated mice. We have repeated this trial with the mouse model and extended the study to include the rat models. Encouragingly, preliminary results from these replication studies suggest there also appears to be an improvement in the function and survival of the retina that has reduced the effect of the RP.

**Future work**

Further analyses of this drug treatment are required to confirm this promising finding. Detailed structural and biochemical studies of the treated retinæ are underway to determine which components of the cellular machinery that deal with damaged proteins might be responsible for the potential improvements.

**Publications**

A paper describing the effect of some drugs on mutant rhodopsin in cell models is in preparation.

A manuscript describing the trials with the animal models will be prepared when the studies are complete.
WIDENING ACCESS TO GENETIC TESTING SERVICES FOR HEREDITARY EYE CONDITIONS

Prof Graeme Black, St Mary’s Hospital, Manchester

The discovery of new genes and mechanisms underlying genetic eye disease has had clear scientific benefits. Families currently request genetic testing in the hope that it will (a) confirm inheritance patterns and risks to family members/offspring and (b) provide information for management and potential treatment in the future. This has generated a clear need for genetic testing to support the management of affected patients and their families.

Thanks to past support by RP Fighting Blindness (RPFB) the North West Regional Molecular Genetics Laboratory (NWRMGL) has established testing for X-linked and autosomal dominant retinitis pigmentosa as well as macular dystrophy. These services are now NHS funded and independent of the Society. This collaboration has demonstrated the clear utility of genetic testing and this has provided the RPFB and its members with a strong influence on prioritisation of genetic testing within the NHS.

However due to factors outside the control of the referring clinicians, issues around equity of access to genetic testing in the UK still exist, and RPFB and the NWRMGL are committed to examining the causes of inequity and working hard to break down the barriers.

In 2010 the NWRMGL commenced a second RPFB funded work programme entitled:

Widening access to genetic testing services for hereditary eye conditions (GR570)

The purpose of this grant was to provide support for on-going work aimed at:

(i) Extending laboratory tests to include new technologies that will broaden the potential for genetic testing to all UK families with an inherited form of retinal degeneration.

(ii) Examining the existing barriers by means of a critical assessment of national referral trends.

Looking back at the first year of our current grant (Grant GR570) we are delighted to report significant progress. In January 2012 the NWRMGL launched an ambitious new test with the potential to simultaneously analyse all genes known to cause retinal degeneration. This test development, validation and successful implementation was jointly funded by RPFB and Fight for Sight who were both acknowledged in a National Press release. Where it is deemed appropriate this test is now available to UK NHS patients via their local referral centres on a not-for-profit basis (again funding for this clinical test is now from local clinical budgets and independent of the Society). We hope that by demonstrating the utility of this high
profile service, we will continue to inform NHS commissioning as to the value of genetic testing to both ophthalmologists and geneticists as well as families affected with genetic eye disease.

In the next two years we look forward to investigating the impact of this new service as well as developing further tests relevant to RPFB members. These are exciting times and with the support of RPFB we will ensure that ophthalmic genetic services within the NHS remain at the forefront of innovation and technology.
MOLECULAR GENETIC STUDIES OF SPACEMAKER, A PROTEIN IMPLICATED IN AUTOSOMAL RECESSIVE (RP25) RETINITIS PIGMENTOSA & UNRAVELLING THE DISEASE MECHANISM FOR PRPF31 RP

Prof Wendy Mustill, Prof Giovanna Alfano & Prof Shomi Bhattacharya, Institute of Ophthalmology, University College London

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). We are working on two related RP 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.

EYS is a major gene located at the RP25 locus of chromosome 6 which is implicated in autosomal recessive retinitis pigmentosa (arRP). It has recently been found that many RP patients from across Europe and Asia have mutations in EYS. This makes EYS an important gene to study in order to understand RP and try to develop a treatment to help RP patients and their families.

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). We have demonstrated that SPAM is present in a layer of the retina of the eye in humans and animals, and we think that SPAM is an important factor in formation of a normal retina structure.

Our recent experiments have been investigating what happens to the protein SPAM when the gene EYS is mutated. We are fortunate to have lots of information on RP patients with EYS mutations, and we have found several different mutations in EYS. We have recreated some of these documented mutations using a molecular biology technique called site directed mutagenesis (SDM). This allows a specific mutation to be introduced to DNA, thus tailoring our experiments to exact patient details.

