RESEARCH ARTICLE

Priorities and trends in the study of proteins in eye research, 1924–2014

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Purpose: To identify the proteins that are relevant to eye research and develop assays for the study of a set of these proteins.
Experimental design: We conducted a bibliometric analysis by merging gene lists for human and mouse from the National Center for Biotechnology Information FTP site and combining them with PubMed references that were retrieved with the search terms "eye" [MeSH Terms] OR "eye" [All Fields] OR "eyes" [All Fields].
Results: For human and mouse eye studies, respectively, the total number of publications was 13 525 and 23 895 and the total number of proteins was 4050 and 4717. For proteins in human and mouse eye studies, respectively, 88.7 and 81.7% had five or fewer citations. The top 50 most intensively studied proteins for human and mouse eye studies were generally in the areas of photoreceptors and phototransduction, inflammation, and angiogenesis, neurodevelopment, lens transparency, and cell-cycle and cellular processes. We proposed selected reaction monitoring assays that were developed in silico for the top fifty most intensively studied proteins in human and mouse eye research.
Conclusions and clinical relevance: We conclude that scientists engaged in eye research tend to focus on the same proteins. Newer resources and tools in proteomics can expand the investigations to lesser-known proteins of the eye.

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1 Introduction

Proteomics is beginning to gain greater attention in the field of eye research, owing to recent advances that have been made in protein chemistry, MS, and bioinformatics [1].

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Abbreviations: **ABCA4**, retinal-specific ATP-binding cassette transformer; **AMD**, age-related macular degeneration; **ARMS2**, age-related maculopathy susceptibility protein 2; **B/D-HPP**, Biology/Disease–Human Proteome Project; **BEST1**, bestrophin-1; **CFH**, complement factor H; **Isl1**, insulin gene enhancer protein ISL-1; **Mitf**, microphthalmia-associated transcription factor; **Pax2**, paired box protein Pax-2; **PAX6**, paired box protein PAX-6; **PDE**, phosphodiesterase; **Pde6b**, rod cGMP-specific 3′,5′-cyclic phosphodiesterase subunit beta; **PEDF**, pigment epithelium-derived factor; **Pou4f2**, POU domain, class 4, transcription factor 2; **RHO**, rhodopsin; **RP**, retinitis pigmentosa; **RPE**, retinal pigment epithelium; **RPGR**, X-linked retinitis pigmentosa GTPase regulator; **Tubb3**, tubulin beta-3 chain; **VEGF-A**, vascular endothelial growth factor-A; **Vsx2**, visual system homeobox 2

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Clinical Relevance

Research on the biology of the eye and underlying molecular mechanisms of eye disease can be advanced through the larger application of state-of-the-art quantification and characterization of protein and proteomes. This study utilized a bibliometric analysis to identify the most intensively studied proteins in human and mouse eye research. Selected reaction monitoring assays have been developed in silico for the top 50 most intensively studied proteins in human and mouse eye research.

Although proteins are an essential link between genotype and phenotype, the mechanisms by which genomic variation is translated to disease phenotypes through proteins is not well understood in general [2]. The level of complexity between the genome and specific phenotypes increases tremendously at the protein level due to protein isoforms, single nucleotide polymorphisms, PTMs, and protein degradation.

The biology and disease-oriented branch of the Human Proteome Project (B/D-HPP) was organized in 2010. The goal of the B/D-HPP is to support "the broad application of state-of-the-art measurements of proteins and proteomes by life scientists studying the molecular mechanisms of biological processes and human disease. This will be accomplished through the generation of research and informational resources that will support the routine and definitive measurement of the process or disease relevant proteins" [2]. Specifically, the B/D-HPP seeks to identify proteins that are relevant to a particular field and generate assays and reagents for these proteins [2]. The dissemination of SRM assays may help accelerate research in many different fields.

Our specific aims were to identify the proteins that have been most intensively studied in eye research and provide new tools for the investigation of the top 50 proteins in human and mouse eye research, respectively. The number of scientific publications was used as the indicator of how intensively a protein was studied in eye research.

2 Materials and methods

In order to identify the proteins that have received the greatest attention in eye research, human, and mouse gene information was retrieved from the National Center for Biotechnology Information FTP site. PubMed references with the search terms "eye" [MeSH Terms] OR "eye" [All Fields] OR "eyes" [All Fields] were downloaded from PubMed. The lists of human and mouse gene were then combined to creative respective lists of proteins for human and mouse eye research, respectively. The earliest publication on PubMed was from 1813, and the earliest reference to a gene was from 1924. There were few publications prior to 1970 (only three for human and 24 for mouse eye research).

PANTHER was used to classify protein function. For the top 50 proteins in human and mouse eye research, respectively, heat maps were used to show the number of publications per year, and STRING was used to examine functional protein networks. NeXtProt was used as the main reference for human proteins and their associated diseases, number of isoforms, variants, and PTMs using gold level criteria. UniProt was used as the main reference for mouse proteins and their associated diseases and number of isoforms. REACTOME and GO were used to identify groups of proteins involved in specific biological pathways studied in human eye research: complement cascade, Wnt signaling, VEGF signaling, apoptosis, visual phototransduction, etc. We did not find published SRM assays for 48 of the top 50 proteins in human eye research and 49 of the top 50 proteins in mouse eye research in the peer-reviewed scientific literature. SRM assays were constructed in silico using Skyline (MacCoss Lab, University of Washington, Seattle, WA), a commonly used theoretical prediction and selection algorithm [3] and following the guidelines for SRM assay development of Kuzyk et al. [4]. None of the SRM assays have been applied in vivo.

3 Results

A total of 4050 proteins were found in human eye studies (Supporting Information Table 1). A total of 4717 proteins were found in mouse eye studies (Supporting Information Table 2). The total number of publications for human and mouse eye studies, respectively, was 13 525 and 23 895. PANTHER was used to classify protein function for the 4050 proteins in human eye studies (Fig. 1A) and 4717 proteins in mouse eye studies (Fig. 1B). The molecular functions and detection of the top 50 human eye proteins in the different tissues and biofluids of the human eye are presented in Supporting Information Table 3. The molecular functions of the top 50 mouse eye proteins are present in Supporting Information Table 4.

PAX-6 was the top among the 50 most intensively studied proteins in both human (Table 1) and mouse (Table 2) eye research. Heat maps showing the frequency of publication per year for the top 50 proteins in human and mouse eye research are shown in Fig. 2A and B, respectively. The functional protein networks of the top 50 most intensively studied proteins in human eye research revealed three clusters representing photoreceptors and phototransduction, inflammation, and angiogenesis, and proteins involved in lens transparency (Fig. 3A). The functional protein networks of the top 50 most intensively studied proteins in mouse eye research

