Genetic Basis of Inherited Macular Dystrophies and Implications for Stem Cell Therapy

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ABSTRACT
Untreatable hereditary macular dystrophy (HMD) presents a major burden to society in terms of the resulting patient disability and the cost to the healthcare provision system. HMD results in central vision loss in humans sufficiently severe for blind registration, and key issues in the development of therapeutic strategies to target these conditions are greater understanding of the causes of photoreceptor loss and the development of restorative procedures. More effective and precise analytical techniques coupled to the development of transgenic models of disease have led to a prolifer growth in the identification and our understanding of the genetic mutations that underly HMD. Recent successes in driving differentiation of pluripotent cells towards specific somatic lineages have led to the development of more efficient protocols that can yield enriched populations of a desired phenotype. Retinal pigmented epithelial cells and photoreceptors derived from these are some of the most promising cells that may soon be used in the treatment of specific HMD, especially since rapid developments in the field of induced pluripotency have now set the stage for the production of patient-derived stem cells that overcome the ethical and methodological issues surrounding the use of embryonic derivatives. In this review we highlight a selection of HMD which appear suitable candidates for combinatorial restorative therapy, focusing specifically on where those photoreceptor loss occurs. This technology, along with increased genetic screening, opens up an entirely new pathway to restore vision in patients affected by HMD.

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INTRODUCTION
Numerous hereditary retinal disorders (HRD) that affect central visual function in humans have been identified. The specialized central region of the retina, the macula, is responsible for central visual acuity and has distinct anatomical and physiological properties. The fovea at the epicentre of the macula contains the highest density of retinal cone photoreceptors and receives its blood supply entirely from the choriocapillaris complex of the choroid. Derived from the optic neuropil from eye development, the photoreceptors share a common embryological origin with and are supported by the retinal pigment epithelium (RPE) [1, 2], which is separated from the choriocapillaris by Bruch’s membrane, a multilayered basement membrane (Fig. 1). Photoreceptors, RPE, and the choriocapillaris are interdependent on each other; photoreceptor dysfunction and degeneration can occur secondary to RPE pathology (e.g., in Best’s macular dystrophy) or be a primary event such as is the case in Stargardt disease (STGD), where RPE pathophysiology resulting from photoreceptor malfunction leads to photoreceptor depletion. Choriocapillaris atrophy and histopathology can be observed after RPE degeneration in various fundus disorders [3, 4]. Such features are characteristic of a heterogeneous subgroup of progressive HRD affecting the macula, which can cause profound central visual loss sufficiently severe enough for blind registration. Blindness substantially impairs an individual’s quality of life [5–8] and places a large burden on healthcare and support services [9]. There is currently no cure for the underlying causes of any of the macular dystrophies, and available treatments are largely palliative.

Much effort has been invested in the development of protective and cell-based therapies for HRD. Some success has been achieved in prolonging the survival of photoreceptors by intraocular application of growth or antiapoptotic factors in humans [10]; however, even with repeated application this approach typically slows the progression but fails to arrest the degenerative process [11]. The transfer of genes encoding for these factors into the eye offers additional protection by modifying the physiology of affected cells; however, this approach is ineffective in cells that are dysfunctional due to an...
underlying genetic mutation. RPE transplantation prevents photoreceptor degeneration in models of RPE dystrophy [12, 13], but not in a photoreceptor degeneration model [14], and it is an inappropriate strategy in humans once irreversible loss of photoreceptors has occurred.

A protracted phase of inner neural retinal remodeling occurs after the depletion of the sensory photoreceptors; however, the initial stages largely involve remodeling of the outer nuclear layer [15, 16]. Prior to the clinical application of any cell replacement strategy, the progress of global retinal remodeling should first be assessed in order to define the key impediments towards visual reconstitution. A sufficient retinal cyto-architecture must exist for successful graft integration to yield improvements in visual function. Functional cell replacement may be complicated by the retraction of interneuron dendrites and axon terminal fields, Müller glial and horizontal cell hypertrophy, and horizontal neurite sprouting towards the inner plexiform layer [15]. Thus the optimal period for cell engraftment within each disease paradigm should be determined. Nonetheless, a therapeutic window of opportunity for cell replacement may exist prior to significant inner retinal remodeling. Such strategies have proved successful in other degenerative regions of the central nervous system; for example, Parkinsonian symptoms have been reduced for up to six months in monkeys following chemical depletion of dopaminergic cells by transplanting monkey embryonic stem cells (ESCs) or ventral mesencephalon [17, 18], and hind limb motor function can be improved in animal models of spinal cord injury following transplantation of olfactory ensheathing cells [19, 20]. Cells grafted into the photoreceptor-depleted outer retina prior to the onset of global remodeling could potentially reconstitute the remaining retinal circuitry, partially restoring visual function.

Here we present the clinical manifestations and genetic correlations of a selection of hereditary macular dystrophies (HMDs) that may be suitable candidates for emerging cell- and gene-based therapy. The main focus of this review is the outer retina and diseases affecting central visual acuity where the primary defect results, either directly or indirectly, in the demise of photoreceptors. Here we concentrate on photoreceptor replacement rather than RPE replacement, although in many cases of HMD the replacement of both tissue types is required. We discuss current research successes, the potential implications and limitations of emerging techniques for visual restoration, potential combinatorial approaches, and possible future directions in this highly enigmatic field.

GENETIC BASIS OF RETINAL DISEASE

Hereditary Macular Dystrophy

Although modifiable lifestyle factors such as smoking and a high body mass index increase the risk of retinal disease, new evidence is emerging that stresses the importance of familial influence and the underlying molecular causes of retinal disease; indeed, few clinical conditions are totally without some genetic influence [21]. Below we describe three major types of HMD and discuss the genetic and environmental contribution to the onset and progression of retinal disease. A summary of other HRDs is presented in Table 1.

A feature of many HMDs, although typically characteristic of Age-Related Macular Degeneration (AMD), is the presence of sub-RPE deposits, or drusen [22–24]. This contributes to disease pathogenesis by cleaving RPE cell attachment to Bruch’s membrane, which causes disruption of membrane transport and subsequent RPE demise [25]. Concomitant local oxidative stress, chronic inflammation, choriocapillaris changes, and neurosensory degeneration occur [26]. Various underlying molecular causes alter individual susceptibility for drusen accumulation and subsequent pathology in HMD; however, drusen and histopathological changes in RPE can also be detected during normal physiological aging [27]. Photoreceptors are continuously renewed by shedding outer segment (OS) distal disks in a circadian fashion [28]. With aging, the heavy metabolic burden of OS phagocytosis and breakdown on the RPE results in incomplete digestion of internalized material and accumulation of autofluorescent lipofuscin, a component of drusen, within RPE lysosomes and endosomes. A major fluorophore of RPE lipofuscin is A2E [29, 30], a minor by-product of the visual cycle [30, 31]. Given its high density of photoreceptors, the macula accumulates the greatest levels of A2E in its RPE. The RPE becomes less efficient in coping with increased toxic lysosomal A2E and other visual by-products, thus contributing to AMD [31]. Although fundoscopically similar, the age of onset, pattern of drusen deposits, and visual course separate normal aging from patients with HMD. AMD, once considered effectively an environmentally exacerbated form of aging, can now be considered another form of HMD with onset later in life.

STGD. STGD is the most prevalent HMD with an estimated incidence of one in 10,000 [32]. It accounts for 7% of all HRD and carries a 25% a priori risk to siblings [21]. The traditional presentation (STGD1) is autosomal recessive with juvenile onset (7-12 years); however, some rarer autosomal dominant forms exist [33–36]. STGD is clinically characterized by RPE and photoreceptor inner segment lipofuscin accumulation, RPE and choroidal vascular atrophy, macular photoreceptor loss, and reactive Müller glial hypertrophy [37, 38] with progressive loss of central visual acuity during the first two decades [21]. Fundus flavimaculatus, characterized by subtly later onset, slower progression, and widespread, symmetrical, deeply localized retinal flecks [39, 40] is understood to be a different manifestation of the same disease.