When we achieved the EYS mutations we wanted, we introduced EYS DNA to cultured cells in vitro via a technique called transfection. We then compared cells which had been transfected with mutant EYS DNA to cells transfected with normal EYS DNA. The results were assessed under a microscope and were quite startling. Cells transfected with normal EYS DNA looked typical, just like cells that had not
undergone transfection. The protein SPAM was present in a diffuse pattern throughout the cell. Cells that had been transfected with mutant DNA, however, had formed aggregates of protein inside the cells, and the cells were dying. This may illustrate how RP patients lose the use of the photoreceptor cells in their retinas when they have an EYS mutation.

The aim of our second project is to develop strategies enabling rescue of retinal degeneration, caused by mutations in PRPF31 (RP11) gene, using a gene therapy approach. The PRPF31 gene encodes a pre-mRNA splicing factor. The splicing is a modification of RNA after transcription (copy of the DNA genetic code), in which introns (non-coding sequences) are removed and exons (coding sequences) are joined together, before synthesis of the protein.

In addition four pre-mRNA splicing factor genes PRPF8, PRPF3, PRP6 and PAP-1 have also been implicated in adRP. Identification of these genes revealed a novel mechanism of photoreceptor degeneration distinct from those operating in other forms of adRP, where mutations have previously been identified in structural proteins and transcription factors. The rod photoreceptor cells are 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.

It has been found that PRPF31 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 RP11 suggests that adRP patients affected by mutations at the RP11 locus have lost one copy of the gene, resulting in a decreased amount of protein synthesis. An interesting feature seen in almost all 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 PRPF31 in the general population. In addition, it was found that people who may otherwise have RP but expressed the PRPF31 allele of 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 PRPF31 may prevent clinical manifestation of the disease in RP patients with normal expression.

Based on our previous findings the main objective of the current project is to develop gene therapy approaches underlying rescue of retinal degeneration using sub-retinal RP11 gene delivery. The idea is to target the photoreceptor cells and to increase their amount of functional protein. To address this point we prepared specific constructs (AAV) carrying a wild-type RP11 functional copy. The constructs have been tested in in vitro cell culture. We are now ready to go ahead testing PRPF31 over-expression in mouse eyes.

Many retinal dystrophy genes have been identified so far, but quite a few are still unknown. Identification of novel retinal dystrophy genes is also one of our goals. We recently came across an uncharacterized new gene, orf90 by bioinformatics
analysis. Preliminary genetics and functional studies suggest orf90 is likely to be a novel putative eye disease gene.

We believe that establishment of gene therapy strategies for one of the major adRP genes (RP11) as well as the identification of a novel eye disease gene would be very important achievements in better understanding and developing novel therapies for retinitis pigmentosa.

We are grateful to RP Fighting Blindness for continued support of our research projects.
CHARACTERISING A NOVEL GENE FOR X-LINKED CONE-ROD DYSTROPHY AND X-LINKED RP

Prof Alison Hardcastle, Dr Michel Michaelides and Prof Mike Cheetham, Postdoctoral fellow Dr Jessica Gardner. UCL Institute of Ophthalmology

Start date 1st August 2012

Retinitis pigmentosa (RP) is a degenerative blinding disease, primarily affecting the rod photoreceptors. A related form of retinal degeneration, cone-rod dystrophy, primarily affects the cone photoreceptors. Genetic studies have resulted in remarkable insight into which genes and molecular pathways are essential for normal vision. This has, in turn, resulted in the development of animal models for testing treatment strategies.

The X-linked forms of retinal degeneration are severe, often presenting in the first decade of life in affected males. Genes on the X-chromosome have already been identified as causing X-linked retinitis pigmentosa (XLRP) or X-linked cone-rod dystrophy (XLCORD), however in some families we have found that none of these previously identified genes are involved. In these families, diagnosed with either XLRP or XLCORD, we have pinpointed a region on the X-chromosome where we believe an as yet unidentified gene causing these conditions must lie.

The purpose of our research is to identify this new gene. Identification of the gene 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 different clinical outcomes of rod-dominant (RP) or cone-dominant (CORD) retinal degeneration.
MODIFICATION OF MUTANT BESTROPHIN-1 TO PREVENT RETINAL DEGENERATION

Dr Forbes Manson, Dr Lisa Swanton, The University of Manchester

Ms Carolina Uggenti started as a PhD student on this project on 1st January 2012 and has made excellent progress in her first 4 months.

