Review Article

# Genes, Proteins and Biological Pathways in Human and Animal Myopia

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## ABSTRACT

Myopia (short-sightedness) is approaching pandemic prevalence of vast socio-economic and public health importance in many Asian communities, with approximately 50% of the world population estimated to be affected by 2050. Several genetic and environmental factors have been implicated in the emergence and progression of myopia. However, what makes this disease so complex is that there is very little overlap in genes linked to Mendelian diseases as listed on the Online Mendelian Inheritance in Man (OMIM) database and single nucleotide polymorphisms in Genome-Wide Association Studies (GWAS). To add a further dimension of complexity to this disease, animal research has implicated many genes and proteins in the development of myopia, again, with no common genes identified across all platforms. What is immediately apparent is the existence of a complex gene-environment relationship and rather than focusing on individual genes, efforts should be redirected to identify biological pathways driving myopic growth. Thus, identifying a molecular fingerprint specific to myopia is essential to ensure early diagnosis and to facilitate the generation of new therapeutic avenues.

## GLOSSARY

A2BP1: ataxin 2-binding protein 1; ABCC10: ATP-binding cassette, sub-family C (CFTR/MRP), member 10; ABCC5: ATP Binding Cassette Subfamily C Member 5; ACTB: beta (β)-actin; ACTC1: actin Alpha Cardiac Muscle 1; ACTR3B: actin Related Protein 3B; ACTR6: ARP6 actin-related protein 6 homolog (yeast); ACTR8: ARP8 actin-related protein 8 homolog (yeast); AFF2: AF4/FMR2 family, member 2; AL: axial length; AMD: age-related macular degeneration; AMPK: AMP-activated protein kinase; ANGPT2: angiopoietin 2; ANO2: anoctamin 2; APLP2: amyloid precursor- like protein 2; APOA1: apolipoprotein A-I; ARHGEF12: Rho guanine nucleotide exchange factor (GEF) 12; ARNTL: aryl hydrocarbon receptor nuclear translocator-like; ARR3: arrestin 3; ATE1: arginyltransferase 1; AY680578: uncharacterised AY680578; B3GNT5: UDP-GlcNAc, betaGal beta-1:3-N-acetylglucosaminyltransferase 5 (B3GNT5); BDES: Beaver Dam eye study; BED: Bornholm eye disease; BGN: biglycan; BICC1: BicC family RNA binding protein 1; BLID: BH3-Like Motif Containing: Cell Death Inducer; BLIMP1: PR domain zinc finger protein 1; BMP2: bone morphogenetic protein 2; BMP3: bone morphogenetic

protein 3; BRCC3: BRCA1/BRCA2-containing complex subunit 3; C1QTNF9B: C1q AndTumor Necrosis Factor Related Protein 9B; C1QTNF9B-AS1: C1QTNF9B antisense RNA 1 (non-protein coding); CABP4: calcium binding protein 4; CACNA1D: calcium voltage-gated channel subunit alpha1 D; CACNA2D4: calcium voltage-gated channel auxiliary subunit alpha2delta 4; CCDC111/PRIMPOL: coiled-coil domain-containing protein 111/Primase and DNA directed polymerase; CCNA2: cyclin A2; CCT5: chaperonin containing TCP1 subunit 5; CD180: CD180 molecule; CD226: CD226 molecule; CDH10: cadherin 10: type II; CDH12: cadherin 12: type II; CDH6: cadherin 6: type II; CHD7: chromodomain helicase DNA binding protein 7; ChEST267a2: uncharacterised gene; ChEST49o10: uncharacterised gene; CHRNG: cholinergic receptor nicotinic gamma subunit; CLEC3A: C-type lectin domain family 3 member A; CLIC2: chloride intracellular channel 2; cM: centimorgan; CNGA2: cyclic nucleotide gated channel alpha 2; CNGA3: cyclic nucleotide gated channel alpha 3; COL1A1: collagen type I alpha 1 chain; COL1A2: collagen type I alpha 2 chain; COMMD3: COMM domain containing 3; CREM: cAMP responsive element modulator; CRMP-62: collapsin response mediator protein of relative molecular mass 62K; CRYBA4: crystallin beta A4; CST3: cystatin C; CSTF3: cleavage stimulation factor subunit 3; CTAG1: cancer/testis antigen 1; CTAG2: cancer/testis antigen 2; CTGF: connective tissue growth factor; CTNND2: catenin delta 2l; CTSH: cathepsin H; CUL3: cullin 3; CXORF1: chromosome X open reading frame 1; CYP26A1: cytochrome P450 family 26 subfamily A member 1; DARPP32: protein phosphatase 1: regulatory subunit 1B; DCN: decorin; DX1: DEAD-box helicase 1; DEPDC4: DEP domain containing 4; DGCR2: DiGeorge syndrome critical region gene 2; DGKD: diacylglycerol kinase delta; DHHC14: zinc finger DHHC-type containing 12; DHX40: DEAH-box helicase 40; DLGAP1: DLG associated protein 1; DLGAP1-AS2: DLGAP1 antisense RNA 2; DLGAP1-AS3: disks large-associated protein 1 (DLGAP1): antisense RNA 3; DLGAP1-AS4: disks large-associated protein 1 (DLGAP1): antisense RNA 4; DLGAP1-AS5: disks large-associated protein 1 (DLGAP1): antisense RNA 5; DPF3: double PHD fingers 3

DRP2: dystrophin related protein 2; DUSP4: dual specificity phosphatase 4; E2F4: E2F transcription factor 4; ECEL1P2: endothelin converting enzyme like 1 pseudogene 2; ECM: extracellular matrix; EDN2: endothelin 2; EDNRB: endothelin receptor type B; EGR1: early growth response 1; EIF1AX: eukaryotic translation initiation factor 1A: X-linked; EIF2: eukaryotic initiation factor 2; ELF1: E74 like ETS transcription factor 1; ELP4: elongatoracetyltransferase complex subunit 4; EMILIN2: elastin microfibrilinterfacer 2; ENK: encephalin; ENO1: enolase 1; EPYC: epiphycan; eQTL: expression quantitative trait loci; ESS2: ess-2 splicing factor homolog; EST: expressed sequence tags; F8Bver/F8: coagulation factor VIII; FA: fatty acid; FCS: functional class scoring; FD: form deprivation; FDM: form deprivation myopia; FMOD: fibromodulin; FMR1: fragile X mental retardation 1; FRAP1/MTOR: FK506-binding protein 12-rapamycin-associated protein 1/mechanistic target of rapamycin kinase; FUNDC2: FUN14 domain containing 2; GAB3: GRB2 associated binding protein 3; GABRA3: gamma-aminobutyric acid type A receptor alpha3 subunit; GALNT1: UDP-N-acetyl-alpha-D-galactosamine; GALNT11: polypeptide N-acetylgalactosaminyltransferase 11; GALNTL5: polypeptide N-acetylgalactosaminyltransferase-like 5; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GAPLINC: gastric adenocarcinoma associated, positive CD44 regulator long intergenic non-coding RNA; GAT1: GABA transporter 1; GDI1: GDP dissociation inhibitor 1; GFRA1: glial cell line-derived neurotrophic factor (GDNF) family receptor, alpha 1; GJD2: gap junction protein delta 2; GNB3: g-protein subunit beta 3; GNG13: g-protein subunit gamma 13; GNG2: guanine nucleotide binding protein (G protein): gamma 2; GOLGA8B: golgin A8 family member B; GOLPH3: golgiphosphoprotein 3; GP1BB: glycoprotein Ib platelet beta subunit; GPR135: G protein-coupled receptor 135; GPR50: g-protein-coupled receptor 50; GRB2: growth factor receptor bound protein 2; GRIA4: glutamate ionotropic receptor AMPA type subunit 4; GRP: gastrin releasing peptide; GTF2H5: general transcription factor IIH subunit 5; GUCA1A: guanylatecyclase activator 1A; GWA: genome-wide association; GWAS: genome-wide association study; H2AFB3: H2A histone family member B3; HDGF: hepatoma-derived growth factor; HIPK3: homeodomain interacting protein kinase 3; HIPPO: serine/threonine-protein kinase, HIPPO; HSP70: heat shock protein 70; HTR3D: 5-hydroxytryptamine receptor 3D; IDS: iduronate 2-sulfatase; IGF1: insulin like growth factor 1;



