PROJECT FINAL REPORT

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Final publishable summary report

Executive summary

Many blinding disorders are caused by mutations in genes preferentially expressed in the photoreceptor cells of the retina. The objective of the AAVEYE consortium has been to develop state-of-art gene transfer tools for the photoreceptor neurons in the retina, and to provide pre-clinical proof-of-concept of gene therapy for severe blinding retinal photoreceptor diseases. Vectors derived from the adeno-associated virus (AAV) have proven to efficiently transduce the retina of animal models and AAV-mediated gene transfer appears to revert retinal pigment epithelium (RPE) defects. The retina represents an ideal target for gene therapy approaches because of the size of the eye, which allows the use of small vector doses, and because of its immuno-privileged environment.

AAVEYE focused, in particular, on two retinal diseases for which no therapies are yet available: Retinitis Pigmentosa (RP) and Leber Congenital Amaurosis (LCA), due to mutations in the *PDE6B* gene and *AIPL1* gene, respectively.
The ultimate goal of the AAVEYE consortium has been to develop gene therapy strategies for the treatment of RP and LCA. The safety and feasibility of the AAV-mediated gene transfer strategy for the treatment of Retinitis Pigmentosa and Leber Congenital Amaurosis has been tested by the AAVEYE consortium for the first time, potentially aiming to the phenotypic and functional rescue of inherited severe photoreceptor degenerations.

The consortium has intended to accomplish such aim through:
1) the development of AAV-based long-term and safe gene therapy to photoreceptors by combining endogenous promoters and AAV serotypes (WP1);
2) the assessment of the impact of AAV-mediated photoreceptor transduction on rescue of visual function in animal models of severe RP and LCA (WP2);
3) the evaluation of the efficacy of combination of gene replacement with adjuvant molecules on photoreceptor survival (WP3), and
4) the characterization of patients with inherited severe photoreceptor diseases to move the gene therapy strategies tested from bench to bedside (WP4).

Summary description of project context and objectives

Many blinding disorders, for which no therapies are currently available, are due to inherited conditions such as Retinitis Pigmentosa (RP, Weleber and Gregory-Evans 2001) and Leber Congenital Amaurosis (LCA, Weleber and Gregory-Evans 2001) that affect the retina.
Over 140 different loci responsible for RP and 10 for LCA (http://www.sph.uth.tmc.edu/Retnet/) have been identified so far. Most of the genes responsible for RP and LCA are expressed in photoreceptors exclusively or in addition to other cell types (Cremers et al. 2002; Weleber and Gregory-Evans 2001). Mutations in many of the genes encoding for proteins involved in phototransduction or in the visual cycle cause inherited retinal degeneration (Weleber and Gregory-Evans 2001). Mutations in *PDE6B* and *AIPL1* are particularly interesting because they are relatively common causes of severe recessively inherited retinal degeneration (*PDE6B* causes RP and *AIPL1* causes LCA). Animal models bearing *Pde6b* and *Aipl1* mutations are available and resemble the disease phenotype observed in humans. bPDE and AIPL1 deficiencies share a common pathogenetic mechanism. *PDE6B* encodes the β-subunit of the rod-specific phosphodiesterase (bPDE). This protein is an essential element in the phototransduction cascade (Ionita and Pittler 2007). Mutations in the *PDE6B* gene have long been linked to autosomal recessive retinitis pigmentosa (Bowes et al. 1990; Danciger et al. 1995) as well as autosomal dominant RP [see

http://www.retina-international.com/sci-news/pdemut.htm]. Six percent of patients with recessive RP have mutations in PDE6B (Bayes et al. 1996). Two naturally occurring mouse models of disease exist, rd1 and rd10 (Chang et al. 2002), both are due to mutations in the rod-specific *Pde6b* gene. The *AIPL1* gene encodes a photoreceptor cell specific chaperone that is hypothesised to be responsible for correct folding or localisation of the bPDE (Liu et al. 2004; Ramamurthy et al. 2004). Mutations in the *AIPL1* gene have been shown to result in Leber's congenital amaurosis, a form of inherited childhood blindness [see http://www.retina-international.com/sci-news/aipl1mut.htm] (Hameed et al. 2000). The two animal models of disease caused by mutations in the *Aipl1* gene are the knockout (Aipl1 -/-) mouse and the hypomorphic mutant (*Aipl1 h/h*) mouse. In the absence of AIPL1, a lack of functional bPDE results in a disruption of the phototransduction cascade and the subsequent loss of the photoreceptor cells. Mutations in *PDE6B* and *AIPL1* are therefore common causes of recessive severe photoreceptor degeneration in humans and mice through a common mechanism involving bPDE activity.

In the case of recessive RP and LCA, supplying a correct copy of the defective gene represents a potential therapeutic strategy for these otherwise untreatable diseases.

To this end, vectors derived from the small AAV are the most promising for somatic retinal gene transfer (Surace and Auricchio 2008) and their safety and efficacy was tested for the first time by PARTNER 2 in the retina of patients affected by LCA due to mutations in the gene encoding RPE65 (Bainbridge et al. 2006; Bennett 2006). A single intraocular injection of AAV results in gene expression sustained for years in photoreceptors or RPE of rodents, dogs, cats and non-human primates (Acland et al. 2005; Acland et al. 2001; Ali et al. 1996; Bennett et al. 1999; Ho et al. 2002; Lotery et al. 2003).

One of the attractive features of AAV as an *in vivo* gene transfer vector is that the surface capsid proteins can be easily interchanged among different AAVs thus creating hybrid vectors with the vector genome (encoding the therapeutic gene) from one serotype, i.e. AAV2, and the capsid from a different AAV, i.e. 1 or 5 (the resulting hybrid vectors being named AAV2/1 or 2/5) (Auricchio 2003). Since capsid proteins are the main determinants of AAV tropism and transduction characteristics, vectors with different capsids have different ability to transduce retinal cells in vivo (Auricchio et al. 2001b; Lotery et al. 2003; Rabinowitz and Samulski 2002; Yang et al. 2002). Based on this, PARTNER 1 recently tested the ability of novel AAV serotypes to transduce the murine retina and more specifically the photoreceptors. PARTNER 1 found that AAV2/8 and 2/7 mediate 6- to 8-fold higher in vivo photoreceptor transduction than AAV2/5, considered so far the most efficient for photoreceptor targeting (Allocca et al. 2007). In addition, following subretinal administration of AAV the rhodopsin promoter allows significantly higher levels of photoreceptor expression than the other ubiquitous or photoreceptor-specific promoters tested (Allocca et al. 2007). This could be crucial for successful treatment of animal models of photoreceptor diseases, a task more challenging than treatment of RPE-specific retinal defects. AAV2/2, 2/1 and 2/4 have been successfully used to treat RPE specific genetic defects including mucopolysaccharidoses (Hennig et al. 2004; Ho et al. 2002), ocular albinism (Surace et al. 2005), RP and LCA due to *MERTK* and *RPE65 gene* deficiencies, respectively (Acland et al. 2005; Acland et al. 2001; Dejneka et al. 2004; Le Meur et al. 2007; Narfstrom et al. 2003; Pang et al. 2006; Smith et al. 2003; Surace et al. 2005). Various centers, including PARTNER 2, are testing the toxicity and tolerability of AAV2/2 vectors in the retina of patients with *RPE65* mutations (Bennett 2006). Gene transfer approaches for photoreceptor-specific diseases have been successful in rare cases (Min et al. 2005; Pawlyk et al. 2005; Zeng et al. 2004) probably due to limitations of the vector system, the nature of photoreceptor-specific retinal degenerations and constrains in photoreceptor accessibility by vectors. The ability of AAV vectors to transduce RPE more efficiently than photoreceptors may be related to the simple monolayer organization of the

