Transcriptome analysis and molecular signature of human retinal pigment epithelium

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Retinal pigment epithelium (RPE) is a polarized cell layer critical for photoreceptor function and survival. The unique physiology and relationship to the photoreceptors make the RPE a critical determinant of human vision. Therefore, we performed a global expression profiling of native and cultured human fetal and adult RPE and determined a set of highly expressed ‘signature’ genes by comparing the observed RPE gene profiles to the Novartis expression database (SymAtlas: http://wombat.gnf.org/index.html) of 78 tissues. Using stringent selection criteria of at least 10-fold higher expression in three distinct preparations, we identified 154 RPE signature genes, which were validated by qRT-PCR analysis in RPE and in an independent set of 11 tissues. Several of the highly expressed signature genes encode proteins involved in visual cycle, melanogenesis and cell adhesion and Gene ontology analysis enabled the assignment of RPE signature genes to epithelial channels and transporters (ClCN4, BEST1, SLCA20) or matrix remodeling (TIMP3, COL8A2). Fifteen RPE signature genes were associated with known ophthalmic diseases, and 25 others were mapped to regions of disease loci. An evaluation of the RPE signature genes in a recently completed AMD genomewide association (GWA) data set revealed that TIMP3, GRAMD3, PITPNA and CHRNA3 signature genes may have potential roles in AMD pathogenesis and deserve further examination. We propose that RPE signature genes are excellent candidates for retinal diseases and for physiological investigations (e.g. dopachrome tautomerase in melanogenesis). The RPE signature gene set should allow the validation of RPE-like cells derived from human embryonic or induced pluripotent stem cells for cell-based therapies of degenerative retinal diseases.

INTRODUCTION

Progressive retinal degenerative diseases, such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP), are major causes of untreatable blindness and have a tremendous social and financial burden on society. As many as 30 million people worldwide are afflicted with AMD, and this diagnosis is expected to increase dramatically in the coming decades because of aging populations (1,2). AMD is an aging-associated multifactorial disease that affects the photoreceptor-retinal pigment epithelium (RPE)–choroid interface in the macula and is caused by the interaction of genetic susceptibility factors and environment (3). The RPE is the source and the target of many retinal degenerative diseases and defects in RPE function can affect the integrity and viability of neighboring cells—primarily photoreceptors (4–6).

The RPE is a polarized monolayer of epithelial cells that separates the neural retina and the choroidal blood supply and forms a highly selective barrier fundamentally important...
for maintaining the health and integrity of the photoreceptors (7,8). This epithelium is derived from neural ectoderm and forms a close anatomical relationship with the photoreceptors, mimicking the neuronal–glial relationship observed in the central nervous system (CNS). In the eye, light–dark transitions and circadian rhythms modulate the RPE transport of nutrients, metabolic waste products, ions and fluid between the choroidal blood supply and the subretinal space surrounding the photoreceptor outer segments (9,10). High metabolic activity and ongoing exposure to light makes the RPE particularly vulnerable to oxidative damage. Not surprisingly, abnormalities in RPE phagocytosis of rods and cones or in the maintenance of the visual cycle can lead to retinal degeneration and photoreceptor cell death (11).

Disease processes affecting RPE/photoreceptor interaction and causing RPE dysfunction have been subjects of intense scrutiny (12–14). In vitro models of RPE have been derived from native and cultured human cells, from fetal and postnatal donor eyes, transformed cell lines and embryonic stem (ES) cells (14–19). Cultured human RPE can be grown in large quantities and used in biochemical and functional assays (18,20) or transplantation studies. However, the value of cultured RPE depends on its ability to recapitulate functional and genetic characteristics of the native tissue. We have previously developed a primary human fetal RPE cell culture model that mimics the normal physiology, function and structure of native fetal and adult RPE, and thus is suitable for a wide range of studies on diseases associated with retina/RPE interactions (10,18,21–23).

The global expression profile of human RPE will be valuable for elucidating its pivotal role in retinal degenerative diseases (24). Hence, we have performed a comparative analysis of transcriptomes from human fetal and adult RPE, primary cultures and commonly used human cell lines and tissues. We report a unique ‘signature’ set of 154 genes whose expression levels distinguish RPE from other tissues or cell types. We also describe a cross-sectional analysis of RPE ‘signature’ genes against an AMD genomewide association study (GWAS) (25) with a goal of identifying candidate genes and pathways relevant to AMD. Ingenuity analysis and RetNet (www.sph.uth.tmc.edu/retnet) were used to analyze RPE signature genes to identify novel candidate genes for RPE disease. Our study provides an important discovery tool for functional investigations of RPE/photoreceptor interaction and establishes a molecular platform to evaluate RPE cells for repair of degenerating retina.

