Genomics and Transcriptomics of Myopia

Subjects: Biochemistry & Molecular Biology

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Myopia is a globally emerging concern accompanied by multiple medical and socio-economic burdens with no well-established causal treatment to control thus far. The study of the genomics and transcriptomics of myopia treatment is crucial to delineate disease pathways and provide valuable insights for the design of precise and effective therapeutics. A strong understanding of altered biochemical pathways and underlying pathogenesis leading to myopia may facilitate early diagnosis and treatment of myopia, ultimately leading to the development of more effective preventive and therapeutic measures.

Myopia molecular genomics GWAS

1. Introduction

Myopia persists as a pressing public health issue globally, with 27% of the population (or 1.45 billion) involved in 2010 and numbers foreseen to rise [1]. The prevalence of myopia among schoolchildren of Asian descent has been rising significantly from 5.8% in 1983 to 21% in 2000 [2]. The rising prevalence of myopia was also reflected in a study of European adults [3].

Quantitatively, human myopia is commonly defined as having a spherical equivalent (SE) refraction of ≤−0.50 diopter (D), and categorized as mild myopia when SE ≤ −3.00 D and moderate myopia between −3.00 D and >−6.00 D. Once SE ≤ −6.00 D, it is categorized as high myopia (HM), in which the axial length (AL) is usually >26 mm [4]. Myopic children usually become more nearsighted as they age, but their refractive error usually stabilizes in their 20s.

Those with HM will be at higher risk of developing pathological complications such as macular degeneration, choroidal neovascularization, chorioretinal atrophy, and retinoschisis which may result in vision deterioration, ultimately leading to blindness, especially in patients that are 60 years old or over [5].

Myopia that is not associated with another disease is known as non-syndromic myopia, while patients with syndromic myopia are predisposed to myopia due to a disease that is usually genetically inherited, which makes a clinical distinction important. Flitcroft and colleagues have reported that variants of genes known to cause syndromic myopia are also present in non-syndromic myopia [6].

Key contributors to myopia may be attributed to the interactions between environmental and genetic factors. Environmental factors such as near-work activities are reviewed and researched again by Hepsen and colleagues to be associated with the onset and progression of myopia [7], and Huang and colleagues concluded a 2% increased chance of developing myopia with every diopter-hour of near work time per week [8]. Genetic factors are also involved in the development and progression of myopia, exhibited by a study showing Asian children being more susceptible to high myopia as compared to their European counterparts by analysis of 20 different myopia-associated loci [9]. The genetic architecture of myopia and currently associated myopia loci, as well as gene-environment interactions and the prediction of myopia via polygenic risk scores have been comprehensively examined previously [10]. Other factors include high educational pressure and limited time outdoors [11]. Limiting educational workloads and increasing time outdoors have been suggested to improve outcomes in the myopic progression of pediatric patients.

Understanding the various factors underpinning myopia may provide valuable insights for the development of therapeutic options for myopia. Therefore, animal myopia models from different species have been proposed to study myopia, each with their pros and cons. Lens-induced myopia (LIM) or form deprivation myopia (FDM) are the two primary ways to induce detectable myopia for better control of environmental factors, and genetically-manipulated animal models, mainly mice, are preferred to study syndromic myopia in humans [12].

Limited treatment options currently available to retard axial elongation in myopia highlights the imperative need for work to be conducted in the field of myopia pharmacogenetics [13][14]. Myopia pharmacogenetics can be used synergistically with precision medicine, which detects at-risk populations, such as the early onset of myopia during childhood, and pathological myopia development during adulthood. Precision medicine has been applied in other fields of ophthalmology, such as in the management of Age-Related Macular Degeneration [15], and has only very recently been applied in the management of pediatric myopia [16].

Molecular biology has been comprehensively reviewed in myopia research to provide perspectives on the genetic basis of ocular development and probable pharmacological candidates for intervention [17]. Many tremendous and exciting advances are taking place within the biological field, facilitated by numerous biotechnological developments and applications [18]. Such advancement has led to the discovery of novel genes that have been implicated in the development and progression of myopia [19], such as in dopamine signaling [20].