STGD dystrophies are mapped to chromosome 1p21-p22.1 and vary in onset, clinical course, and severity. This is likely due to allelic mutations, largely missense, producing a continuum of disease pathology and presentation [21, 41, 42].
| Macular Dystrophy                          | Occurrence         | Age of onset | Chromosome (locus)                          | Gene                                |
|-------------------------------------------|--------------------|--------------|--------------------------------------------|-------------------------------------|
| Achromatopsia (AR)                        | 1:30,000           | Congenital   | 2q11 (ACHM2)                               | CNGA3, CNGR3                        |
| Blue cone monochromatic color blindness    | <1:100,000         | Congenital   | Xq28                                       | Red cone pigment, green cone pigment |
| (AD, cross-linked recessive)               |                    |              | Xg21-q22 (ACHM3)                           |                                     |
| Progressive cone dystrophy (AD)           | nk                 | First to fourth decade | 6p21-6cen; 6p21.1; Xp11.4; Xq27           | RDS/peripherin, GUCA1A, CNGA3        |
| AMD (AD)                                  | 1:250 (Blindness prevalence 8.7%) | From fifth decade onwards | 1q24-q25 (ARMD1); 1p (ARMD2); 1q31 (ARMD3); 1q32 (ARMD4); 10q11 (ARMD5); 19p13.3 (ARMD6); 10q26 (ARMD7); 10q26 (ARMD8); 19p13.3-p13.2 (ARMD9); 9q32-q33 (ARMD10); 20p11.2 (ARMD11); 3pter-p21; 6q25.1; 11q12-q13.1 | FBLN6, ABCR, FBLN5, Y402H, ERCC6, RAXL1, HTRA1, LOC387715, C3, TLR4, CST3, CXCR1, ESR1, E4 variant of ApoE, SERPING1, HLA, VEGF |
| Best vitelliform (AD)                      | Rare <1:200,000    | Early childhood to middle age | 11q13                      | VMD2                                |
| Adult vitelliform (AD)                     | nk                 | Fourth or fifth decade | 6p21.2-cen (RP-7) | RDS, RDS; PRPH2 |
| Butterfly-shaped dystrophy (AD)            | nk                 | Third or fourth decade | 6p21.2-cen (RP-7) | RDS, RPOH2 |
| Central areolar choroidal dystrophy (AD)   | nk                 | nk            | 17p13-p12                                  | EEFEMP1                             |
| Doyne honeycomb dystrophy (AD)            | nk                 | nk            | 2p16-p21                                   |                                    |
| Late-onset retinal dystrophy               | <1:2000            | Fourth to fifth decade | 11q23.3                                  | C1 QTNF5                           |
| North Carolina (AD)                       | nk                 | Infancy       | 6q14-q1.63 (MCDRI1)                       | nk                                  |
| Pseudoaxanthoma elasticum (AD/AR)         | 1:25,000-50,000    | 10 years to adulthood | 16p13.1                                  | ABCC6, TIMP5                        |
| Sorsby fundus dystrophy (AD)              | <1:2000            | Fourth and fifth decades when CNV develops | 22q12.1-q13.2 | ELOV4, TIMP5 |
| STGD3/STGD4 (AD)                          | <1:2000            | First or second decades | 6cen-q14 (STGD3); 4p (STGD4)             |                                    |
| STGD1 (AR)                                | 1:10,000           | 6–20 years    | 1p21-p22.1 (STGD1)                        | ABCA4                               |

Abbreviations: AD, autosomal dominant; AMD, Age-Related Macular Degeneration; AR, autosomal recessive; CNV, choroidal neovascularization; nk, not known.
The adenosine triphosphate–binding cassette (ABC) transporters function to aid adenosine triphosphate–dependent translocation of substrates across cell membranes and are implicated in human inherited disorders. Deletion of the ABC subfamily A (ABCA) member four (ABCA4, alternatively ABCR) gene [43, 44], which encodes for the rod OS protein Rim (RnM) [45, 46], functions in the transmembrane transport of vitamin A derivatives to the RPE and accounts for 60% of STGD cases [47]. Mutations in ABCA4 have been identified in retinitis pigmentosa (RP) and cone-rod dystrophy [48–51]. ABCA4 mutations are also associated with all juvenile HMD, recessive RP, and cone-rod degeneration [47, 50].

Aber+/− mice display biochemical, physiological, and ultrastructural changes with delayed rod dark adaptation, delayed clearance of all-trans-retinaldehyde (all-trans-RAL), increased levels of PE in rod OS, accelerated A2E accumulation in the RPE, thickening of Bruch’s membrane, and visual loss [52, 53]. When raised in total darkness, A2E accumulation in Aber+/− mice is completely inhibited, indicating that the rate of STGD progression in humans might be slowed by limiting light exposure [54].

The two dominant forms are genetically distinct from recessive STGD, mapped to chromosome 6cen-q14 (STGD3) and 4p (STGD4). These affect the elongation of very long-chain fatty acid-like gene four (ELOVLA4), expressed abundantly in photoreceptors [55]. A knock-in mouse model of a five base pair deletion in ELOVLA4 was recently reported [56]. Mice heterozygous for this mutation show photoreceptor degeneration, while the homozygous variety have abnormally compacted outer epithelium, lack key hydrophobic components of the stratum corneum affecting permeability barrier function, and die shortly after birth.

**Sorsby Pseudoinflammatory Fundus Dystrophy.** Sorsby pseudoinflammatory fundus dystrophy (SFD) is a highly penetrant, rare, autosomal dominant condition characterized by loss of central vision due to extracellular matrix abnormalities in Bruch’s membrane and bilateral choroidal neovascularization (CNV). This disrupts choroidal nutrient and metabolite transport leading to atrophy of the neural retina [57, 58]. Onset is typically 40–50 years of age [59, 60]. Fine drusen-like deposits, atrophic lesions at the macula [21], and lipid deposits at the RPE/Bruch’s membrane interface are observed [22]. This is usually complicated by CNV and associated hemorrhage leading to a disciform macular scar [61]. The peripheral retina is also affected, resulting in night blindness (nyctalopia).

SFD shares several features with late-stage AMD, resulting in SFD becoming an accepted AMD genetic model [62]. Whilst AMD has highly complex and largely unknown etiology, SFD is a single-gene disorder that occurs due to mutations in the TIMP3 gene on chromosome 22 [63–65]. Its product is an RPE enzyme important in extracellular matrix regulation [66, 67], and the presence of a mutant form may affect retinal protein turnover [61]. Eight mutations are described, seven affecting the coding sequence in exon 5; six are missense, introducing a novel unpaired cysteine residue into the C-terminal domain [61, 62, 66, 68–70]. Other mutations introduce a stop codon at position 139 [71] or a single base (A) in the splice acceptor site of exon 4 and 5 (CAO to CAAG) [72]. A region of the TIMP3 C-terminus appears particularly vulnerable; three mutations are found between residues 166–168 with two others (172 and 181) in close proximity [61]. The serine-to-cysteine substitution of residue 181 [Ser181Cys] in exon five is the causal mutation in the majority of Sorsby’s families tested in the United Kingdom.

In SFD, mutant TIMP3 accumulates in the RPE and Bruch’s membrane, prompting the disease process [65, 73, 74]; TIMP3 overexpression can induce apoptosis in several cell types including RPE [75]. Unlike other TIMP family members, TIMP3 binds to the extracellular matrix via its C-terminal, the site of all known SFD mutations [61, 76–78]. Whereas mice with targeted deletion of TIMP1 show CNV [79], homozygous TIMP3-null mice do not exhibit an obvious retinal phenotype [80]. A knock-in mouse carrying a Ser156Cys mutation in the orthologous murine TIMP3 gene shows clinical features of human SFD, including abnormalities and elevated TIMP3 in Bruch’s membrane and RPE [68, 81], providing an experimental system in which to investigate SFD pathophysiology. The range of mutations is limited in SFD, meaning that genetic analysis makes diagnosis quick and reliable, although the underlying disease process remains untreatable.

**Age-Related Macular Degeneration.** AMD is the leading cause of blindness in the developed world over 60 years of age and accounts for 50% of blind registration [82–87]. Incidence of AMD rises exponentially with age [88]. While its prevalence is increasing in the United Kingdom, blind registration from cataract, glaucoma, and optic atrophy has declined [89]. There are two main forms of AMD: an early form characterized by degenerative changes in the RPE and accumulation of drusen, and a late form that manifests with geographic atrophy alongside sub-RPE and subretinal CNV [90]. Therapies to prevent and treat AMD are limited. High-dose antioxidant vitamins and metabolic therapies can reduce the progressive visual loss in patients with early AMD and, in the case of metabolic therapy, the regression of drusen; however, the absolute reduction in new cases is small [91–93]. Although environmental factors are known to play a role in disease pathology and progression, epidemiological and family-based studies provide convincing evidence for a genetic basis for AMD, with inheritance thought to be polygenic [94–100]. Despite its prevalence, the etiology and pathogenesis of AMD remains poorly understood. Susceptibility loci (chromosome 1q32 confers the greatest risk of AMD [112]; the CFH Y402H haplotype appears to play a role have been identified [104–108], some correlating with AMD pathology more strongly than others; more than 100 different proteins are associated with AMD deposits [108].

The complement system, involved in our immune defense against foreign antigens, is implicated in AMD [109–111]. Complement factor H (CFH) inhibits the complement cascade that ensures the system is directed against pathogens and not the body’s own tissues, and is a major susceptibility gene in AMD. Complement components are found around drusen and the RPE-choroid interface [110], and abnormal macular dysregulation of the complement cascade in RPE and Bruch’s membrane appears to cause unrestrained complement activation and drusen formation [112]. The CFH Y402H haplotype on chromosome 1q32 confers the greatest risk of AMD [113]; however, multiple polymorphism variants in caucasian AMD patients have been determined [104, 114–118]. Y402H is not a primary indicator in Japanese patients, however [119], nor in the Chinese population in which this polymorphism is associated specifically with neovascular AMD [120]. The classical complement pathway is largely regulated by the SERPING1 gene product, and a common polymorphism in SERPING1 was recently identified as a causal factor in AMD [121]. Interestingly, a CFH haplotype with a deletion in CFHR1 and CFHR3 elicits an independent decreased risk of AMD [113].