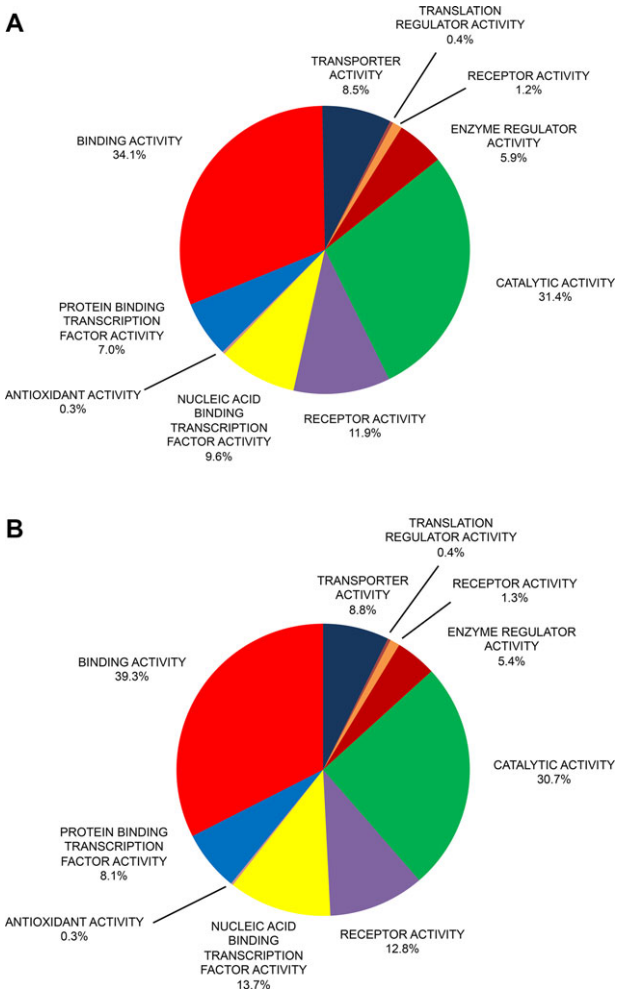

Figure 1. Pie diagram of protein functions in (A) human and (B) mouse eye research classified by PANTHER.

revealed three clusters that represented photoreceptors and phototransduction, neurodevelopment, and cell-cycle and cellular processes (Fig. 3B).

We further examined the overlap between the 50 most intensively studied proteins in human and mouse eye research. There were 15 proteins that were common to both human and mouse eye studies: paired box protein PAX-6, vascular endothelial growth factor A, rhodopsin, alpha-crystallin A chain, retinoid isomerohydrolase, peripherin-2, pituitary homeobox 2, tyrosinase, lens fiber major intrinsic protein, alpha-crystallin B chain, gap junction alpha-8 protein, cone-rod homeobox protein, cellular tumor antigen p53, rod cGMP-specific 3′,5′-cyclic phosphodiesterase subunit beta, and homeobox protein SIX3.

The least-studied proteins comprised a large proportion of the proteins in both human and mouse eye studies, as mentioned above. Of the 4050 proteins in human eye studies, the percentages of proteins with 5, 4, 3, 2, or 1 citation(s) were 2.5, 3.8, 7.6, 17.6, and 57.2%, respectively. Of the 4717 proteins in mouse eye studies, the percentages of proteins with 5, 4, 3, 2, or 1 citation(s) were 3.2, 4.4, 8.3, 16.0, and 49.8%, respectively. In other words, 88.7% of proteins in human eye studies and 81.7% of proteins in mouse eye studies had five or fewer citations.

To facilitate the use of MS for the quantification of these top proteins, we have proposed SRM assays for the top 50 proteins in human and mouse eye research as presented in Supporting Information, Tables 5 and 6. The list of the top proteins as characterized by a bibliometric approach corresponds to what Van Eyk has called "popular proteins." A complementary approach is to identify "priority proteins" based upon biological pathways. Biological pathways that are currently under intensive investigation in eye research include the complement cascade, Wnt signaling, VEGF signaling, apoptosis, visual phototransduction, degradation of extracellular matrix, cell response to hypoxia, oxidative stress-induced senescence, ERK activation, signaling by the TGF-beta receptor complex, and the inflammasome. A provisional list of 1416 "priority proteins" is shown in Supporting Table 7. Only 16 of the top 50 most intensively studied human eye proteins overlapped with the provisional list of priority proteins.

4 Discussion

In the present study, we identified over 4000 proteins that have been studied in human eye research and over 4700 proteins that have been studied in mouse eye research. There were nearly 80% more scientific publications for proteins in eye research for mouse than for humans. The underlying reason for the difference is not clear, but one could speculate that mouse eye proteins have been more frequently studied due to the greater available of eye tissues from mice than from humans. The ten most intensively studied proteins in human and mouse eye research are discussed below.

Paired box protein PAX-6 (PAX6) has been the most intensively studied protein in both human and mouse eye studies. PAX6 plays a multi-level role in the morphogenesis of the eye, especially in the development of the lens, cornea, and retina [5]. PAX6 is a transcriptional factor that binds with DNA through interactions with two N- and C-terminal domains, termed PAI and RED, respectively. Three isoforms of PAX6 are produced via alternative splicing. The ratio between the canonical form, isoform 1, and isoform 5a varies among tissue types [5]. PTMs of PAX6 include phosphorylation and ubiquitination. Multiple variants have been reported in PAX6. Mutations in *Pax-6* are associated with small eye (Sey) in mouse [6] and aniridia (partial or complete absence of the iris) in humans [7].

Myocilin is a 504 amino acid glycoprotein that was initially identified because it is induced in the eye by glucocorticoid treatment [8, 9]. Myocilin is found in the trabecular meshwork, cornea, lamina cribosa, ciliary body, iris, vitreous, retina, optic nerve, and aqueous humor [10]. The structure

Table 1. The 50 most studied proteins in human eye research[a), b)]

UniProt ID	Gene	Protein name	Citations	Functions	Associated diseases	Isoforms, PTMs, variants
P26367	PAX6	Paired box protein PAX-6	204	Transcription factor	Aniridia; Peters anomaly; foveal hypoplasia 1; keratitis hereditary; coloboma of iris, choroid, and retina; coloboma of optic nerve; bilateral optic nerve hypoplasia; aniridia, cerebellar ataxia, and mental deficiency	2 isoforms 1 PTM 85 variants
Q99972	MYOC	Myocilin	198	Regulates the activation of different signaling pathways; secreted glycoprotein	Glaucoma 1, open angle, A; glaucoma 3, primary congenital, A	1 isoform 2 PTMs 148 variants
P36955	SERPINF1	Pigment epithelium-derived factor	180	Neurotrophic protein; inhibits angiogenesis	Osteogenesis imperfecta 6	1 isoform 5 PTMs 38 variants
P15692	VEGFA	Vascular endothelial growth factor A	138	Growth factor that plays a role in angiogenesis, vasculogenesis, and endothelial cell growth	Microvascular complications of diabetes	17 isoforms 6 PTMs 21 variants
P08603	CFH	Complement factor H	132	Cofactor in alternative complement pathway	Basal laminar drusen; complement factor H deficiency; hemolytic uremic syndrome atypical 1; macular degeneration, age-related, 4	2 isoforms 51 PTMs 255 variants
O76090	BEST1	Bestrophin-1	108	Forms calcium-sensitive chloride channels	Vitelliform macular dystrophy 2; retinitis pigmentosa 50; adult onset vitelliform macular dystrophy; Bestrophinopathy, autosomal recessive; vitreoretinochoroidopathy, autosomal dominant	3 isoforms 0 PTMs 172 variants
P78363	ABCA4	Retinal-specific ATP-binding cassette transporter	96	Inward-directed retinoid flipase	Stargardt disease 1; fundus flavimaculatus; macular degeneration, age-related, 2; cone-rod dystrophy 3; retinitis pigmentosa 19	1 isoform 10 PTMs 479 variants
Q92834	RPGR	X-linked retinitis pigmentosa GTPase regulator	94	Plays a role in ciliogenesis	Retinitis pigmentosa 3; retinitis pigmentosa and sinorespiratory infections with or without deafness; cone-rod dystrophy, X-linked 1; macular degeneration, X-linked, atrophic	6 isoforms 4 PTMs 155 variants
P0C7Q2	ARMS2	Age-related maculopathy susceptibility protein 2	87	Retina homeostasis	Macular degeneration, age-related, 8	1 isoform 0 PTMs 7 variants
P08100	RHO	Rhodopsin	87	Key role in visual process	Retinitis pigmentosa 4; night blindness, congenital stationary, autosomal dominant 1	1 isoform 13 PTMs 111 variants