RPE as opposed to the photoreceptors that are higher in number and architectonically organized in rows (Clarke et al. 2000) which may represent a physical barrier to their transduction. The mechanism of photoreceptor loss suggests that gene transfer efficiency may be a limiting factor for photoreceptor rescue. RP initially affects the peripheral retina, resulting in the degeneration of rods, while cones and central vision are preserved at this stage (Dryja 2001). With the progression of the disease, the cones also degenerate (rod–cone degeneration) suggesting a non cell-autonomous mechanism of cell death. Non-autonomous patterns of degeneration are similarly observed in those inherited retinal diseases affecting primarily cones, as cone-rod dystrophies, suggesting a general mechanism of degeneration in inherited retinal diseases (Weleber and Gregory-Evans 2001). Therefore, widespread photoreceptor transduction is desirable to prevent detrimental effects from non-transduced photoreceptors. To this end, the combination of AAV2/8 and the rhodopsin promoter or endogenous promoters may allow successful treatment of severe photoreceptor diseases.
Considering the severe nature of some photoreceptor degenerations like those due to bPDE and AIPL1 deficiencies, combinatorial therapies aiming at supplying the correct copy of a mutant gene and at decreasing the progression of photoreceptor degeneration acting on mechanisms downstream of the defective gene could be desirable. The efficacy of several neurotrophic molecules or of small molecule drugs (Frasson et al. 1999; Pawlyk et al. 2005; Pearce-Kelling et al. 2001; Takano et al. 2004; Yamazaki et al. 2002) has been tested in animal models of photoreceptor degeneration.

Based on this context, the objectives of the AAVEYE consortium (and the milestones due) have been:

1- To optimize AAV-mediated gene transfer to photoreceptors for treatment of bPDE and AIPL1 deficiencies, through combination of AAV serotypes and endogenous promoters.

Milestone 1: Levels of bPDE expression in mice with AAV2/8 using endogenous promoters.

Milestone 2: Levels of AIPL1 expression in mice with AAV2/8 using endogenous promoters.

Milestone 3: Expression level of bPDE in Non Human Primates (NHPs).

Milestone 4: Expression levels of AIPL in NHPs.

Milestone 5: Feasibility of using endogenous promoters to drive expression of bPDE and AIPL1.

2- To test the efficacy of AAV-mediated photoreceptor gene transfer in murine models of *Pde6b* and *Aipl1* gene deficiencies.

Milestone 6: Complete production of vectors necessary for gene transfer to murine models.

Milestone 7: First report on outcome of photoreceptor survival after gene transfer in models of retinal degeneration.

Milestone 8: Final report of efficiency of photoreceptor survival after gene transfer in models of retinal degeneration.

3- To combine AAV-mediated gene replacement with molecules prolonging photoreceptor survival, like the neurotrophic factor GDNF, Ca^{++} channel blockers and RNA interference of the polycomb protein Bmi1.

Milestone 9: Report on tumor suppressor action on photoreceptor survival.

Milestone 10: Long-term photoreceptor survival by combined gene replacement therapy with GDNF.

Milestone 11: Long-term photoreceptor survival induced by combined gene replacement therapy with Nilvadipine.

Milestone 12: Synergism between Bmi1 inhibition or Rb treatment and *PDE6B/AIPL1* gene transfer.

Milestone 13: Report on micro-array analysis to identify survival pathways.

4- To characterize at the molecular and clinical levels RP and LCA patients to identify candidates to move AAV-mediated *PDE6B* and *AIPL1* gene transfer from bench to bedside.

Milestone 14: Molecular characterization of patients available from beginning of project.
Milestone 15: Report of clinical characterization of *PDE6B* patients, the optimal time interval for gene therapy and the recommended clinical tests before gene therapy.
Milestone 16: Report on the clinical characterisation of *AIPL1* patients, the optimal time interval for gene therapy and the recommended clinical tests before gene therapy.

Description of the main S&T results/foregrounds

During the AAVEYE granting period the PARTNERS have achieved the following objectives:

Workpackage 1

Optimization of AAV-mediated photoreceptor gene transfer for gene therapy of bPDE and AIPL1 deficiencies

Objectives

The goal of WP1 was to obtain AAV-mediated photoreceptor gene transfer that could mimic physiological levels of bPDE and AIPL1 expression. For this purpose, the PARTNERS used AAV2/8 vectors (that have the highest levels of photoreceptor gene transfer among a series of AAV serotypes tested, Allocca *et al.* 2007) in combination with fragments of the endogenous *PDE6B* and *AIPL1* 5' regulatory regions, based on the assumption that endogenous promoters should reproduce more faithfully the expression levels and timing of a transgene than exogenous regulatory regions.

Description of work

The viral vector based on adeno-associated virus serotype 8 (AAV2/8) has been identified as the most efficient to transfer genes to photoreceptors (PR). The Partners demonstrated that this AAV2/8 ability is retained across various species (mice and pigs).
This is a major achievement in the field as: i) diseases requiring gene transfer to PR are a major cause of blindness; ii) the vectors available before our discovery were unsatisfying in terms of rescue of PR-specific diseases in animal models.
In addition, PARTNER 1 described a strategy to tailor tightly and efficiently therapeutic gene expression to PR based on exploiting small non-coding RNAs (miRNAs), which are physiologically expressed in retinal PR cells. The results from these studies pertain to WP1 and are reported in the following publications: Karali *et al.,* 2011; Mussolino *et al*., 2011 (see PDFs attached)
As there was a report that the vector could spread outside the eye, PARTNER 2 looked at the presence of gene transfer to the brain. Six dogs (4 young, 2 adults) were injected subretinally with AAV2/8 vectors carrying a marker gene under control of a ubiquitous promoter. Examination of the retina showed widespread expression of the transgene, as expected for this

vector. There was evidence of limited vector spread outside the eye, with transgene expression apparent in the optic nerve and optic chiasm and sporadic expression in the brain. Therefore, the vector system can effectively transfer genes to the photoreceptor cells in rodents and large animals. Moreover, it appeared that spread of the vector to the brain is not a major problem and is unlikely to substantially affect the feasibility of retinal gene therapy using AAV2/8 vectors.

In parallel to the demonstration that AAV2/8 is an efficient vector for gene transfer in the retina of models of severe retinal degeneration, the PARTNERS tested different sequences of the *PDE6b* promoter in order to drive efficient PDE6b expression specifically in photoreceptors. The promoter sequence had been previously characterized by the group of Farber (Di Polo and Farber, 1995; Di Polo et al. 1997), but not for gene transfer purposes. PARTNERS 3 identified a sequence that allows inducing high and rapid expression of the transgene. This rod-specific promoter sequence is the shortest (149 bp) allowing efficient gene transfer and is thus of great interest for AAV vector design in general since it gives more space for the transgene of interest and good expression.

In conclusion, the PARTNERS:

- demonstrated that the viral vector based on adeno-associated virus serotype 8 (AAV2/8) is the most efficient to transfer genes to photoreceptors;
- demonstrated that the AAV2/8 vector system can effectively transfer genes to the photoreceptor cells in rodents and large animals;
- described a strategy to tailor tightly and efficiently therapeutic gene expression to PR based on exploiting small non-coding RNAs (miRNAs), which are physiologically expressed in retinal PR cells;
- reported that spread of the vector to the brain is not a problem and is unlikely to substantially affect the feasibility of retinal gene therapy using AAV2/8 vectors;
- identified a rod-specific promoter sequence that allows inducing high and rapid expression of the transgene, allowing efficient gene transfer.