Oxidative stress pathways are also causally involved [122]. Schmidt and colleagues identified a coding change
[Ala69Ser] in the LOC387715/ARMS2 gene on chromosome 10q26 as the second major identified susceptibility allele [123]. They report that genetic susceptibility in combination with a modifiable lifestyle factor conveys a significantly higher risk than either factor alone. The population-attributable risk is 36% for LOC387715/ARMS2 and 43% for Y402H, however combining LOC387715 and cigarette smoking increases risk to 61% [123]. The LOC387715/ARMS2 protein product colocalizes with the mitochondrial outer membrane in mammals [106]; thus, the Ala69Ser mutation presumably contributes to AMD pathogenesis by affecting mitochondrial function. Polymorphisms of other immune response genes, such as human leukocyte antigen, are also implicated after observation of strong human leukocyte antigen immuno-reactivity in drusen [109, 124]. Variants of complement cascade component factor B and complement component two are protective against AMD [105]. Several genes implicated in the etiology of various HMD have been examined in AMD. Carrier relatives of STGD are more likely to develop AMD [125], and genetic similarities may exist between the two [43]; however, it remains controversial as to whether the ABCA4 gene plays a pathogenic role [43, 44]. The high level of ABCA4 polymorphism across individuals makes it difficult to determine any responsibility for ABCA4 in AMD [21]; however, its involvement is possible in a small number of cases [107]. A2E, which accumulates in the STGD eye, is also found in the RPE in AMD and perturbs efflux of cholesterol from RPE endosomes/lysosomes, causing cholesterol and cholesteryl ester deposit accumulation [61, 126, 127]. Initially identified as a causative factor in Doyne’s disease, a mutation in the extracellular matrix protein Fibulin 3 gene [128] led to the correlation of missense mutations in Fibulin 5 in 1.7% of 402 AMD cases in the United States [129]. These contribute to AMD by causing reduced Fibulin five and elastin production—a key Bruch’s membrane component—but were not causal [129]. The TIMP3 mutation in SFD has not correlated with AMD [66, 130], although distribution of the TIMP3 enzyme product in the AMD Bruch’s membrane is significantly higher than age-matched controls, remains in active form, and is associated with drusen patches [76]. Late-onset retinal degeneration (LORD) is a rare autosomal dominant disorder that has striking parallels with AMD. Histopathology reveals thick sub-RPE deposits that result in RPE dysfunction and photoreceptor loss [24, 131–133]. A Ser163Arg mutation in the CTRPs/C1QTNF5 gene is reported to cause approximately 50% of documented LORD cases [134]. As yet it is unknown whether mutations in CTRP5 influence susceptibility to AMD. Apolipoprotein E (ApoE) and β-amyloid aggregates, associated with Alzheimer’s disease, have been found in AMD-related macular drusen [135, 136]. Some studies implicate the ApoE ε4 allele as a protective factor which delays disease onset, while the ε2 allele accelerates disease onset and progression [137, 138]. However, although there is currently no significant association between Alzheimer’s disease and AMD [139], a recent study supports some relationship between cognitive function and dementia with early AMD in older individuals [140]. Mutations in the Bestrophin gene, responsible for vitelliform macular dystrophy (Best’s disease) show a small, nonsignificant correlation with AMD [141, 142]. The identification of vascular endothelial growth factor (VEGF) polymorphisms in late-stage AMD has led to the use of optical coherence tomography-guided anti-VEGF (OCT-VEGF) treatments which slow disease progression by treating CNV [143]. They do not, however, alter the underlying pathophysiology, and atrophic macular changes can still progress despite successful CNV treatment. A naturally occurring mouse model with autosomal recessive late-onset severe retinal degeneration mapped to the Mdm1 gene on mouse chromosome 10 was recently reported, but is not associated with the human Mdm1 ortholog or AMD [144]. The Sod1ťťť and ApoE knock-in mice show the most similarities to the human clinical manifestation of AMD such as good disease models [145, 146]. In conclusion, the most prominent genetic factors identified to date in the etiology of AMD are the Y402H variant of CFH, LOC387715, and SERPING1, which are found in more than 60% of cases [121]. Cell Replacement Strategies While modifiable lifestyle factors present an increased risk of retinal disease to an individual already expressing high-risk alleles, there is clear evidence that genetics plays an important role in the occurrence and development of HMD. Understanding the inheritance and genetic basis of these retinal diseases will undoubtedly provide new treatment platforms for restorative therapy. Gene therapy, whether corrective [147–150] or to overexpress various neuroprotective substances [153] either on its own or combined with stem cell therapy, has enormous potential in these diseases, although it may be less applicable to those cases where the dystrophy is advanced at birth or where perturbed retinal and cortical development has compromised visual function [151]. Stem cell therapy could be used in two main ways, namely by enhancing endogenous repair using stem cells that secrete growth factors or deliver drugs utilizing the innate attraction of diseased structures to various stem cell populations, or by exogenous cell replacement strategies. In the case of AMD, the latter strategy could be a useful therapeutic option depending on integrity of the inner retina, Bruch’s membrane, and choriocapillaris, and provided that problems associated with the synaptic integration between transplanted photoreceptors and the host retina can be overcome. Various sources of cells and methods of transplantation have been used in the attempt to replenish the retina with functional photoreceptors or healthy RPE following the degeneration of host cells in assorted animal models of retinal degeneration with various degrees of success [152–157]. In the best case, subretinal transplantation of embryonic and postnatal day 1 (P1) mouse retinal cells into murine models of retinal degeneration showed greatest integration, differentiation, and synaptic connectivity within host tissue when already committed to a photoreceptor fate while still morphologically immature. Increases in pupil sensitivity in this study correlated with the number of incorporated Nrl− donor cells, a result that was not achieved with transplanted proliferating or stem cells [157]. Recent reports describe the successful generation of photoreceptor precursors from ESCs using defined protocols incorporating factors that are known to promote forebrain development, retinal progenitor specification and photoreceptor induction [158–161]. Similarly, protocols for RPE production from ESCs are being refined, and functional studies on human ESC-derived RPE demonstrate their physiological viability [162]. Small numbers of opsin- and rhodopsin-positive cells can be observed after subretinal transplantation of spontaneously differentiated human ESCs (hESCs) into neonatal and adult rat eyes with no tumor formation observed up to 18 weeks post-transplant [163]. More recently, retinal progenitors derived from hESC were subretinally transplanted into Cpxťťť, a model of Leber congenital amaurosis (LCA) [164]. Grafted cells integrated within the outer nuclear layer; displayed layer-specific expression of opsin, rhodopsin, and recoverin; and restored the light response in previously

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unresponsive recipients, even though grafted cells expressing photoreceptor-specific markers did not develop OSs. These results highlight the value of hESC as a potentially unlimited source of specialized cell types for transplantation; however, issues do remain regarding their use, including efficient direction of differentiation towards a required lineage and elimination of undifferentiated ESCs from the cell population intended for transplantation in order to minimize the risk of tumorigenesis.

The ETDRS chart, originally used in the Early Treatment of Diabetic Retinopathy Study, has become the standard distance visual acuity as measurement in clinical research [165]. More recently, modest improvements in visual acuity as measured using the ETDRS chart was reported after transplantation of fetal neural retina with its RPE in a patient with dominant RP [166]. These authors recently published their results from an additional fetal retina-RPE transplant clinical trial conducted on six RP and four AMD patients [167]. In this latest trial, 70% of transplant recipients showed improvements in visual acuity, and postoperative loss of RPE pigmentation was not associated with changes in visual outcome [167]. In one patient, improvement in visual acuity and light sensitivity was maintained for 6 years. It is difficult to discern, however, whether observed improvements are due to graft functionality, graft-derived factors, or surgical manipulation eliciting improvements in visual function [168, 169]. The development of an artificial Bruch’s membrane substitute or use of scaffold to maximize the adhesion and safe surgical delivery of the RPE sheets or tissue under the retina may enhance cell replacement strategies [158, 170] provided there is access to functional choriocapillaris. The absence of just one of these vital elements will cause a sequence of events affecting retinal viability and hinder graft integration and function.

In summary, although cell transplantation results are encouraging, only a small proportion of grafted cells (in the best case 0.4% of sorted P1 Nrl+ photoreceptor precursors) integrated within the correct lamina and differentiated appropriately in a model of retinal degeneration [158]; therefore, the efficiency of retinal reconstitution via cell replacement remains a challenge. Encouraging incorporation of grafted photoreceptor precursors is ineffective without a functional RPE/Bruch’s membrane/choriocapillaris complex. Studies over the past 10 years have shown that alogeneric RPE largely resists attachment to aged Bruch’s membrane in vitro and where successful attachment and proliferation has been achieved, long-term survival is poor [171–173]. Early studies in humans demonstrated that long-term survival of transplanted fetal retina with its RPE (up to 6 months) could be achieved in RP patients, but with no improvement in visual function [174]. Similar studies in AMD patients yielded mixed results; transplantation of fetal or adult RPE [175–178] improved that late-AMD is less conducive for graft survival with patients showing signs of rejection within 3 months [174]. RPE transplanted alongside aggregate retinal transplants or photoreceptor sheets into RP and AMD patients proved the long-term safety and survival of grafts but again patients showed no improvement in visual function [179–181].

**Revolutionary Developments in the Stem Cell Field: Induced Pluripotency**

As highlighted in the above section, hESCs have been shown to differentiate along photoreceptor and RPE lineages using growth factors that have roles in forebrain development and early and late retinal fate specification. In one very recent study, hESC-derived photoreceptor cells were shown to settle into the appropriate layers and express markers of differentiated rod and cone cells upon intraocular injection into animal models of retinal disease [164]. These are undoubtedly promising results, but many substantial shortcomings of hESC differentiation still await resolution. Among these are the ethical issues associated with the use of embryonic tissue, the apparently embryonic/fetal phenotype of the cells derived during hESC differentiation [183], and the potential of tumorigenesis arising from the presence of undifferentiated progenitors remaining in culture; however, the principal problem is one of immune rejection of differentiated cells after transplantation into the patient. Pioneering work carried out in the last 3 years suggests that immune rejection issues may be overcome by creating embryonic-like stem cells from somatic cells of adult individuals through a process called induced pluripotency [184–186]. This process, which is held as one of the most seminal discoveries in the stem cell field, was first reported by Yamanaka’s group [187] and involves overexpression of four key genes (Oct4, Sox2, Klf4, and c-Myc) in murine fibroblasts, resulting in their conversion into cells that resemble ESCs in terms of morphology, gene expression, growth, and differentiation capabilities and are now named induced pluripotent stem cells (iPSCs). This work was soon supplemented by the generation of human iPSCs by two groups, one of whom [186] showed that adult human dermal fibroblasts can also be reprogrammed by overexpression of OCT4, SOX2, KLF4, and c-MYC, while another [188] made use of a slightly different set of factors (OCT4, SOX2, LIN28, and NANOG) to reprogram both fetal and adult human fibroblasts. Since these initial papers there has been remarkable progress in the field, aimed largely at increasing the efficiency of the iPSC generation protocol and replacing the initial retroviral vectors that were used to transfected fibroblasts with OCT4, SOX2, KLF4, and c-MYC. Two approaches to this latter problem have been used with varying degrees of success. The use of nonintegrating retroviruses that maintain transient expression of the reprogramming factor genes from episomes that do not integrate into the host cell genome has shown some potential to generate iPSCs, albeit with lower efficiency than is achievable with integrating retroviruses [189]. Very recently, a protocol using recombinant proteins for induction of pluripotency has been published, which suggests that future methods for the production of iPSCs may be much simpler than those of the initial publications in this area [190].