(*Continued*)

Table 1. Continued

UniProt ID	Gene	Protein name	Citations	Functions	Associated diseases	Isoforms, PTMs, variants
P02489	CRYAA	Alpha-crystallin A chain	77	Plays a role in transparency and refractive index of the lens	Cataract 9, multiple types	1 isoform 13 PTMs 28 variants
Q15582	TGFBI	Transforming growth factor-beta-induced protein ig-h3	77	Binds to type I, II, and IV collagens	Corneal dystrophy, epithelial basement membrane; corneal dystrophy, Groenouw type 1; corneal dystrophy, lattice type 1; corneal dystrophy, Thiel-Behnke type; corneal dystrophy, Reis-Bucklers type; corneal dystrophy, lattice type 3A; corneal dystrophy, Avellino type	1 isoform 33 PTMs 110 variants
Q16518	RPE65	Retinoid isomerohydrolase	63	Roles in production of 11-cis retinal and visual pigment regeneration	Leber congenital amaurosis 2; retinitis pigmentosa 20; autosomal dominant retinitis pigmentosa with choroidal involvement	1 isoform 9 PTMs 106 variants
O15537	RS1	Retinoschisin	62	Involved in cell adhesion processes during retinal development	Retinoschisis juvenile X-linked 1	1 isoform 5 PTMS 99 variants
Q92743	HTRA1	Serine protease HTRA1	60	Serine protease	Macular degeneration, age-related 7; cerebral arteriopathy with subcortical infarcts and leukoencephalopathy, autosomal recessive	1 isoform 0 PTMs 34 variants
Q9NZN9	AIPL1	Aryl-hydrocarbon-interacting protein-like 1	58	May be involved in protein trafficking and/or protein folding and stabilization	Leber congenital amaurosis 4	5 isoforms
Q12948	FOXC1	Forkhead box protein C1	54	Regulator of cell viability and resistance to oxidative stress	Axenfeld-Rieger syndrome 3; iridogoniodysgenesis anomaly; Peters anomaly	1 isoform 5 PTMs 37 variants
Q00604	NDP	Norrin	54	Involved in canonical Wnt signaling pathway; role in retinal neovascularization	Norrie disease; vitreoretinopathy, exudative 2	1 isoform 5 PTMs 75 variants
Q16678	CYP1B1	Cytochrome C450 1B1	53	Heme-thiolate monooxygenase	Peters anomaly; glaucoma 3, primary congenital, A; glaucoma, primary open angle; glaucoma 1, open angle, A	1 isoform 2 PTMs 101 variants
Q04671	OCA2	P protein	52	Role in melanin synthesis, melanosome maturation	Albinism, oculocutaneous, 2	3 isoforms 5 PTMs 209 variants
P06400	RB1	Retinoblastoma-associated protein	51	Tumor suppressor	Childhood cancer retinoblastoma; bladder cancer; osteogenic sarcoma	1 isoform 30 PTMs 201 variants

(*Continued*)

Table 1. Continued

UniProt ID	Gene	Protein name	Citations	Functions	Associated diseases	Isoforms, PTMs, variants
P23942	PRPH2	Peripherin-2	50	Involved in outer segment disk morphogenesis	Retinitis pigmentosa 7; retinitis punctata albescens; adult-onset vitelliform macular dystrophy; patterned dystrophy of retinal pigment epithelium; choroidal dystrophy, central areolar 2; cone-rod dystrophy; retinitis pigmentosa; macular degeneration	1 isoform 2 PTMs 46 variants
P82279	CRB1	Protein crumbs homolog 1	49	Role in photoreceptor morphogenesis	Retinitis pigmentosa 12; Leber congenital amaurosis 8; pigmented paravenous chorioretinal atrophy	5 isoforms 82 PTMs 301 variants
P01137	TGFB1	Transforming growth factor beta-1	46	Multifunctional; involvement in proliferation, differentiation, etc.	Camurati–Engelmann disease	1 isoform 12 PTMs 40 variants
Q8WWY3	PRPF31	U4/U6 small nuclear ribonucleoprotein Prp31	46	Involved in pre-mRNA splicing	Retinitis pigmentosa 11	3 isoforms 9 PTM 40 variants
Q99697	PITX2	Pituitary homeobox 2	46	Involved in cell proliferation, morphogenesis	Axenfeld-Rieger syndrome 1; iridogoniodysgenesis 2; Peters anomaly; ring dermoid of cornea	3 isoforms 1 PTM 47 variants
P07320	CRYGD	Gamma-crystallin D	44	Component of lens	Cataract 4, multiple types	1 isoform 0 PTMs 21 variants
P14679	TYR	Tyrosinase	43	Involved in formation of pigments	Albinism, oculocutaneous, 1A; albinism, oculocutaneous, 1B	2 isoforms 6 PTMs 225 variants
P30301	MIP	Lens fiber major intrinsic protein	43	Water channel	Cataract 15, multiple types	1 isoform 4 PTMs 41 variants
P02511	CRYAB	Alpha-crystallin B chain	42	Component of lens	Myopathy, myofibrillar, 2; cataract 16, multiple types; myopathy, myofibrillar, fata infantile hypertonic, alpha-B crystallin-related; cardiomyopathy, dilated 1II	1 isoform 7 PTMs 23 variants
P02649	APOE	Apolipoprotein E	42	Mediates the binding, internalization, and catabolism of lipoprotein particles	Hyperlipoproteinemia 3; Alzheimer disease 2; sea-blue histiocyte disease; lipoprotein glomerulopathy; familial hypercholesterolemia	1 isoform 9 PTMs 46 variants

(*Continued*)

Table 1. Continued

UniProt ID	Gene	Protein name	Citations	Functions	Associated diseases	Isoforms, PTMs, variants
P48165	GJA8	Gap junction alpha-8 protein	42	Channel activity	Cataract 1, multiple types	1 isoform 0 PTMs 82 variants
P17302	GJA1	Gap junction alpha-1 protein	39	Gap junction protein that acts as regulator of bladder capacity	Oculodentodigital dysplasia; oculodentaldigital dysplasia, autosomal recessive; syndactyly 3; hypoplastic left heart syndrome 1; Hallermann-Streiff syndrome; atrioventricular septal defect 3; craniometaphyseal dysplasia, autosomal recessive	1 isoform 26 PTMs 113 variants
P51810	GPR143	G-protein coupled receptor 143	39	Receptor for tyrosine, L-DOPA, and dopamine	Albinism ocular 1; nystagmus congenital X-linked 6	1 isoform 1 PTM 70 variants
Q02846	GUCY2D	Retinal guanylyl cyclase 1	39	Possible functional role in rod/cone photoreceptors	Leber congenital amaurosis 1; cone-rod dystrophy 6	1 isoform 3 PTMs 128 variants
Q96CV9	OPTN	Optineurin	39	Roles in maintaining Golgi complex, membrane trafficking, exocytosis	Glaucoma 1, open angle, E; glaucoma, normal pressure; amyotrophic lateral sclerosis 12	3 isoforms 8 PTMs 60 variants
O43186	CRX	Cone-rod homeobox protein	37	Transcription factor, upstream of several photoreceptor-specific genes	Leber congenital amaurosis 7; cone-rod dystrophy 2; retinitis pigmentosa	1 isoform 0 PTMs 47 variants
P04637	TP53	Cellular tumor antigen p53	37	Tumor suppressor	Esophageal cancer; Li-Fraumeni syndrome; squamous cell carcinoma of the head and neck; lung cancer; papilloma of the choroid plexus; adrenocortical carcinoma; basal cell carcinoma 7	9 isoforms 34 PTMs 1706 variants
P01375	TNF	Tumor necrosis factor	35	Proinflammatory cytokine	Psoriatic arthritis	1 isoform 5 PTMs 18 variants
P35913	PDE6B	Rod cGMP-specific 3′,5′-cyclic phosphodiesterase subunit beta	34	Role in transmission and amplification of the visual signal	Retinitis pigmentosa 40; night blindness, congenital stationary, autosomal dominant 2	3 isoforms 2 PTMs 104 variants