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IL18: interleukin 18; IL9R: interleukin 9 receptor; IRX1: iroquoishomeobox 1; IRX2: iroquoishomeobox 2; KCNQ5: potassium voltage-gated channel subfamily Q: member 5; KERA: keratocan; KLH24: kelch like family member 24; KLHL6: kelch like family member 6; KMT2C: lysine methyltransferase 2C; LAMA1: laminin subunit alpha 1; LAMA2: laminin subunit alpha 2; LAMP3: lysosomal associated membrane protein 3; LIM: lens-induced myopia; LINC01895: long intergenic, non-protein coding RNA 1895; LOC100394842: protein phosphatase Slingshot homolog 2; LOC100399806: endothelin-converting enzyme-like 1; LOC100896985: uncharacterized LOC100896985; LOC157627: long intergenic non-protein coding RNA 599 (LINC00599); LOC399959: uncharacterized LOC399959; LOC425969: loricrin-like; LPIN2: lipin 2; LRPAP1: LDL receptor related protein associated protein 1; LUM: lumican; MAGEA10: MAGE family member A10; MAP6D1: MAP6 domain containing 1; MCAM: melanoma cell adhesion molecule; MCF2L2: MCF.2 cell line derived transforming sequence-like 2; MDH: malate dehydrogenase; MECP2: methyl-CpG binding protein 2; MFN1: mitofusin 1; MIPEP: mitochondrial intermediate peptide; MIR124-1: microRNA 124-1; MIR3924: microRNA 3924; MIR4660: microRNA 4660; MIR6718: microRNA 6718; MKP-2: MAPK phosphatase 2; MPP1: membrane palmitoylated protein 1; MS/MS: tandem mass spectrometry; MSRA: methionine sulfoxidereductase A; MSX2: mshhomeobox 2; MTM1: myotubularin 1; mTOR: mechanistic target of rapamycin; MYH13: myosin heavy chain 13; MYL12A: myosin light chain 12A; MYL12B: myosin light chain 12B; MYO1D: myosin ID; MYOM1: myomesin 1; NET1: neuroepithelial cell transforming 1; NFIL3: nuclear factor: interleukin 3 regulated; NOG: noggin; NSA2: NSA2: ribosome biogenesis homolog; NSDHL: NAD(P) dependent steroid dehydrogenase-like; NSF: N-ethylmaleimide sensitive factor: vesicle fusing ATPase; NT_025307.28: uncharacterised transcript; NT_025307.29: uncharacterised transcript; OGFRL1: opioid growth factor receptor like 1; OMIM: octanlinemendelian inheritance in man; OPN1LW: opsin 1 (cone pigments): long-wave-sensitive; ORA: over-representation analysis; OSBP2: oxysterol binding protein 2; OSBPL6: oxysterol binding protein like 6; OXPHOS: oxidative phosphorylation; P4HA2: prolyl 4-hydroxylase subunit alpha 2; PAX6: paired box 6; PCDH15: protocadherin 15; PDE3A: phosphodiesterase 3A; PDGFRA: platelet derived growth factor receptor, alpha; PDPK1: 3-phosphoinositide dependent protein kinase 1; PDZD2: PDZ domain-containing protein 2; PEDF: pigment epithelium-derived factor; PGAM1: phosphoglyceratemutase 1; PIK3R2: phosphoinositide-3-kinase regulatory subunit 2; PIMT: protein-L-isoaspartate (D-aspartate) O-methyltransferase; PKC1: protein kinase C 1; PKM1: pyruvate kinase M1; PKM2: pyruvate kinase M2; PNUTL2: septin 4; POAG: primary open angle glaucoma; POLS/TENT4A: DNA polymerase sigma/terminal nucleotidyltransferase 4A; PPFIA2: PTPRF interacting protein alpha 2; PPP1R3B: protein phosphatase 1 regulatory subunit 3B; PPP2CA: protein phosphatase 2 catalytic subunit alpha; PRKRIR: protein-kinase, interferon-inducible double stranded RNA dependent inhibitor, repressor of (P58 repressor); PRRG3: proline rich and Gla domain 3; PRSS56: protease, serine 56; PSARL: presenilin associated rhomboid like; PSMD14: proteasome 26S subunit: non-ATPase 14; PTCHD2/DISP3: dispatched RND transporter family member 3; PTPRQ: protein tyrosine phosphatase: receptor type Q; PTPRR: protein tyrosine phosphatase: receptor type R; PVALB: parvalbumin; QTL: quantitative trait loci; RAB11B: RAB11B: member RAS oncogene family; RAB22A: RAB22A: member RAS oncogene family; RAB39B: RAB39B: member RAS oncogene family; RASGRF1: Ras protein specific guanine nucleotide releasing factor 1; RCBTB1: RCC1 and BTB domain containing protein 1; RDH5: retinol dehydrogenase 5; RENBP: renin binding protein; RGR: retinal G protein coupled receptor; RGS6: regulator of G-protein signaling 6; RHOG: ras homolog family member G; RhoGDI: Rho GDP dissociation inhibitor (GDI) alpha; RORB: RAR related orphan receptor B; RP1L1: retinitis pigmentosa 1 like 1; RPE: retinal pigment epithelium; RPLP0: ribosomal protein lateral stalk subunit P0; RRH: retinal pigment epithelium-derived rhodopsin homolog; SAG: S-antigen visual arrestin; SARS: seryl-tRNAsynthetase; SCO2: cytochrome c oxidase assembly protein; SE: spherical equivalence; SHQ1: H/ACA ribonucleoprotein assembly factor; SIX4: SIX homeobox 4; SIX6: SIX homeobox 6; SLC39A5: solute carrier family 39 member 5; SLITRK2: SLIT and NTRK like family member 2; SNP: single neucleotide polymorphism; SNTB1: syntrophin beta 1; SOX2OT: SRY-box 2 (SOX2) overlapping transcript; SPH: sphere; SPRY3: sprouty RTK signaling antagonist 3; SPTBN1:



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spectrin beta, non-erythrocytic 1; STARD3NL: STARD3 N-terminal like; SYBL/VAMP7: vesicle associated membrane protein 7; SYT1: synaptotagmin 1; TEX28: testis expressed 28; TGIF: transforming growth factor beta (TGFB) induced factor homeobox; TGIF1: TGFB induced factor homeobox 1; TJ: tight junction; TKTL1: transketolase like 1; TMLHE: trimethyllysine hydroxylase, epsilon; TPM3: tropomyosin 3; TRIM23: tripartite motif containing 23;

TRIM29: tripartite motif containing 29; UCHL1: ubiquitin C-terminal hydrolase L1; UHRF1BP1L: UHRF1 binding protein 1 like; URP: heterogeneous nuclear ribonucleoprotein U like 2/; UTP20: small subunit processome component 20 homolog I; UTS2D: urotensin 2 domain containing; VBP1: von Hippel-Lindau (VHL) binding protein 1; VCD: vitreous chamber depth; VIP: vasoactive intestinal peptide; WASH6P: WAS protein family homolog 6 pseudogenel; WT1: Wilmstumor 1; XRCC2: X-ray repair cross complementing 2; YEATS2: YEATS domain containing 2; ZC3H11A: zinc finger CCCH-type containing 11A; ZFHX1B: zinc finger E-box binding homeobox 2; ZFR: zinc finger RNA binding protein; ZIC2: zic family member 2; ZNF275: zinc finger protein 275; ZNF644: zinc finger protein 644; ZWINT: ZW10 interactor.

## INTRODUCTION

Myopia (short-sightedness) is the greatest risk factor for blindness and other visual impairments [2-4]. The myopia epidemic has been associated with several lifestyle factors including increasing education, lack of outdoor activity and excessive near-work [3,5]. Myopia is prevalent in more than 86% of adults [6] and 84% of children [7] in developed countries such as Taiwan and Poland, respectively. In communities with high educational standards, the prevalence of myopia is as high as 95% in university students [8], indicating that environmental factors such as reading and education influences myopia development. Myopia also tends to be familial [9] where an individual with myopia has a strong likelihood of also having a myopic parent [9-15]. Evidence for an environmental contribution in refractive error development has grown exponentially since 1975 when the first Singapore and Australian studies appeared (reviewed in [16]) suggesting that both genetics and environmental components are likely to contribute to the development of myopia.

## EVIDENCE FOR A GENETIC CONTRIBUTION

Genetic linkage studies have confirmed Mendelian inheritance of 25 myopia loci for which the underlying molecular basis is not known. Further attempts have utilised Genome-Wide Association Studies (GWAS) to determine whether a set of genetic variants (ie. single-nucleotide polymorphisms) in individuals within a population are associated with the observed changes in myopic axial growth or Spherical Equivalent (SE) refraction. These studies have identified genetic sequence variations in several potential candidate genes that may offer genetic susceptibility to myopia. Interestingly, this genetic susceptibility can only account for between 8-50% of the variation of the condition [17-20], fuelling the long-standing nature vs nurture debate. Given that myopia is prevalent in homogenous genetic populations (ie. Eskimo/Inuit [21-27], and that visual experience can be manipulated in animals using occlusion [28-30], environmental factors are well accepted as playing a major influence on the development of myopia and high myopia.

Animal models of experimental myopia illustrate the impact of manipulating the visual environment. Both negative lens wear and Form Deprivation (FD) induce changes in gene expression changes which parallel axial elongation, increased vitreal volume and thinning of the retina and choroid [30-33]. These morphological changes are similar to those seen in profound human myopia [34,35]. Indeed animal studies have proved vital to understanding the molecular fingerprint of refractive development with the adaptive response to environmental visual manipulations showing remarkable similarities to differential gene pathways in humans [36,37]. Large-scale transcriptomic and proteomic animal studies have implicated hundreds of Differentially Expressed Genes (DEGs) and Differentially Abundant Proteins (DAPs) as causative of environmentally-induced myopic growth; however, it is rare that a single gene or protein is solely responsible for a disease or phenotypic variation. This is particularly noteworthy in biological systems built on redundant genomes susceptible to genetic drift [38,39], especially those that are susceptible to post-translational modifications and epigenetic regulation [40-44]. Furthermore, pathway enrichment analysis is also proving to be an invaluable tool in interpreting how these DEGs and DAPs interact with each other within a complex biological system.

Despite the extensive amount of non-correlative data from human and animal studies, commonalities in genes and proteins in human and animal myopia exist, suggesting that the biological basis of refractive error development is conserved across species (Figure 1) [36].

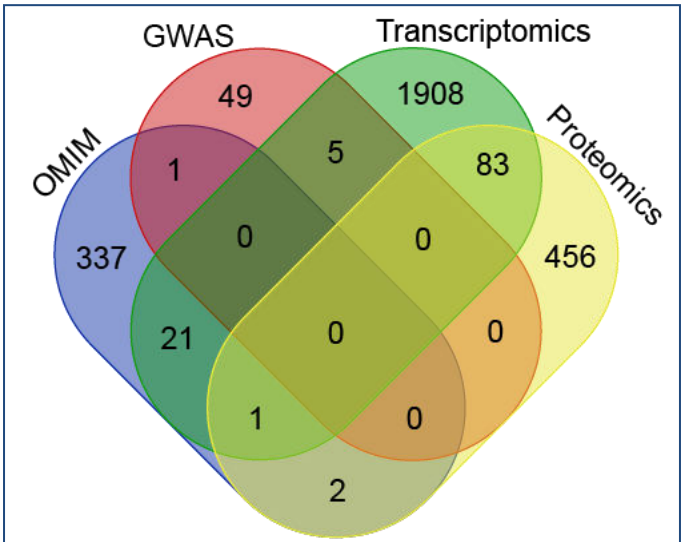


**Figure 1:** Venn diagram showing the total number of common genes identified in Mendelian diseases of myopia, GWAS and animal transcriptomic and proteomic studies. Genes associated with mendelian myopia were downloaded from the OMIM database (https://www.omim.org/). Genes significantly ($p \leq 5*10-8$) associated with myopia susceptibility as identified in GWAS were downloaded from the GWAS Catalog (https://www.ebi.ac.uk/gwas/). Differentially expressed genes and differentially abundant proteins were collected from Riddell & Crewther [36] and original papers where possible. Animal genes and proteins were converted to human orthologs to aid comparisons.