RESULTS

Human RPE ‘gene signature’

We generated global expression profiles of native fetal and adult human RPE, and of fetal primary cultures and compared these with transcriptomes of adult transformed RPE cell lines and of other human tissues (Fig. 1). Principle component analysis (PCA) and hierarchical cluster analysis were first used to evaluate similarities or differences in gene expression between samples from primary cultures and native RPE. The hierarchical clustering dendrogram based on principal components of 30 samples demonstrates that native human tissues (fnRPE and anRPE) and cultured cells (fcRPE and ARPE-19) cluster separately regardless of the sample source (Fig. 2A). In contrast, biological (n = 4) or technical replicates (ARPE-19; n = 8) in each RPE group cluster together. More than 50% of the total variability in expression data is included in PC1, PC2 and PC3 (Fig. 2B, C and see legend). Visual inspection of PC1 versus PC2 (Fig. 2B) and PC2 versus PC3 (Fig. 2C) plots reveals distinct clusters separating the four different RPE preparations.

To identify an expression profile that distinguishes human RPE from other cell types, we compared the expression of native adult and fetal RPE and primary cultures of fetal RPE against 78 different human tissues and cell cultures (26).

The relative expression (rEx) values (see Materials and Methods) revealed a set of 154 highly expressed genes (171 probe sets) in anRPE, fnRPE and fcRPE (Fig. 3A and B). We call these ‘signature’ genes as they together provide a unique profile of RPE functions. Gene ontology (GO) analysis further identified several critical functional groups significantly over-represented in the ‘signature’ genes (P < 0.005). These include (i) vision, perception of light and vitamin A metabolism (e.g. CRX, EFEMP1, RPE65, SFRP3, SLX3, TIMP3, BEST1, RDH11, RBPI); (ii) response to stimulus and sensory perception (e.g. AHR, CDH3, GJA1, ENPP2, PITPN4); (iii) oxidoreductase activity (e.g. PCYOX1, STCH, ALDH1A3, CDO1, BDH2, FAIDS1); (iv) pigment biosynthesis and melanin biosynthesis [e.g. GPR143, TTRP1, dopachrome tautomerase (DCT), SILV]; (v) phagocytic activity (LAMP2, VDP, GULPl); (vi) transporter activity (e.g. SLC39A6, SLC4A2, SLC16A1, SLC16A4) (Fig. 3C and Table 1).

Based on the rEx levels, the 154 RPE ‘signature genes’ in anRPE, fn RPE, fcRPE and ARPE preparations can be clustered into four groups (Fig. 4 and Supplementary Material, Table S1). Cluster 1 consists of genes that are on average three times more highly expressed in native fetal compared with the native adult RPE. These genes are involved in extracellular matrix (ECM) formation, tissue remodeling, cytoskeleton reorganization and trafficking, and can be used as sentinels for cell culture-induced alterations in gene expression. Cluster 2 identifies genes whose expression levels are high and relatively...
unchanged among the four RPE preparations; these include genes involved in visual cycle, pigment biosynthesis, transporter activity and cell signaling. Cluster 3 is similar to Cluster 2, but with lower levels of gene expression. Cluster 4 includes an important group of 17 genes that exhibit 26–87 times lower expression in ARPE-19 cells when compared with native and fetal cultured RPE. Functional groups (GO terminology) represented in this cluster include (i) transporters; (ii) growth factors and transcriptional regulators; (iii) signaling proteins and (iv) visual cycle components.

Validation of RPE ‘signature’ genes

Expression levels of RPE signature genes were validated by qRT-PCR in preparations from donor RPE (n ≥ 2) and in a panel of human tissues and cell cultures from native fetal retina, native and cultured fetal choroid, brain, melanocytes, colon, intestine, kidney, liver, lung, trachea, calu-3 cells, a tissue-mix and testes. The mean rEx for each gene by qRT-PCR in fetal-cultured RPE, adult-cultured RPE/ARPE-19, fetal native RPE and adult native tissue showed a significant correlation (P < 0.0001) with the microarray data in each RPE sample type. The correlation coefficient is 0.74 for cultured fetal RPE, 0.94 for the adult cultured/ARPE-19, 0.83 for fetal native RPE, and 0.76 for native adult tissue.