2. Significance of Genetic Analyses

The central dogma of molecular biology states that biological information is encoded by DNA and the genetic information is transcribed to messenger RNA. Messenger RNA is then translated into proteins [21]. However, other factors can influence the production of proteins. Epigenetics investigates the changes in gene expression associated with alterations in the chromosome, while the DNA sequence remains unchanged. This gives more information regarding the interaction of genetic and environmental factors [22]. Transcriptomics, on the other hand, involves analyzing which of the genes encoded in DNA are turned on or off and to what extent. Transcriptomics is a continuum that complements genomics, filling the gap between genomics and proteomics in the precision medicine

era. The two key contemporary techniques employed in transcriptomics include RNA-sequencing (RNA-seq) to examine the quantity and sequences of RNA in a sample using next-generation sequencing (NGS), and microarray, which detects thousands of genetic sequences simultaneously [23].

3. Techniques of Genomics and Transcriptomics

3.1. Genomics

The term ‘genomics’ was coined to define the study of genes and their functions [24][25]. This has given rise to the revolutionary Human Genome Project in 1990, which cataloged the genetic sequences of the human body [26]. The genome includes chromosomal and extrachromosomal DNA material, consisting of both coding and non-coding regions.

Linkage analysis, previously the main, powerful analysis method for the identification of genes involved in disease etiology, can be conducted in conjunction with NGS or whole exome sequencing approach [27].

At present, genome-wide association study (GWAS) is the main research approach used to identify genomic variants (such as single nucleotide polymorphism [SNP], a genomic variant at a single base position in the DNA) that are statistically associated with a risk for a disease or a particular trait. GWAS results can be applied in many settings, such as helping to gain insights into a phenotype’s underlying biology, calculating genetic correlations, making clinical risk predictions, and informing drug development programs about risk factors and health outcomes. To validate GWAS results, a meta-analysis is usually used to reduce the chance of false positives; quantitatively, different populations from similar ancestry are used to run GWAS again to confirm the signals. Other methods include targeted re-sequencing of the regions of interest, custom SNP Chip analysis, and microarray.

In terms of bench work for validation, Polymerase Chain Reaction (PCR) is used to amplify small segments of DNA to attain large quantities of DNA [28]. It is the most common DNA amplification method used and it facilitates the analysis of genes involved in myopic development and progression. PCR is performed with a thermostable DNA polymerase [29]. Southern blotting involves covalently binding DNA fragments to membranous support, which can then be used for hybridization [30]. This helps in the detection of specific DNA sequences in complex samples of DNA, which can be applied in the context of myopia genomics. Northern blotting is similar to Southern blotting, but it involves the detection of RNA sequences instead of DNA sequences and was therefore used heavily in the field of transcriptomics [31].

Microarray greatly advances the study of differential genetic expressions by allowing for the analysis of thousands of genetic sequences [32]. A probe is used to detect the presence of complementary genetic sequences and therefore genetic variants.

3.2. Transcriptomics

RNA-seq is a sophisticated molecular technique that sheds light on gene expression in cells in physiological and pathological states [33]. This aids in the understanding of the molecular mechanisms underlying myopic refractive status. For example, RNA-seq has led to the discovery that the retina is likely able to distinguish between hyperopia and myopia [34].

4. Applications of New Technologies in Myopia Research

Big data analytics can reveal correlations in large amounts of raw genetic data and calculates the risk contributed by environmental factors leading to the development of myopia in order to help clinicians make data-informed and clinically significant decisions. Myopia research has been applied successfully in many cases. The first successful applications were in GWAS studies reported by Nakanishi and colleagues in an Asian population, as well as Solouki and colleagues in a European population [35][36]. Studies also identified specific genes that are heavily influenced by the environment, with education being a salient contributing factor. GWAS studies in the human population emphasized the role of the retina in the generation of myopic eye growth signaling and supported the notion that refractive errors are caused by a light-dependent retina-to-sclera signaling cascade.