Together these developments suggest that it is now possible to derive iPSCs free of transgenes and with perhaps reduced risk of tumorigenesis, which was observed in the initial animal-based studies (20–30%) associated with reactivation of c-MYC in adult tissues [191]. Caution is required, however, for the small molecules and chemicals that are required for reprogramming because they may promote global epigenetic and genetic modifications, which may compromise the safety aspect of these cells.

The ability to generate iPSCs readily allows us to progress to one of the principal applications envisaged for this technology, which is the generation of human disease models and potential correction of genetic disease. For example, iPSC technology was combined with gene therapy to correct the mutant human sickle cell anemia allele [192]. The corrected iPSC generated in this study were induced to differentiate to hematopoietic progenitors that were subsequently transplanted into the murine model of sickle cell anemia, leading to functional recovery and providing a “proof of principle” demonstration for future human therapeutic application. Similarly, transplantation of murine iPSC-derived neural progenitors into animal models of Parkinson’s disease led to improved behavior 4 weeks after transplantation [193]. Furthermore, iPSCs

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have been generated from other species, including the rhesus macaque, which is by far one of the most relevant primate models for human disease [194].

To date, a large number of iPSC lines from various diseases such as amyotrophic lateral sclerosis [195]; Parkinson’s disease [196]; type I diabetes [197]; spinal muscular atrophy [198]; adenosine deaminase deficiency-related severe combined immunodeficiency (ADA-SCID); Shwachman-Bodian-Diamond syndrome; Gaucher disease; and type III, Duchenne, and Becker muscular dystrophy [199] have been reported.

Creation of iPSC lines on its own is not sufficient and needs to be combined with techniques that have and will be developed in human ESC/iPSC for efficient and directed differentiation towards the desired functional cell type. In view of this, it is important to determine whether iPSCs differentiate into various phenotypes in a manner similar to ESCs, and, although a couple of studies have suggested that this may be the case under in vitro conditions [200–202], in vivo functional studies in animal models need to be performed to fully investigate the potential of iPSC lines for both disease modeling and cell transplantation.

### Potential Applications and Limitations of iPSCs for Understanding and Treatment of Retinal Disease

Derivation of iPSCs from patients with diseases causing outer retinal degeneration, alongside in vitro gene correction with a robust differentiation method for producing photoreceptor and RPE cells from these cells, could provide a source of autologous cells for transplantation. A very recent report has shown that human iPSC can be differentiated into photoreceptor cells with efficiency similar to that of human ESCs [203], suggesting that already established protocols for human ESCs can be transferred directly to iPSCs. Of equal importance is the derivation of iPSCs from patients with retinal disease for creation of disease models, which provides an invaluable opportunity to investigate disease pathogenesis and treatment that has not been possible before. Although there are successful animal models of retinal degeneration, most of these models mimic modulation of one or at most two genes [204]. Using iPSCs isolated from affected patients provides new opportunities to complete the current gaps in our understanding [205], especially for disorders such as AMD where multiple genes are likely to play a role in disease initiation and progression as highlighted above (see Age-Related Macular Degeneration) and where animal models that mimic the disease perfectly are difficult to create due the polygenic nature of the disease. Such cells can be used to devise genetic tests for risk prediction and diagnosis as well as testing and design of new drugs that may have an impact on photoreceptor and RPE cell degeneration.

Generation of photoreceptor and RPE cells from patient-specific iPSC lines overcomes the need for immunosuppression and will likely reduce the risk of rejection of grafted tissue within the neurally depleted region. However, in diseases where a single underlying genetic mutation is causing cellular dysfunction or death, one caveat in generating iPSCs from such patients will be the generation of populations of cells that carry the same mutation. Targeting host photoreceptors with gene therapy to supply growth factors or antiapoptotic genes has already been tested in animal models of degenerative retinal disease in addition to antiangiogenic factors in animal models of neovascular retinal disease, with no adverse morphological, inflammatory, or vascular effects [206]. It can therefore be envisaged that expression of wild-type ABCA4 in a mouse model of STGD was shown to transduce up to 20% of photoreceptors in the injected region and substantially reduce disease-associated lipofuscin accumulation [206].

It can be therefore envisaged that expression of wild-type ABCA4 in iPSCs derived from well-characterized STGD patients with ABCA4 mutations and their further differentiation to RPE and photoreceptor cells could potentially elicit some clinical improvement. Conversely, in cases where differing clinical manifestations of HRD arise from the same gene mutation (for example, adult vitelliform, central areolar choroidal, and butterfly-shaped dystrophies all arise from a mutation in the RDS gene on the short arm of chromosome six (Table 1)), the same corrective gene therapy might be applied to patients spanning more than one dystrophy, eliciting wider clinical impact. Care should be exercised with this approach, however, because overexpression of particular genes may carry the additional risk of unwanted and unpredicted physiological effects. In addition, applications of this approach for other single-gene disorders will depend very much on the size of the gene of interest because vectors have limited packing capacity, which may convey limitations in terms of the size of the defect that could be fixed. This approach will not work in genetic diseases associated with dominant negative effect such as STGD patients with ELOVL4 mutations or SFD patients with TIMP3 [207, 208]. In these cases, gene correction at the iPSC stage is likely to be the only means of gene correction. There are already developments in this field mainly applied to hESCs using zinc finger nucleases [209]; however, this method has to be established and tested in human iPSCs and in multiple loci with high efficiency before this approach can be envisaged as a tool in cell and gene therapy.

Correction of gene defects in other retinal disorders such as AMD already associated with multiple genes, polymorphisms, and environmental factors, however, is unlikely using the iPSC approach, and in this case the usefulness of iPSC technology will mainly rely on the development of screening tools that will enable identification of individuals with high-risk of advanced AMD prior to retinal degeneration.

### Translation: Challenges Ahead?

Efficient tissue delivery, graft integration, and synaptic connection with host circuitry remain as issues for the successful clinical translation of cell-based restorative therapies [210]. In patient terms, those affected by severe macular degeneration may be more willing to consider the option of translational human surgery. There is rarely any HRD-associated cognitive impairment, which means that informed consent could be performed with the patients prior to any translational research. Exploiting HMD with well characterized causal mutations is an excellent starting point for the translation of gene therapy combined with iPSC cell-replacement strategies from bench to bedside. For example, STGD is relatively well characterized and elicits quite common and clinically relevant, and it primarily affects the macula, thus allowing potentially simple surgical replacement of macular cells with genetically-corrected iPSCs.
Cell replacement requires the stable and appropriate non-disruptive integration of functional cells within the correct lamina, the formation of synapses with appropriate interneurons, and the correct electrophysiological and transmitter responses to light to enable the transmission of discernable visual responses to the cortex. It also requires the long-term survival and viability of grafted cells. Variables in the transplantation procedure in humans include the source of cells, age of the donor, transplantation method, and stage of host disease progression [211, 212]. The presence of xenogeneic molecules in protocols used to direct differentiation and integration in vitro will limit clinical use. Similar to the use of hESCs, driving patient-specific stem cells towards a desired lineage in appropriate numbers remains a challenge. Recent advances in differentiating stem cells to photoreceptors have been achieved, meaning there are already some methodologies in place for generating the cells of interest [160, 161, 213], and the results of the study by MacLaren et al. provides information about the developmental time point to which we should drive cellular differentiation to encourage graft integration [158]. Although much work needs to be done to enhance the incorporation of grafted cells, it may be that in some patients only a small number of integrated cells are needed for functional restoration. In one study, the onset of obvious visual symptoms in glaucoma patients was reported only after loss of 25-35% of ganglion cells [214]. It should be noted here that the possibility of correcting the underlying etiology in the end stage of disease by iPSCs, gene correction, and transplantation is attractive, but the comprehensiveness of this approach is dependent on the viability of other vital support retinal components. Patients affected by perturbed retinal cytoarchitecture will be less amenable to this therapy, making clinical assessment of these parameters by in vivo imaging and electrophysiology vital.

The continued identification of candidate genes and suitable delivery vehicles is key. Research into the molecular genetics of HRD is at an early stage, making disease classification on the basis of molecular pathology difficult. Patients previously have been divided by phenotype; however, the importance of accurately genotyping individual cases must be realized. This is likely currently complicated by expense or the lack of a well identified gene defect with an available test for exploitation.

Additionally, HRDs are inherited due to numerous types of mutations (loss of function, gain of function) and are clinically and genetically heterogeneous [215]. Mutation screening in STGD patients has led to the identification of 400 sequence variations in the ABCA4 gene [216–219]. In early-onset HRD, genetic tests are able to be conducted more readily; however, this becomes problematic in the case of AMD where, due to the late onset, parents of affected individuals are often deceased and their offspring yet to be affected [220]. In representative animal models of HRD, the efficiency of gene correction techniques can be tested [53, 81], and this will be further enhanced by the availability of patient-derived iPSCs.