Thus the aim of this review is to outline the evolution of human genomic research and animal transcriptomic and proteomic research and explain the importance of assessing the collective changes in genes and proteins (ie. biological processes) in the adaptive response to environmentally-induced myopia.

## EVIDENCE FROM GENETIC LINKAGE STUDIES AND GWAS

Myopia can be considered a heterogenous condition, with family-based linkage approaches highlighting that myopia may follow Mendelian inheritance patterns; Autosomal Dominant (AD), Autosomal Recessive (AR), and X-Linked Recessive (XLR) inheritance. Linkage analysis has allowed for the identification of 25 myopia loci (MYP1-MYP3, MYP5-MYP26) along with several potential candidate genes responsible for the disease. GWAS have aided in assessing several additional susceptible loci and Single Nucleotide Polymorphisms (SNPs) for refractive error development. These studies are reviewed below.

**MYP1:** The first of the genetic linkage studies on myopia was conducted in when Bartsocas and Kastrantas [45] demonstrated a convincing X-linked pedigree of myopia inheritance in which five myopic grandsons were descendent from three myopic brothers. Although within this family, some of the females had mild myopia not requiring corrective glasses. This inheritance pattern was also shown in a population of Asian Indians in which only males were affected by myopia [46] suggesting that the pedigree pattern was consistent with X-linked recessive inheritance, with full penetrance. The 1.25Mb genomic interval was identified on chromosome Xq28 which contains multiple candidate genes for high-grade myopia including cancer/testis antigen 2 (CTAG2), growth factor receptor bound protein 2 (GRB2)-associated protein 3 (GAB3) membrane palmitoylated protein 1 (MPP1), coagulation factor VIII (F8Bver/F8), FUN14 domain containing 2 (FUNDC2), von Hippel-Lindau (VHL) binding protein 1 (VBP1), ras-related protein Rab-39B (RAB39B), chloride intracellular channel 2 (CLIC2), trimethyl lysine hydroxylase, epsilon (TMLHE), vesicle associated membrane protein 7 (SYBL/VAMP7), interleukin 9 receptor (IL9R), sprouty homolog 3 (SPRY3) and CXYorf1 (WAS protein family homolog 6, pseudogene (WASH6P). These finding were not confirmed in a later, larger scale study [47] nor have they been found to be involved in the pathogenesis of the MYP1 phenotype in the Asian Indians pedigrees [46].

Other genes within this locus were shown to be associated with high myopia. Variants in the opsin gene were observed in the Bornholm Eye Disease (BED) phenotype [48-50]. A 28-kb intergenic deletion was observed between a BRCA1-BRCA2-containing complex 3 (BRCC3) gene in males on top of an 8-kb intergenic duplication between SPRY3 and TMLHE genes [49]. In both pedigrees (ie. BED & Minnesota), as well as a UK sample, copy numbers of the gene, testis expressed 28 (TEX28), was significantly reduced by half compared to female controls. Other candidate genes within the MYP1 locus (CTAG1, CTAG2, MPP1, CLIC2, H2AFB3, TMLHE, SPRY3, SYBL (VAMP7), NT_025307.28, NT_025307.29, AFF2, CXORF1, MECP2, MTM1, RENBP, GABRA3, GDI1, MAGEA10, NSDHL, TKTL1, and ZNF275) or adjacent to the Xq28 locus (FMR1, IDS, SLITRK2,



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SLITRK2) were found not to be associated with the disease status [49].

In Han Chinese males, sequence analysis of the GPR50, PRRG3, CNGA2, and BGN genes failed to detect any mutations. These genes did however show sequence variations that could alter gene function [51]. Additionally, two rare variants in the OPN1LW were detected in the proband using sequencing [52] suggesting a role for colour discrimination [53] and cone phototransduction [54] in high-myopia.

In fact, individuals with Xq28 duplication syndrome manifested with myopia, autism spectrum disorder, cognitive impairment, and behavioural problems [55,56].

**MYP2:** The MYP2 locus (18p11.31) has been identified in several multigenerational families in the USA [57] and Hong Kong Chinese [58] with an autosomal pattern of inheritance of high myopia (greater than -6D). However this same locus has not been identified in the Beaver Dam Eye Study [59], or in a UK [60], Japanese [61], Amish or Ashkenazi Jew [62] population of high myopes. Further haplotype analysis identified an interval of 0.8cM between markers D18S63 and D18S52 [63] and later revised to a 2.2cM interval between D18S52 and D18S481 [64] to be significantly associated with high myopia. Genes mapping to the extended region include DLGAP1, DLGAP1-AS2, DLGAP1-AS3, DLGAP1-AS4, DLGAP1-AS5, GAPLINC, LINC01895, MIR6718, MYL12A, MYL12B, MYOM1 and TGIF1. However associations between TGIF, EMILIN2, MYL12B, DLGAP1, LPIN2, MYL12A, MYL12B, MYOM1 and high myopia were not supported in subsequent validation studies [64-67]. LPIN2 was also identified as a potential candidate gene implicated in myopia with 11 nucleotide variants detected in both highly myopic and unaffected individuals however these polymorphisms did not result in functional changes in the protein [68]. Polymorphisms in the LAMA1 gene showed contradictory results. Sasaki et al. [69] did not identify any significant LAMA1 SNPs associated with high myopia. Comparatively, Zhao et al. [70] showed that the polymorphism rs2089760, located in the promoter region of LAMA1, was associated with high myopia in a Chinese population.

Interestingly, a female with de novo deletion of the short arm of chromosome 18 displayed marked phenotypic features including developmental delay, facial dysmorphism and myopia suggesting that genes on chromosome 18 may predispose individuals to developing myopia. The 143 genes located in this proband primarily code for proteins involved in regulation of expression and transcription processes, cellular growth, regulation of chromosome division (mitosis), carbohydrate and triglycerides metabolism, and cell adhesion [71].

**MYP3:** Young et al. [57] demonstrated a link between autosomal dominant high-grade myopia (-6.0D) and chromosome 12q21-q23 in a large German/Italian family. The locus was later refined to 12q21-q21.31 [72-74] and later confirmed by whole-genome SNP-based linkage [47,75]. Although, Li et al. [47] noted that the interval of 9.94cM (12q21.31-22) in their study encompassed a multiethnic dataset of Caucasian, Asian & African-American participants. Indeed, Farbrother et al. [60] and Ibay et al. [62] did not find significant associations between this locus and familial high myopia. This could possibly be due to the different refractive error characteristics used in the analysis (ie. sphere (SPH) and SE). The original 30.1cM interval on 12q21-q23 extended from marker D12S1684 to D12S317 and contained approximately 229 genes. Whereas the refined interval narrowed to a composite region with only 34 genes. Within this region, several candidate genes were identified such as insulin growth factor 1 (IGF1), lumican (LUM), fibromodulin (FMOD), decorin (DCN), synaptotagmin 1 (SYT1) and keratocan (KERA). Mutant lumican transgenic mice displayed significantly larger axial length measures compared to wild-type mice, possibly by disrupting the formation of collagen fibrils resulting in changes to the elasticity and tension of the sclera [76].

Interstitial deletion of chromosome 12q15-q23 has been shown to result in corneal/scleral abnormalities but not in altering axial length suggesting that the gene responsible for myopia may not be located within this deleted locus, even with the loss of several important structural genes such as DCN, LUM and KERA [77]. Polymorphisms in proteoglycan genes at MYP3 (FMOD, DCN, LUM, KERA, and epiphycan (EPYC)) were also not associated with high myopia in a Chinese population [78] or in two US families [64,79], and hence, are unlikely to be major contributors to the genetic predisposition to high myopia. Polymorphisms in IGF1 did show strong association with high grade myopia as measured by SPH and SE [80] with the

functional consequence of this variation more likely to have downstream direct effects on regulating gene expression rather than a direct factor in myopia development. Other genes linked to high-grade myopia within the MYP3 region include UHRF1BP1L, PTPRR (PTPRQ), PPFIA2, DEPDC4, ACTR6, and UTP20. These genes are more likely to regulate the expression of other genes that contribute to axial growth [81].

**MYP5:** The MYP5 locus was identified as autosomal dominant in a multigenerational English/Canadian family by Paluru et al. [82] but as autosomal recessive in a UK family [60]. Haplotype analysis refined the critical interval to a 7.71cM region on 17q21-q22, between markers D17S787 and D17S1811. Out of the 536 genes in this region, efforts have focused on validating only a few of these genes. Polymorphisms in the main candidate gene, COL1A1 were identified to be strongly associated with high myopia in a Japanese [83] and Caucasian [84] populations. Although contradictory studies suggest that COL1A1 is weakly associated with the disease and is not a high genetic risk factor for myopia [84-91]. No other candidate genes within this loci have been suggested or validated thus far.

**MYP6:** While considerable efforts have been made to identify a susceptibility locus for high-myopia, Stambolian et al. [92] identified a susceptibility locus related to mild/moderate myopia (> -1.0D) in American families of Ashkenazi Jewish descent. The findings indicated that a region on chromosome 22 (22q12), at marker D22S685, was strongly linked to mild myopia. These findings were later confirmed in the Beaver Dam Eye Study [93] and in a large multiethnic cohort [46]. One gene within this region suggested to be implicated in myopia development is cytochrome c oxidase assembly protein (SCO2). A heterozygous nonsense mutation in the SCO2 gene was identified in a family of European decent (average spherical refractive error of -22D). These finding were then validated in a mouse model of Lens Induced Myopia (LIM) [94], confirming that SOC2 localises in the retina and sclera but both mRNA and protein were significantly reduced in experimental myopia.