(*Continued*)

Table 1. Continued

UniProt ID	Gene	Protein name	Citations	Functions	Associated diseases	Isoforms, PTMs, variants
O75695	RP2	Protein XRP2	33	GTPase-activating protein	Retinitis pigmentosa 2	1 isoform 4 PTMs 51 variants
Q08397	LOXL1	Lysyl oxidase homolog 1	33	Active on elastin and collagen substrates	Exfoliation syndrome	1 isoform 7 PTMs 34 variants
P05231	IL6	Interleukin-6	32	Multifunctional cytokine; induces acute phase response	Rheumatoid arthritis systemic juvenile	1 isoform 3 PTMs 32 variants
P61812	TGFB2	Transforming growth factor Beta-2	32	Suppresses interleukin-2 dependent T-cell Growth	Loeys-Dietz syndrome 4	2 isoforms 8 PTMs 47 variants
Q92781	RDH5	11-cis retinol dehydrogenase	32	Catalyzes final step in biosynthesis of 11-cis retinaldehyde, the universal chromophore of visual pigments	Retinitis punctata albescens	1 isoform 1 PTM 50 variants
O95343	SIX3	Homeobox protein SIX3	31	Transcriptional regulator	Holoprosencephaly 2	1 isoform 0 PTMs 30 variants
Q9GZR5	ELOVL4	Elongation of very long chain fatty acids protein 4	31	Elongates saturated and monosaturated very long chain fatty acids	Stargardt disease 3	1 isoform 1 PTM 38 variants
Q9NZR4	VSX1	Visual system homeobox 1	31	Binds to the locus core region of the red/green visual pigment gene cluster	Corneal dystrophy, posterior polymorphous, 1; keratoconus 1; craniofacial anomalies and anterior segment dysgenesis syndrome	8 isoforms 0 PTMs 39 variants
Q9UI36	DACH1	Dachshund homolog 1	31	Transcription factor involved in regulation of organogenesis	—	4 isoforms 6 PTMs 84 variants
O60313	OPA1	Dynamin-like 120 kDa protein, mitochondrial	30	Role in mitochondrial fusion and regulation of apoptosis	Optic atrophy 1; dominant optic atrophy plus syndrome	2 isoforms 4 PTMs 142 variants

a) Protein function(s), disease states, isoforms, PTMs, and variants based upon NeXtProt entries using gold level criteria. Disease states, isoforms, PTMs, and variants reported for NeXtProt-curated entries.
b) It should be noted that although this list comprises 50 proteins, there are actually many more proteins due to isoforms and variants noted in the last column.
RPE65, retinoid isomerohydrolase.

Table 2. The 50 most studied proteins in mouse eye research[a),b)]

UniProt ID	Gene	Protein name	Citations	Functions	Associated diseases in mice	Isoforms
P63015	Pax6	Paired box protein Pax-6	653	Transcription factor involved in development of eye and other organs	Defects in Pax6 cause condition of small eye (Sey) with lack of eyes and nasal primordial	3 isoforms
P23440	Pde6b	Rod cGMP-specific 3′,5′-cyclic phosphodiesterase subunit beta	298	Role in transmission and amplification of visual signal	Defects in Pd36b are cause of a retinal degeneration	2 isoforms
P15409	Rho	Rhodopsin	244	Photoreceptor required for vision at low light intensity	—	1 isoform
Q61412	Vsx2	Visual system homeobox 2	162	Role in specification and morphogenesis of sensory retina	Defects in Vsx2 are cause of ocular retardation (OR(J)), a disease with microphthalmia, retinal destruction, and absence of optic nerve	1 isoform
Q91ZQ5	Rpe65	Retinoid isomerohydrolase	144	Role in visual pigment regeneration	Defects in Rpe65 cause light damage susceptibility (LDS) of the retina	1 isoform
Q9ERD7	Tubb3	Tubulin beta-3 chain	137	Major constituent of microtubules	—	1 isoform
Q08874	Mitf	Microphthalmia-associated transcription factor	132	Transcription factor for genes that play essential roles in cell differentiation, proliferation, and survival	Defects in Mitf cause microphthalmia (mi)	9 isoforms
P61372	Isl1	Insulin gene enhancer protein ISL-1	129	Regulates promoters of insulin, glucagon, and somatostatin genes	—	2 isoforms
Q63934	Pou4f2	POU domain, class 4, transcription factor 2	127	Transcription factor	—	1 isoform
P32114	Pax2	Paired box protein Pax-2	126	Transcription factor	Renal-coloboma syndrome	2 isoforms
Q62226	Shh	Sonic hedgehog protein	117	Intercellular signal essential for various patterning events during development	—	1 isoform
P12658	Calb1	Calbindin	115	Buffers cytosolic calcium	—	1 isoform
P24622	Cryaa	Alpha-crystallin A chain	113	Contributes to transparency and refractive index of the lens	—	2 isoforms
P70677	Casp3	Caspase-3	112	Role in activation cascade of caspases involved in apoptosis	—	1 isoform
P15499	Prph2	Peripherin-2	111	May function as adhesion molecule in outer segment disks	Responsible for retinal degeneration slow (Rds)	1 isoform
Q00731	Vegfa	Vascular endothelial growth factor A	104	Growth factor involved in angiogenesis, vasculogenesis, and endothelial cell growth	—	6 isoforms
P48432	Sox2	Transcription factor SOX-2	95	Transcription factor for some genes involved in embryonic development	—	1 isoform

(*Continued*)

Table 2. Continued

UniProt ID	Gene	Protein name	Citations	Functions	Associated diseases in mice	Isoforms
P48437	Prox1	Prospero homeobox protein 1	89	Transcription factor involved in developmental processes	—	1 isoform
P54846	Nrl	Neural retina-specific leucine zipper protein	88	Transcription factor involved in expression of several rod-specific genes	—	1 isoform
P11344	Tyr	Tyrosinase	87	Involved in formation of pigments such as melanins	Defects in Tyr results in various forms of albinism	1 isoform
Q62233	Six3	Homeobox protein SIX3	87	Transcriptional regulator	—	2 isoforms
O54751	Crx	Cone-rod homeobox protein	87	Transcription factor that transactivates a sequence upstream of several photoreceptor-specific genes	—	1 isoform
P20612	Gnat1	Guanine nucleotide-binding protein G(t) subunit alpha-1	86	Modulator or transducer of various transmembrane signaling systems	—	1 isoform
Q9QXZ9	Opn4	Melanopsin	85	Photoreceptor required for regulation of circadian rhythm	—	2 isoforms
Q08331	Calb2	Calretinin	84	Calcium-binding protein	—	1 isoform
Q9Z2E5	Atoh7	Protein atonal homolog 7	83	Transcription factor involved in the differentiation of most ganglion cells	—	1 isoform
P03995	Gfap	Glial fibrillary acidic protein	81	Class-III intermediate filament	—	2 isoforms
P21275	Bmp4	Bone morphogenetic protein 4	81	Induces cartilage and bone formation	—	1 isoform
P25322	Ccnd1	G1/S-specific cyclin-D1	78	Phosphorylates and inhibits members of the retinoblastoma (RB) protein family		1 isoform
P08553	Nefm	Neurofilament medium polypeptide	78	Component of neurofilaments	—	1 isoform
O35602	Rax	Retinal homeobox protein Rx	77	Regulates initial specification of retinal cells and/or their subsequent proliferation	—	1 isoform
Q02248	Ctnnb1	Catenin beta-1	73	Downstream component of canonical Wnt signaling pathway	—	1 isoform