Workpackage 2

Gene replacement therapy in *Pde6B* and *Aipl1* murine mutants

Objectives

The overall goal of WP2 was to test the efficacy of state-of-the-art AAV-mediated photoreceptor gene transfer in animal models of *Pde6b* and *Aipl1* deficiencies as well as providing proof-of-principle of gene replacement therapy for severe photoreceptor diseases due to *PDE6B* and *AIPL1* mutations.

Description of work

The PARTNERS showed that subretinal administrations of AAV2/8, which has high PR gene transfer ability, results in morphological PR protection in animal models of Retinitis Pigmentosa and Leber congenital amaurosis type 4 (LCA4). Indeed, PARTNER 1 observed that neonatal subretinal injections of AAV2/8 carrying the correct copy of the genes mutated in the two forms of inherited retinal degenerations (PDE6B mutated in RP and AIPL1

mutated in LCA4) significantly slowed the fast PR degeneration present in the retinae of these two murine models. The data produced by PARTNER 1 are contained in the following publications: Allocca *et al*., 2011; Testa *et al*., 2011 (see PDFs attached).
For the treatment of Leber congenital amaurosis type 4, PARTNER 2 produced AAV2/8 vectors carrying the *AIPL1* gene. After subretinal administration of AAV2/8.CMV.AIPL1 in *Aipl1* knock-out mice, which have an extremely rapid retinal degeneration, there was production of AIPL1 protein, an improvement in retinal function and a survival of photoreceptor cells for at least 6 weeks post-treatment, which is about 4 times longer than the natural photoreceptor survival in these animals. The AAV2/8.RK.AIPL1 construct that drives AIPL1 expression in the photoreceptors only, was tested in a manner similar to the study above. PARTNER 2 could show in *Aipl1*$^{-/-}$ mice that this vector supplemented the AIPL1 and restored the PDE production and localisation. There was a rescue of retinal function and a prolongation of photoreceptor survival for up to 5 months. As photoreceptor specific expression of the transgene adds to the safety aspects of a novel gene therapy vector, PARTNER 2 has chosen to use the AAV2/8.RK.AIPL1 vector in a planned clinical trial testing the safety and efficacy of AAV2/8-mediated gene therapy for LCA4. The results of the AIPL1 gene replacement studies by PARTNER 2 are published in Tan *et al*. (2009) and Sun *et al*. (2010) (see PDFs attached).
An AAV2/8 vector carrying the therapeutic transgene controlled by the *PDE6b* promoter was tested by PARTNER 3 for restoring gene function in a severe form of an animal model of retinitis pigmentosa, the *Rd10* mouse which presents defective *Pde6b* expression. PARTNER 3 observed that the gene therapy approach allows correcting the expression of the missing protein, rescuing a large number of photoreceptors and, more importantly, restoring retina function. Such rescue can be achieved when the vector was administrated just before the initiation of cell death, when the photoreceptors start to degenerate (inner segments already reduced) indicating that this therapeutic approach may be efficient in human even with altered photoreceptors.
In conclusion the PARTNERS demonstrated that:

- administration of AAV2/8 carrying the correct copy of the genes mutated (*PDE6B* mutated in RP and *AIPL1* mutated in LCA4) significantly slows down the fast photoreceptors degeneration and restores retina function.

Workpackage 3
Combined therapies to restore vision in *Pde6b* and *Aipl1* murine mutants.

Objectives
WP1 and WP2 aimed to develop a clinically relevant viral vector to efficiently target photoreceptors. In the case that gene transfer, in the most severe forms of Pde6b and Aipl1 deficiency, is not be sufficient to markedly delay photoreceptor death, combined strategies were envisaged using gene transfer of the wild-type gene together with treatment with additional components such as the neurotrophic factor GDNF, calcium blockers, or activation of tumor suppressors controlling photoreceptor differentiation.

Goal of this workpackage was to test the potential synergistic effect of neurotrophic molecules, Ca^{++}-channel blockers and cell-cycle manipulation with gene replacement on photoreceptor survival in *Pde6B* and *Aipl1* deficient models.

Description of work

In order to improve the therapeutic efficacy obtained by gene therapy in the rd10 mouse model of retinitis pigmentosa, the PARTNERS coupled to subretinal administrations of AAV2/8 methods to slow down retinal degeneration such as: i) dark-rearing; ii) systemic administrations of calcium inhibitors; iii) intraocular gene delivery of a neurotrophic factor. All these strategies are known to slow PR loss either in this (i and ii) or in other rodent models of PR degeneration (iii). Unfortunately, none of these combinations resulted in higher therapeutic efficacy than gene therapy alone. Thus, the consortium proposed intraocular AAV2/8-mediated delivery of the correct copy of the PDE6B gene mutated in this form of RP as the gene therapy strategy of choice. These results pertain to WP3 and are contained in the following publication: Allocca *et al*., 2011 (see PDF attached).

To develop a gene therapy protocol for RP40, PARTNER 2 injected AAV2/8 vectors carrying the PDE6B gene subretinally into mouse models of disease. Treatment resulted in a modest replacement of PDE protein in the treated, but not the untreated eyes of the PDE deficient mice. However, this did not lead to a significant improvement of photoreceptor survival. Further development of the treatment strategy using neurotrophics or small molecule inhibitors of the ion channels did not yield a substantial improvement of the therapeutic effect. However, preliminary results suggested that genetic inhibition of the calcium channels may prolong the survival of the photoreceptors and rescue the photoreceptors and this line of research is continuing.

In parallel to these studies, PARTNER 3 identified a new cellular pathway that leads to photoreceptor death. PARTNER 3 observed that several cell cycle proteins are expressed before the state of photoreceptor death. Using genetic models or drug testing *in vitro* on cultured retina explants, PARTNER 3 showed that the inhibition of certain cell cycle proteins rescues transiently or markedly both rods and cones depending on the molecular target. Different vectors were then produced in order to attempt to delay photoreceptor death during retinal degeneration of the *Rd10* mouse by interfering with some cell cycle protein functions. None of these vectors showed efficient rescue even when combined with the therapeutic AAV2/8- PDE6b vector. However, a pharmacological approach showed promising results *in vitro* by inhibiting Cdks, but several developments are still necessary to be performed to deliver efficiently this compound into the eye during a long period.

In summary, these series of experiments clearly show that cell cycle proteins play a major role in the execution of the photoreceptor death process in several models of retinal degeneration. Moreover, Cdk proteins appear to be a realistic potential therapeutic target, whereas Bmi1 protein, which was thought at the beginning to be a valid candidate, appears difficult to control. This is probably because Bmi1 participates to histone and DNA modifications, which are also necessary to re-arrange in order to restore a normal gene expression pattern. In parallel PARTNER 3 identified that Bmi1 prevents Müller cells from regenerating the

photoreceptor layer. In the absence of Bmi1, Müller cells can de-differentiate and migrate to the photoreceptor layer, their final fate remaining to be determined. This observation also opens a new field of investigation to manipulate these cells via Bmi1 to generate new photoreceptors. Of course, for genetic diseases, an adequate gene replacement strategy remains to be undertaken to restore a visual function. The vectors developed in the present study show the feasibility of using the AAV2/8 vector with a specific photoreceptor promoter for restoring gene function in a severe form of retinitis pigmentosa. These gene therapy results consolidate similar observations obtained recently with the same vector in various models, including large animals, rendering this vector a good candidate for human applications.

A manuscript entitled "Retinal degeneration depends on Bmi1 function and re-initiation of cell cycle machinery" Zencak, D., Chen, D., Ekström, P., Tanger, E., Bremner, R., van Lohuizen M., Arsenijevic, Y., is in revision (submitted to PNAS).