The bestrophin-1 protein is altered at a genetic level in several retinal degeneration diseases collectively known as bestrophinopathies. In the current research we will characterise the different cellular mechanisms underlying the bestrophinopathies and test a range of therapeutic chemicals to try to restore the normal function of bestrophin-1.

Alterations to proteins can cause them not to fold properly and as a result the cellular machinery may degrade them or fail to send them to the correct location. To study this process in cultured cells the bestrophin-1 protein has to be made in sufficiently high quantities in a relevant cell type that we can grow in the laboratory. Bestrophin-1 is normally present in the pigmented layer of the retina called the retinal pigmented epithelium (RPE) and we have unsuccessfully tried to use five different types of RPE cell to make bestrophin-1. To overcome this issue we have used a new model cell system that has been modified so that it is possible to add any gene of interest to the same position in the cell’s DNA and express it at high levels in a controlled manner. We will insert normal and different disease-causing versions of bestrophin-1 into this model cell system so we can track how the different bestrophin-1 proteins are made. We have made the necessary DNA constructs that are required to insert the different versions of the bestrophin-1 gene into the recipient cells, have optimised the conditions for introducing the DNA constructs into the cells, and have determined the antibiotic concentration that is necessary to isolate the cells that have successfully integrated the introduced genes. Following on from this preliminary work we have generated three cell lines that permanently express the gene that we inserted. These are currently being analysed by using fluorescently-tagged antibodies to determine the protein’s cellular location.

We are just about to perform the first experiments to determine whether the altered versions of bestrophin-1 that cause disease have a different turn-over rate in the cell. To do this we will label the cells with radio-activity and then follow how the labelled bestrophin-1 protein disappears over time. This will provide important data on whether particular parts of the cell machinery process normal and disease-causing versions of the bestrophin-1 protein in different ways.
Rhodopsin is the rod cell photoreceptor pigment that is responsible for vision in dim light conditions. The light–sensitive rhodopsin pigment is formed when the protein (opsin) combines with a small molecule called 11-cis retinal. Mutations in the rhodopsin gene are one of the most frequent causes of Autosomal Dominant Retinitis Pigmentosa (ADRP). Most of these mutations destabilise rhodopsin and prevent it from folding correctly. Accumulation of misfolded rhodopsin ADRP mutants in rod cells is thought to be the trigger for death cell. Our goal is to understand why (and how) these rhodopsin ADRP mutants misfold, and to identify new ways to stop this from happening. We are also conducting experiments to examine how the defective mutant rhodopsin is distributed in cells and characterising the proteins (gatekeepers) that interact with misfolded rhodopsin mutants. This RP Fighting blindness grant has allowed us to develop methods and characterise rhodopsin ADRP mutants as follows:

We have inserted the mutant rhodopsin genes into the chromosome of cells. We have also tagged the mutant rhodopsin genes with a fluorescent protein called GFP. This allows us to visualize rhodopsin as it is made and trace its progress to the cell surface. We have now developed methods to observe this process in live cells.

Unlike normal rhodopsin, many misfolded ADRP mutants get trapped in the interior of cells. When 11-cis retinal is present, some ADRP rhodopsin mutants can reach the cell surface. The trapped misfolded mutant rhodopsin proteins are recognised and retained by ‘gatekeeper’ proteins that bind tightly to these mutant rhodopsins. The interaction is tight enough such that both proteins can be isolated together. Our results thus far suggest that these gatekeeper bind more tightly to the most severely misfolded mutants. We are now trying to examine these interactions in more detail and are attempting to identify additional gatekeeper proteins.

We have found that a small group of rhodopsin ADRP mutants can fold (are rescued) and form pigment when made in the presence of 11-cis retinal. We predict that these mutants will also fold, to some level, in rod cells. However, these folded pigments remain defective compared to normal rhodopsin. These remaining defects may explain why RP still develops in individuals carrying these mutations.

In a further set of experiments we have shown that some rhodopsin ADRP mutants can be repaired by bridging two regions of the rhodopsin protein. These repaired mutants also do not form long-lasting associations with the gatekeeper proteins. Importantly, repaired mutants act more similar to normal rhodopsin. These results
suggest that there may be other ways to stabilise rhodopsin ADRP mutants, perhaps using small molecule drugs.