(*Continued*)

Table 2. Continued

UniProt ID	Gene	Protein name	Citations	Functions	Associated diseases in mice	Isoforms
P02340	Tp53	Cellular tumor antigen p53	72	Tumor suppressor	—	1 isoform
P17208	Pou4f1	POU domain, class 4, transcription factor 1	71	Probable transcription factor	—	1 isoform
Q02067	Ascl1	Achaete-scute homolog 1	70	Transcription factor	—	1 isoform
P20444	Prkca	Protein kinase C alpha type	70	Calcium-activated, phospholipid- and diacylglycerol (DAG)-dependent serine/threonine-protein kinase	Expression of mutant form UV25 causes malignant transformation of cells	1 isoform
P23927	Cryab	Alpha-crystallin B chain	69	May contribute to transparency and refractive index of lens	—	1 isoform
P46414	Cdkn1b	Cyclin-dependent kinase inhibitor 18	67	Regulator of cell-cycle progression	—	1 isoform
Q60867	Neurod1	Neurogenic differentiation factor 1	64	Transcriptional activator	Neurod1 null mice are deaf and die shortly after birth	1 isoform
P09803	Cdh1	Cadherin-1	63	Calcium-dependent cell adhesion protein	—	1 isoform
P70447	Neurog2	Neurogenin-2	62	Transcriptional regulator	—	1 isoform
P49919	Cdkn1c	Cyclin-dependent kinase inhibitor 1C	57	Inhibitor of several G1 cyclin/CDKcomplexes and mitotic cyclin B-CDC2		2 isoforms
Q08481	Pecam1	Platelet endothelial cell adhesion molecule	56	Cell adhesion molecule needed for leukocyte transedothelial migration	—	4 isoforms
P51180	Mip	Lens fiber major intrinsic protein	55	Water channel	Defects in Mip cause autosomal dominant cataract	1 isoform
P15105	Glul	Glutamine synthetase	53	Essential for proliferation of fetal skin fibroblasts	—	1 isoform
P97474	Pitx2	Pituitary homeobox 2	52	Involved in cell proliferation and morphogenesis	Mice embryos lacking isoform Ptx2c show left-right patterning defects and severe development abnormalities	5 isoforms
P28236	Gja8	Gap junction alpha-8 protein	52	Component of gap junction	—	1 isoform
Q9CXV0	Isl2	Insulin gene enhancer protein ISL-2	52	Transcriptional factor	—	1 isoform
P51491	Opn1sw	Short-wave-sensitive opsin 1	50	Visual pigment	—	1 isoform
P20443	Sag	S-arrestin	50	Binds to photoactivated-phosphorylated rhodopsin	—	1 isoform

a) Protein function(s), disease states, and isoforms based upon UniProt entries.
b) It should be noted that although this list comprises 50 proteins, there are actually many more proteins due to isoforms and variants noted in the last column.

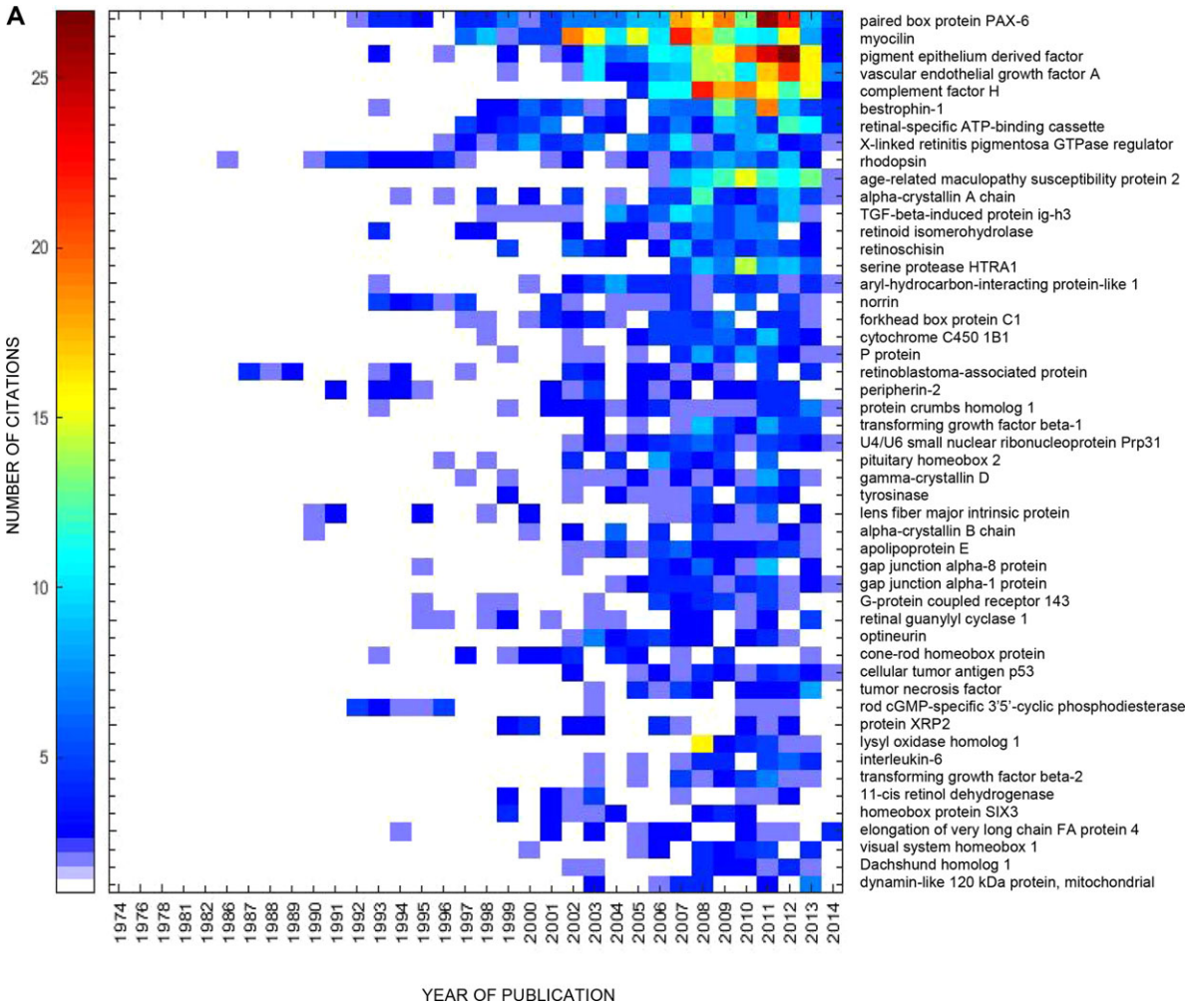

Figure 2. (A) Heat map of the 50 most studied proteins in human eye research, 1974–2014. The first publication associated with both a gene and eye for human research in PubMed appeared in 1974. (B) Heat map of the 50 most studied proteins in the mouse eye research, 1924–2014. The first publication associated with both a gene and eye for mouse research in PubMed appeared in 1924. The heat map does not represent a full year for 2014, but only what was published on PubMed by 10/20/14.

of myocilin includes a signal peptide sequence for cleavage as a secreted protein and a C-terminal olfactomedin domain. Over 70 glaucoma-associated variants have been identified in myocilin, of which >90% are located in exon 3 that codes for the olfactomedin domain [9]. Most myocilin variants that contain an amino acid substitution are not secreted but accumulate within the endoplasmic reticulum as homo- or heterodimers [9]. The function of myocilin is not well understood [9, 10].