**MYP7, MYP8, MYP9 and MYP10:** The MYP7-MYP10 loci were identified by Hammond, Andrew, Mak, and Spector in a study encompassing dizygotic (DZ) twin pairs. The regions identified to be associated with myopia included chromosomes 11p13 (MYP7), 3q26 (MYP8), 4q12 (MYP9), and 8p23 (MYP10) which were supported in a later study [95]. Stambolian et al. [95] also refined the loci intervals to D3S1262 to D3S3053 on chromosomes 3q26 and D8S1130 to D8S1469 on chromosome 8p23. Although chromosome 11p13 and 8p26 were found to be associated with myopia, later reports suggested no evidence of linkage of these regions with myopia [96,97]. However, Andrew et al. [98] did replicate the initial linkage to 3q26 and identified SNPs in 2 potential candidate genes (MFN1 & SOX2OT) and in the PSARL gene region (LAMP3, MCF2L2, B3GNT5, KLHL6, KLH24, YEATS2, MAP6D1, PSARL, ABCC5 and HTR3D).

Interestingly, individuals with proximal 11p deletion syndrome (P11pDS) [99], interstitial deletion in the long arm of chromosome 11 [100], interstitial deletion of region 3p26 [101] or proximal deletion of chromosome 4q12-21.21 [102] displayed marked phenotypic features including brain abnormalities, developmental delay, facial asymmetry, and severe myopia (-10D). These deletion studies indicate that there are genes in these regions that regulate, or are directly involved in, the development of myopia. Some of the potential candidate genes within the 11p13 region include CSTF3, ELP4, HIPK3, PAX6, WT1 [103] however, only PAX6 has been determined to be associated with myopic development [12,104] leaving a substantial number of genes unvalidated. GWAS in a French population provided evidence for the association of chromosome 8p23 with high myopia, with potential candidate genes MIR124-1, MIR4660, MSRA and PPP1R3B [105].

**MYP11:** The MYP11 locus was identified as an autosomal dominant form of high myopia in a large Chinese family. The linkage region maps to a 20.4cM interval on chromosome 4q22-q27, between markers D4S1578 and D4S1612 [106] which overlapped the linkage peaks 4q21-q22 [73], 4q24 [47], 4q25 [107] and 4q27-q28 [95] identified in later GWAS and meta-analyses. One potential candidate gene, (Retinal Pigment Epithelium-Derived Rhodopsin Homolog, RRH) was sequenced but no mutations were identified in the gene suggesting that this gene was not a causative factor in myopia development [106]. No other candidate genes have been identified thus far however, a number of Expressed Sequence Tags (ESTs) are located within this region, one of which

(BI480957) has been reported to be expressed in the native human Retinal Pigment Epithelium (RPE) [107].

**MYP12:** The locus on chromosome 2q37.1 was discovered by Paluru, Nallasamy, Devoto, Rappaport, and Young in a large USA family of Northern European decent. The link between MYP12 and high myopia was supported in subsequent studies [47,108,109] although many additional regions surrounding 2q37.1 have been implicated in the condition [47,109]. Individuals with 2q partial trisomy exhibit ocular abnormalities including reduced visual function and severe myopia (-6.0D) [110]. Two candidate genes, S-antigen (SAG) and Diacylglycerol Kinase-Delta (DGKD), were sequenced however these genes were found not to be a causative factor in myopia [111]. Although GWA studies have identified CHRNG, ECEL1P2 (20) and PRSS56 [112] to be significantly associated with myopia but no other genetic variants within this region were found [109].

**MYP13:** The MYP13 locus was identified in a Chinese family for which 6 of the males had high myopia ranging from -7D to -16D that seemed to be inherited in an X-linked recessive pattern [113]. Red-green colour vision defects were also prominent in this family however it was found not to co-segregate with the myopia phenotype. Haplotype analysis identified conserved changes between DXS1210 and DXS8057 on chromosome Xq23-q25 (interval of 25cM (14.9Mb)) [113]. The same lab confirmed these findings and extended the identified locus to Xq25-q27.2 between DXS1001 and DXS8043 [114] with no potential candidate genes identified.

**MYP14:** Wojciechowski et al. [115] performed linkage analysis in a population of Ashkenazi Jewish families and identified an 11Mb region on chromosome 1p36 between markers D1S552 and D1S1622 which was later refined to 1p36.1 [73]. Two candidate genes have been proposed; FRAP1/MTOR and PTCHD2/DISP3. FK506 binding protein-rapamycin complex-associated protein 1 (FRAP1/MTOR) was strongly associated with corneal curvature but not myopia [116]. Whereas, the gene, dispatched RND transporter family member 3 (PTCHD2/DISP3), was found to be strongly associated with SE [112].

**MYP15:** A critical region of 2.67cM on chromosome 10q21.1 was found to be strongly associated with high-grade myopia in a large Hutterite family from South Dakota [117]. GWAS confirmed a strong association for this region with high myopia, identifying four potential candidate genes (BicC family RNA binding protein 1 (BICC1) (20), ZW10 interactor (ZWINT), microRNA 3924 (MIR3924) [105] and protocadherin 15 (PCDH15)), although direct sequencing of the coding regions of these genes did not reveal myopia-implicated mutations.

**MYP16:** Lam et al. [118] investigated the genetic component of autosomal dominant high myopia in a family of Hong Kong Chinese. Haplotype analysis indicated the linkage region to be 5p15.33-p15.2 with a 17.45cM interval which was confirmed in a later study [47]. Five potential candidate genes (IRX2, IRX1, POLS/TENT4A, CCT5, and CTNND2) within this region were screened for sequence variants with several polymorphisms identified however no associations between these variants and high myopia were found [118]. Subsequent analysis of the CTNND2 in a Asian population confirmed the association between CTNND2 and high myopia, but suggested that this SNP may associate with moderate myopia in the Chinese population [119,120].

**MYP17 (formally MYP4):** The MYP17 locus was identified in a population of French and Algerian families to be associated with an autosomal dominant form of high-grade myopia (-6D) [121]. The analysis indicated that the 11.7cM linkage region was on chromosome 7q36, from marker D7S798 to D7S2423 with D7S2423 located close to the telomere [49,121]. However, many studies have not been able to reproduce these findings. Rather, the most significant locus associated with high-myopia on chromosome 7 seemed to be 7q11.23-7q21.2 [74], 7q21-q22 [82,93,95], 7p14 [73] and 7p15 [60,96,122]. Although no candidate genes have been identified from this locus, an individual with chromosome 7q36.1q36.2 triplication presented with developmental delay, growth retardation and severe myopia [123]. Candidate genes in the triplicated region include GALNTL5, GALNT11, KMT2C, XRCC2, and ACTR3B [123] suggesting that epigenetic transcriptional activation, cell structure and the Notch signaling pathway may be implicated in myopia. Interestingly, the symbol MYP4 was originally used for the locus on 7p15 but has been renamed to MYP17 [122].

**MYP18:** This locus was associated with autosomal recessive inheritance of extreme myopia (-13D) and axial growth (28.36mm) in three siblings from a Chinese family. Fine

mapping and haplotype analysis provided strong evidence for a 25.23Mb region between markers D14S984 and D14S999 on chromosome 14q22.1-q24.2. The clinical presentation was similar in the affected siblings and included a tigroid fundus, a circular choroidal defect around the optic disc and a mild reduction in the amplitude of cone response, although all patients had normal colour vision and normal thickness of the retinal layers [124]. Potential candidate genes in this linkage interval include guanine nucleotide binding protein (G protein), gamma 2 (GNG2), G protein-coupled receptor 135 (GPR135), SIX homeobox 4 (SIX4), and regulator of G-protein signaling 6 (RGS6). Interestingly, GWAS has also identified associations between the gene involved in eye development, SIX homeobox 6 (SIX6), and high myopia (20).

**MYP19:** Ma et al. [125] investigated the genetic component of autosomal dominant high myopia (-11.6D) in a Chinese family. Linkage analysis indicated the linkage region to an approximately 11.69cM (14.14Mb) interval between D5S2096 and D5S1986 on chromosome 5p13.3-p15.1 which was later confirmed by Abbott et al. (2012). Six candidate genes were also assessed including cadherin 6, type II (CDH6), cadherin 10, type II (CDH10), cadherin 12, type II (CDH12), PDZ domain-containing protein 2 (PDZD2), Golgi phosphoprotein 3 (GOLPH3), and zinc finger RNA binding protein (ZFR). These candidate genes have roles in cell adhesion, intracellular signal transduction, protein trafficking, and DNA/RNA binding activities which are some of the functions most likely to be associated with myopia. However, mutation analysis of these genes did not reveal any disease-causing mutation within these genes [124].

**MYP20:** A GWA study of ~500K SNPs were assessed in a population of Han Chinese with high myopia (-12D) [125]. After adjusting for genomic control, gender, and age, the study identified 34 SNPs with a highly significant association between SNP rs9318086 at chromosome 13q12.12 and high myopia. Four of the most significant SNPs associated with myopia included rs9318086 and rs1886970 located in intron 10 and intron 14, respectively, of the mitochondrial intermediate peptide gene (MIPEP). And rs7325450 and rs7331047 located in intron 4 of the C1q and Tumor Necrosis Factor Related Protein 9B (C1QTNF9B) gene [126].

**MYP21:** Exome sequencing and segregation analysis identified a missense mutation in the ZNF644 gene located at 1p22.2 to be responsible for the autosomal dominant inheritance of high myopia (-6D) in a Han Chinese population [127], US cohort [128] and Chinese population [129-131]. All the family members reached high myopia by age 7 (-11D to -20D) with three of the elderly family members presented with thinning of the RPE and the choriocapillaries [127].