In conclusion, in this workpackage the PARTNERS demonstated that:

- treatment strategy using neurotrophic factors or small molecule inhibitors of the ion channels does not yield a substantial improvement of the gene therapy therapeutic effect, therefore intraocular AAV2/8-mediated delivery of the correct copy of the gene mutated is the gene therapy strategy of choice.

In addition,

- a new cellular pathway that leads to photoreceptor death has been identified
- a gene implicated in preventing retinal regeneration has been identified.

Workpackage 4

Molecular and clinical characterisation of RP/LCA patients with *PDE6B* and *AIPL1* mutations.

Objectives

In view of future potential applications of gene therapy, the AAVEYE consortium performed the molecular and clinical characterization of RP/LCA patients with *PDE6B* and *AIPL1* mutations.

Description of work

In view of future potential applications of gene therapy, the AAVEYE consortium performed the molecular and clinical characterization of RP/LCA patients with *PDE6B* and *AIPL1* mutations.

PARTNER 1 characterized at the molecular level series of patients with sporadic or recessive RP as well as LCA who were followed up at the Second University of Naples Department of Ophthalmology. PARTNER 1 identified 10 LCA patients with mutations in the *AIPL1* gene, thus then defined as LCA4 patients, as well as 4 RP patients carrying mutations on both *PDE6B* alleles. The patients with PDE6B mutations presented with typical RP and severe PR degeneration throughout the retina, according to a rod-dominated disorder. The patients with LCA4 had early onset severe retinal dystrophy with marked macular degeneration. However

in most of the Italian LCA4 patients including older individuals, perifoveal PR appears to be spared by the degenerative process suggesting that these PR can be the target of gene therapy. PARTNER 1 results on the LCA4 patients have been published in Testa *et al*., 2011 (PDF attached).

Approximately 400 DNA samples from the early-onset severe retinal degeneration patient database of Moorfields eye hospital were screened (by PARTNER 2) for mutations in a range of retinal dystrophy genes. PARTNER 2 was able to identify seven LCA patients with mutations in *AIPL1* that are likely to be disease-causing, but no patients with *PDE6B* mutations were identified. Direct sequencing led to the identification of 29 novel *AIPL1* sequence variants, and computer analysis suggests that 6 of these variants are likely to be disease-causing mutations. The ages of identified AIPL1-deficient patients ranged from 2 years to 36 years. Although the LCA phenotype is characterised by early onset severe visual loss in our patient series, some of the retinal structure and retinal function appeared to be preserved in some of the patients. This phenomenon was most marked in the youngest patient in our cohort, who had a measurable visual acuity. In this subject assessment of electrophysiology revealed reduced cone photoreceptor function, but rod responses were within normal limits. These data suggested that there are patients who have a reasonable window of opportunity for LCA4 gene therapy in childhood. A manuscript describing the characterisation of the AIPL1-deficient patients at Moorfields Eye Hospital is submitted for publication (Tan *et al*., 2011).

To evaluate whether a gene therapy approach can be proposed to patients affected by the PDE6b mutations, PARTNER 3 screened RP and LCA patients in Switzerland. No individuals were identified for PDE6b mutations, but one LCA4 patient was detected. PARTNER 3 enlarged the patient cohort by including families from Tunisia with the collaboration of the Service of Ophthalmology in Tunis (Prof. L. El Matri) and identified 2 patients with the same mutation, and 6 showing only one variant. The characterization of the patient phenotype revealed a massive alteration of the retina with the presence of cysts/oedemas and vitreous attachment leading to retina detachment. Gene therapy for these patients appears very difficult if the therapy is not undertaken at a very early age, and by targeting a large portion of the retina. Indeed, if the retina is only treated locally, we can expect that the cyst formation in the non-treated area with retina attachment on the vitreous will deteriorate the treated region. It thus appears that PDE6b patients are not eligible for a first gene therapy clinical trial with the AAV2/8 vector to show the safety and (some) efficacy of the treatment.

In conclusion, panels of DNA samples from patients diagnosed with severe, early-onset retinitis pigmentosa and Leber's congenital amaurosis, available from the Eye Hospitals in London, Naples and Lausanne, have been screened at ASPER Biotech, Tartu, Estonia (PARTNER 4). 575 DNA samples have been screened during the project for mutations in the *PDE6B* or the *AIPL1* genes using the arRP gene chip or the LCA gene chip, respectively. The arRP gene chip tests for the presence of 594 known disease-associated sequence variants in any of 18 genes, including 26 sequence variants in *PDE6B*. The LCA gene chip tests for the

presence of 641 known disease-associated sequence variants in any of 13 genes, including 40 sequence variants in *AIPL1*. Mutations in *PDE6B* and *AIPL1* identified using the gene chips were confirmed with direct sequencing by other PARTNERS. The analysis results showed that a *PDE6B* mutation has been identified in ca 2 % of DNA samples tested for arRP chip (5 DNAs out of 278) and an *AIPL1* mutation has been identified in ca 4% of DNA samples tested for LCA chip (11 DNAs out of 283). The arRP gene chip tests for the presence of 594 known disease-associated sequence variants in any of 18 genes, including 26 sequence variants in *PDE6B*. From 278 arRP patient DNAs, 116 mutations in 13 genes were identified. 4 mutations in five different DNAs were identified in *PDE6B* gene. A table, describing arRP patient DNA genotypes is included as a Table 1. The LCA gene chip tests for the presence of 641 known disease-associated sequence variants in any of 13 genes, including 40 sequence variants in *AIPL1*. From 283 LCA patient DNAs, 115 sequence variants from 13 genes were identified. 6 different mutations in eleven DNAs were identified in *AIPL1* gene. A table, describing LCA patient DNA genotypes is included as a Table 2.

Table 1.

Number of DNA samples	Gene	Exon	nucleotide change	amino acid change	Result
1	**CNGA1**	8	947C>T	S316F	T/A
5	**CRB1**	2	614T>C	I205T	TC/AG
1	**CRB1**	6	1313G>A	C438Y	GA/CT
3	**CRB1**	7	2306_2307G>A	R769H	GA/CT
1	**CRB1**	7	2555T>C	I852T	CT/AG
1	**CRB1**	7	2671T>G	C891G	TG/A-
1	**CRB1**	9	3074G>T	S1025I	A/T
1	**CRB1**	9	3122 T>C	M1041T	CT/AG
2	**CRB1**	7	2307C>T	R769R	T/A
1	**CRB1**	1	55_58insT	I20fs	AT/GA
1	**CRB1**	9	2843G>A	C948Y	A/T
1	**MERTK**	11	1618G>A	E540K	GA/CT
1	**PDE6A**	1	304C>A	R102S	A/T
4	**PDE6A**	3	647A>G	N216S	AG/CT
4	**PDE6A**	5	878C>T	P293L	CT/GA
1	**PDE6A**	9	1171G>A	V391M	GA/CT
2	**PDE6B**	**3**	**655T>C**	**Y219H**	TC/AG
1	**PDE6B**	**4**	**811G>A**	**E271K**	GA/CT
1	**PDE6B**	**12**	**1580T>C**	**I527P**	TC/AG
1	**PDE6B**	**14**	**1798G>A**	**D600N**	A/T
2	**PNR/NR2E3**	2	166G>A	G56R	GA/CT
1	**PNR/NR2E3**	4	361G>A	E121K	A/T
2	**PROM1**	12	1348_1355insT	Y452fs	T/A
2	**RDH12**	2	184C>T	R62X	CT/GA
2	**RDH12**	3	193C>T	R65X	T/A
1	**RDH12**	3	194G>A	R65Q	GA/CT