Pigment epithelium-derived factor (PEDF) is a secreted glycoprotein that belongs to the serpin (serine protease inhibitor) family [11]. PEDF has heparin- and collagen-binding sites, and an unusual asymmetric distribution of charged amino acid residues, with basic and acidic regions on the opposite poles of the protein [12, 13]. PEDF has neurotrophic and anti-angiogenic effects [11] and provides protection against oxidative stress in diabetic retinopathy [14]. PEDF inhibits retinal neovascularization induced by vascular endothelial growth factor [15].

Vascular endothelial growth factor-A (VEGF-A), a member of the vascular permeability factor/VEGF family, is a disulfide-bonded dimeric glycoprotein that plays a central role in angiogenesis [16]. VEGF-A has 17 isoforms that arise from alternative promoter usage, alternative splicing, and alternative initiation. The VEGF-$A^{164/165}$ isoform, named after the total number of amino acid residues in mouse and human proteins, respectively, has been most intensively studied because of its role in angiogenesis [16]. VEGF-A binds with VEGF receptors 1 and 2, two high affinity tyrosine kinase receptors [17], and with neuropilin-1 [18]. Neutralization of VEGF-A with ranibizumab, a recombinant monoclonal antibody, was shown to prevent visual loss in neovascular AMD [19]. Other antibodies against VEGF-A have shown similar effects in treatment of AMD.

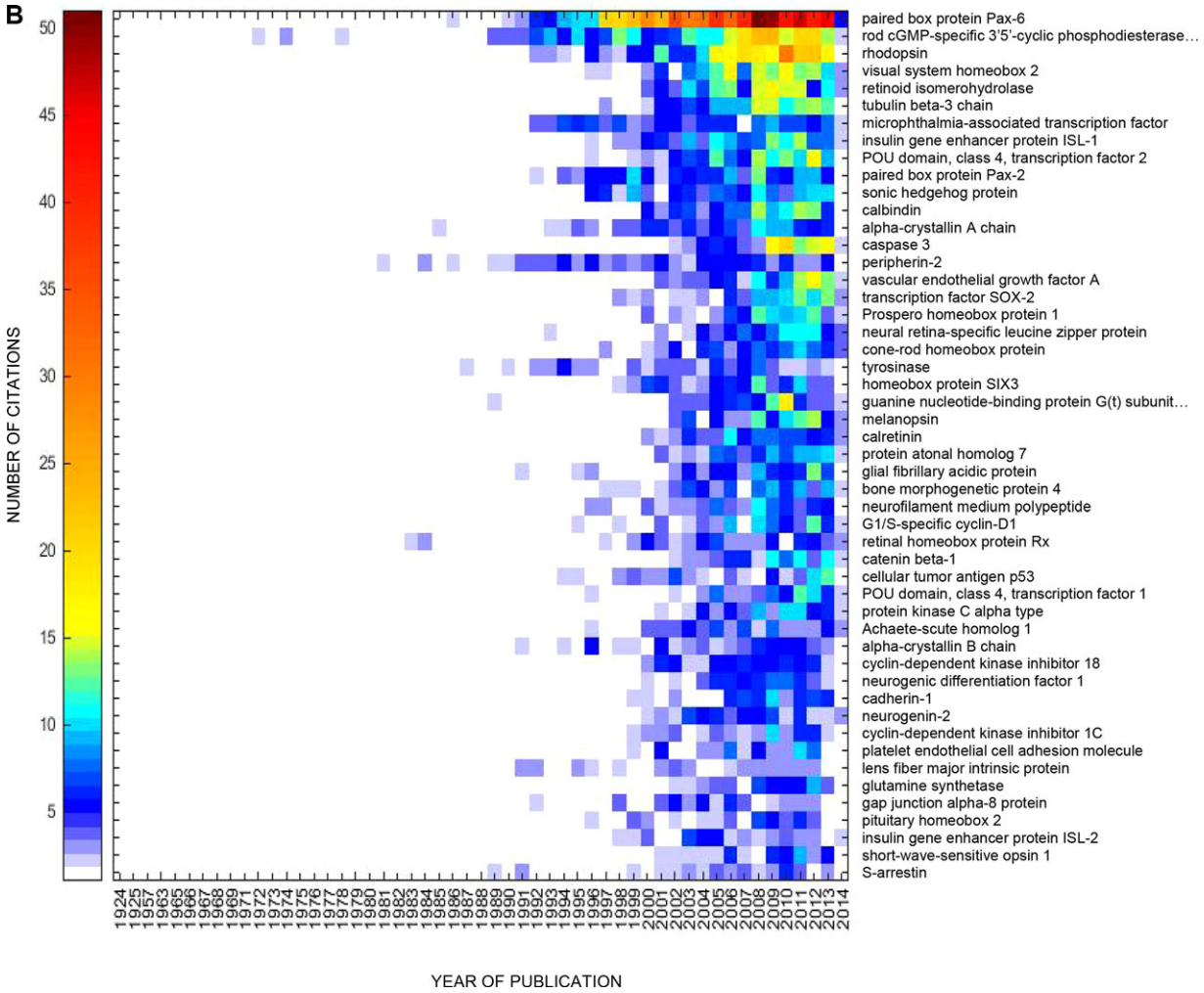

Figure 2. *Continued.*

Complement factor H (CFH) is a 1213 amino acid glycoprotein that plays a central role in the complement system. The complement system of innate immunity is involved in cellular integrity, microbial killing, immune surveillance, tissue homeostasis, and mediation of inflammatory responses [20]. Complement is involved in the recognition of diseased or damaged host cells, regulation of cellular immune responses, and interaction with the coagulation cascade.

CFH plays a role in limiting complement-mediated damage to healthy host cells [21]. CFH has multiple-binding sites, including those for C3b, heparin, C-reactive protein, and sialic acid. Two variants of CFH, Y402H, and I62V, are strongly associated with the risk of AMD [22]. Immunohistochemical studies have demonstrated that CFH is present within vascular lumens and perivascular spaces around large blood vessels, in the choriocapillaris, intercapillary septa, Bruch's membrane, and in large choroidal vessels and stroma in eyes with AMD [23].

Bestrophin-1 (BEST1) is a 585 amino acid transmembrane protein that is involved in intracellular calcium signaling [24]. There are three isoforms that arise through alternative splicing. BEST1 is most strongly localized in the cytosol close to the basolateral membrane of the retinal pigment epithelium (RPE) [24]. Mutations in BEST1 cause a variety of retinal degenerations, the best known being Best's vitelliform macular dystrophy, or Best's disease. Mutations in BEST1 are associated with increased accumulation of lipofuscin, a yellow aging-associated pigment, in the RPE, but the underlying pathophysiology is not well understood [25].