**MYP22:** An autosomal dominant form of high myopia (-20D) was identified in Chinese family spanning 4 generations [132]. Exome sequencing within this family identified a missense mutation in the CCDC111/PRIMPOL gene located on 4q35.1. This variation was found to only segregate with myopia in this family and was absent in 270 non-myopic Chinese individuals [132]. Although, due to the variation in severity of the clinical phenotype among the affected individuals, the authors concluded that environmental influences were likely to contribute to the aetiology in the family.

**MYP23:** Extreme myopia (-17D) in 3 consanguineous Saudi Arabian families was associated with loss of function mutations in the LRPAP1 and CTSH genes on chromosome 4p16.3 [133]. The mutations identified in this family segregated fully with myopia and were not identified in 210 Saudi exome files or in the SNP databases of the 1000 Genomes Project or Exome Variant Server. Later studies confirmed the role of LRPAP1 in early onset myopia [129,134,135].

**MYP24:** Guo et al. [136] performed whole-genome linkage analysis on a Chinese family with severe myopia (ranging from -5D to -25D) and identified chromosome 12q13.3 as significantly associated with the disease. Two SNPs rs774033 and rs10122 in the SLC39A5 gene segregated fully with disease in the family and was not found in 1,276 population-matched controls [136]. Variants in SLC39A5 were found to be associated with early-onset high myopia [129] and high myopia (<-6D) [134,137,138]. Interestingly, individuals with 12q13.2-q13.3 Microdeletion Syndrome present with neurodevelopmental delay, facial dysmorphia bone malformations and severe myopia. Further analysis revealed deletion of a total of 26 genes. Expression analysis revealed a 50% reduction in expression of these genes [137].

**MYP25:** This locus was identified in a Chinese family with severe myopia (ranged from -6D to -20D) [139]. Linkage

analysis identified 4 candidate regions on chromosomes 1, 5, 6, and 21. Whole-exome sequencing identified a missense mutation in the prolyl 4-hydroxylase subunit alpha 2 (P4HA2) gene located on chromosome 5q31.1, that segregated fully with disease in the family and was not found in 626 population-matched controls or in public variant databases [139]. Mutations in P4HA2 were shown to result in decreased mRNA expression and protein abundance in Caucasian individuals with mild-moderate myopia [140].

**MYP26:** Early-onset myopia in three large Chinese families was found to be significantly linked to marker DXS986 on chromosome Xp11.1-q13.3 [141] similar to earlier reports linking marker DXS6800 on chromosome Xq13-q21 to high myopia in an Amish family [95]. Whole-exome sequencing identified a missense mutation in the arrestin 3 (ARR3) gene which was found to segregate fully with disease in each of the families and which was not found in 192 controls or in public variant databases. Analysis of the whole-exome data did not identify mutations in other high myopia- or retinal disease-associated genes [141].

On the other hand, several studies have found other candidate loci using linkage disequilibrium analysis. A candidate locus for high myopia was located outside the MYP2 region on chromosome 18q12.2 at marker D18S0301i with the gene GALNT1 (UDP-N-acetyl-alpha-D-galactosamine), closest to this microsatellite marker [61]. Paluru et al. [79] presented evidence of suggestive linkage to chromosome 1q23-32 (markers D1S484, D12S1583, and D12S79). Chromosome 11p exhibited significant linkage of myopia in a Caucasian population [142]. Individuals with interstitial 3p12.3-13 deletion presented with myopia suggesting that genes at this loci may be important to the development of myopia [143]. Yang et al. [124] noted that linkage to regions of chromosomes 11 and 17 could not be excluded in a Chinese family. Finally, evidence for linkage of ocular axial length to 14q32 was found by Zhu et al. [74] which is a locus to which a recessive form of isolated microphthalmia has been mapped [144].

Although, these studies can only account for up to 50% of the variation in myopia, it is important to note that there is lack of significant sharing of cis-expression quantitative trait loci (eQTLs) between blood and other tissues [145,146], making it necessary to analyse the transcriptome in affected tissue (ie. retina/RPE/choroid) to validate the findings from these GWAS and genetic linkage studies. What is immediately apparent is that the genes associated with myopia susceptibility are closely linked to those involved in neurodevelopment and dysregulations of these genes result in subsequent cognitive impairments, highlighting a potential avenue for research into this disorder. All in all, these studies have been crucial in understanding the genetic basis of myopia development even though environmental factors and epigenetic changes may eventually prove more relevant to the aetiology of myopia.

## ADDITIONAL LOCI ASSOCIATED WITH MYOPIA SUSCEPTIBILITY (GWAS)

In addition to the linkage disequilibrium analysis, SNP studies in humans have identified several potential myopia susceptibility loci and genes (Supplementary Table 1), however few variants have been validated in animal or other studies. Studies using GWAS have identified several high-risk variants on many loci in specific populations.

**Asian:** The most widely studied myopic individuals are from Asian populations. This population is targeted as the prevalence of myopia is high, with up to 95% of university students within this population diagnosed with myopia (< -0.50D) [8]. Interestingly, GWAS has revealed that the genetic variations associated with myopia across many Asian populations also differ. In Japanese populations, the 11q24.1 locus and two candidate genes, BLID and LOC399959, in particular have been found to be associated with high myopia (<-6D) [147]. However these associations were not significant in a population of Han Chinese [148]. In a population of Han Chinese, the 13q12.12 region was found to be associated with high myopia, particularly as the rs9318086 and rs1886970 are in intron 10 and intron 14, respectively, of MIPEP gene. Indeed, rs9318086 may also affect the following genes; C1QTNF9B (also called RP11-45B20.2), and C1QTNF9B antisense RNA 1 (non-protein coding) (C1QTNF9B-AS1) [125]. Several SNPs within 4q25 were also found to be strongly associated with high myopia (≤-8D) with the most significant SNP being rs10034228 which is not within, or adjacent to any known genes. However, the predicted protein sequence for which the SNP is located is highly similar to that of the tubulin family [149] highlighting a role for structural proteins in the myopia phenotype. In a meta-analysis of studies in Singapore,

China (Sichuan), Hong Kong and Japan, 2 loci were found to be associated with severe myopia (AL ≥26mm or SE of at least -6D) with genetic variations within zinc finger E-box binding homeobox 2 (ZFHX1B; also known as ZEB2; rs13382811) and Syntrophin, Beta 1 (SNTB1; rs6469937) the likely causative mutations. Khor et al. [150] also assessed the mRNA levels of ZEB2 and SNTB1 using myopic mice and found that these genes were both significantly downregulated in myopic retina/RPE compared with naive control retina/RPE [150]. These data provide support for the involvement of these genes in playing a role in myopic eye growth at the transcriptome level.

**Caucasian:** The genetic variants in Caucasian populations of European ancestry have shown commonalities to Asian-based population studies in the mechanisms controlling ocular growth. Solouki et al. [151] focused on 31 SNPs spread across four loci on chromosomes 15q14, 14q24, 1q41 and 10p12.3 in five separate cohorts RS-I, RS-II, RS-III and the Erasmus Rucphen Family Study from The Netherlands, and a twin study from the United Kingdom. Meta-analysis of the combined discovery and replication cohorts showed a significant association between refractive errors (as measured by SE) and the locus on 15q14. The identified locus on 15q14 is within an intergenic region near the genes GJD2 (39 kb from rs634990 at its 3′ end), ACTC1 (74 kb from rs634990 at its 3′ end) and GOLGA8B (180 kb from rs634990 at its 5′ end). It may be that the SNPs within the regulatory elements at 15q14 may promote transcription of genes in the area and increase the production of ACTC1 and GJD2 mRNA.

In another study on a population of British twins [152], SNPs in the RASGRF1 promoter region at chromosome 15q25 were significantly associated with high myopia (based on SE). The strongest evidence of association was found for rs939658 and rs8027411 with an extra copy of the myopia susceptibility allele (G and T for the two SNPs, respectively) altering the SE by 0.15D. These varients fall within the transcription initiation site of RASGRF1. Validation of this gene in mice knockdown models showed that the lack of expression in RASGRF1 resulted in a morphologically normal retina but caused pronounced deficiencies in the lens, photoreception and visual sensory processes without any other obvious sensory impairments [153,154]. In Ashkenazi Jews, three loci were found to be associated with myopia (≤ -1D) eg. 6q22-q24, 11p14-q14 and 20p12-p11 [155].

In the Beaver Dam Eye Study (BDES; Wisconsin, USA), there was strong evidence of myopia linkage to chromosome 22q11 (rs737923) which is adjacent to the gene, ess-2 splicing factor homolog (ESS2). ESS2 has been shown to modulate transcription factors and forms part of the splicesome complex, suggesting that ESS2 may function in regulating mRNA processing of genes involved in the myopia pathology [156]. Other studies utilising the BDES population have discovered linkage between SE and loci 7q36 [49] which is consistent with findings by Naiglin et al. [121] in French and Algerian families. Additionally, chromosome 7q15, 2q12, 4q26, 4q31, 6q15, 12q24, 2q25, 16q24 provided evidence of linkage to refraction in the BDES [49,91].

While most studies have focused on the association between SE or SPH and myopia, Mishra et al. [157] have shown corneal curvature to be highly associated with myopia susceptibility. Corneal curvature was found to be linked to rs2114039 in platelet derived growth factor receptor alpha (PDGFRA) and rs2444240 in tripartite motif containing 29 (TRIM29) in individuals of European ancestry within an Australian population.

Other loci linked to myopia susceptibility include 2q37.2-37.3, [108], 1p31.3, 2q33.1, 3q29, 10p11.21-11.22, 6q13-16.1,5q35.1-35.2, 7q11.23-21.2 [75], 5q14.2, 6q14.1, 10q25.3, 14q32, 6q15 [74], 4q21-22, 7p14 [73], 1p23-24, 7q21, 12q24, 20q13, and 8p22-23 [95]. Loci on chromosome 11 and 17 are also likely to offer susceptibility to myopia in humans. Other candidate genes located on 22q (outside MYP6 locus) were assessed for polymorphisms and association with myopia. DGCR2, GP1BB, CRYBA4, and PVALB with rs2071861, rs2239832 and rs2009066 SNPs of CRYBA4 showing the most significant association to high myopia in southern Chinese [158]. More recently, five SNPs in the potassium voltage-gated channel subfamily Q member 5 (KCNQ5) gene were associated with high myopia susceptibility in a Chinese population [159].