Hierarchical clustering of tested samples (Fig. 5) demonstrates a distinct segregation of RPE samples (shown above the yellow line) from 14 other tested tissues, as revealed by the expression of 150 signature genes. The qPCR levels of RPE signature genes (Supplementary Material, Table S1) segregate into two major clusters according to the level of variation of their rEx between native and cultured RPE groups and within each RPE group. Cluster 1 includes ‘commonly expressed RPE genes’ that are, for the most part, three to four orders of magnitude more highly expressed in the RPE samples relative to the validation set. The dashed box in Cluster 2 indicates genes that are ≈100-fold more highly expressed in native RPE (fetal and adult) when compared with cultured RPE and with the validation set. In contrast, the expression levels of ‘commonly expressed RPE
genes’ are consistently high in almost all RPE preparations (excluding ARPE19; dotted box, Cluster 1) and therefore are not substantially affected either by culturing or by the choice of model (fetal versus adult or native versus cultured). We suggest that these genes can be used as RPE markers.

Culturing RPE cells can alter the expression of ‘signature’ genes. To evaluate this further, we calculated the relative decrease in expression for all signature genes in AC (ARPE-19) and FC RPE relative to adult RPE. In both cases, the median decrease is \(\approx 3\)-fold. The expression of a given gene was considered unchanged if it was similar to native adult RPE expression. However, some genes express at drastically lower levels (up to 1000-fold lower) in ARPE-19, but not in FC RPE (Supplementary Material, Table S1). In ARPE-19, 74 of 150 of the signature genes are expressed at lower levels when compared with adult native RPE. In comparison, only 34 of 150 are expressed at reduced level in FC RPE when compared with adult native RPE.

Differential expression of selected RPE genes was validated by immunoblot analysis. Protein levels of TYRP1, BEST1, CDH3, CRX, CHRNA3, RPE65 were determined in fetal RPE cultures (three donors) and ARPE-19 cell cultures (Fig. 6A). As predicted by qRT-PCR and microarray analysis, protein levels of TYRP1 were similar between the RPE models, whereas the levels of other proteins, including BEST1, CDH3, CRX, CHRNA3, RPE65, were dramatically reduced in ARPE-19 cultures. Immunoblot analyses also demonstrated high expression of RPE65, BEST1, SILV1, CHD3, CHRNA3 and SERPFI1 proteins in RPE when compared with other tissues tested (Fig. 6B).

**Cross-sectional analysis of the RPE signature genes against AMD–GWAS**

Early changes in AMD include RPE dysfunction (27). To check the potential contribution of RPE-enriched ‘signature’ genes to AMD, we examined \(~2.5\) million genotyped and imputed single nucleotide polymorphisms (SNPs) in 2157 AMD cases and 1150 controls (28). Among these SNPs, we focused on those with at least 1% minor allele frequency and within 100 kb of the 5’ and 3’ end of each of the 154 RPE ‘signature’ genes, resulting in a set of 33 096 SNPs for evaluation. For each of these, we examined the association with AMD in the GWAS data set and compared the observed \(P\)-values with their chance expectations (assuming none of the variants are associated with AMD; Fig. 7). The most significant association maps near the *TIMP3* gene (rs5754221, \(P = 5 \times 10^{-11}\)), and other potentially interesting signals, are observed near *GRAMD3* (rs4836255, \(P = 3 \times 10^{-4}\)), *PITPNA* (rs17821234, \(P = 4 \times 10^{-4}\)) and *CHRNA3* (rs11072791, \(P = 6 \times 10^{-4}\)). We note that genotyping of additional AMD case–control samples (25) indeed validated the association of SNPs near *TIMP3* with AMD (\(P = 10^{-11}\)).

In addition to these four SNPs near 48 other genes show slight association with AMD at a \(P\)-value of \(<0.01\) (Table 2) and may be the candidates for further examination, given the convergence of gene expression data (reported here) and the genetic association data (from the GWAS). The functional classification of these 48 genes by DAVID (29) revealed 18 genes with a signal sequence at N terminus (Fig. 8). All 18 have a central hydrophobic region (red), N-terminal hydrophilic region (green) and a C-terminal flanking region (blue). Notably,
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coding regions of these genes include many variants that potentially could contribute to protein misfolding.

In a separate analysis, we utilized a catalog of SNPs [called expression quantitative trait loci (eQTLs)] known to be associated with expression levels of specific genes (30). From this catalogue, we selected a list of 44 SNPs (Supplementary Material, Table S2) associated with expression levels of some of the genes in the RPE signature set \( (P < 10^{-7}) \). Four of these SNPs exhibited nominal association with AMD at \( P < 0.05 \) (compared with two expected by chance); these eQTLs are rs12150474 (associated with expression of PHACTR2 at \( P < 10^{-7} \) and with AMD with \( P = 0.007 \)); rs7105701 (RAB38 with \( P < 10^{-7} \); AMD with \( P = 0.01 \)); rs1483539 (LGALS8 with \( P < 10^{-8} \); AMD with \( P = 0.03 \)) and rs2449517 (LAPTM4B with \( P < 10^{-8} \); AMD with \( P = 0.04 \)).