Methods we have developed, in part, during this funding period have contributed to a publication: ‘Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy of G Protein-Coupled Receptors. Joseph Goncalves, Markus Eilers, Kieron South, Chikwado A. Opefi, Philippe Laissue, Philip J. Reeves and Steven O. Smith. Methods in Enzymology (in press).’
IDENTIFYING NOVEL GENE MUTATIONS IN INHERITED RETINAL DEGENERATIONS

Dr Stephanie Halford, University of Oxford

The retina, the light sensitive tissue in the eye, is a complex structure consisting of several layers of neurons which are connected by synapses. However, the only neurons that are directly sensitive to light are the photoreceptor cells, the rods and cones as well as a small subset of retinal ganglion cells. The rods and cones are the cells responsible for vision, the rods respond to dim light and provide black and white vision and the cones respond to bright light and give colour vision. The human retina contains approximately 120 million rods and 6 million cones, the cones are located in the centre, the macula, and the rods more in the periphery.

Inherited retinal dystrophies, leading to either partial or total blindness, affect approximately 1 in 2500-3500 people, but many more suffer from age-related macular degeneration (AMD) in later life with 25% of people over the age of 70 affected by AMD. This diverse group of disorders includes retinitis pigmentosa (RP) and various macular degenerations. Patients with RP present with narrowing visual fields and night blindness, while those with diseases of the macula lose their central vision first. Currently the online database RetNet (http://www.sph.uth.tmc.edu/RetNet/) lists 224 chromosomal locations that are involved in various types of retinal disease. The gene underlying the disease has been identified in 183 of them. Comparison of these figures to those for 1995, 55 regions and 20 genes, shows that although massive strides have been made in the last 10-15 years in identifying the regions associated with disease and the causal genes more work is still required.

Dysfunction or death of rod and cone photoreceptors is the primary cause of blindness in the vast majority of these retinal degenerative diseases. The protein products of many of the disease genes that have already been identified have well defined and specific functions in the photoreceptors, for example proteins involved in processing light information into a visual image. Therefore a catalogue of the specific genes that are expressed in the photoreceptor cells will provide a comprehensive database of candidates for retinal degenerations and will help our understanding of how photoreceptor cells develop, function and are maintained.

We have compiled such a catalogue of genes by utilizing our unique access to mice lacking rod and cone photoreceptors. Comparison of the genes expressed in the retina of normal mice with those lacking rods and cones has enabled us to establish a database of genes that are expressed in rods and cones. As well as identifying genes with a known role in the retina we have identified a subset of novel genes with no previously known retinal function. We are currently characterising these novel genes and determining in what types of cells they are functional. We are also assessing their role in various retinal degenerations of unknown origin to determine if
mutations in these genes give rise to retinal disease, in turn improving patient screening and guiding future treatment of these conditions.
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 are continuing to acquire human iris tissue, which we receive as a by-product of glaucoma surgery and from donor eyes which has been consented for research. We are using this tissue to derived immature (progenitor) cells. Unlike other types of progenitor cells, iris derived cells cannot be grown in the laboratory indefinitely therefore we will continue to generate iris progenitor cells throughout the duration of this project. In order to manipulate these cells into becoming like photoreceptor (light-sensitive) cells, we have been genetically modifying the cells with a transcription factor known as cone rod homeobox (Crx). This small piece of genetic material is specific to photoreceptor cells. Crx acts as a switch to turn on development of these highly specialised light sensitive cells. We have been using Crx in the hope that it will be sufficient to drive our cells to look and function as photoreceptors. We are continuing to analyse the results of these experiments.

Using healthy pieces of retina we have optimise the model of retinal damage, which we propose to utilise in order to study cell integration and migration. Using a drug, we have produced cell death which is specific to the light sensitive retinal cells. This pattern of cell death mimics that seen in retinitis pigmentosa. We have now found the optimum concentration and exposure time to produce photoreceptor cell death while preserving the viability of the other cells in the retina. The next step for this part of the project will be to grow the iris progenitor cells with these pieces of diseased retina, in order to determine if the cells can become part of the retina.
Transplantation of cells into the eye attached to scaffolds has 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 does not degrade. 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. To maximise cell attachment we have modified the surface of the scaffolds. In our experiments we found that changing the surface by simple chemical modification can increase both cell attachment and viability. The next step for this part of the project will be to optimise how we can deliver these scaffolds safely into the eye and to determine how well the scaffolds are tolerated within the eye.