Using genome-wide survival analysis, Kiefer and colleagues performed the GWAS meta-analysis from the 23andMe database and identified 22 significant associations with the age of onset of myopia with 45,771 European participants. The associations in total explained 2.9% of the variance in myopia age of onset and pointed toward multiple genetic factors involved in the development of myopia. The associations also suggested that complex interactions between extracellular matrix remodeling, neuronal development, and visual signals from the retina may underlie the development of myopia in humans [37]. A more recent study suggests that mutations in the cone opsin genes were shown to account for about 4.6% of the variance in common myopia [38]. Following studies contributed by Tedja et al. combined the Consortium for Refractive Error and Myopia (CREAM) and 23andMe, expanding the study sample to 160,420 individuals from a mixed ancestry population, in addition to independent replication in 95,505 participants from the UK BioBank. System comparisons were conducted and showed a high genetic correlation between Europeans and Asians (>0.78, $p = 2.48 \times 10^{-7}$). Expression experiments and comprehensive in silico analyses identified retinal cell physiology and light processing as prominent mechanisms and identified functional contributions to refractive-error development in all cell types of the neurosensory retina, RPE, vascular endothelium, and extracellular matrix. Newly identified genes implicate novel mechanisms such as rod-and-cone bipolar synaptic neurotransmission, anterior-segment morphology, and angiogenesis. Thirty-one loci resided in or near regions transcribing small RNAs, thus suggesting a role for post-transcriptional regulation [39]. All these would be good bases for hypotheses to design animal studies for further validation and pharmaceutical target identification.

Breakthroughs in the field of myopia research involve the application of molecular techniques and experimental animal models. For example, the use of PCR and Southern blotting has led to the discovery that the chick does not possess a functional M1 muscarinic acetylcholine receptor [40]. Such findings imply that muscarinic antagonists, which prevent the progression of myopia in the chick, either work through another muscarinic receptor subtype, most likely M4, or through non-specific or non-receptor mechanisms.

Another successful application is a study conducting drug screening for myopia control [41]. Researchers modified the lumican gene with a morpholino oligomer in zebrafish embryos, allowing the establishment of an excessive sclera expansion animal model for screening 642 compounds approved by the U.S. FDA. Effective compounds were then applied in form deprivation myopia models mice and the Syrian hamster. The study reported that MMP inhibitors are potential candidates for the treatment of myopia by targeting the sclera.

In addition to pharmacological interventions, effective optical interventions, informed by genetics, are already available [42]. Rappon et al. reported that the discovery of mutations in the cone opsin gene *OPN1LW* led to Bornholm Eye Disease (BED) as reported by McClements and colleagues [43]. Hagen and colleagues also reported that polymorphisms of the *OPN1LW* gene may play in role in non-syndromic common myopia [44]. The characterized biological function of the mutant genes [45] led to the development of diffusion optics technology (DOT) lenses (designed to reduce retinal contrast), which were shown to slow myopia progression by 74% in a multicenter, randomized, and controlled double-masked trial [16].

Leveraging on harvested eye tissue from animal myopia models, differentially-expressed single genes analyzed from RNA-seq, along with right bioinformatic analysis methods, studies could elucidate the underlying mechanism of light-dependent signaling in the retina for myopic eye growth. Utilizing a short myopia induction period of 1–3 days in the chick model, Riddell N. et al. used GSEA and reported subtle shifts in structural, metabolic, and immune pathway expression in the retina, which were correlated with eye size and refractive changes induced by lens defocus [46]. Another study also utilized New World primates, common marmosets, which were treated with either negative (-5D) or positive (+5D) lenses for either a short period (10 days) or a long time (5 weeks) to induce refractive error. QIAGEN's IPA software and database were used to identify biological functions (GO categories), which are significantly affected by the changes in gene expression induced by the optical defocus in the retina. IPA revealed that the primate retina responds to defocus of different signs by activation or suppression of largely distinct pathways. Twenty-nine genes that were differentially expressed in the marmoset retina in response to imposed defocus are localized within human myopia quantitative trait loci (QTLs), suggesting functional overlap between genes differentially expressed in the marmoset retina upon exposure to optical defocus and genes causing myopia in humans [34].

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