The large number of potential susceptibility loci and SNPs not only raises interesting questions about the role of genetics in the development of myopia, but of the robustness of the results and statistical power of these studies. The phenotypic variance



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of refractive error varies by study from 3% (20), 8% [19], 12% (18; 20), 25-35% [160], to 50% [17] As noted by Klein et al. [161], these studies need to be taken with a great deal of caution as it is likely that most historic genome-wide scans based on STR markers and linkage approach were underpowered to detect true effects and also very prone to false-positive findings. Additionally, the complexities of associating a SNP with a specific disease process provides little assurance that these associations will occur for differing ethnic and/or genetically distinct populations.

## DEG, DAPS AND OVER-REPRESENTATION ANALYSES IN ANIMAL 'OMIC' STUDIES

Over the last decade there have been many large discovery type 'omic' studies examining the transcriptomic and proteomic basis of environmentally altered eye growth in animal models of refractive error development (summarised in Supplementary Table 2). The most common is the chicken as the rapid eye growth observed in response to visual manipulation (eg. with form deprivation myopia, FDM) reaches severe myopic levels (-9D) in 5 days [162], and develops to as much as -20D after 2 weeks [28-30,163]. In contrast, primates and mice require much longer periods to produce smaller degrees of FDM (marmoset: 4.5 weeks, -8D, [164]; macaque: 17 weeks, -5D, [165]; mice 56 days, -2.5 [166]. Regardless of the animal model used in large transcriptomic and proteomic studies on refractive error development, all have identified hundreds of differentially expressed genes and proteins as potential candidate genes for environmentally altered eye growth.

**Chicken:** One of the first transcriptomic studies [167] in chickens utilised microarray technology to assess differentially expressed genes in retina/RPE of the chick model of FDM. A list of 15 genes were differentially expressed after 6h of Form Deprivation (FD) with two growth factors (BMP2 and CTGF) identified as genes most likely to play a role in the onset of myopia. In contrast, signaling molecules (IL18, VIP, URP), MKP-2, and EDNRB were considered to be more likely to play a role in maintaining ocular growth after 3 days of FD [167]. Interestingly, Rada and Wiechmann[168] measured changes in the immune-related transcriptome in response to prolonged FD and FD recovery. Of the 14 immune-related genes identified, ATH and ovotransferrin were upregulated after 1 day and 4 days of recovery, respectively. The rationale behind the use of an immune system microarray was unclear as no evidence of immune regulation in myopia was available in the literature at the time, however more recently, the immune system has been implicated in myopic growth control in response to lens-induced defocus [169,170] and FD [171]. The majority of the DEGs that were downregulated in Rada and Wiechmann [168] were not immune-related and encoded for transcription factors (ZFHX1B, c-Jun), ribosomal protein RPLP0, GAPDH, structural proteins (DCN, TPM3), kinase PDPK1, and molecular chaperone HSP70. This suggests that the immune system may be involved in ocular growth, but it is not the only biological process contributing to the development of myopia. This was further supported in a proteomic study which identified altered abundances of ovotransferin (and three other proteins, APOA1, CST3 and purpurin) after 7 days of lens-induced ametropia in chick vitreous [172].

Gene expression in the lens-induced model presents a different profile. In response to 24h of lens-induced defocus with +6.9D lenses, 123 genes were found to be differentially expressed [173]. Of these, glucagon, ZENK (EGR1), RHOG, and CD226 were identified as potential candidate genes. In response to stronger powered lenses (±15D), thousands of genes were differentially expressed. Of these, DUSP4 responded to plus-lens wear and BMP2, VIP, UTS2D, NOG, MYH13, OSBPL6, PDE3A responded to negative lens wear [174]. It is apparent that a majority of the DEGs in response to ±10D lenses are neurotransmitters (glutamate, glycine, GABA, acetylcholine), neuropeptides, and clock/circadian rhythms genes [174]. However, when submitting these genes to Over-Representation Analyses (ORA), the response to lens-induced defocus with stronger powered lenses (±15 D) were interpreted to be involved in biological pathways such as nervous system development/function, cell signaling, small molecule biochemistry, the cell cycle, cell-to-cell signaling/interaction, cellular movement, and metabolism of amino acids and carbohydrates [174]. This suggests that the analysis criteria (ie. differential gene expression vs ORA) greatly influences the interpretation of the results. Riddell et al. [170] also demonstrated good concordance with past microarray studies. DEGs in response to lens-induced myopia (-10D) were mainly involved in phototransduction, neurogenesis, response to stress, and cell structure while DEGs in response to lens-induced

hyperopia (+10D) were related to protein dimerization, inflammation, cell development and metabolism. Interestingly, many common genes were present in both normal development and in both lens groups. These genes were involved in circadian rhythm (NFIL3, ARNTL), and phototransduction (BLIMP-1, GUCA1A, CNGA3, NET1, CACNA2D4), not unexpectedly suggesting a role for circadian rhythm and phototransduction in the development of refractive errors [170].

It is well established and accepted that retinal signals drive ocular growth without the need for cortical input [175-177]. However due to the highly heterogenous nature of the retina, it is unclear whether certain cell types, ie. amacrine cells, are responsible for myopia development. Whole transcriptome analysis was performed on one such cell type, amacrine cells [178], as these cells had been reported to release several factors implicated in ocular growth [179-187]. A total of 128 DEGs were identified after 24h of minus lens wear and 58 DEGs after 24h of plus lens wear. Several candidate genes were proposed (ANGPT2, ChEST267a2, ChEST49o10, CYP26A1, DHHC14, DPF3, GNG13, GFRA1, GTF2H5, CD180, LOC425969, MSX2, PRKRIR, RAB22A) and validated. Although these genes were differentially expressed in the amacrine cell layer, and may be associated with a role in eye growth regulation, these genes could also represent indirect responses to changes in phototransduction, visual distortion and global eye size induced by lens wear [178].

While most 'omic' studies have focused on identifying retinal genes and proteins, Yu et al. [172] aimed to assess DAPs in chick vitreous. Their proteomic analysis revealed four DAPs associated with changes in ocular growth in response to positive and negative lenses. These DAPs were found to be interact indirectly with lipid metabolism pathways and cholesterol metabolism. Pathway analysis also revealed nicotinic acid, one of the water-soluble B vitamins, as a compound potentially modulating these proteins.

In contrast to transcriptomic evidence, experimental myopia in chick produced several DAPs. In response to 7 days of LIM using -10D lens, Lam, Li, Lo, Guggenheim, and To [188] identified dynamin-1, villin 1, tubulin, DDX1, Nuclear RNA Helicase (DEAD Family) Homologue-Rat, DRP2, CRMP-62, SARS, Septin 6, PGAM1 and Similar to Natural Killer Cell Enhancing Factor Isoform to be significantly differentially expressed [189], while Ras related protein Rab-11B, S-antigen retina and pineal gland (arrestin), 26S proteasome non-ATPase regulatory subunit 14 (PSMD14), β-tubulin, peroxiredoxin 4 and ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) were significant during 24h of lens-induced recovery [188]. By comparison, CRMP-62, B-creatine kinase, γ enolase, tubulin α-1 chain, vimentin and APOA1 were significant during FDM [190]. It is plausible that signaling induced cytoskeleton remodelling involving axonal outgrowth, neuronal growth, and actin/tubulin reorganization at the retinal level would be expected during the myopic eye growth but also, likely that these changes might be altering the rate of developmental processes in ocular growth as suggested by Riddell, Faou, and Crewther [191].

While proteomics has revealed several potential proteins implicated in myopia development, not many have considered the effect of epigenetic regulation and post-translational modifications on protein abundance and function. One such study by Chen et al. [192] analysed the retinal phosphoproteome in a lens-induced (-10D) myopic chick model using titanium dioxide ($TiO_2$) enrichment and nano-LC-Triple TOF MS/MS analysis [192]. Out of the 1,631 proteins (560 phosphoproteins) identified in the myopic chick retina, 45 were upregulated and 30 were downregulated during myopic eye growth. This approach also identified several acetylated retinal proteins including carbonic anhydrase, ubiquitin carboxyl-terminal hydrolase, fatty acid-binding protein, nucleophosmin, 40S ribosomal protein S12 and histone H1x. Acetylated proteins indicate which proteins are actively responding to internal and external perturbations, and have been suggested to interfere with metabolic processes and energy homeostasis [193] as well as representing a type of epigenetic marker. Taken together, identifying these modifications would make for a clearer understanding of the retinal proteome in response to myopic growth, particularly as malfunctioning acetylation machinery can lead to diseases such as cataracts [194,195], neurodegenerative diseases [196] and developmental delay [197,198]. The presence of acetylated histones also suggests to us that the control of myopic eye growth may be under metabolic and/or epigenetic regulation.