Number of DNA samples	Gene	Exon	nucleotide change	amino acid change	Result
1	**RDH12**	6	806_810del 5bp (CCCTG)	A269fs	G/G
1	**RGR**	6	700G>A	A234T	GA/CT
6	**RGR**	6	722C>T	S241F	CT/GA
2	**RGR**	IVS6+5	IVS6+5 a --> g	SPLICE	AG/TC
1	**RLBP1**	5	677T>A	M226K	T/A
1	**RPE65**	4	272G>A/C	R91Q/P	A/T
1	**RPE65**	7	700C>T	R234X	T/A
3	**RPE65**	9	963 T>G	N321K	TG/AC
2	**TULP1**	IVS2	IVS2+1G>A	SPLICE	A/T
1	**USH2A**	4	688G>A	V230M	GA/CT
1	**USH2A**	8	1434G>C	E478D	CG/CG
5	**USH2A**	10	1663C>G	L555V	CG/GC
12	**USH2A**	12	2137G>C	G713R	GC/CG
7	**USH2A**	13	2276G>T	C759F	GT/CA
1	**USH2A**	13	2282C>G	P761R	CG/GC
2	**USH2A**	13	2299delG	E767fs	GA/CA
1	**USH2A**	21	4560C>T	I1520I (polymorphism?)	CT/GA
1	**USH2A**	30	5975A>G	Y1992C	AG/CT
2	**USH2A**	30	5858C>G	A1953G	CG/GC
5	**USH2A**	34	6587G>C	S2196T	CG/-
3	**USH2A**	41	7685T>C	V2562A	TC/AG
5	**USH2A**	47	9262G>A	E3088K	AG/CT
2	**USH2A**	50	9815C>T	P3272L	T/A
1	**USH2A**	54	10712C>T	T3571M	CT/GA
1	**USH2A**	63	12445T>C	W4149R	TC/AG
1	**USH2A**	63	12334T>C	W4149R	TC/AG
2	**USH2A**	71	15377T>C	I5126T	T-/AG
1	**USH2A**	71	15433G>A	V5145I	GA/CT

Table 2.

Number of DNA Samples	Gene	Exon	nucleotide change	amino acid change	Result
3	**AIPL1**	**3**	**401A>T**	**Y134F**	**AT/TA**
1	**AIPL1**	**6**	**IVS5-10_786 del**	**SPLICE**	**CA/GC**
5	**AIPL1**	**6**	**834G>A**	**W278X**	**A/T**
2	**AIPL1**	**6**	**905G>T**	**R302L**	**GT/CA**
1	**CEP290**	IVS3	180+1G>T	splice	GA/CT
1	**CEP290**	9	613C>T	R205X	CT/GA
1	**CEP290**	IVS12	1066-1G>A	SPLICE	GA/CT
1	**CEP290**	14	1219_1220delAT	M407fs	AG/AG
1	**CEP290**	15	1593C>A/G	Y531X	CA/GT
12	**CEP290**	IVS26	2991+1655A>G	C998X	G/C
1	**CEP290**	28	3166_3176ins1A	I1058fs	TA/GT
2	**CEP290**	36	4723G>T	K1575X	AT/TA
2	**CEP290**	41	5668G>T	G1890X	T/A
1	**CEP290**	43	5932C>T	R1978X	CT/A
2	**CEP290**	43	5941G>T	E1981X	T/CA
1	**CRB1**	1	55_58insT	I20fs	AT/GA
1	**CRB1**	2	614T>C	I205T	TC/AG
1	**CRB1**	7	2234C>T	T745M	CT/GA
1	**CRB1**	7	2401A>T	K801X	-/TA
2	**CRB1**	7	2555T>C	I852T	TC/AG
1	**CRB1**	7	2671T>G	C891G	TG/AC
4	**CRB1**	9	2843G>A	C948Y	GA/CT
1	**CRB1**	9	3074G>T	S1025I	G!A/C!T
1	**CRB1**	9	3307G>A/C	G1103R	AG/CT
3	**CRB1**	11	3879G>A	W1293X	GA/CT
1	**CRX**	2	121C>T	R41W	CT/GA
2	**CRX**	2	196G>A	V66I	GA/CT
1	**CRX**	3	268C>T	R90W	T/A
2	**CRX**	3	425A>G	Y142C	AG/TC
1	**CRX**	3	472G>A	A158T	GA/CT
1	**GUCY2D**	2	121C>T	L41F	CT/GA
1	**GUCY2D**	2	307G>A	E103K	GA/CT
1	**GUCY2D**	2	delC 387/388/389	FS	unclear
1	**GUCY2D**	2	450G>A	W150X	GA/CT
5	**GUCY2D**	4	1093C>T	R365W	CT/GA
1	**GUCY2D**	6	1537C>T	L513F	CT/GA
1	**GUCY2D**	8	1724C>T	P575L	CT/GA
1	**GUCY2D**	10	1978C>T	R660X	CT/GA
1	**GUCY2D**	10	2101C>T	P701S	T/A
3	**GUCY2D**	12	2302C>T	R768W	CT/GA
1	**GUCY2D**	15	2849C>T	A950V	CT/GA

Number of DNA Samples	Gene	Exon	nucleotide change	amino acid change	Result
2	**GUCY2D**	15	2943del G	FS	GT/CG
1	**LCA5**	5	835C>T	Q279X	CT/GA
1	**LCA5**	8	1151delC	P384fs	A/T
1	**LRAT**	2	217_218delTA	M73fs	G/G
1	**MERTK**	11	1618G>A	E540K	GA/CT
1	**MERTK**	17	2214del	C738fs	G/C
1	**RDH12**	1	57_60del	P20delfs	CA/TG
5	**RDH12**	2	184C>T	R62X	T/A
1	**RDH12**	3	194G>A	R65Q	GA/CT
2	**RDH12**	6	806_810 del 5bp (CCCTG)	A269fs	CG/AG
1	**RPE65**	3	131G>A	R44Q	GA/CT
1	**RPE65**	4	272G>A	R91Q	A/T
2	**RPE65**	5	394G>A	A132T	GA/CT
2	**RPE65**	9	963T>G	N321K	TG/AC
1	**RPE65**	int1	11+5G>A	SPLICE	GA/CT
1	**RPE65**	14	1451G>A	G484D	A/T
1	**RPGRIP1**	IVS5+1	800+1G>A	SPLICE	GA/CT
1	**RPGRIP1**	11	1447C>T	Q483X	CT/GA
1	**RPGRIP1**	14	1767G>T	Q589H	GT/CA
1	**RPGRIP1**	14	1793G>A	R598Q	A/T
3	**RPGRIP1**	21	3341A>G	D1114G	AG/TC
2	**RPGRIP1**	23	3719G>A	G1240E	GA/CT
1	**SPATA7**	5	322C>T	R108X	T/A
1	**SPATA7**	11	1183C>T	R395X	CT/GA
2	**TULP1**	IVS2	IVS2+1G>A	SPLICE	GA/CT
1	**TULP1**	12	1204G>T	E402X	GT/CA
1	**TULP1**	15	1511_1521delTGCA GTTCGGC	L504fs	C/A

Potential impact and the main dissemination activities and exploitation of results

Potential impact

Discoveries typically begin at “the bench” with basic research — in which scientists study disease at a molecular or cellular level — then progress to the clinical level, or the patient's “bedside.” The AAVEYE proposal has been an example of this type of research; the results obtained by this proposal could have an immediate impact on patient health, by using the preclinical data obtained in the animal models as starting point for the development of clinical trials.