Retinal-specific ATP-binding cassette transformer (ABCA4) is in the family of ABC transporters, a ubiquitous set of integral membrane proteins present in all living organisms [26]. ABCA4 has two transmembrane domains, two nucleotide-binding domains (ATP-binding cassettes), and two extracellular domains [26]. ABCA4 is located in the disk margins of photoreceptor outer segments. The reason for the restricted localization of ABCA4 within the rod outer segments is not clear [26]. ABCA4 seems to play a role in the clearance of all-*trans*-retinal from disk membranes after photoexcitation of rhodopsin [26]. Mutations in ABCA4 are

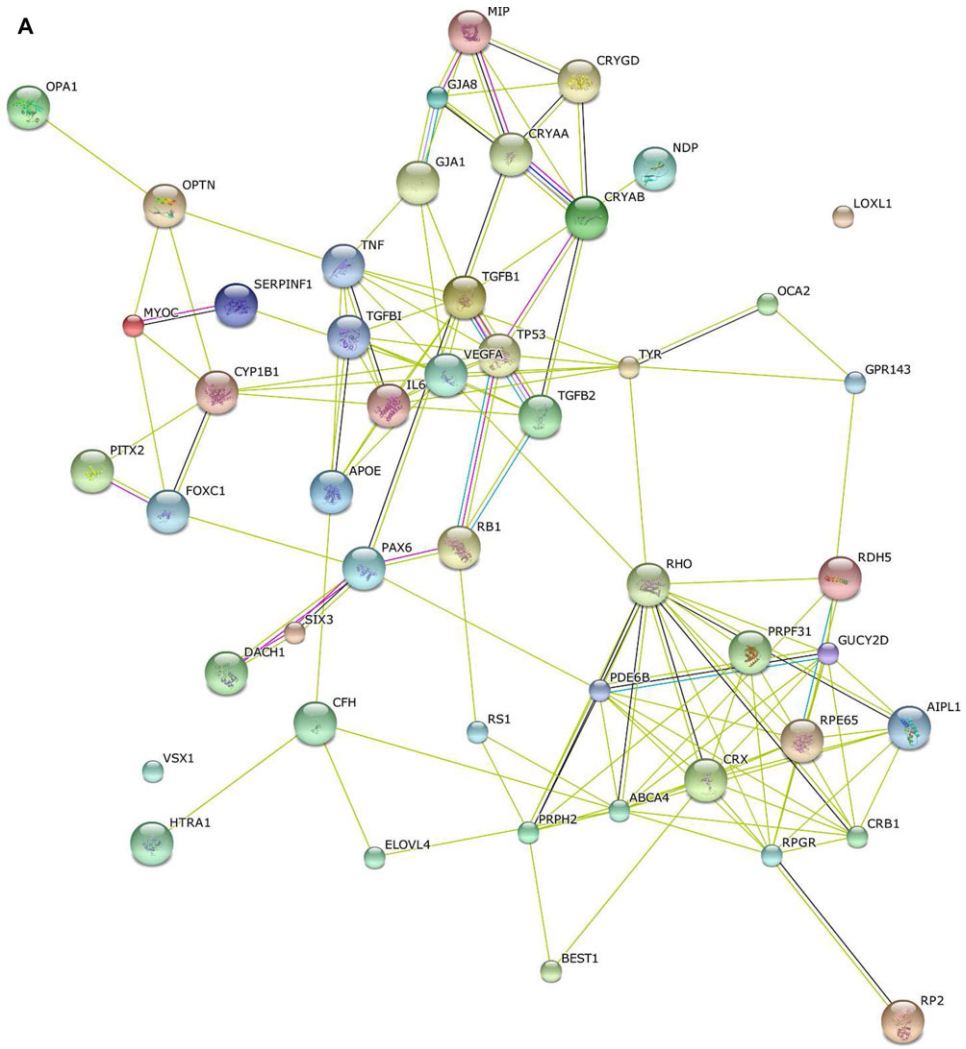

Figure 3. Functional protein networks among the top 50 most studied proteins for (A) human and (B) mouse eye.

associated with Stargardt disease [27], fundus flavimaculatus [28], cone-rod dystrophy, and a form of retinitis pigmentosa [26].

X-linked retinitis pigmentosa GTPase regulator (RPGR) is a 1020 amino acid protein that has six isoforms arising from alternative splicing. Isoform 6, or $RPGR^{ORF15}$ is highly expressed in photoreceptors and is implicated in retinal disease. RPGR contains a glycine/glutamic-acid rich domain near the C-terminal end that accounts for up to 80% of RPGR mutations [29]. RPGR is found in centrioles, ciliary axonemes, and microtubular transport complexes [29]. RPGR plays a role in microtubular transport through the ciliary structures that connect the inner and outer segments of photoreceptors [29, 30]. X-linked forms of cone-rod dystrophy, cone dystrophy, and macular atrophy have been associated with $RPGR^{ORF15}$ mutations.

Age-related maculopathy susceptibility protein 2 (ARMS2) is a 107 amino acid protein that has been implicated in AMD. *ARMS2*, a recent gene in evolution, is present only in humans and higher primates [31]. No homologous gene has been annotated in lower vertebrates or other organisms [32]. ARMS2 has nine predicted phosphorylation sites but no remarkable structural motifs. Recent studies show there are two isoforms of ARMS2: isoform A, the canonical form and isoform B that arises as a splice variant [33, 34]. The function of ARMS2 is not well understood. ARMS2 has been localized to retina and RPE [35]. The A69S risk variant of ARMS2 is strongly associated with AMD [22]. Since ARMS2 is in strong linkage disequilibrium with serine protease HTRA1, it is unclear whether ARMS2, HTRA1, or both proteins are involved in the pathogenesis of AMD.

Rhodopsin (RHO), a visual pigment found in rod photoreceptors in the retina, is essential for the process of vision. RHO is a member of the G-protein-coupled-receptor family. The structure of RHO includes a transmembrane protein moiety, opsin, which contains a ligand-binding site for retinal on the extracellular side of the transmembrane bundle [36]. The absorption of photons causes the isomerization of 11-*cis* retinal to all-*trans* retinal, conformation changes in rhodopsin, and downstream signal transduction [36].