One concern might be whether inheritance of HRD will affect gene therapy potential; for example, STGD is recessive and the mutations give variable penetration and expressivity. However, recessive models have been more amenable to gene therapy by restoration of the wild-type form of mutant genes. Photoreceptor rescue was achieved in 1996 by Bennett and colleagues who used adenovirus to introduce wild-type PDE6β in the rd mouse [221], an effect that was improved by the use of second-generation adenovirus and then lentivirus [222, 223]. The results of three independent human retinal RPE65-replacement trials, published recently, demonstrate the safety of subretinal vector delivery in humans and show that gene therapy in advanced cases of human LCA could induce modest improvements in vision [148–150, 224]. Two of the studies [148, 149] used the ETDRS chart and an obstacle course combined with various other tests; however, only one reported electroretinography results [149]. Each study utilized recombinant AAV-2 vector delivered subretinally. In the study by Maguire et al. in Philadelphia [148], human RPE65 carrying a chicken β-actin promoter was introduced into three patients, two with homozygous missense and one with null mutation in RPE65. Patients reported improvements in vision at 2 weeks and measured improvements in pupillometry and nystagmus frequency over several weeks, concomitant with improvements in confidence and time taken to complete an obstacle course in all subjects [148]. The London study reported by Bainbridge et al., [149] delivered the human RPE65 gene under a human RPE65 promoter in three LCA patients with RPE65 missense mutations. Unlike the first study, only one patient showed visual improvement as measured by ETDRS alongside significantly improved retinal sensitivity, dark-adapted periometry, and dramatic improvements in mobility through an obstacle course (from 77 to 14 seconds). Electroretinography results however, were not improved [149]. The third study reported treatment of three young patients with LCA. All three patients showed significantly increased visual sensitivity in vector treated retinal regions, yet the kinetics of the newly restored retinoid cycle in rod and cone photoreceptors was slow [150]. In all studies there were no adverse systemic effects. The safety of the technique has thus been demonstrated, though visual improvements were modest, which suggests that perhaps treatment needs to occur at an earlier stage in the disease course or in combination with cell-replacement therapy.

It is important to show that iPSCs can be generated from older patients and whether patients at later stages of disease are amenable to combinatorial therapy. Bainbridge et al. proposed that the patient who benefited from gene therapy did so due to being treated while at a less advanced stage than their counterparts [149], highlighting the importance of understanding the optimal window of opportunity for genetic intervention. It cannot be ruled out, however, that observed differences between the studies may have occurred due to the use of different promoters, the subretinal delivery of varying amounts (150 µl vs. 1,000 µl), or slight variations in methodology.

Furthermore, although the eye and subretinal space in particular is relatively immunologically privileged, the immune response may be exacerbated and affect graft survival in HRD cases where the blood retinal barrier is compromised [11, 211]. There is evidence that AMD has an inflammatory element, and avoiding any element of immune rejection would be vital in terms of disease recurrence. Use of iPSCs would avoid an immune response to the donor cells themselves, but genetically engineered modifications are being made to viral vectors to limit the immune response using this approach [225].

Questions and Future Directions

Whilst developing the potential of iPSCs as a therapeutic strategy overcomes many of the issues associated with the use of hESCs (ethics, immunorejection), there are some very important control experiments that must be performed before translation of iPSC-based cell therapies to the clinic can become a reality. Fundamental questions include: Are these cells identical to hESCs? Do they have a normal karyotype? Can we efficiently differentiate iPSCs to photoreceptors and RPE? Given recent successes in generating photoreceptor precursors and functional RPE from hESCs, one would imagine...
similar results could be achieved with iPSCs. There is already evidence that iPSCs can differentiate into derivatives of the three germ layers [186]; however, it remains unknown whether retroviral transduction has any long lasting effects or whether cells might be reactivated under stress or with a lack of instructive cues, leading to the presence of potentially oncogenic cells. In light of this last comment, virus-free, nonintegrating plasmid reprogramming has been demonstrated in embryonic mouse fibroblasts [226], albeit at low efficiency compared with retroviral methods. These nonviral iPSCs were free from transgene integration in host chromosomes, demonstrated a capacity to differentiate towards all three germ layers, and could produce chimeric mice when injected into blastocysts. It is clear that while HMD with clearly identified etiology and a common causal mutation are the best candidates for combinatorial iPSC/gene/transplantation therapy, much work remains to be done to transform the current course of action for HMD for such therapy to become a reality in the near future. Improved knowledge of the mechanisms and molecular basis of HMD alongside continued safety evaluation of the long-term action and consequences of gene therapy and cell restoration will allow us to move towards tailoring safe, more specific and efficient restorative therapies for patients affected by this heterogeneous group of currently incurable diseases.

**REFERENCES**

1. Mund ML, Rodrigues MM. Embryology of the human retinal pigment epithelium. In: Zinn RM, Marmor MF, eds. The Retinal Pigment Epithelium. London, Harvard University Press, 1979:45–52.

2. Moshiri A, Close J, Reh TA. Retinal stem cells and regeneration. Int J Dev Biol 2004;48:1003–1014.

3. Korte GE, Reppucci V, Henkind P. RPE destruction causes choriocapillary atrophy. Invest Ophthalmol Vis Sci 1984;25:1135–1145.

4. Armstrong JD, Meyer D, Xu S, Ellevsig JL. Long-term follow-up of Stargardt’s disease and fundus flavimaculatus. Ophthalmology 1998; 105:448–457.

5. Rovner BW, Zisselman PM, Shmuely-Dulitzki Y. Depression and disability in older people with impaired vision: a follow-up study. J Am Geriatr Soc 1996;44:181–184.

6. Stuck AE, Walthert JM, Nikolau T et al. Risk factors for functional status decline in community-living elderly people: a systematic literature review. Soc Sci Med 1999;48:445–469.

7. Grisso JA, Kelsey JL, Strom BL et al. Risk factors for falls as a cause of hip fracture in women. The Northeast Hip Fracture Study Group. N Engl J Med 1991;324:1326–1331.

8. Williams RA, Brody BL, Thomas RG et al. The psychosocial impact of macular degeneration. Arch Ophthalmol 1998;116:514–520.

9. Lanchoney DM, Maguire MG, Fine SL. A model of the incidence and consequences of choroidal neovascularization secondary to age-related macular degeneration. Comparative effects of current treatment and potential prophylaxis on visual outcomes in high-risk patients. Arch Ophthalmol 1998;116:1045–1052.

10. Sieving PA, Caruso RC, Tao W et al. Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF delivered by encapsulated cell intraocular implants. Proc Natl Acad Sci USA 2006;103:3896–3901.

11. Harvey AR, Hu Y, Leaver SG et al. Gene therapy and transplantation in the CNS repair: the visual system. Prog Ret Eye Res 2006;25:449–489.

12. Li L, Turner JE. Inherited retinal dystrophy in the RCS rat: prevention of photoreceptor degeneration by pigment epithelial cell transplantation. Exp Eye Res 1988;47:911–917.

13. Lopez R, Gouaras P, Kjeldbye H et al. Transplanted retinal pigment epithelium modifies the retinal degeneration in the RCS rat. Invest Ophthalmol Vis Sci 1989;30:586–593.

14. Le L, Sheedlo JH, Turner JE. Retinal pigment epithelial cell transplants in retinal degeneration slow mice do not rescue photoreceptor cells. Invest Ophthalmol Vis Sci 1993;34:2141–2145.

15. Mare RC, Jones BW, Watt CB et al. Neural remodeling in retinal degeneration. Prog Ret Eye Res 2003;22:607–655.

16. Li ZY, Klajvin II, Milam AH. Rod photoreceptor neurite sprouting in retinitis pigmentosa. J Neurosci 1995;15:5429–5438.

17. Collier TJ, Sortwell CE, Elsworth JD et al. Embryonic ventral mesencephalic grafts to the substantia nigra of MPTP-treated monkeys: feasibility relevant to multiple-target grafting as a therapy for Parkinson’s disease. J Comp Neurol 2002;442:320–330.

18. Takahashi J, Takagi Y, Saiki H. Transplantation of embryonic stem cell-derived dopaminergic neurons in MPTP-treated monkeys. Methods Mol Biol 2009;482:199–212.

19. Plant GW, Christensen CL, Oudega M, Bunge MB. Delayed transplantation of olfactory ensheathing glia promotes sparing/regeneration of supraspinal axons in the contused adult rat spinal cord. J Neurotrauma 2003;20:1–16.

20. Sasaki M, Li B, Lankford KL et al. Remyelination of the injured spinal cord. Prog Brain Res 2007;161:419–433.

21. Black GMC, Genetics for Ophthalmologists: The Molecular Genetic Basis of Ophthalmic Disorders. Hatchwell E, ed. London, UK: Remedica Publishing, 2002.

22. Capon MR, Marshall H, Kraft J et al. Sorbs’ fundus dystrophy: a light and electron microscopic study. Ophthalmology 1989;96:1769–1777.

23. Green WR, Enger C. Age-related macular degeneration histopathologic studies: the 1992 Lorenz E. Zimmerman Lecture. Ophthalmology 1993;100:1519–1535.

24. Kunze C, Elsner AE, Beauscourte E et al. Spatial extent of pigment epithelial detachments in age-related macular degeneration. Ophthalmology 1999;106:1830–1840.

25. Enzmann V, Yolcu E, Kaplan HJ et al. Stem cells as tools in regenerative therapy for retinal degeneration. Arch Ophthalmol 2009;127:563–571.