Therefore, the consortium has established collaborations with important clinical researchers in each Partner state to provide patients, to assess clinical phenotypes, in order to determine

markers and clinical progression that will be fundamental knowledge to evaluate the efficacy of a future therapy.
The PARTNERS already had close associations with patient organizations (eg PARTNER 1: Retina Italia, Italian Association Leber's Congenital Amaurosis; PARTNER 2: British Retinitis Pigmentosa Society, Foundation Fighting Blindness; and PARTNER 3: Swiss patient association Retina Suisse), this collaboration with patients association allowed the consortium to rapidly disseminate results to patient organizations, to raise awareness of the state-of–art of new therapeutic approaches and to recruit new patients for molecular analysis and future clinical trials.

The potential of the results emerging from this proposal could impact:

1) the design of novel AAV vectors and the application of this vector system for the treatment of diseases that affect the photoreceptor cells of the retina.

2) the development of combinatorial therapies for severe photoreceptor diseases by testing alternative strategies with growth factors in order to increase therapeutic window and potential. The results from this objective will open new perspectives for the use of combinatorial therapies summing the effect of gene replacement with adjuvant molecules for these as well as other CNS diseases.

3) the possibility to properly treat millions of patients suffering severe retinal degenerations due to photoreceptor demise. AAVEYE consortium have paved the way for future gene therapy trials in humans for severe retinal degenerations by studying the clinical outcome of photoreceptor degenerations in humans and identifying target patients for the gene therapy strategies selected.

4) the fine tuning of therapies for diseases affecting other parts of the central nervous system, being the retina an accessible part of the CNS and given the similarities in viralvector transduction between the retina and the CNS.

The scientific results of the Project have been disseminated as follows:

- Data have been discussed in lab meetings, conference calls, closed meetings and open conferences.

- Data have been used to generate hypothesis for further research, for diagnostic possibilities, and therapeutic potential.

- Data have been published in the scientific literature.

- Results of the project have also been presented in international conferences and meetings.

- A public Website has been developed and is online (www.aaveye.eu). Scope of the website is two-fold: to favour dissemination of the project to the scientific community and lay public, and to facilitate inter-communication among PARTNERS and accelerate work

progression. This site provides information concerning the objectives, participants, achievements, publications, meetings, employment opportunities and other pertinent information regarding the overall project. The target audience will not only be academic researchers, but also patient groups, industry, biotechnology, and training institutions.
To this end, the site consists of a public area and a private area that is password protected (**username**: PARTNERS; **password**: europa4). In the public area, which is easily accessible for any internet user, a first section (What is AAVEYE?) provides general information on the focus of the project, the diseases of interest to the consortium, and the aims and impact of the project in terms of EU scientific and societal objectives. A more specific description of the project workpackages and their objectives is also provided together with a general overview of each partner making up the consortium and his/her role in the project, the key personnel and publications acknowledging AAVEYE. Links to other websites, relevant events and participation to meetings and symposia is also available.
Finally, a separate section (Internal Use) with sensitive data (such as the TA, the contract and terms) is restricted to the PARTNERS of the consortium and is password protected.
The AAVEYE management team has also given particular attention to keep the website up to date with the consortium annual meetings, including the PPTs presented by each partner and Minutes of each meeting, and with the publications acknowledging AAVEYE. The PPTs, Minutes of the meetings and full articles of AAVEYE publications are again restricted to the PARTNERS and the EU and therefore are password protected.

Communication within the consortium flowed steadily. Participants have frequently been in contact with each other, via email or skype, to exchange ideas, discuss about the advancement of research and guarantee that it was focused towards the objectives, and to make strategic

decisions (when necessary) regarding scientific planning and financial aspects of the programme.

Exchange among PARTNERS has also been facilitated by the fact that PARTNERS have been invited to the respective research institutes to give formal lectures. As an example, **PARTNER 3** gave a seminar at UCL (**PARTNER 2**) in June 2009. Also **PARTNER 1** was invited at UCL in November 2009 and gave a seminar entitled "Gene therapy for inherited retinal dystrophies: from viral vector to patient". In February 2010, a senior Research Fellow of **PARTNER 2**'s laboratory was invited to Lausanne (**PARTNER 3**) to discuss data on retinal gene therapy in the lab and the clinic in a public seminar. In addition, **PARTNER 2** gave a seminar at TIGEM (**PARTNER 1**) in June 2011, entitled "Development of photoreceptor transplantation strategies for the treatment of retinal degeneration".

Moreover, **PARTNERS 1 and 2** have cooperated in another EU project (CLINIGENE-NoE). The aim of the CLINIGENE consortium, consisting of 38 PARTNERS, was to create a European network (EC-NoE) for the advancement of clinical gene transfer and therapy by fostering the interaction of all stakeholders in the field in order to facilitate and help harmonize ethical, quality, safety, efficacy and regulatory issues.
In addition, **PARTNERS 2 and 3** have both participated to the Retina Suisse Conference in Fribourg (Switzerland) in June 2009 and to the documentary film *Malvoyants: lueurs d'espoir*, broadcasted on the Télévision Suisse Romande (see sections Seminars and Conference Attendance and Science Dissemination and the Media for major details), where they both also contributed to the design, other than the science, of the documentary film.

Main dissemination activities during the project

Seminars and Conference Attendance

2011

January 20th-23th – ARVO Asia annual meeting, Singapore (Invited speaker) **[Ali, PARTNER 2]**

February 4th – Swiss Stem Cell Networking meeting, Lausanne, Switzerland (Poster) **[Schouwey, PARTNER 3]**

March 3th - European Workshop for Rheumatology Research, Amsterdam, Netherlands - "Gene therapy of inherited retinal diseases" (Invited speaker) **[Auricchio, PARTNER 1]**

May – ASGCT annual meeting, Seattle, USA (Conference) **[Ali, PARTNER 2]**

May 6th-10th – Association for Research in Vision and Ophthalmology meeting, Fort-Lauderdale, USA (Poster) **[Schouwey, PARTNER 3]**

May 6th-10th – Association for Research in Vision and Ophthalmology meeting, Fort-Lauderdale, USA (Poster) **[Ihm, PARTNER 3]**

May 21th - The American Society of Gene and Cell Therapy meeting 2011 (ASGCT), Seattle, WA - A. Manfredi, M. Karali, A. Puppo, E. Marrocco, A. Gargiulo, E. M. Surace, S. Banfi

and A. Auricchio. "MicroRNA-based transgene regulation in the retina" (Poster). Winner of ASGCT Travel Grant Award **[Auricchio, PARTNER 1]**

June 24th - UniStem Center, University of Milan, Italy - "Terapia genica e cellulare delle degenerazioni retiniche ereditarie" (Lecture) **[Auricchio, PARTNER 1]**

September 7th-10th – International Society for Ocular Cell Biology meeting, Vancouver, USA (Invited speaker) **[Arsenijevic, PARTNER 3]**

September 12th - Congresso della Societa' Italiana di Genetica Umana, Seconda Universita' degli Studi di Napoli, Napoli, Italy - "Retinopatie ereditarie: nuove frontiere terapeutiche" (Invited speaker) [Auricchio, PARTNER 1]

October 1st - 2nd Course in Eye Genetics, Bologna, Italia - "Gene therapy for Leber's Congenital Amaurosis" (Lecture) **[Auricchio, PARTNER 1]**

October 29th - European Society of Gene and Cell Therapy meeting, Brighton, UK - "Gene therapy with AAV vectors" (Invited speaker) **[Auricchio, PARTNER 1]**

October 29th - European Society of Gene and Cell Therapy meeting, Brighton, UK - (Invited speaker) **[Ali, PARTNER 2]**

October 30th Epigenetics and the Inheritance of Acquired State, Boston, USA (Invited speaker) **[Arsenijevic, PARTNER 3]**

2010

January 28th-29th - Swiss Eye Research Meeting 2010 (SERM2010), Biel, Switzerland - Promises and challenges of gene therapy for inherited retinal diseases (Lecture). **[Surace, PARTNER 1].**