Role of DCT in RPE physiology

Epithelia are characterized by the asymmetric distribution of plasma membrane proteins. This polarity fundamentally contributes to a range of functions that allow the epithelium to support the health and integrity of surrounding cells. The present data show that DCT is highly expressed in human RPE (Table 1; Supplementary Material, Fig. S1). Previous studies have indicated a role for this gene product in pigment development and the modulation of cell responses to oxidative stress (31,32). In Figure 9A, we used a lentivirus system to deliver specific shRNA to reduce DCT levels (clone 38) by \( \approx 75\% \) in hfRPE. A similar reduction was observed in two additional experiments. This treatment caused a significant reduction in the transepithelial resistance (TER) of confluent monolayers from 842 ± 222 to 328 ± 171 V cm² (\( n = 6 \); \( P < 0.05 \)). A comparison of Fig. 9C and F show that transduction of hfRPE cells with DCT38 clone shRNA dramatically reduced intracellular DCT levels (Fig. 9F).

Reduction of DCT levels also led to a significant reorganization of fully polarized RPE cytoskeleton. For example, a comparison of Figure 9D and G show that the apical localization of ezrin is totally disrupted with an apparent loss of its normal apical membrane polarity. Finally, Figure 9E and H show RPE F-actin fibers are disrupted to a more diffuse pattern throughout the cells. These data indicate that DCT, a highly expressed human RPE signature gene, is critical for the maintenance of normal epithelial phenotype.

DISCUSSION

The RPE is fundamentally important for retinal development and function, and is a critical focus of retinal degenerative diseases and therapeutic intervention. Although RPE is functionally distinct from other epithelial cells and its pathophysiology is under intense investigation, relatively little is known about the set of genes that distinguish the RPE phenotype. The gene expression profile of a cell should reflect its morphological and functional specificity as well as molecular and physiological signaling pathways. The present study provides, for the first time, a specific gene expression signature of normal human RPE. We generated global expression profiles of human RPE (native and cultured cells) and identified 154
genes that exhibit 10-fold or higher expression when compared with the median of Novartis data set of various transcriptomes. Somewhat lesser stringent criteria of 5-fold or higher expression increased the list of RPE genes to 919 probe sets. We suggest that the 154 highly expressed genes, reported here, can serve as a ‘unique’ functional signature of RPE and can discriminate it from other epithelia or cell types. Because of RPE’s relevance to retinal disease, the RPE ‘signature’ gene set is of value for identifying candidate genes for genetic analysis or physiological studies. Ingenuity pathway analysis, together with the RetNet database (www.sph.uth.tmc.edu/retnet/home.htm), revealed 17 RPE signature genes that are involved in ocular disorders (TYRP1, SIL1, BEST1, COL8A2, EFEMP1, LOXL1, SERPING1, BMP4, VEGFA, TIMP3, CHRNA3, PRNP, RPE65, CRX, GPNMB, CDH1, CDH3). In addition, our analysis of RPE signature genes identified a number of newly discovered disease-associated genes. For example, GRP143 was not included by ingenuity in the list of disease-associated genes, but mutations in this gene were reported to cause X-linked ocular albinism (OA1) (33–35). Another example is a discovery of two SNPs in the LOXL1 gene, recently associated with strong genetic risk for pseudoexfoliation (PEX) syndrome and PEX glaucoma and involved in the formation of choroidal neovascularization (36,37). Using the RetNet database (http://www.sph.uth.tmc.edu/retnet/), we also identified 25 of the RPE signature genes within the critical genomic region for retinal degenerative disease loci (Table 3). The disease-causing genes within these loci have not been identified, but the signature genes should be considered as possible candidates, given the critical functional interactions between the RPE and the neural retina. For example, neuroglycan C plays an important role in retinal development and is found to be up-regulated in a mouse model of retinal degeneration (38). In addition, PTPRG might be a candidate for AMD (GWAS P = 0.00065; Table 2). Another interesting example is the disease-associated locus MCDR3 (macular dystrophy, retinal 3) that includes RPE signature genes SCAMP1 and RHOBTB3. These two genes play a major role in regulating cell traffic.

Figure 4. Cluster analysis performed on the profiles of 154 RPE-specific genes (171 probe sets) determined from microarray analysis on adult native RPE (AN) tissues, native fetal tissues (FN), fetal cultured RPE (FC) and ARPE-19 (AC). (A) Gene clusters (Cl 1–Cl 4) reflect different relative expression (rEx) patterns of the RPE-specific genes for each of the four RPE preparations. (B) Each horizontal colored band represents mean rEx of a single gene in each RPE preparation with the color-bar, showing the numerical rEx value. The cluster dendrogram on the right-hand side of the heat map groups the genes into the clusters represented in (A). (C) Log–log plot of signature gene-rEx of fetal native (FN - vertical axis 0-600 of rEx values) and adult native (AN - horizontal axis 0-600 of rEx values) RPE. Genes above the unity line have a higher expression level in fetal native compared with adult native RPE.
endocytosis and exocytosis (39,40), and mutations in these genes could disrupt the polarity of RPE and function leading to retinal (photoreceptor) degeneration.