Once again we thank RP Fighting Blindness for their invaluable support of this work.
GR564 (B1/9/08) – Identification of genes causing autosomal recessive retinal disease exploiting novel loci derived from autozygosity mapping of consanguineous pedigrees

GR569 (B1/10/09) - The pursuit of novel genes causing autosomal recessive retinal dysfunction from loci identified through autozygosity mapping techniques in consanguineous families

Professor A Webster, Moorfields Eye Hospital, London

GR564 and GR569 are two grants 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 in humans due to mutation of known genes. This is vital in order to understand which disorders are best candidates for gene-replacement, and cell-replacement therapies and which metrics of retinal dysfunction and structure can be used to assess efficacy, ii) to determine novel genes causing retinal dysfunction and/or degeneration. There are many more such genes still to find, and each one gives insights into human retinal physiology and pathology. 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, and has been built up over many years, with valuable contributions in the past from the charity. Moreover, the technology available to investigate the patients including imaging, electrophysiology and psychophysical testing 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.

The study includes the appraisal of clinical data as well as experiments in the molecular genetic laboratory. Since the start of the first grant in February 2010, 131 families with recessive inherited retinal disease have been ascertained and analysed using genome-wide SNP scans. Families were either consanguineous in which one or more affecteds were assumed to be autozygous for a chromosomal segment harbouring the disease gene, or included multiply affected siblings to non-related parents, in which case, traditional linkage analysis was applied. Many families linked to known genes. Those unlinked, were anlayed further using high-throughput sequencing (HTS) in collaboration with the UCL Genetics Institute. This technique allows the simultaneous determination of the majority of genes in the human genome. Through this approach two novel genes have been discovered, each leading to further insights into human retinal function. KCNJ13 is a gene encoding a potassium channel, which when absent causes abnormal development and later
degeneration of the retina. *PLA2G5* is a gene encoding an enzyme acting on phospholipids, which when mutated leads to the accumulation of debris beneath the human retina causing a specific condition known as 'benign fleck retina syndrome'.

The same strategy has identified *TSPAN12*, a gene encoding a protein involving cell-signalling, as a cause of recessive vitreoretinopathy. Moreover, through work on this project, families with mutations in known genes including *OAT, GRM6, RDH5, KCNV2, RDH12, LRAT, C2orf71* have been identified and studied clinically to give further insights into human retinal disease, each leading to a publication.

The resource, now, is more extensive and powerful than before, due at least in part to the investment and support of the charity. Many novel genes have been highlighted by the strategy above and are being investigated to determine the consequence of their mutation in patients. It is highly likely that further insights will be gained from this burgeoning resource in the future.
MECHANISMS OF PHOTORECEPTOR DEGENERATION IN CHOROIDEREMIA

Prof Clare Futter, Institute of Ophthalmology, UCL

Choroideremia (CHM) is an RP-related disease where the photoreceptors, the cell layer that supports the photoreceptors (the retinal pigment epithelium-RPE) and the choroid that provides the blood supply to the RPE degenerate. CHM patients show gradual retinal degeneration and vision loss by middle age. CHM is caused by mutation in Rep1, which is required for the function of a family of proteins called Rabs. Rabs govern how proteins are moved from one part of the cell to another, a process termed membrane traffic, and this traffic regulates the biogenesis of the different parts of the cell. Many of the causes of RP are mutations which cause key proteins required for retinal survival/vision to be trafficked incorrectly and thus CHM can be used as a model for multiple types of RP.

This project is about to begin. We have generated various mouse models of CHM and shown that loss of Rep1 leads to the progressive loss of photoreceptors. Cell death can occur by a number of different mechanisms and in this project we aim to identify the mechanism of photoreceptor cell death in our CHM models. We are using well-established assays of cell death pathways to analyse the mechanism of photoreceptor cell death using preserved retinal sections and retinal explants that can be maintained in culture for two weeks. In parallel we are analysing potential membrane traffic defects in the photoreceptors that may be responsible for the activation of cell death pathway(s). We are focussing on the traffic of the visual protein, rhodopsin, because i) rhodopsin is critical for vision ii) rhodopsin is produced by photoreceptors in huge quantities and must be correctly trafficked in order to function, iii) the high levels of rhodopsin produced make it comparatively easy to measure, iv) defects in rhodopsin traffic are found in several forms of RP and v) defective rhodopsin traffic has previously been linked with photoreceptor cell death. To directly link defects in rhodopsin traffic with photoreceptor cell death we will inhibit known regulators of traffic in retinal explants and determine whether this induces cell death in photoreceptors and, if so, whether the same death pathway is activated as that that occurs in vivo in our models of CHM.