February 6th - GMRC Human Genetics Unit, Edinburgh, Scotland - Gene therapy for retinal dystrophies (Invited speaker) **[Ali, PARTNER 2]**

March 4th-7th - 1st World Congress on Controversies in Ophthalmology (COPHy), Prague, Czech Republic - The development of AAV vectors for retinal gene therapy: Advantages and limitations (Lecture) In: Diagnosis and Therapy of Pediatric Retinal Dystrophies (Lecture) **[Surace, PARTNER 1]**

March 31st - British Society of Gene and Cell Therapy, Egham, UK - Gene therapy for treatment of Leber congenital amaurosis (Session Chair) **[Ali, PARTNER 2]**

May 2th - Canadian Gene Therapy and Vaccines Symposium, Montreal, Canada - "Gene therapy of inherited retinal diseases" (Invited speaker) **[Auricchio, PARTNER 1]**

May- ASGCT annual meeting, Hannover, Germany (Invited speaker) **[Ali, PARTNER 2]**

June 10th - Congresso Nazionale S.I.O.P., Napoli, Italia - "Terapia genica delle distrofie retiniche ereditarie" (Invited speaker). **[Auricchio, PARTNER 1]**

June 12th - Association “Information Recherche Rétinite Pigmentaire” (IRRP), XXVIth National Meeting, Chambéry, France – Patients association (Invited speaker) **[Arsenijevic, PARTNER 3]**

June – BRPS Members conference 2011, London, UK (Invited speaker) **[Ali, PARTNER 2]**

June 27th - Retina International, Stresa, Italy - “Genes and Gene Therapy” (Keynote lecture) **[Auricchio, PARTNER 1]**

July – Retina International World congress 2010, Hamburg, Germany (Invited speaker) **[Ali, PARTNER 2]**

September 24th - The Swiss Society for Neuroscience symposium "Viral Strategies in Brain Research" 2010, Zurich, Switzerland (Conference) **[Arsenijevic, PARTNER 3]**

September 29th -30th - Annual meeting of the Danish Society of Medical Genetics, thema "Genetics and Vision”, Knudshoved, Denmark (Conference) **[Arsenijevic, PARTNER 3]**

October 14th - Institute for Ophthalmic Research, University of Tübingen, Tübingen, Germany (Seminar) **[Arsenijevic, PARTNER 3]**

October 22th - European Society of Gene and Cell Therapy meeting, Milan, Italy, “AAV vectors for gene therapy of retinal degeneration” (Invited speaker) **[Auricchio, PARTNER 1]**

October 22th - European Society of Gene and Cell Therapy meeting, Milan, Italy, (Invited speaker) **[Ali, PARTNER 2]**

November – Retina 2011, Dublin, Ireland (Meeting) **[AJ Smith, PARTNER 2]**

November 9th - Centre for Regenerative Medicine "Stefano Ferrari" University of Modena and Reggio Emilia, Modena, Italy (Seminar) **[Arsenijevic, PARTNER 3]**

2009

February 20th - Neuro-Ophthalmology Update Course, Madrid, Spain - *Gene therapy of retinal diseases* (Invited speaker) **[Auricchio, PARTNER 1]**

March 9-11th - Telethon Convention 2009, Riva del Garda, Italy - *Advancement in AAV–mediated retinal gene transfer for inherited retinal diseases* (Invited speaker). **[Auricchio, PARTNER 1]**

March 11th - Centre for Life, Newcastle University, Newcastle, UK - *Gene therapy for retinal dystrophies* (Invited speaker). **[Ali, PARTNER 2]**

April 15th - International Congress on Neuronal Ceroid Lipofuscinoses, Hamburg, Germany - *Clinical trial of gene therapy for inherited retinal degeneration* (Invited speaker). **[Ali, PARTNER 2]**

April 22nd - 8th Master in Clinical Genetics, International School of Pediatrics Science, Genova, Italy - *Trasferimento genico per malattie retiniche* (Invited speaker). **[Auricchio, PARTNER 1]**

May 13th - Dept. of Biological and Technological Research (DiBIT), San Raffaele Hospital, Milano, Italy – *Expanding the utility of AAV vectors for gene therapy of human inherited diseases* (Lecture) **[Auricchio, PARTNER 1]**

May 27th - The American Society of Gene and Cell Therapy meeting 2009 (ASGCT), San Diego, CA - P. Colella, C. Iodice, U. Di Vicino, A. Auricchio. "AAV-mediated Gene transfer of the Erythropoietin derivatives in models of Retinal Degenerative Diseases" (Invited speaker) Winner of ASGCT Travel Grant Award **[Auricchio, PARTNER 1]**

May 3th–7th – ARVO Annual Meeting, Ft Lauderdale, USA (Invited speaker) **[Tan, PARTNER 2]**

May 3th–7th – ASGT Annual Meeting, San Diego, USA (Invited speaker) **[Ali, PARTNER 2]**

May 3th–7th – ASGT Annual Meeting, San Diego, USA (Invited speaker) **[Arsenijevic, PARTNER 3]**

June 1st-2nd - Vision of Children Foundation International Symposium, San Diego, CA, USA - *Advancements in AAV-mediated gene transfer for inherited retinal diseases* (Invited speaker) **[Auricchio, PARTNER 1]**

June 12th - Retina Suisse Conference, Fribourg, Switzerland - *Development of novel therapies for inherited retinal degeneration* (Session Chair) **[Ali, PARTNER 2]**

June 12th - Retina Suisse Conference Fribourg, Switzerland - *Perspectives d'essais thérapeutiques en Suisse* (Lecture). **[Tran, PARTNER 3]**

June – BRPS Members Conference 2009, London, UK (Invited speaker) **[Ali, PARTNER 2]**

June – Retina International World congress 2009, Stresa, Italy (Invited speaker). **[Ali, PARTNER 2]**

September 4th - International Workshop adeno-associated viral vectors, Nantes, France – *Development of AAV vectors for retinal degeneration* (Session Chair). **[Ali, PARTNER 2]**

September – *In vivo* Applications of Recombinant AAV meeting, Nantes, France (Invited speaker) **[AJ Smith, PARTNER 2]**

October 6th – International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy - *AAV-mediated gene transfer to the retina and liver for human inherited diseases* (Lecture). **[Auricchio, PARTNER 1]**

November 21st-25th - ESGT Combined Meeting, Hannover, Germany - *AAV-mediated gene transfer to the retina: from bench to bedside* (Invited speaker). **[Auricchio, PARTNER 1]**

November 21st-25th - ESGT Combined Meeting, Hannover, Germany (Poster) **[Philippe, PARTNER 3]**

November 22nd - European Society of Gene and Cell Therapy, Hannover, Germany - *Gene therapy for treatment of Leber congenital amaurosis due to defects in AIPL1* (Session Chair) **[Ali, PARTNER 2]**

November 27th - UCL Institute of Ophthalmology, Genetics and Molecular Therapy, London, UK - *Gene therapy for inherited retinal dystrophies: from viral vector to patient* (Lecture) **[Auricchio, PARTNER 1]**

December 3rd - Foulkes Foundation Annual Meeting, London, UK - *Gene therapy for retinal dystrophies* (Invited speaker) **[Ali, PARTNER 2]**

2008

September 29th - Dept. of General Pathology, Universita' degli studi di Napoli Federico II, Napoli, Italy - *AAV-mediated gene transfer for human inherited diseases* (Lecture). **[Auricchio, PARTNER 1]**

November 11-16th - The European Society of Gene Therapy Annual Meeting, Bruges, Belgium - *Gene therapy for retinal diseases* (Invited speaker). **[Auricchio, PARTNER 1]**

November 11-16th - The European Society of Gene Therapy Annual Meeting, Bruges, Belgium - *Serotype- dependent packaging of large genomes in adeno-associated viral vectors result in efficient in vivo gene delivery* (Invited speaker). **[Auricchio, PARTNER 1]**

Publications

In preparation/submission/in press

Tan, M.H., MacKay, D.S., Cowing, J., Tran, H.V., Smith, A.J., Dev-Borman, A., Henderson, R., Moradi, P., Russell-Eggitt, I., Robson AG, Cheetham ME, Thompson DA, Webster AR, Michaelides M, **Ali, R.R**., Moore, A.T. (2011) Leber Congenital Amaurosis Associated with *AIPL1* Variants: Challenges in Ascribing Disease Causation, Clinical Characteristics, and Implications for Gene Therapy. Submitted for publication - PLoS ONE.