A surprisingly large number of genes (currently 32) in the RPE signature set have been implicated as potential markers for different types of cancers, and therefore may be critical for the regulation of important RPE functions, including proliferation, migration or signaling. For example, prostaglandin D2 synthase (PTGDS) is a key enzyme in arachidonic acid metabolism and is repressed in premalignant stages of oral epithelial cancers (41). This enzyme is a melanocyte marker that is also elevated in retinal detachments and associated with open-angle glaucoma (42). Syndecan-2 is associated with AMD (Table 2) and found to be over expressed in hepatocellular carcinomas, colon carcinomas, and is involved in the suppression of lung carcinoma metastasis (43,44). Podoplanin (PDPN) is a novel marker for human well-differentiated keratinizing squamous cell carcinomas of the epithelium (45,46) and dendritic sarcomas (47). It is also a candidate disease gene for Leber congenital amaurosis (Table 3). Mutations in ADAM9 (Table 2) have been implicated in the pathogenesis retina/RPE attachment in cone-rod dystrophies (48). In addition, frizzle-related protein 5 (SFRPS5) is a known inhibitor of the WNT pathway and plays a crucial role in the development of human cancers and is a candidate gene for X-linked retinal dystrophies (49,50).

Cluster analysis is an important tool for distinguishing the genetic architecture of RPE models. For example, Fig. 4 (Clusters 2 and 3) summarizes a set of genes that are expressed at approximately the same level across all native and cultured tissues. These genes, although expressed at two different levels, are all highly expressed when compared with the Novartis transcriptome and invariant with developmental stage or culture conditions. Therefore, we suggest that they represent a kernel of genes minimally required for RPE phenotype. In addition, we found a group of RPE genes (n = 26) that are significantly under expressed in ARPE-19 cultured cells when compared with native tissue and primary culture (Fig. 4A, Cluster 4). Previously, it has been shown that these transformed cell lines lack functional characteristics of native RPE. For example, they have relatively low TER, no visible pigmentation and practically no apical microvilli (51,52).

The genes showing low ARPE19 expression can be grouped into the following functional categories: (i) transporter activity; (ii) growth factors and transcriptional regulators; (iii) ECM formation and tissue remodeling; (iv) retinoic and fatty acids metabolism and (v) formation of tight junctions, trafficking and melanogenesis. Not surprisingly, the lack of expression...
of these proteins can significantly alter normal function of RPE cells (53–57). For example, mice with deletion of \textit{ALDH1A3} (Cluster 5), a key factor regulating synthesis of retinoic acid, die just after birth due to altered epithelial–mesenchymal development (58). A reduced level of \textit{COL8A2} could affect formation of ECM by RPE, which in turn deregulates ability of the cell to proliferate and differentiate (53). Lack of \textit{GPR143} affects melanosomal biogenesis and trafficking leading to the X-linked ocular albinism (OA1) in humans (33,35,59). Reduced expression of these genes in ARPE-19 is probably due to a combination of factors including contamination of the primary cultures by fibroblasts, an excessive number of passages and further de-differentiation compared with primary cultures of fetal human RPE.

Many of the genes in the signature set are differentially expressed between native fetal and adult RPE (Fig. 4A, Cluster 1). This expression difference, confirmed by PCR, is particularly high for the following genes located well above the unity line in Figure 4C: \textit{DCT}, \textit{GPR143}, \textit{SOSTDC1}, \textit{COL8A2}, \textit{FOXD1}, \textit{SILV} and \textit{FGFR2}. Mutations in \textit{COL8A2} gene are linked to Fuchs’ endothelial dystrophy and posterior polymorphous dystrophy (60). Mutations in \textit{FGFR2} gene are associated with a variety of CNS disorders such as Crouzon syndrome, Pfeiffer syndrome and Craniosynostosis. Several of these genes may be developmentally important and related to pigment synthesis. Mutations of \textit{GPR143} can affect pigment production in the eye and cause optic changes associated with albinism (35,59) (vide supra). The \textit{DCT} gene product is another example of an enzyme involved in melanin biosynthesis that contributes to RPE homeostasis by detoxifying DOPA-derived metabolites (61). Modulation of \textit{DCT} levels by siRNA substantially affects proliferation in cortical neural progenitor cells (62) and is involved in multidrug resistance (63,64).