26. Sipky BD, Hinton DR. Aging of retina and retinal pigment epithelium. In: Lim JI, ed. Age-Related Macular Degeneration. New York: Marcel Dekker, Inc; 2002:1–14.

27. Bok D. The retinal pigment epithelium: a versatile partner in vision. J Cell Sci Suppl 1993;17:189–195.

28. Sparrow JR, Boulton M. RPE lipofuscin and its role in retinal pathophysiology. Exp Eye Res 2005;80:595–606.

29. Travis GP, Golczak M, Moise AR et al. Diseases caused by defects in the visual cycle: retinoids as potential therapeutic agents. Annu Rev Pharmacol Toxicol 2007;47:469–512.

30. Rattner A, Nathans J. Macular degeneration: recent advances and therapeutic opportunities. Nat Rev Neurosci 2006;7:860–872.

31. Belacharski, PA. Fundus flavimaculatus. In: Newsome, DA, ed. Retinal Dystrophies and Degenerations. New York: Marcel Dekker, Inc; 2002:14–17.

32. Bhatia PP, Berns LA. Dominant inheritance of Stargardt’s disease. J Am Ophthalmol Assoc 1988;59:112–117.

33. Puech B, Hache JC, Tierut P et al. X-shaped macular dystrophy with flavimaculatus flecks. Ophthalmologica 1989;199:146–157.

34. Perez MF, Maumenee IH, de la Cruz Z et al. Autosomal-dominant fundus flavimaculatus. Ophthalmology 1990;97:798–809.

35. Monsour AM. Long-term follow-up of dominant macular dystrophy with flecks (Stargardt’s). Ophthalmologica 1992;205:138–143.
Restorative therapy for macular dystrophy