**Rodent:** Although responses to visual manipulations are slow in mice, the benefit of using this species is the large availability of

genetic backgrounds, making them ideal to use in disease studies with known genetic susceptibility. In a recent study [198], mice of varying genetic backgrounds (129S1/svlmj, A/J, C57BL/6J, CAST/EiJ, NOD/ShiLtJ, NZO/HlLtJ, PWK/PhJ, and WSB/EiJ) were assessed to determine the role of genetic variation and gene expression in baseline refractive errors, and in the development of refractive errors. On average, C57BL/6J were emmetopic, CAST/EiJ, NZO/HlLtJ, PWK/PhJ, and WSB/EiJ mice exhibited a range of hyperopia (ie. +10.6±2.2 D to +22±4.0 D) at baseline. Mice strains that were generally myopic prior to FD were A/J, NOD/ShiLtJ, and 129S1/svlmj (ie. -3.5±3.6 D to -21.2±3.9 D). The range of baseline refractive errors in these mice offers great support for the role of genetics in refractive error development as it can be inherited as a quantitative trait. When these mice were FD for 3 weeks, transcriptomic analysis revealed a total of 2,302 retinal genes that were differentially expressed across all 8 mice strains. Of these, 793 genes upregulated in the strains that were hyperopic at baseline and 1,509 genes were upregulated in the strains that were myopic at baseline. Genetic background was also found to influence the animal's susceptibility to FD with the CAST/EiJ and NZO/HlLtJ strains more susceptible to developing high and low myopia, respectively, even though these strains had a baseline hyperopic refraction. Differential expression of 643 genes were found to be positively correlated with the susceptibility to myopia, whereas expression of 1,274 genes was negatively correlated with the susceptibility to myopia. Furthermore, 714 genes were found to be common between baseline refractive eye development and susceptibility to myopia in mice suggesting that genetic background plays an important role in susceptibility to myopia, particularly in baseline hyperopic mice.

The large number of genes differentially expressed across the 8 strains of mice indicated that several biological processes were involved in baseline refractive error including regulation of neurogenesis, neuron migration, regulation of DNA methylation, visual perception,synaptic vesicle endocytosis, regulation of protein kinase B, regulation of transcription and translation, covalent chromatin modification, insulin receptor signaling, dendrite morphogenesis, and response to oxidative stress. In particular, negative refractive errors were associated with activation of mTOR, EIF2, AMPK, β-adrenergic, and dopamine-DARPP32 feedback signaling pathways and suppression of HIPPO and RhoGDI signaling pathways. Additionally, several biological processes were involved in the susceptibility to myopia including regulation of signal transduction, cell-cell adhesion, transcription, translation, protein transport, and lysosome organization. Myopia susceptibility was also linked to suppression of mTORsignaling, EIF2 signaling, protein kinase A signaling, D-myo-inositol-5-phosphate metabolism, cholesterol and choline biosynthesis, as well as with activation of amyloid processing, HIPPO signaling, PTEN signaling, and PPARα/RXRα signaling pathways. Several pathways were also associated with both baseline refractive development and susceptibility to myopia including EIF2 signaling, protein kinase A signaling, regulation of eIF4 and p70S6K signaling, mTOR pathway, AMPK signaling, HIPPO pathway, axonal guidance signaling, synaptic long-term depression, dopamine-DARPP32 feedback signaling, RAR signaling pathway, synaptic long-term potentiation, CREB signaling in neurons, NRF2-mediated oxidative stress response, melatonin signaling, relaxinsignaling, β-adrenergic signaling, sumoylation pathway, PTEN signaling, ephrin receptor signaling, eNOS signaling, opioid signaling pathway, and α-adrenergic signalling [199]. These findings highlight that there are different mechanisms involved in maintaining normal ocular growth compared to those accelerating ocular growth.

One of the first studies to assess large transcriptome changes in mice was conducted by Brand, Schaeffel, and Feldkaemper [200]. Mice with the C57BL/6 background were FD for a period of 30min to 6h. Expression of 16 genes was found to be affected after 30 min whereas 4h of FD resulted in 27 differentially expressed genes. After 6h of darkness and 6h of light (ie. 12h), the number of up and down regulated genes was balanced, with 10 genes being up-regulated and 11 being down-regulated. Of these genes, EGR1 and cfos were suppressed in response to FD [200]. Although several genes were identified by Brand et al. [200] none were consigned to a specific biological pathway in their pathway analysis. However, most of the genes identified after 30mins and 4h of FD can be shown to be involved in cellular development, whereas DEGs at 24h of FD can be associated with DNA replication, recombination, nucleic acid metabolism, and small



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molecule biochemistry [200] suggesting that dysfunction in growth signals may underlie myopia development.

Following 2 weeks of FD in C57BL/6J mice, 54 miRNAs and 261 mRNAs were identified as significantly differentially expressed [201]. Most of these genes were over-represented in biological processes related to intermediate filament organization, scaffold protein binding, detection of stimuli, visual perception, eye development, phototransduction, calcium ion homeostasis, G protein-coupled receptor, cell projection, and structural molecule activity. In contrast, Tkatchenko et al. [201] did not find any significantly expressed miRNAs in FD mouse sclera but 53 miRNAs were differentially expressed in FD mouse retina. A total of 135 mRNA targets were found for 21 out of 53 differentially expressed miRNAs[202], which encoded for proteins primarily involved in synaptic function, cellular growth, proliferation, nervous and visual systems development. Interestingly, many miRNAs seem to target at least one transcription factor suggesting that the development of FDM involves a highly integrated genetic network of large-scale changes in the retina.

Quantitative proteomics on myopic (-15D) C57BL/6J mice revealed a more refined view of the mechanism involved in ocular growth. Of the 58 DAPs identified, most were involved in the GABAergic signaling pathway (GAT1, NSF, clatherin, dynein 1). Other DAPs were related to oxidative phosphorylation (OXPHOS), energy metabolism, structural pathways, cell differentiation and proliferation, DNA damage [203].Whereas, differentially abundant alpha-A-crystallin, crystallin beta A1 and crystallin beta A2 were significant in FD C57BL/6J mice [204].

**Primate:** One of the first transcriptomic studies utilised microarray technology to assess whole transcriptome changes in myopic retina of macaques subject to FD by lid fusion [205]. After several weeks of FD, a total of 119 DEGs were identified; 19 were negative correlation with the difference in depth of the vitreous chamber between closed and open eye whereas 100 DEGs showed a positive correlation with Vitreous Chamber Depth (VCD). Several genes were found to correlate with axial elongation (LOC157627, ARHGEF12, APLP2, PNUTL2, ZNF275, DHX40, AY680578, CCNA2, cyclin B1, cyclin B2, E2F4, HDGF, VIP) The majority of the DEGs are known to be involved in cell proliferation and nucleic acid metabolism [205].

More recently, sequencing analysis in marmosets wearing ±5D lens for 10 days to 5 weeks has revealed several DEGs underlying refractive development. Interestingly, the response to plus and minus lense resulted in differential expression of different genes, with very little overlap between the two lens conditions. In marmosets, nine coding genes (ZC3H11A, TRIM23, STARD3NL, RCBTB1, PPP2CA, LOC100394842, CUL3, COMMD3, ACTR8) and four long noncoding RNAs (LOC103794697, LOC100396694, LOC100394543, LOC100392587) exhibited sign-dependent expression. However, three genes (PIK3R2, OGFRL1, and NSA2) were specific to plus-lens defocus and six genes (CLEC3A, CREM, LOC100399806, LOC100896985, MCAM, PRSS56) specific to negative-lens defocus [205]. Again, as suggest by Riddell et al. [169], there are clearly notable bidirectional responses to positive and negative lens defocus. After 10 days of negative lens wear, the primary pathways altered included glycogen degradation, ephrin and reelin signaling, biosynthesis of spermine and choline, while prolonged lens wear (i.e. 5 weeks) involved activation of ß-adrenergic signaling and suppression of cAMP-mediated signaling, protein kinase A signaling, calcium signaling, androgen signaling, and dopamine-DARPP32 feedback signaling, among several other pathways. However, 10 days of +5D lens wear primarily involved phenylalanine degradation and RANK, SAPK/JNK, NGF, and gap junction signaling, while prolonged lens wear (ie. 5 weeks) involved activation of EIF2, Notch, JAK/Stat, oncostatin M, somatostatin receptor 2, interleukin, CNTF, CREB, α-adrenergic, integrin, and ceramide signaling as well as suppression of apoptosis and aldosterone signaling [206].

Interestingly, the 29 DEGs identified by Tkatchenko et al. [206] were located within 24 QTLs found to be associated with myopia in the human population suggesting a strong link between known human SNPs and myopia susceptibility. Indeed, when meta-analysing combined refractive error 'omic' data from different platforms and species, these differences were bidirectional [36]. Additionally, DEGs can give initial insights to the gene/proteins that are changing dramatically between the two conditions, however DEGs do not necessarily mean that the protein product is related to the disease condition. Genes can

be subject to mutations in their coding sequence and/or post-translational modifications (ie. phosphorylation or acetylation) which can subsequently affect the functioning of the gene without affecting its expression level [207]. Hence, focusing on DEGs could potentially hamper the discovery of disease-related genes in 'omic' studies. To overcome these limitations, Functional Class Scoring (FCS) approaches considers all genes within a study and discards the use of arbitrary cut-offs as all gene expression changes are considered regardless of the degree of change between samples [208-210].

**Tree shrew:** In sclera of lens-induced myopic tree shrew, several DAPs were identified, most of which function in cell adhesion, cytoskeleton, transcriptional regulation, and Extra Cellular Matrix (ECM) structural proteins (ie. PEDF, COL1A1, thrombospondin 1, COL1A2, GRP 78, thrombospondin 1) [211]. In the recovery period, there were fewer proteins that differed significantly. Of these, most were proteins involved in collagen synthesis, such as colligin, keratocan, collagen 12 α1 [211] and thrombospondin 1 [210]. Two proteins (annexin A1 and receptor of activated PKC1) were significantly upregulated during LIM and during recovery compared with normal eyes [212]. These findings confirm the understanding that cell-matrix adhesions, cytoskeleton and transcriptional regulation are involved in controlling scleral remodelling during myopia development and recovery but are not the primary mechanism driving eye growth.

**Guinea pig:** In sclera of FD guinea pigs, DEGs were mainly associated with muscle development, structural processes glutamate signaling, and ion transport [213].Whereas in retina of minus lens guinea pigs, 8 DAPs (ACTB, ENO1, MDH, PKM2, PIMT, EIF1AX, RAB11B, PKM1) were associated with myopic growth [214].Crystallines (αA-, αB-, βA3/A1-, βA4- and βB2) were also more likely to be differentially expressed in FD guinea pig sclera, particularly between FD and FD recovery [215].