The present experiments (Fig. 9) indicate a novel function for \textit{DCT} in maintaining epithelial polarity and tight junction integrity. The shRNA-induced decrease in \textit{DCT} protein expression significantly decreased the total tissue resistance, which in RPE is mainly determined by the resistance of the paracellular (tight junction) pathway (65). Dissolution of epithelial junctions is associated with proliferation and migration and is a precursor of epithelial to mesenchymal transitions, a hallmark of the progression to cancer (65). The reorganization of the cytoskeleton and the loss of polarity following the decrease in \textit{DCT} levels further support this notion. This \textit{RPE} signature gene joins several recently identified micro-RNAs enriched in \textit{RPE} (65) that help maintain a quiescent and polarized state throughout the life of the organism.

Recent linkage and association studies have revealed a number of single nucleotide or other genetic variants that...
exhibit major (CFH region at 1q32 and ARMS2 region at 10q26) or minor (C2/CFB, C3, CFI, ABCA4) contributions to AMD susceptibility (66). A number of additional loci were recently suggested to exhibit significant genetic association in a GWAS (25); however, their relevance to AMD would require functional validation. Our cross-sectional analysis that examined SNPs near the 154 RPE signature genes for association in the AMD–GWAS data set revealed four genes, including TIMP3. We also identified three additional genes such as CHRNA3, GRAMD3 and PITPN4 that deserve further investigations for their potential role in AMD etiology. CHRNA3 encodes the nicotinic cholinergic receptor alpha 3, a member of the nicotinic acetylcholine receptor family, which plays an important role in calcium regulation, neuronal development and cognitive functions (67,68). Mutations in this gene lead to dysfunction associated with various neurodegenerative disorders, including Alzheimer’s disease, Parkinson’s, epilepsy and autism. In RPE, deregulation of Ca\textsuperscript{2+} signaling could significantly impair overall cell physiology, for example, leading to abnormal fluid absorption, or to the abnormal secretion of different growth factors, including VEGF, leading to the development of neovascular AMD (69,70).

Further bioinformatic analysis (71) of the 48 RPE signature genes that showed nominal association with AMD revealed...
similar signal peptide sequences in 18 of the encoded proteins (Fig. 8; 72–74). There is growing evidence that signal peptides play a major role in controlling protein sorting and trafficking in the endoplasmic reticulum [ER (75–77)]. Accumulation of mild folding variants of the proteins due to polymorphic variations/mutations leads to the aggregation of misfolded proteins, increased ER stress and eventual cell degeneration. For example, late-onset autosomal dominant retinal macular degeneration (L-ORMD), which phenotypically resembles AMD, is caused by mutations in C1QTNF5, a short-chain collagen gene expressed in the RPE. It has been proposed that mutant CTRP5 is misfolded, retained in the ER and subjected to degradation leading to RPE dysfunction (78). The phenotype of L-ORMD is similar to Sorsby's fundus dystrophy caused by mutations in TIMP3. In both cases, ER stress and abnormal cell adhesion cause cell degeneration and a failure to clear cellular debris from under the RPE, which suggests the possibility of immune attack—as seen in AMD (79).

As RPE is thought to be a critical target for AMD, numerous investigations have focused on regenerating or replacing damaged RPE from ES cells or from iPS cells. Several human ES lines can be induced to develop the RPE phenotype (80–82) and one of these has been used in transplantation experiments to rescue visual function in RCS rats (83). However, in the absence of a molecular signature, it is difficult to assess which in vitro generated RPE lines will retain appropriate function after transplantation. The RPE signature gene set can therefore be a valuable tool in regenerative medicine for validating the progress of RPE differentiation, propagation and maintenance. For clinical trials, it would be critical to confirm that RPE cell lines derived from hES cells exhibit an expression profile comparable with the native RPE. We suggest that the signature gene set can be used to monitor the development to RPE phenotype and, together with functional tests such as polarity and physiology (18,84), can determine appropriate cell lines for transplantation and rescue experiments.

In conclusion, we have described a specific gene signature of human RPE based on extensive analysis of native and cultured cells. Our analysis of the 154 RPE signature gene set provides a wealth of information for biological studies, reveals candidate genes for retinal/macular diseases and suggests potential molecular markers for assessing the integrity and function of RPE for cell-based therapies.