37 Stargardt K. Über familiare, progressive Degeneration in der Makula-gegend des Auges. Albrecht von Graefes Arch Klin Exp Ophthalmol 1901;71:534–549.
38 Birnbach CD, Järveläinen M, Possin DE et al. Histopathology and immunocytochemistry of the neurosensory retina in fundus flavimaculatus. Ophthalmology 1994;101:1211–1219.
39 Franceschetti A. Über tapeto-retinale Degeneration im Kindesalter. In: Sautter H, ed. Entwicklung und Fortschrift in der Augenheilkunde. Enke, Stuttgart: 1963;107–126.
40 Franceschetti A, Francois J. Fundus flavimaculatus. Arch Ophthalm Rev Gen Ophthalmol 1965;25:505–530.
41 Kaplan J, Gerber S, Larget-Piet D et al. A gene for Stargardt’s disease (fundus flavimaculatus) maps to the short arm of chromosome 1. J Biol Chem 1997;272:10303–10310.
42 Gerber S, Rozet JM, Bonneau D et al. A gene for late-onset fundus flavimaculatus with macular dystrophy maps to chromosome 1p13. Am J Hum Genet 1995;56:396–399.
43 Allikmets R. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nat Genet 1997;17:122.
44 Stone EM, Webster AR, Vandenburg K et al. Allelic variation in ABCR associated with Stargardt disease but not age-related macular degeneration. Nat Genet 1998;20:328–329.
45 Illing M, Molday LL, Molday RS. The 220-kDa rim protein of retinal rod OSs is a member of the ABC transport superfamily. J Biol Chem 1997;272:10303–10310.
46 Azarian SM, Travis GH. The photoreceptor rim protein is an ABC transporter encoded by the gene for recessive Stargardts-disease (ABCR). FEBS Lett 1997;400:247–252.
47 Koememko RK. The gene for Stargardt disease, ABCA4, is a major retinal gene: a mini-review. Ophthalmic Genet 2003;24:75–80.
48 Martinez-Mir A, Bayes M, Vilageliu L et al. A new locus for autosomal recessive retinitis pigmentosa (RP19) maps to 1p13–1p21. Genomics 1997;40:142–146.
49 Martinez-Mir A, Paloma E, Allikmets R et al. Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene (ABCR). Nat Genet 1999;18:11–12.
50 Cremers FP, van de Pol DJ, van Driel M et al. Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt disease gene ABCR. Hum Mol Genet 1998;7:355–362.
51 Mauger A, Klevering BJ, Rohrschneider K et al. Mutations in the ABCA4 (ABCR) gene are the major cause of autosomal recessive cone-rod dystrophy. Am J Hum Genet 2000;67:960–966.
52 Weng J, Mata NL, Azarian SM et al. Insights into the function of Rin protein in photoreceptors and etiology of Stargardt’s disease from the phenotype in abcr knockout mouse. Cell 1999;98:13–23.
53 Radu RA, Mata NL, Nusnowitz S et al. Treatment with isotretinoin inhibits lipofuscin accumulation in a mouse model of recessive Stargardt’s macular degeneration. Proc Natl Acad Sci USA 2003;100:4742–4747.
54 Mata NL, Weng J, Travis GH. Biosynthesis of a major lipofuscin fluorophore in mice and humans with ABCR-mediated retinal and macular degeneration. Proc Natl Acad Sci USA 2000;97:7154–7159.
55 Donoso LA, Frost AT, Stone EM et al. Autosomal dominant Stargardt-like macular dystrophy; founder effect and reassessment of genetic heterogeneity. Arch Ophthalmol 2001;119:564–570.
56 Vasireddy U, Uchida Y, Salem N et al. Loss of functional ELOVL4 depletes very long-chain fatty acids (VLCFAs) in retinal rods and in human RPE cells. J Biol Chem 2003;278:16778–16785.
57 Langton KP, Mckee MD, McKee A. Localization of the functional domains of human tissue inhibitor of metalloproteases-3 and the effects of a Stargardt’s dystrophy mutation. J Biol Chem 1998;273:16778–16781.
58 Lee MH, Dodds P, Verma V et al. Tailoring tissue inhibitor of metalloproteases-3 to overcome the weakening effects of the cysteine-rich domains of tumor necrosis factor-alpha converting enzyme. Biochem J 2003;371:369–376.
59 Yamada E, Tooe T, Yamada H et al. TIMP1 promotes VEGF-induced neovascularization in the retina. Histol Histopathol 2001;16:87–97.
60 Lec J, Waterhouse P, Sanchez OH et al. Spontaneous air space enlargement in the lungs of mice lacking tissue inhibitor of metalloproteases-3 (TIMP3). J Clin Invest 2001;108:817–829.
61 Weber BH, Lin B, White KE et al. A mouse model for Stargardt’s dystrophy. Invest Ophthalmol Vis Sci 2002;43:732–739.
62 Leibowitz HM, Krueger DE, Maunder LR et al. The FRMD1 gene (FRMD1) is mutated in recessive Stargardt macular dystrophy. Hum Mol Genet 2000;9:1945–1951.
63 Della NG, Campochiaro PA, Zack DJ. Localization of TIMP-3 mRNA expression to the retinal pigment epithelium. Invest Ophthalmol Vis Sci 1996;37:1921–1924.
64 Felbor U, Doepner D, Schneider U et al. Evaluation of the gene encoding the tissue inhibitor of metalloproteinases-3 in various macular degenerations. Invest Ophthalmol Vis Sci 1997;38:1054–1059.
65 Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biophys Acta 2000;1477:267–283.
66 Felbor U, Stohr H, Amann T et al. A novel Ser156Cys mutation in the tissue inhibitor of metalloproteinases-3 (TIMP3) in Sorsby’s fundus dystrophy with unusual clinical features. Hum Mol Genet 1995;4:2415–2416.
67 Jacobson SG, Cideciyan AV, Reganath G et al. Night blindness in Sorsby’s fundus dystrophy reversed by vitamin A. Nat Genet 1995;11:27–32.
68 Jacobson SG, Cideciyan AV, Bennett J et al. Novel mutation in the TIMP3 gene causes Sorsby fundus dystrophy. Arch Ophthalmol 2002;120:376–379.
69 Langton KP, Mckee N, Curtis A et al. A novel tissue inhibitor of metalloproteinases-3 mutation reveals a common molecular phenotype in Sorsby’s fundus dystrophy with unusual clinical features. Hum Mol Genet 1998;10:179–182.
70 Chong NH, Alexander RA, Gin T. TIMP-3, collagen, and elastin immunohistochemistry and histopathology of Sorsby’s fundus dystrophy. Invest Ophthalmol Vis Sci 2000;41:3898–902.
71 Tabata Y, Iwashiki Y, Kamimura K et al. A novel splice site mutation in the tissue inhibitor of the metalloproteases-3 gene in Sorsby’s fundus dystrophy with unusual clinical features. Hum Mol Genet 1998;10:179–182.
72 Chong NH, Alexander RA, Gin T. TIMP-3, collagen, and elastin immunohistochemistry and histopathology of Sorsby’s fundus dystrophy. Invest Ophthalmol Vis Sci 2000;41:3898–902.
73 Langton KP, Mckee MD, McKee A. Localization of the functional domains of human tissue inhibitor of metalloproteases-3 and the effects of a Sorsby’s dystrophy mutation. J Biol Chem 1998;273:16778–16781.
74 Lee MH, Dodds P, Verma V et al. Tailoring tissue inhibitor of metalloproteases-3 to overcome the weakening effects of the cysteine-rich domains of tumor necrosis factor-alpha converting enzyme. Biochem J 2003;371:369–376.
75 Yamada E, Tooe T, Yamada H et al. TIMP1 promotes VEGF-induced neovascularization in the retina. Histol Histopathol 2001;16:87–97.
76 Evans J, Probability of blindness and partial sight in England and Wales 1990–1991: studies on medical and population subjects. No 57. London: HMSO, 1995.
77 Balatsoukas DD, Simola C, Parisi A et al. Visual handicap in south-east England. J R Coll Surg Edinb 1995;40:49–51.
78 Owen CG, Fletcher AE, Donoghue M et al. How big is the burden of visual loss caused by age related macular degeneration in the United Kingdom? Br J Ophthalmol 2003;87:312–317.
79 la Cour M, Kjaergaard JF, Nissen MH. Age-related macular degeneration: epidemiology and optimal treatment. Drugs Aging 2002;19:19–41.
80 Lotery A, Trump D. Progress in defining the molecular biology of age-related macular degeneration. Hum Genet 2007;122:219–236.
81 Evans J, Wormald R. Is the incidence of registrable age-related macular degeneration increasing? Br J Ophthalmol 1996;80:9–14.
82 Arnold JJ, Sarks SH. Extracts from “clinical evidence”: age related macular degeneration. BMJ 2000;321:741–744.
83 Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with...
vittamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol 2001;119:1417–1436.
92 Feher J, Kovacs B, Kovacs I et al. Improvement of visual functions and fundus alterations in early age-related macular degeneration treated with a combination of acetyl-L-carnitine, n-3 fatty acids, and coenzyme Q10. Ophthalmologica 2005;219:154–166.
93 Feher J, Kovacs B, Kovacs I et al. Metabolic therapy for early treatment of age-related macular degeneration. Ovit Heft 2007;148: 2259–2268.
94 Smith W, Mitchell P. Family history and age-related maculopathy: the Blue Mountains Eye Study. Aust N Z J Ophthalmol 1998;26: 203–206.
95 Hymann L, Neborsky R. Risk factors for age-related macular degeneration: an update. Curr Opin Ophthalmol 2002;13:171–175.
96 Meyers SM. A twin study on age-related macular degeneration. Trans Am Ophthalmol Soc 1994;92:775–845.
97 Klein ML, Francis PJ. Genetics of age-related macular degeneration. Ophthalmol Clin North Am 2003;16:567–574.
98 Seddon JM, Santangelo SL, Book K et al. A genomewide scan for age-related macular degeneration provides evidence for linkage to several chromosomal regions. Am J Hum Genet 2003;73:780–790.
99 Seddon JM, Cote J, Page WF et al. The US twin study of age-related macular degeneration: relative roles of genetic and environmental factors. Am J Ophthalmol 2005;140:321–327.
100 Hammond CJ, Webster AR, Snieder H et al. Genetic influence on early-related maculopathy: a twin study. Ophthalmology 2002; 109:730–736.
101 Klein ML, Schultz DW, Edwards A et al. Age-related macular degeneration. Clinical features in a large family and linkage to chromosone 1q. Arch Ophthalmol 1998;116:1082–1088.
102 Weeks DE, Conley YP, Tsai HJ et al. Age-related maculopathy: an expanded genomewide scan with evidence of susceptibility loci within the 1q31 and 1q25 regions. Am J Ophthalmol 2001;132: 682–692.
103 Weeks DE, Conley YP, Mah TS et al. A full genome scan for age-related maculopathy. Hum Mol Genet 2000;9:1329–1349.
104 Klein RJ, Zeiss C, Chew EY et al. Complement factor H polymorphism in age-related macular degeneration. Science 2005;308: 385–389.
105 Gold B, Merriam JE, Zermant J et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. Nat Genet 2006;38:458–462.
106 Kanda A, Chen W, Othman M et al. A variant of mitochondrial protein LOC387715/ARMS2, not HTRA1, is strongly associated with age-related macular degeneration. Proc Natl Acad Sci USA 2007;104:16227–16232.
107 Stone EM, Braun TA, Russell SR et al. Missense variations in the EFEMP1 gene LOC387715 genes and susceptibility to age-related maculopathy: a two-stage case–control study. Lancet 2001;38:1828–1834.
108 Conley YP, Jakobsdottir J et al. CFH, ELOVL4, PLEKHA1, and LOC387715 genes and susceptibility to age-related maculopathy: AREDS and CHS cohorts and meta-analyses. Hum Mol Genet 2006; 15:3206–3218.
109 Schmidt S, Hauser MA, Scott WK et al. Cigarette smoking strongly modifies the association of LOC387715 and age-related macular degeneration. Am J Hum Genet 2006;78:852–864.
110 Ennis S, Jomary C, Mullins R et al. Association between the SERPINE1 gene and age-related macular degeneration: a two-stage case–control study. Orv Hetil 2007;148:761–766.
111 Choi YP, Shirtcliffe H, Smiddy WE et al. Novel association of the apolipoprotein gene are associated with age-related macular degeneration. Eye 2008;22:768–776.
112 Lakkaraju A, Finnemann SC, Rodriguez-Boulan E. The lipidusin fluorescence A2E perturbs cholesterol metabolism in retinal pigment epithelial cells. Proc Natl Acad Sci USA 2007;104:11026–11031.
113 Curtico CA, Presley JB, Malek G et al. Stentifed and unesterified cholesterol in drusen and basal deposits of eyes with age-related maculopathy. Exp Eye Res 2005;81:731–741.
114 Stone EM, Lotery AJ, Munier FL et al. A single EFEMP1 mutation associated with both Malattia Leventinese and Dryne hombcomb retinal dystrophy. Nat Genet 1999;22:199–202.
115 Ennis S, Braun TA, Russell SR et al. Missense variations in the fibulin 5 gene and age-related macular degeneration. N Engl J Med 2004;351:346–353.
116 de la Paz MA, Pericak-Vance MA, Lemon F et al. Exclusion of TIMP3 as a candidate locus in age-related macular degeneration. Invest Ophthalmol Vis Sci 1997;38:1060–1065.
117 Duvail J, McKechnie NM, Lee WR et al. Extensive subretinal pigment epithelial deposit in two brothers suffering from dominant retinitis pigmentosa. A histopathological study. Graefes Arch Clin Exp Ophthalmol 1986;224:299–309.
118 Milam AH, Curtico CA, Cideciany AV et al. Dominant late-onset retinal degeneration with regional variation of sub-retinal pigment epithelium deposits, retinal function, and photoreceptor degeneration. Ophthalmology 2000;107:2256–2266.
119 Jacobson SG, Cideciany AV, Wright E et al. Phenotypic marker for early disease detection in dominant late-onset retinal degeneration. Invest Ophthalmol Vis Sci 2001;42:1892–1899.
120 Hayford C, Shu X, Cideciany AV et al. Mutation in a short-chain collagen gene, CTRP5, results in extracellular deposit formation in late-onset retinal degeneration: a genetic model for age-related macular degeneration. Hum Mol Genet 2003;12:2657–2667.
121 Klaver CC, Klifffen M, van Duijn CM et al. Genetic association of apolipoprotein E with age-related macular degeneration. Am J Hum Genet 1998;63:200–206.
122 Anderson DH, Talaga KC, Rivest AJ et al. Characterization of beta amyloid assembles in drusen: the deposits associated with aging and age-related macular degeneration. Exp Eye Res 2001;73:887–896.
123 Hughes AE, Orr N, Esfandary H et al. A common CHF haplotype, with deletion of CHFR1 and CHFR3, is associated with lower risk of age-related macular degeneration. Nat Genet 2006;38:1173–1177.
124 Hageman GS, Anderson DH, Johnson LV et al. A common haplotype in the complement regulatory gene factor H (CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci USA 2005;102:7227–7232.
125 Edwards AO, Ritter Iii R, Abel KJ et al. Complement factor H polymorphism and age-related macular degeneration. Science 2005;308: 421–424.
126 Zareparsi S, Branhman KE, Li M et al. Strong association of the Y402H variant in complement factor H Gene with susceptibility to age-related macular degeneration. Arch Ophthalmol 2006;124:1201–1205.
127 Lau L, Cheng C, Gao Q et al. Association of the Y402H polymorphism in the Y402H variant in complement factor H Gene and neovascular age-related macular degeneration in Chinese patients. Invest Ophthalmol Vis Sci 2006;47:3242–3246.
128 Ennis S, Jamory C, Mullins R et al. Association between the SERPINE1 gene and age-related macular degeneration: a two-stage case–control study. Lancet 2001;38:1828–1834.
129 Allikmets R, Seddon JM, Bernstein PS et al. Evaluation of the Best gene in patients with age-related macular degeneration and other maculopathies. Hum Mol Genet 1999;10:449–453.
130 Lotery AJ, Munier FL, Fishman GA et al. Allelic variation in the VMD2 gene in best disease and age-related macular degeneration. Invest Ophthalmol Vis Sci 2000;41:1291–1296.
Radtke ND, Aramant RB, Seiler MJ et al. Vision change after sheet transplantation of intact sheets of foetal neural retina with its retinal pigment epithelium in retinitis pigmentosa patients. Am J Ophthalmol 2002;133:544–550.

Algrve PV, Berglin L, Gourns P et al. Transplantation of RPE in age-related macular degeneration: observations in disciform lesions and dry RPE atrophy. Graefes Arch Clin Exp Ophthalmol 1997;235: 149–158.

Algrve PV, Berglin L, Gourns P et al. Transplantation of foetal retinal pigment epithelium in age-related macular degeneration with subfoveal neovascularization. Graefes Arch Clin Exp Ophthalmol 1994; 232:707–716.

Vugler A, Lawrence J, Walsh J et al. Embryonic stem cells and retinal degeneration. Curr Gene Ther 2007; 1510:12–1517.

Vugler A, Lawrence J, Walsh J et al. Generation of induced pluripotent stem cells from dermal fibroblasts. Proc Natl Acad Sci USA 2006; 103:12769–12774.

Vugler A, Lawrence J, Walsh J et al. The functional analysis of phagocytosis by human embryonic stem cell-derived RPE cells using a novel human retinal assay. Mol Vis 2009; 15:283–295.