Zencak D, Chen D, Ekström P, Tanger E, Bremner R, van Lohuizen M, **Arsenijevic Y**. Retinal degeneration depends on Bmi1 function and re-initiation of cell cycle machinery. PNAS, in revision

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Allocca, M., Manfredi, A., Iodice, C., Di Vicino, U., **Auricchio, A.** (2011) AAV-mediated gene replacement either alone or in combination with physical and pharmacological agents results in partial and transient protection from photoreceptor degeneration associated with βPDE deficiency. *Invest. Ophthalmol. Vis. Sci*. 52(8):5713-9.

Carvalho LS, Xu J, Pearson RA, Smith AJ, Bainbridge JW, Morris LM, Fliesler SJ, Ding XQ, **Ali RR** (2011). Long-term and age-dependent restoration of visual function in a mouse model of CNGB3-associated achromatopsia following gene therapy. Hum Mol Genet 20:3161-75.

Colella P, Iodice C, Di Vicino U, Annunziata I, **Surace EM**, **Auricchio A** (2011). Non-erythropoietic erythropoietin derivatives protect from light-induced and genetic photoreceptor degeneration. Hum Mol Genet.

Karali, M., Manfredi, A., Puppo, A., Marrocco, E., Gargiulo, A., Allocca, M., Corte, M.D., Rossi, S., Giunti, M., Bacci, M.L., Simonelli, F., **Surace, E.M.,** Banfi, S., **Auricchio, A.** (2011) MicroRNA-restricted transgene expression in the retina. *PLoS One 6*, e22166.

Mussolino, C., della Corte, M., Rossi, S., Viola, F., Di Vicino, U., Marrocco, E., Neglia, S., Doria, M., Testa, F., Giovannoni, R., Crasta, M., Giunti, M., Villani, E., Lavitrano, M., Bacci, M.L., Ratiglia, R., Simonelli, F., **Auricchio, A.**, **Surace, E.M.** (2011). AAV-mediated photoreceptor transduction of the pig cone-enriched retina. Gene Ther *18*, 637-645.

Testa, F., **Surace, E.M.,** Rossi, S., Marrocco, E., Gargiulo, A., Di Iorio, V., Ziviello, C., Nesti, A., Fecarotta, S., Bacci, M.L., Giunti, M., Della Corte, M., Banfi, S., **Auricchio, A**., Simonelli F. (2011). Evaluation of Italian patients with leber congenital amaurosis due to AIPL1 mutations highlights the potential applicability of gene therapy. Invest Ophthalmol Vis Sci *52*, 5618-5624.

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Simonelli, F., Maguire, A.M., Testa F, Pierce EA, Mingozzi F, Bennicelli, JL, Rossi S, Marshall, K, Banfi S, **Surace, E.M.**, Sun J, Redmond, TM, Zhu X, Shindler KS, Ying G-S, Ziviello C, Acerra C, Wright JF, Wellman McDonnell J, High KA, Bennett J, **Auricchio, A.** (2010). Gene Therapy for Leber's Congenital Amaurosis is Safe and Effective Through 1.5 Years After Vector Administration. *Mol Ther* 18(3): 643-650.

Sun, X., Pawlyk, B., Xu, X., Liu, X., Bulgakov, O.V., Adamian, M., Sandberg, M.A., Khani, S.C., Tan, M.H., Smith, A.J., **Ali, R.R.**, Li T (2010). Gene therapy with a promoter targeting both rods and cones rescues retinal degeneration caused by AIPL1 mutations. *Gene Ther* 17(1): 117-131.

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Maguire AM, High, KA, **Auricchio A**, Wright JF, Pierce EA, Testa F, Mingozzi F, Bennicelli JL, Ying G-S, Rossi S, Fulton A, Marshall KA, Banfi S, Chung DC, Morgan JIW, Hauck B, Zelanaia O, Zhu X, Raffini L, Coppieters F, De Baere E, Shindler KS, Volpe NJ, Surace EM, Acerra C, Lyubarsky A, Redmond TM, Stone E, Sun J, Wellman McDonnell J, Leroy BP, Simonelli F, Bennett J (2009). Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose escalation trial. *Lancet* 374(9701): 1597-1605.

Smith AJ, Bainbridge JW, **Ali RR** (2009). Prospects for retinal gene replacement therapy. *Trends Genet* 25(4): 156-165.

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Tran HV, Schorderet DF, Kostic C, Munier FL, **Arsenijevic Y** (2009). Thérapie génique des rétinopathies héréditaires: premiers résultats. *Rev Med Suisse* 5: 118-123.

Patient Associations

Patient Organizations. We work closely with a number of patient organizations. Members of the team speak regularly to lay audiences at regional and national meetings of the British Retinitis Pigmentosa Society as well as Retina International, which is an international organization composed of many national patient groups (**PARTNER 2**).

Website. We have developed an extensive website that provides patients and their families with information about the research developed in the AAVEYE project. It includes FAQs and downloadable videos and links to support organizations, such as the British Retinitis Pigmentosa Society (**PARTNER 2**).

Science Dissemination and the Media

Patient information leaflets. We receive many inquiries about our work, particularly after media coverage, and have therefore developed information leaflets that are used to provide information [**PARTNER 2**]

Schools. Participation in annual open days and lectures on gene therapy for school children organized by the Gene Therapy Advisory Committee (Department of Health) and the British Society of Gene Therapy. Provision of short-term summer work experience in my laboratory for local sixth formers (participation in Nuffield Science Bursary Scheme) [**PARTNER 2**]

University. University of Lausanne, School of Medicine, Master I & II September-December 2010 Lausanne, Switzerland **[PARTNER 3]**

Medical schools. Creation of the Option Course "Gene and Vision" for students in 3rd year and Master degree in Medicine (35 hours/year) [**PARTNER 3]**.

Radio interviews
Radio Suisse Romande: Emission Impatience. 30 September 2009 Switzerland **[PARTNER 3]**
Radio Suisse Romande: Emission Impatience. 7 September 2010, Switzerland **[PARTNER 3]**

Television. January 14th 2009 – Documentary film: *Malvoyants: lueurs d'espoir* broadcasted on the Télévision Suisse Romande, TSR1, 36.9º Magazine Santé (participation and assistance in the design of the documentary film) [**PARTNERS 2 and 3]**.

Seminar and course. Centre for Regenerative Medicine "Stefano Ferrari" University of Modena and Reggio Emilia, 9 November 2010 Italy **[PARTNER 3].**

Other

Sponsoring
AAVEYE has sponsored the Eye and Brain session of the ESGCT 2009 Annual Meeting in Hannover, Germany (22/11/09) (**PARTNER 1**).
AAVEYE has sponsored the Eye and Brain session of the ESGCT 2010 Annual Meeting in Milan, Italy (22/10/10) (**PARTNER 1**).

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