### MATERIALS AND METHODS

#### Native tissues and cell culture

This research followed the tenets of the Declaration of Helsinki and the guidelines of NIH Institutional Review Board and written informed consent was obtained from the GWAS subjects. Human fetal eyes (gestation, 16–18 weeks) were obtained from Advanced Bioscience Resources (Alameda, CA, USA) and human adult eyes were obtained from Analytical Biological Services, Inc. (Wilmington, DE, USA). Human adult native RPE (anRPE) were obtained from four donors of Caucasian descent (age 64–89 years old) within 24 h of death (postmortem time <12 h). Human fetal native RPE (fnRPE), retina and human fetal choroid (hfCH) were isolated and fnRPE were cultured on Primaria® flasks as described previously (18). For immunofluorescence localization or fluid transport experiments, cells were cultured on human ECM-
coated transwells (Corning Costar, 0.4 μm pores, polyester membrane). ARPE-19, a spontaneously transformed RPE cell line, was maintained under culturing conditions identical to fetal RPE primary cultures. The initial experimental design included separate samples of RPE grown on flasks (passage P0) or inserts (passage P1) coated with ECM. As no significant difference was observed between expression profiles of the cells grown on flasks or inserts (data not shown), we merged the two data sets for subsequent analysis.

**Protein analysis**

RPE, retina or choroid cells were lysed in RIPA buffer (Sigma-Aldrich, St. Louis, MO, USA) containing a proteinase inhibitor cocktail (Roche, Indianapolis, IN, USA). Protein extracts (10–15 μg) were electrophoresed using 4–12% Bis–Tris NuPAGE gel and blotted onto nitrocellulose membranes (Invitrogen, Carlsbad, CA, USA). The blots were incubated with antibodies against human BEST1, TYRP1 (Abcam, Figure 9. DCT silencing in hfRPE cultures grown on cell culture inserts using lentiviral-mediated transduction of shRNA. (A) Semi-quantitative evaluation of western blots of DCT after transduction with different shRNA clones. Labels indicate different clones: NT—non-targeting construct and 38–42 are DCT targeting shRNA clones. After quantification of band intensities and normalization to tubulin, DCT protein expression shRNA transduced cells were calculated relative to that of the cells transduced with NT shRNA (100%). (B) Transepithelial resistance measurements of confluent hfRPE monolayers grown on inserts transduced with DCT38 shRNA clones and compared with an NT construct controls (P < 0.05; n = 6). (C–H) Representative immunohistochemistry staining of hfRPE cells expressing shRNA directed against DCT (F, G, and H) and NT control shRNA (C, D, and E). Lower part of each panel is an en face view of maximum intensity projection (MIP) through the z-axis. Top part of each panel is a cross-sectional view through the z-plane. Lowest part of DAPI signal (dotted white lines) delineates the basal membrane. White arrowheads point to hfRPE apical surface. Red: DCT (C, F), ezrin (D, G), actin (E, H). Blue: DAPI-stained nuclei; green: ZO-1 indicates tight junction location separating apical and basolateral membranes. Transduction of hfRPE cells with DCT38 shRNA dramatically reduced the DCT levels inside cells (F), reduced and disorganized ZO-1 localization (F and G), and disrupted F-actin fibers to a more diffuse pattern with apical localization (H).
**Table 3.** Twenty-five candidate RPE signature genes found in loci associated with retinal disease

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<th>Candidate RPE genes</th>
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*a*Information about disease loci collected from RetNet: www.sph.uth.tmc.edu/retnet/  
*b*Chromosome location of disease loci.

Immunocytochemistry

hrRPE cultures on cell culture inserts (Transwell; Corning Costar) transduced with MISSION lentiviral particles were fixed for 30 min in 4% formaldehyde–PBS on ice, washed three times with PBS, and permeabilized for 30 min with 0.2% Triton X-100–PBS. The cells were washed three times with PBS, stained with antibodies against DCT (1:1000, ProteinTech), ezrin (1:500, Abcam), ZO-1 (1:1500, Invitrogen) overnight at 4°C in blocking solution, following by incubated with Alexa Fluor conjugated secondary antibodies (1:1000, Invitrogen) for 2 h and mounting with Vectashield medium containing DAPI (VectorLabs). F-actin was stained with Texas Red phalloidin (Molecular Probes). Stained images were imaged for microscopy (Axioplan 2 with Axiovision 3.4 software with ApoTome; Carl Zeiss Meditec, Inc., Dublin, CA, USA). Negative controls were performed with omission of primary antibodies.

**Lentivirus transduction**

Lentiviruses have the unique ability to infect nondividing cells. MISSION™ (Sigma) lentiviral system was used to deliver specific short-hairpin RNAs (shRNA) in hrRPE cells to mediate the levels of DCT expression. Target hrRPE cells were seeded in a 24-well insert (2 × 10^5/well), grown to confluence × 105/g, grouped to confluence and cultured for 4–6 weeks. Hexadimethrine bromide (8 μg/ml) was added to increase the efficiency of lentiviral transduction (Sigma), and all the transductions were performed at minimum effective multiplicity of infection of 2. The use of lentivirus shRNA resulted in 98% transduction efficiency. Viral medium was removed after 24 h of transduction and DCT protein levels were measured by western blots a week later. Immunocytochemistry staining was performed 3 weeks after the transduction. The functional effects on intact monolayers were evaluated by measurement of TER using EVOM (Precision Instruments).