Vegter HK, van der Steen JF, van Laarhoven MC et al. Induction of pluripotent stem cells from skin fibroblasts of patients with 15q11-q13 microdeletion syndrome. Nature Biotechnol 2007; 25:79–89.

Volkert D, Feng W, Duncan J et al. Correction of the retinal dystrophy phenotype of the RCS rat by viral gene transfer of Metk1. Proc Natl Acad Sci USA 2001;98:12584–12589.

Maguire MA, Simonelli F, Pierce EA et al. Safety and efficacy of gene transfer for Leber’s congenital amaurosis. N Engl J Med 2008; 358:2240–2248.

Bainbridge JW, Smith AJ, Barker SS et al. Effect of gene therapy on visual function in Leber’s congenital amaurosis. N Engl J Med 2008; 358:2231–2239.

Cideciyan AV, Aleman TS, Boye SL et al. Human gene therapy for RPE65 isomerase deficiency: a phase I study. Graefes Arch Clin Exp Ophthalmol 2004;12:1159–1165.

Silverman MS, Hughes SE. Photoreceptor rescue in the RCS rat without pigment epithelium transplantation. Curr Eye Res 1990;9: 183–191.

Del Priore LV, Geng L, Tezel TH et al. Extracellular matrix ligands promote RPE attachment to inner Bruch’s membrane. Curr Eye Res 2007;32:707–716.

Tezel TH, Kaplan HJ, Del Priore LV. Fate of human retinal pigment epithelial cells seeded onto layers of human Bruch’s membrane. Invest Ophthalmol Vis Sci 1999;40:467–476.

Giraud S, Giraud S, Vugler A et al. Impaired RPE survival on aged submacular human Bruch’s membrane. Exp Eye Res 2005;80:235–248.

Radlde ND, Seiler MJ, Aramant RB, Petry HM, Widell DJ. Transplantation of intact sheets of foetal neural retina with its retinal pigment epithelium in retinitis pigmentosa patients. Am J Ophthalmol 2002;133:544–550.

Algrve PV, Berglin L, Gourns P et al. Transplantation of RPE in age-related macular degeneration: observations in disciform lesions and dry RPE atrophy. Graefes Arch Clin Exp Ophthalmol 1997;235: 149–158.

Algrve PV, Berglin L, Gourns P et al. Transplantation of foetal retinal pigment epithelium in age-related macular degeneration with subfoveal neovascularization. Graefes Arch Clin Exp Ophthalmol 1994; 232:707–716.

Tao S, Young C, Redenti S et al. Survival, migration and differentiation of retinal progenitor cells transplanted on micro-machined poly(methyl methacrylate) scaffolds to the subretinal space. Lab Chip 2007;7:695–701.

Lamba DA, Karl MO, Wore CB et al. Efficient generation of retinal progenitor cells from human embryonic stem cells. Proc Natl Acad Sci USA 2006;22:103:12769–12774.

Lowry WE, Richter L, Yachocki E et al. Generation of human induced pluripotent stem cells from dermal fibroblasts. Proc Natl Acad Sci USA 2008;105:2883–2888.

Park HJ, Lerou PH, Zhao R et al. Generation of human-induced pluripotent stem cells. Nat Protoc 2008;3:1180–1186.

Takahashi K, Tanabe K, Ohnuki M et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–872.

Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–676.

Yu J, Vodyanik MA, Smuga-Otto K et al. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007;318: 1917–1920.

Yu J, Hu K, Smuga-Otto K et al. Human induced pluripotent stem cells free of vector and transgene sequences. Science 2009 [Epub ahead of print].

Zhou H, Wu S, Joo YJ et al. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 2009;4:381–384.

Oktika K, Ichisaka T, Yamanaka S. Generation of germ-line-competent induced pluripotent stem cells. Nature 2007;448:313–317.

Hanna J, Wermig M, Markoulaki S et al. Treatment of sickle cell anemia mouse model with iPSC cells generated from autologous skin. Cell 2007;131:861–872.

Wermig M, Zhao JP, Pruszak J et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the forebrain and improve symptoms of rats with Parkinson’s disease. Proc Natl Acad Sci USA 2006;103:5856–5861.

Liu H, Zhu F, Yong J et al. Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. Cell Stem Cell 2008;3: 587–590.

Dimos JT, Rodolfa KT, Niakan KK et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 2008;321:1218–1221.
197 Tateishi K, He J, Taranova O et al. Generation of insulin-secreting islet-like clusters from human skin fibroblasts. J Biol Chem 2008; 283:31601–31607.

198 Ebert AD, Yu J, Rose FF et al. Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature 2009;457:277–280.

199 Park IH, Arora N, Huo H et al. Disease-specific induced pluripotent stem cells. Cell 2008;134:877–886.

200 Choi KD, Yu J, Smuga-Otto K et al. Hematopoietic and endothelial differentiation of human induced pluripotent stem cells. Stem Cells 2009;27:559–567.

201 Karumbayaram S, Novitch BG, Patterson M et al. Directed differentiation of human-induced pluripotent stem cells generates active motor neurons. Stem Cells 2009;27:806–811.

202 Schenke-Layland K, Rhodes KE, Angelis E et al. Reprogrammed mouse fibroblasts differentiate into cells of the cardiovascular and hematopoietic lineages. Stem Cells 2008;26:1537–1546.

203 Hirami Y, Osakada F, Takahashi K et al. Generation of retinal cells from mouse and human induced pluripotent stem cells. Neurosci Lett 2009;458:126–131.

204 Tuo J, Bojanowski CM, Zhou M et al. Murine ccl2/cx3cr1 deficiency results in retinal lesions mimicking human age-related macular degeneration. Invest Ophthalmol Vis Sci 2007;48:3827–3836.

205 Lensch MW, Daley GQ. Scientific and clinical opportunities for modeling blood disorders with embryonic stem cells. Blood 2006;107:2065–2072.

206 Kong J, Kim SR, Binley K et al. Correction of the disease phenotype in the mouse model of Stargardt disease by lentiviral gene therapy. Gene Ther 2008;15:1311–1320.

207 Grayson C, Molday RS. Dominant negative mechanism underlies autosomal dominant Stargardt-like macular dystrophy linked to mutations in ELOVL4. J Biol Chem 2005;280:32521–32530.

208 Li Z, Clarke MP, Barker MD et al. TIMP3 mutation in Sorsby's fundus dystrophy: molecular insights. Expert Rev Mol Med 2005;7:1–15.

209 Lombardo A, Genovese P, Beaumont CM et al. Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. Nat Biotechnol 2007;25:1296–1306.

210 Jones BW, Watt CB, Frederick JM et al. Retinal remodeling triggered by photoreceptor degenerations. J Comp Neurol 2003;464:1–16.

211 Lund RD, Ono BJ, Keegan DJ et al. Retinal transplantation: progress and problems in clinical application. J Leukoc Biol 2003;74:151–160.

212 Boulton M, Roumowski M, Wess T. Ageing of the retinal pigment epithelium: implications for transplantation. Graefes Arch Clin Exp Ophthalmol 2004;242:76–84.

213 Vugler A, Carr AJ, Lawrence J et al. Elucidating the phenomenon of HESC-derived RPE: anatomy of cell genesis expansion and retinal transplantation. Exp Neurol 2008;214:347–361.

214 Kerrigan-Baumrind LA, Quigley HA, Pease ME et al. Number of ganglion cells in glaucoma eyes compared with threshold visual field tests in the same persons. Invest Ophthalmol Vis Sci 2000;41:741–748.

215 Sullivan LS, Daiger SP. Inherited retinal degeneration: exceptional genetic and clinical heterogeneity. Mol Med Today 1996;2:380–386.

216 Rozet JM, Gerber S, Souied E et al. Spectrum of ABCR gene mutations in autosomal recessive macular dystrophies. Eur J Hum Genet 1998;6:291–295.

217 Mauger A, van Driell MA, van de Pol DJ et al. The 2588GC mutation in the ABCR gene is a mild frequent founder mutation in the Western European population and allows the classification of ABCR mutations in patients with Stargardt disease. Am J Hum Genet 1999;64:1024–1035.

218 Rivera A, White K, Stohr H et al. A comprehensive survey of sequence variation in the ARCA4 (ABCR) gene in Stargardt disease and age-related macular degeneration. Am J Human Genet 2000;67:800–813.

219 Simonelli F, Testa F, de Crecchio G et al. New ABCR mutations and clinical phenotype in Italian patients with Stargardt disease. Invest Ophthalmol Vis Sci 2000;41:892–897.

220 Michaelides M, Hunt DM, Moore AT. The genetics of inherited macular dystrophies. J Med Genet 2003;40:641–650.

221 Bennett J, Tanabe T, Sun D et al. Photoreceptor cell rescue in retinal degeneration (rd) mice by in vivo gene therapy. Nat Med 1996;2:649–654.

222 Kumar-Singh R, Farber DB. Encapsidated adenovirus minichromosome-mediated delivery of genes to the retina: application to the rescue of photoreceptor degeneration. Hum Mol Genet 1998;7:1893–1900.

223 Takahashi M, Miyoshi H, Verma IM et al. Rescue from photoreceptor degeneration in the rd mouse by human immunodeficiency virus vector-mediated gene transfer. J Virol 1999;73:7812–7816.

224 Koenekeop RK. Successful RPE65 gene replacement and improved visual function in humans. Ophthalmic Genet 2008;29:89–91.

225 Borras T. Recent developments in ocular gene therapy. Exp Eye Res 2003;73:643–652.

226 Okita K, Nakagawa M, Hyenjong H et al. Generation of mouse induced pluripotent stem cells without viral vectors. Science 2008;322:949–953.