Taken together this study will not only increase our understanding of how photoreceptors die in choroideremia and forms of RP characterised by defective rhodopsin traffic, but may also identify novel targets of therapeutic intervention in these diseases.
PHOTORECEPTOR REPLACEMENT THERAPY AND GLIOSIS: DEVELOPMENT OF STRATEGIES TO IMPROVE CELL INTEGRATION

Dr Rachael Pearson and Prof Robin Ali, Institute of Ophthalmology, UCL

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 and became integrated within the recipient retina and developed into mature photoreceptors (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. In another project we have spent much time trying to improve the transplantation procedure itself. This has led to improvements sufficient to permit restoration of scotopic vision in animal models, which we have recently reported in the journal Nature (Pearson et al., Nature, 2012).

These exciting results now 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 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 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 the success of photoreceptor transplantation. It also suggests that some types of disease may be more amenable to treatment by transplantation than others. However, it would appear that it may be possible to repair even severe degeneration by photoreceptor transplantation.
Last year we established the **RP Fighting Blindness Centre For Gene Therapy** at UCL Institute of Ophthalmology. The centre provides core infrastructure support for our gene therapy clinical trials programme.

There are currently 120 genes that have been identified that lead to various forms of inherited retinal dystrophy. More than 100 of these genes can carry loss-of-function mutations and could therefore be targets for gene supplementation therapy. In order to decide which gene defects are the most suitable targets for the first gene therapy approaches, various parameters must be considered. These include disease severity and prevalence, the availability and suitability of animal models of disease, and the efficacy of the treatment in proof-of-concept studies in animals. Severity of disease in particular is an important consideration, as on the one hand a rapid loss of photoreceptor cells severely limits the window of opportunity for treatment, but on the other hand allows an efficient read-out of treatment efficacy in a clinical trial.

Over the past decade, we have developed gene supplementation therapy protocols in animal models for over 10 different forms of retinal dystrophy and we have conducted a clinical trial of **RPE65** gene therapy for Leber congenital amaurosis (LCA) type 2. Over the next 5 years we aim to feed the pipeline of therapies that we have developed in animal models into a growing portfolio of clinical trials.

We are focusing on new clinical trials for the following disorders:

1. **RPE65** gene therapy for LCA type 2
2. **AIPL1** gene therapy for LCA type 4
3. **GUCY2D** gene therapy for LCA type 1
4. **CNGB3** gene therapy for achromatopsia type 3
5. **RDH12** gene therapy for LCA type 13
6. **RPGR** gene therapy for XRP3

Our strategy is to begin our initial studies in rare, but probably most amenable disorders, such as LCA2 and move towards more common, but more complicated disorders for which to develop treatments, culminating in the development of gene therapy for X-linked RP caused by defects in **RPGR**

We are now expanding the infrastructure and expertise to take this pipeline of therapies through to clinical trials. Through support from RP Fighting Blindness, National Institute of Health Research and The Medical Research Council we are increasing the clinical expertise, regulatory support, clinical trials infrastructure, including trial co-ordinators, clinical research assistants and toxicology support.
required to conduct several new clinical trials of gene in the next 5 years. The clinical grade vector for the first of these new trials is being produced at the gene therapy vector facility at University College London, which is now fully functional.

Our main achievements this year have been:

1) Completion of our RPE65 trial – we will report on the long term follow up of 12 patients later this year
2) Development of protocols for our next clinical trial. The first of a set of ethics applications will be submitted to the regulatory authorities (GTAC) in the next couple of months.
3) We have obtained considerable additional funding for the gene therapy programme from National Institute of Health Research and The Medical Research Council
4) James Bainbridge secured an NIHR Professorship with support for AIPL gene therapy
5) Accreditation of the UCL clinical grade gene therapy vector manufacturing facility by the Medicines and Healthcare Products Regulatory Agency (MHRA).
6) Recruitment of a full time Gene and Cell Therapy Manager, Dr Prateek Buch (p.buch@ucl.ac.uk). One of Prateek’s major duties is patient engagement
7) Recruitment of clinician-scientist Dr Koji Nishiguchi to assist with development of clinical trials protocols. Koji is an experienced vitreoretinal surgeon and has a PhD in molecular genetics of RP from Harvard Medical School.
8) Recruitment of a Regulatory Filing Manager, Dr Stuart Beattie. Stuart has industry experience of developing gene therapy. Until recently he was working at Amsterdam Molecular Therapeutics.
9) Retina Patient Day. This year we also held our first patient conference. Patients with retinal degeneration and their families gathered in London on April 21st to attend the first UCL/Moorfields Biomedical Research Centre for Ophthalmology “Retina Patient Day”. Over 250 attendees had the opportunity to interact with more than 40 clinicians and scientists from Moorfields Eye Hospital and UCL Institute of Ophthalmology Department of Genetics, who provided updates on their research into developing gene and cell treatments for blinding retinal conditions. The centrepiece of the “Retina Patient Day” was a unique opportunity for the attendees to interact with dozens of clinicians and scientists, which we arranged in four broad ways: information stalls, a chance to ‘meet the doctor’, ‘meet the scientist’ and ‘meet the counsellor,’ an artist’s workshop, and most importantly, over 30 poster presentations explaining our research in terms that attendees could relate to. Our group presented their posters to explain various area of gene and cell therapy research. We answered many excellent questions put to us by the attendees, who were keen to understand more about their conditions and about the research into treatments. We aim to hold a “Retina Patient Day” every year. Patient engagement is an essential part of developing an effective translational research programme. Our team not only look forward to further opportunities to explain our work to patients and their families, but to involve and learn from those who may benefit from it in the future. To register interest in attending next year’s “Retina Patient Day” please contact Heather Kneale at mo.therapy@ucl.ac.uk
PATHOLOGY AND TREATMENT OF PROMININ1 (PROM1)-MEDIATED RETINITIS PIGMENTOSA

Prof Shin-ichi Ohnuma, UCL Institute of Ophthalmology

Retinitis pigmentosa (RP) is a term used to describe a group of eye disorders, which are clinically and genetically diverse. Death of photoreceptor cells, which occurs in RP, 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.

Frame shift and some missense mutations in the gene Prominin 1 (PROM1) cause both autosomal-recessive and autosomal-dominant retinitis pigmentosa associated with lipofuscin accumulation and macular dystrophy. However, there is no effective treatment because of the lack of known pathological mechanisms. In order to examine the detailed molecular function of PROM1-mediated retinal disease, a PROM1 knockout mouse was constructed in collaboration with Prof Toru Kondo.

The PROM1−/− mice did not show any obvious early developmental defect with normal retinal development. However, when raised under normal light conditions the mutant mice develop rapid photoreceptor degeneration, accompanied with increased lipofuscin. This is in contrast to when they are raised in the dark, where the retina remains normal with no photoreceptor degeneration. This led to the hypothesis that PROM1 functions in the visual cycle.

We have performed some trials of potential drugs in some other models of macular degeneration (in ABCA4 knock out mice) which also show increased lipofuscin, with promising results. A decrease in A2E (a major component of lipofuscin) was detected by a technique called UPLC-mass spectrometry, with no adverse effect on the function of the retina as measured by electroretinogram (ERG). We will test these compounds in our PROM1 mouse model in the near future.

Another phenotype of the Prom1 knockout mouse appears to be decreased fertility, particularly affecting male sperm production. While we are continuing to expand our Prom1 knockout mouse colony, we have also started using another model organism to increase our knowledge of Prom1 mediated RP. *Xenopus leavis* (African clawed frog) is an excellent model system with the advantage of rapid development, allowing for much faster results. *Xenopus leavis* was initially used to describe the development of the visual system. We are currently over-expressing PROM1 (Xenopus, human and mutant PROM1) in Xenopus by injecting DNA into the developing Xenopus embryo and analysing the results. At the same time we are blocking the translation or splicing of PROM1 by injecting antisense oligos (morpholinos). Together with raising the Xenopus embryos under different lightning conditions.
conditions while over expressing or knocking down levels of PROM1, this combination of approaches will give us insights into the exact pathological mechanism of PROM1 mediated RP.

Furthermore, recent studies of the molecular properties of PROM1 indicate that it may be involved in multiple pathological mechanisms of photoreceptor degeneration, relating to reactive oxygen, hyperoxia, cilia abnormality, and the formation of drusen deposits, plus defects in the vascular system. We will investigate these potential roles of PROM1 in photoreceptor degeneration.

This project will determine the molecular pathological mechanisms underlying the PROM1-mediated retinitis pigmentosa and this will highlight potential novel therapeutics.