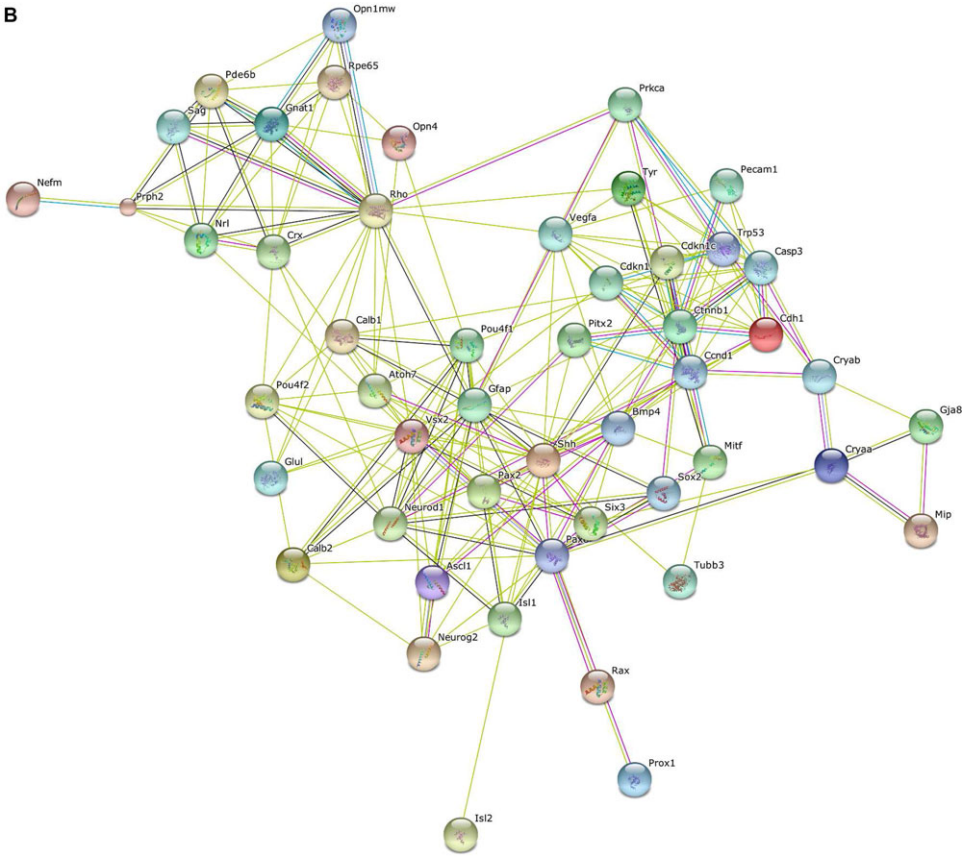

Figure 3. *Continued.*

Mutations in rhodopsin are associated with congenital stationary night blindness and retinitis pigmentosa (RP) [37].

Rod cGMP-specific 3′,5′-cyclic phosphodiesterase subunit beta (Pde6b) has been extensively studied in mouse models for autosomal recessive retinitis pigmentosa [38]. Rod phosphodiesterase (PDE) is a membrane-associated protein that consists of two catalytic subunits, rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit alpha and Pde6b, and two gamma inhibitory subunits [38]. PDE plays a role in phototransduction by hydrolyzing the cGMP second messenger. Natural mouse models with the *Pde6b* mutations have been used to evaluate pharmacological treatments and gene therapy for protecting photoreceptors from apoptosis [38].

Visual system homeobox 2 (Vsx2) is a transcription factor that controls the morphogenesis of the eye [39, 40]. Vsx2 is a 361 amino acid protein that contains a 60 amino acid homeodomain, or DNA-binding module composed of three alpha helices [39]. Mutations in Vsx2 are associated with microphthalmia in humans [39, 40] and mice [41].

Retinoid isomerohydrolase (Rpe65) is an RPE-specific protein that plays an important role in the visual cycle by converting all-*trans* retinyl esters to 11-*cis*-retinol [42]. Rpe65 is bound to smooth endoplasmic reticulum in RPE cells, but the exact mechanism of this binding is unclear [42]. In humans, mutations of Rpe65 are associated with Leber's congenital amaurosis, recessive RP, fundus albipunctatus, and autosomal dominant RP with choroidal involvement [43].

Tubulin beta-3 chain (Tubb3) is a component of microtubules. Microtubules form the cytoskeleton and consist of heterodimers of alpha- and beta-tubulin. Tubulin has a wide range of PTMs, including acetylation, phosphorylation, detyrosination, polyglycylation, and polyglutamylation [44]. Tubb3, one of six tubulins found in mammals, has expression mainly limited to neurons [45]. In humans, *TUBB3* mutations are associated with congenital oculomotor nerve hypoplasia and later-onset peripheral axon degeneration [45].

Microphthalmia-associated transcription factor (Mitf), a member of the family of basic helix-loop-helix leucine-zipper microphthalmia-related transcription factors, is a regulator of melanocytes and has pleiotrophic roles in RPE cells, mast cells, and osteocytes [46]. There are nine isoforms of Mitf. PTMs of Mitf include phosphorylation, sumoylation, and ubiquitination. The target genes for Mitf include those involved in pigmentation, cell cycle, survival, motility and invasion, metabolism, and oxidative stress [47]. Defects in Mitf cause microphthalmia in mice.

Insulin gene enhancer protein ISL-1 (Isl1), a transcription factor of the LIM-homeodomain protein family, is a 349 amino acid that is essential in development of many cell types, including retina [48]. Isl1 has two tandemly arrayed LIM domains near the N terminus that mediate protein–protein

interactions and an adjacent homeodomain that binds DNA [49]. ISl1 regulates promoters of insulin, glucagon, and somatostatin genes.

POU domain, class 4, transcription factor 2 (Pou4f2) is a 411 amino acid transcription factor that is expressed in developing and adult retinal ganglion cells [50]. Pou4f2 is one of three members of the POU4F family, all of which are expressed only in ganglion cells of the retina [51]. Selective ablation of *Pou4f2* had no impact on long-term survival of retinal ganglion cells in adult mice [51]. Pouf4f2 has been of interest in glaucoma research, since glaucomatous optic atrophy is characterized by a progressive loss of retinal ganglion cells.

Paired box protein Pax-2 (Pax2) is a transcription factor that is required for optic fissure closure in the developing eye [52]. Astrocytes, the earliest glial cell population in optic nerve development, play a role in retinal angiogenesis and formation of the brain-retinal barrier [53]. Pax2 mutations are associated with a renal-coloboma syndrome that involves the eye, ear, CNS, and urogenital tract in humans and mice [54, 55].

A bibliometric analysis conducted in 2011 showed that about three-quarters of protein research focuses on the 10% of proteins that were known before the human genome was mapped [56]. Most of the diseases or processes associated with the ten most intensively studied proteins of the human eye are related to development or single gene disorders such as retinitis pigmentosa. Some of the proteins that are being investigated, such as ARMS2, have gained recent attention mainly because of strong disease associations at the genetic level. As noted by the investigators of the 2011 bibliometric analysis, scientists have an apparent reluctance to work on unknown or lesser known proteins. The reasons for this are unclear but may possibly have to do with greater risk in grant applications, as it is harder to explain rationale and significance for proteins that have unknown functions [56]. In addition, the intensity with which certain proteins were studied was related to the availability of chemical probes for the particular protein [56]. The present analysis corroborates the observation that most proteins of the eye have not been well studied: over 57% of proteins in human eye research and nearly 50% of proteins in mouse eye research had one citation only. A limitation of this study is that many older references may have had less stringent quality control than are currently used in claiming identification of proteins. Another limitation is that the proteins identified as "priority proteins" for human eye research will likely grow and change in the future. What we have proposed here is a starting point based upon some of the most intensely studied biological pathways in human eye research.

Recent advances in proteomics, bioinformatics, and MS instrumentation should help expand scientific investigations to lesser known proteins in eye research. Discovery work on proteomes of specific tissues and cell types has been greatly facilitated by data-dependent acquisition approach using Orbitrap mass spectrometers [57] or data-independent approaches (e.g. SWATH) [58]. Targeted methods for selective protein quantitation such as SRM most often use triple quadrupole mass spectrometers. SRM assays were recently used for quantification of a large number of human tear proteins [59]. The SRM assays proposed in the present paper for human and mouse eye proteins can be applied for protein quantification. All instruments can quantitate proteins without the need for antibodies or specific chemical probes, although these can employed when increased sensitivity is required to deal with dynamic range constraints [60, 61]. Protein interaction studies can be used to determine binding partners and infer their functional networks [62, 63]. Many strategies are available to identify and quantify different PTMs, such as phosphorylation, *O*-GlcNAcylation, and glycosylation [64–66]. Specific isoforms and variants arising from single nucleotide polymorphisms can be targeted by SRM assays. Application of these new advances should help both discovery and hypothesis-based research about the rich diversity of proteins involved in biological processes of the eye and vision and in health and disease. These newer tools will help scientists investigate proteins that remain "hidden in plain sight" [56].

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