**RNA profiling**

RNA was extracted from human tissues and cells using RNAasy Kit (Qiagen, Valencia, CA, USA) or total RNA isolation kit (Superarray Biosciences, Frederick, MD, USA). The panel of human tissues and cell cultures in this study included brain, melanocytes, colon, intestine, kidney, lung, trachea, testes, liver, calu-3 cells and a tissue mix, and were obtained commercially (Ambion First Choice Survey). Concentration and quality of RNA was determined using Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and/or Nano drop spectrophotometer (Wilmington, DE, USA). All samples had A(260)/A(280) ratios of the total RNA >2.0, and the ratio of 28S/18S ribosomal RNA bands was more than 1.8. The purity of RPE preparations was confirmed by measuring transcript levels of rhodopsin. We also confirmed the absence of several choroid-specific transcripts (S100A4, RGS5, ACTA2, ACTN1) in RPE samples. The absence of cross-contamination was confirmed in retina and choroid samples from the same eye by measuring RPE65 transcript levels. For the RNA Affymetrix chip analysis, we used The Vanderbilt Functional Genomics Shared Resource (FGSR). For each sample, the RNA integrity was indicated by an RIN number ranging from 0 to 10, with higher numbers indicating higher quality and we used samples with RIN >7. All four RPE groups (FC, AC, FN and AN) were definitively distinguished by the microarray analysis. Supplementary Material, Figure S1 shows that the RPE tissues are relatively indistinguishable from each other, but most importantly they are all clearly segregated from the other tissue types throughout the body. The relative uniformity of mean gene expression, from tissue to tissue, and their low variance indicates that the data are not limited by relatively small sample size.

The cDNA, reverse-transcribed from total RNA, was used to generate biotinylated cRNA with a BioArray High Yield RNA Transcript Labeling Kit (Affymetrix, Santa Clara, CA, USA). Fifteen micrograms of fragmented cRNA were hybr-
dized to expression microarrays (human GeneChips U133A plus 2.0 array, Affymetrix).

The signal intensity for each of 54 675 probe sets on the Affymetrix Human U133 plus 2.0 chips was calculated using GeneChip® Operating Software 1.4 (Affymetrix). Affymetrix probe set signal intensities were median normalized, i.e. divided by the median of each chip, and log10 transformed. Normalization and statistical analysis were carried out using the MSCL Analysts Toolbox (http://abs.cit.nih.gov/MSCLtoolbox/), a microarray analysis package that consists of custom-written scripts in the JMP statistical discovery software (SAS Institute, Cary, NC, USA), and developed by two of the co-authors (P.J.M., J.J.B.). Data were collected under Genomics Institute of Novartis (NICHD/NIH) for helpful discussion of protein signal sequences. (NICHD/NIH) for helpful discussion of protein signal sequences.

Comparison of RPE genes to AMD–GWAS data
To examine possible association of RPE ‘signature’ genes to AMD, we identified SNPs within 100 kb of the 5’ and 3’ ends of the RPE ‘signature’ genes and evaluated their association with macular degeneration in a recently completed AMD–GWAS (89). The GWAS data included 2157 AMD cases and 1150 controls, each examined on 324 067 SNPs using Illumina Human 370CNV BeadChips. An additional ~2.2 million markers arrays were imputed using HapMap genotypes and were also examined (90). A total of 33 096 SNPs near 154 RPE signature genes were examined, corresponding to a Bonferroni significance threshold of 1.5 \times 10^{-6}. The 33 096 correspond to 4305 independent tag SNPs. To identify additional SNPs that may be implicated in AMD pathogenesis, we also evaluated false discovery rates (91) and inspected quantile–quantile plots for all SNPs.

eQTL analysis
A database of expression quantitative trait loci, obtained by GWA analysis of SNPs with gene expression levels in lymphoblastoid cell lines (30), was searched for regulatory SNPs associated with RPE ‘signature’ genes. The evidence for association between each of these potential regulatory SNPs and AMD was then evaluated based on the data of Chen et al. (25). The Dixon et al. data consist of a catalog of association between SNPs and transcripts generated by examining lymphoblastoid cell lines from ~400 children.

SUPPLEMENTARY MATERIAL
Supplementary Material is available at HMG online.

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REFERENCES