**3.6 Tilapia:** A less studied animal model of refractive error is the tilapia. Jostrup et al. [216] induced myopia by FD in 3-month old tilapia for 4 weeks. Proteomic analysis revealed only three proteins as responding to 4 weeks of FD including annexin A5, gelsolin, and chaperonin-containing TCP-1 theta subunit. These proteins have not been identified in any other proteomic study indicating possible differences in the response to FD in tilapia compared to other animal models FD.

## BIOLOGICAL PATHWAYS IN MYOPIA

Both human GWAS and animal studies have identified many candidate genes and proteins as implicated in the control of refractive myopia. The large number of single candidate genes and proteins differentially expressed in these studies cannot account for most of the observed morphological changes in both clinical myopia and animal models of refractive error development.

As shown in recent studies summarised in Supplementary Table 2, both ORA and FCS analysis have provided greater understanding of the mechanisms involved in refractive error development, driving the need to identify pathway-level changes as they seem to offer more interpretable and reproducible results [36,208].

Interestingly, the genes and proteins identified in both human and animal studies converge into similar biological pathways such as cell structure, cell-cell communication, neurotransmission, retinoic acid metabolism, ion transport, energy metabolism, immune system and eye development [18,36,152,217]. Hence it is surprising that only a few studies [36,37,169-171,191,205] used FCS analysis in identifying biological pathways associated with refractive error development. These studies have highlighted that abnormal axial growth during FD ie. reduced luminance modulation, is accompanied by significant changes in retinal signal transduction at the mRNA and protein level. In turn, reduced downstream signal transmission to bipolar and ganglion cells (and more importantly back to the RPE) are secondary mechanisms involved in myopic eye growth. This altered state of retinal function also impacts on the availability of energy (via mitochondria) as phototransduction involves active ion transport and is a high-energy consuming process following reduced luminance modulation as seen in FD. Additionally, these biological processes activate a complex cascade of events designed to maintain the health and function of the eye, including complement activation, oxidative stress, antioxidant regulation and epigenetic regulation that when impaired, ultimately leads to the observed morphological changes seen in experimental myopia.

**GWAS:** Human GWAS have identified many SNPs that are considered representative of myopia susceptibility (see Supplementary Table 1). More recently, these studies have characterised these genes into groups based on similarities in their biological and molecular function. The first GWAS to take this approach identified candidate genes with functions in neurotransmission (A2BP1, GJD2, GRIA4, RASGRF1), ion transport and maintenance of membrane potential (CACNA1D, CHRNG, KCNQ5, MYO1D), retinoic acid metabolism (CYP26A1, RDH5, RORB), extracellular matrix remodeling (LAMA2, BMP2, BMP3) and eye development (CHD7, SIX6, PRSS56, ZIC2) [20]. Cheng et al. [218] and Riddell and Crewther [36] expanded on this, highlighting a role for BMP signaling, WNT signaling, metabolism, ion transport, organogenesis, embryogenesis, collagen and gap junction in their meta-analyses. More recently, functional categorisation of genes associated with high myopia [19] identified 66 gene sets, of which 83% were eye-related pathways. The most significant pathways were 'abnormal photoreceptor inner segment morphology', 'thin retinal outer nuclear layer', 'detection of light stimulus', 'nonmotile primary cilium' and 'abnormal anterior eye segment morphology'. Similarly, these pathways were also found to be significant for syndromic myopia [219]. Four genes (RGR, RP1L1, RORB and GNB3) were common in the first 3 pathways and seven genes (ANO2, RP1L1, GNB3, EDN2, RORB and CABP4) were highlighted as the most likely causal genes in high myopia. Tissue enrichment analysis also implicated the retina as the most significant tissue in high myopia, and that light-driven visual signals are the initiating factors that drive myopia development - a conclusion reached from animal studies many years ago [220,221].

**Animal studies:** There is clear indication that the expression profiles responding to visual manipulation differ to those involved in myopia progression. However, the question remains as to which biological pathways are involved in the development and progression of myopia. There is a clear consensus that the initial signal to initiate myopic growth begins at the photoreceptors [221] followed by effector proteins that are secreted throughout the retina and RPE in response to visual stimuli [167] which then activates a signaling cascade throughout the retina resulting in ocular growth.

While several DEGs and DAPs have been identified in animal models of myopia (Supplementary Table 2), most have used ORA to assess the biological functions of these genes (as reviewed above). However, not many studies have taken on second-generation pathway analysis which is able to identify subtle gene expression changes within a full expression dataset.

Whole transcriptome pathway analysis of 'omic' studies of refractive errors to ascertain the biological mechanisms driving eye growth are limited. In optical defocus models of myopia (±10D), ORA analysis of DEGs has shown that the genes function in protein dimerization activity, inflammatory responses, cell development and metabolism. Such differentially expressed gene measures of myopia progression are indeed indicative of cell responses associated with wound healing, stress and adherans junction morphology [170]. However, when the analysis is broadened to take in the complete dataset with no arbitrary cut-off, structural (ECM and TJs), metabolic (FA, TCA, mitochondrial) and immune-related pathways appear more strongly up-regulated during myopia induction in chick [170]. In the FD model, these pathways differ. Chicks FD for up to 72h display altered gene expression in pathways involved in mitochondrial energy metabolism and 'one carbon pool by folate'. Whereas, long-term FDM induction (ie. 10 days) has been shown to involve several pathways including mitochondrial energy metabolism, neurotransmission, ion transport, and immune pathways. Interestingly, FD recovery was found to involve suppression in bile acid metabolism [171]. These data suggest that the mechanisms driving eye growth are dependent on the type and duration of visual manipulation although similar biological processes are present across lens type and duration including cell/tissue development, cell signaling and metabolism. Furthermore, these secondary mechanisms are essential in supporting ocular growth changes and retinal homeostasis during reduced luminance modulation.

When considering positive and negative lens types, second-generation pathway analyses have identified additional biological pathways. Riddell et al. [191] have also identified significant differences in abundance of proteins involved in ion and vascular homeostasis and signal transduction in response to negative lens wear. By comparison, positive lenses were more associated with nucleocytoplasmic transport [191]. This is not

surprising as lens-wear is characterised by blur and changes in phototransduction, ocular growth rate and thickness of the choroid. Furthermore, Riddell et al. [37] showed that the differentially abundant proteins following 6h and 48h of negative and positive lens-wear were highly correlated to several human diseases characterized by abnormal electroretinograms, photophobia, arrhythmia and nyctalopia at 6h, and physiological stress, cholesterol homeostasis, and melanin at 48h. While proteins related to age-related age-related macular degeneration (AMD), primary open angle glaucoma (POAG), cataract and choroidal neovascularization were significant after 48h of negative-lens wear [37].

Interestingly, only one group has provided complementary transcriptomic and proteomic datasets for the effects of LIM [37,170,191] and FDM [171,222] on animal models of refractive errors. Although there were significant differences in genes and proteins within the transcriptome and the proteome analysis in response to experimental refractive errors, there was very little concordance in results. The main assumption with large-scale proteomic studies using MS/MS is that alterations in transcript levels are highly correlated with protein levels. However, changes at the mRNA level do not always parallel changes at the protein level. Post-translational modifications of proteins (ie. acetylation) may also be involved in regulating eye growth in refractive myopia. It is well-documented that proteins are greatly influenced by translational efficacy, codon usage/bias, mRNA stability, protein stability and post-translational modifications resulting in a lack of correlation between mRNA and protein abundances[223-225]. Therefore, animal 'omic' studies should be considered along with these assumptions, particularly as a recent meta-analysis has illustrated very little commonalities between the transcriptome and proteome profiles of the myopic retina [36].

## SUMMARY

Vast effort has been made to identify the genetic susceptibility to myopia. What is immediately apparent from syndromic myopia is its comorbidity with developmental delay and intellectual disability suggesting that myopia is most likely a result of impaired neurodevelopment. This is not surprising as several studies have indicated that a number of developmental processes are dysregulated in response to visual manipulations, making this a possible avenue to explore in future research [36,169-171].

It is also evident that negative defocus and FD induces a stronger response at the gene level compared to positive lens wear [170,171,206], and that genetic susceptibility plays a role in myopic development in animal models [199]. Thus, these data reveal that hyperopic and myopic defocus affect the expression of largely different genes and proteins in the retina, and that post-translational modifications should be considered in these large-scale studies. While very few genes are affected by both hyperopic and myopic defocus, there are commonalities in the pathways with which these genes are involved in [36] suggesting that similar developmental mechanisms are involved in ocular growth. Although candidate proteins showed some overlap with both transcriptome studies and GWAS candidate genes, it seems that the most significant interaction is between the proteome and transcriptome[36].

It is also important to note that changes in the mRNA level are not always translated into changes in protein content. It has been found that alterations in mRNA abundance are often poorly correlated with changes in protein levels, particularly in complex biological systems [40-44]. One factor for the discordant levels of mRNA to protein may relate to the possibility that at any point in time, the concentration of mRNA reflects a homeostatic process aimed at maintaining a certain level of protein in tissues where many interacting factors and feedback loops are at play. This makes it important to assess both transcriptomic and proteomic changes and eventually microRNA and epigenetic changes within these systems. Additionally, it may be how these molecules interact with the environmental ionic changes that accompany FD or optical defocus-induced reduction in luminance modulation that produces changes in ocular volume and refractive error, that would give a better understanding of the complex biological response to refractive error development. This is particularly important as expression changes are conserved across species regardless of their DEGs and DAPs profiles suggesting that there are similar biological mechanisms driving eye growth [36]. Thus, it would be short-sighted to assume that individual genes (eg. dopamine, VIP) and genetic variants play a primary role in refractive error development. Rather, it is how genes within a biologically redundant organism function together that



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predicts the phenotypic outcome. In fact, it may be that this complex disease is not controlled by genetics, rather that the genes react to environmental factors and the induced epigenetic changes.

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