

Nutritional Genomics in Primary Open-Angle Glaucoma

Asensio-Marquez E. M.¹, Ortega-Azorin C.¹, Zanon-Moreno V.^{1, 2, *}

¹Department of Preventive Medicine and Public Health and CIBER Physiopathology of Obesity and Nutrition, School of Medicine, University of Valencia, Valencia, Spain

²Ophthalmic Research Unit “Santiago Grisolia”, Dr. Peset University Hospital, Valencia, Spain

Email address:

vicente.zanon-moreno@uv.es (Zanon-Moreno V.)

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Abstract: Primary open-angle glaucoma (POAG) is an optic neuropathy characterized by a high intraocular pressure (IOP), an alteration of the optic nerve head and a loss of visual field. POAG is one of the main causes of blindness worldwide, currently with no cure. POAG is a multifactorial disease that involves both genetic and environmental factors, so that the development of the glaucomatous disease is determined not only by the individual effect of each of these factors, but also by the joint effect of the interaction between all of them. To study the interactions between these factors and their association with POAG is a very hard task, but we can approach this issue investigating the genetics-nutrition relationship and the effect of this binomial on the glaucomatous pathogenesis by means of the Nutritional Genomics. The influence of nutrition on glaucomatous pathogenesis has been studied since long time ago. For example, it is well known the role of vitamins in eye health. However, the interaction of genetic and nutritional factors and their effect on glaucomatous optic neuropathy are investigated recently. In this article we review the genetics of primary open-angle glaucoma, as well as various risk factors for this disease, including nutritional factors. Also, we review the articles studying the interaction of all these factors (genetics and nutritional) in relation to this optic neuropathy.

Keywords: Primary Open-Angle Glaucoma, Genetics, Nutrigenetics, Nutrigenomics, Nutrition, Diet

1. Introduction

Primary open-angle glaucoma (POAG) is the most common form of glaucoma, representing more than 50% of cases of this disease in developed countries. It is characterized by high intraocular pressure, optic nerve atrophy and loss of visual field [1]. This disease is asymptomatic in its early stages and the vision is lost gradually.

According to the World Health Organization 2010 data, glaucoma is the second cause of blindness in the world (8% of the cases) and the third cause of visual impairment (2% of the cases) [2]. It is estimated that glaucoma affects more than 66 million people worldwide [3]. The economic and social repercussions are so important that, nowadays, glaucoma represents a public health problem. About 8.4 million people were bilaterally blind from primary glaucoma in 2010, a figure that is expected to increase to 11.1 million in 2020 [4].

The prevalence of glaucoma shows large fluctuations, between the lowest value in Eskimos residing in Alaska (0.06 %) and the highest value found in African-derived people living in the Caribbean (7.1 to 8.8 %) [5]. Depending

on the authors, its value ranges from 0.4 to 0.8 % in populations with no family history and between 3.5 to 19%, if any. The variation in the prevalence of POAG should be quantified by taking into account age, gender or race. In studies by racial group, the prevalence is higher in black population (4.2%) than in white population (2.1%) or Asian population (1.4%). If we study the prevalence by age, the value increases proportionally with age for each racial group [6, 7]. It has been observed in white population a doubling in POAG prevalence per decade, that is to say, although black population has the highest prevalence in all ages, the proportional increase with age is greater in white population. Considering gender, the results are controversial. There are studies where prevalence is higher in men [8, 9], others in women [10, 11] and others have found no differences [7, 12], and therefore the results in relation to this risk factor are inconclusive. It would be interesting for future studies to delve into the effect of this factor in the risk of POAG [6].

2. Risk Factors

One of the most important risk factors for POAG is the

intraocular pressure (IOP). IOP regulates the secretion and outflow of aqueous humor. This fluid is produced by the ciliary body in the posterior chamber of the iris and flows towards the anterior chamber. It leaves the anterior chamber by the iridocorneal angle or cameral sinus, goes through the trabecular meshwork and flows into Schlemm's canal. The aqueous humor provides nutrients to the iris, lens and cornea [3]. In POAG the iridocorneal angle is not blocked but the aqueous outflow decreases. The alteration of the aqueous humor outflow causes an increase in IOP, which may cause stress in retinal ganglion cells and a decrease of axonal transport. As a result, the cells could die by deprivation of neurotrophic factors. Glial cells in the optic nerve head are activated in response to elevated IOP. Activated astrocytes synthesized molecules that lead to degradation and remodelling of the extracellular matrix. These changes have a biomechanical effect on the optic nerve head, increasing the stress in retinal ganglion cell axons. All this process produces alterations in the visual field [13].

The cause of primary open-angle glaucoma is still unknown, therefore the main goal of treatment for POAG is trying to slow down its progression, maintaining the visual function as long as possible. Untreated patients with high IOP has seven times more incidence of visual field loss after twenty years of monitoring than patients with normal IOP [14]. Therefore, treatments are aimed at reducing IOP and prevent that the damage to the optic nerve continues.

The hypotensive treatments include medical treatment, laser surgery and incisional surgery. The success of the treatment will depend on proper diagnosis and its effectiveness and enforcement. The first goal sought in the choice of treatment is the reduction, between 20 and 50%, of the initial IOP at which the damage occurred. The desired amount depends on the initial IOP and the extent of damage to the optic nerve and visual field in diagnosis. The IOP level

to be achieved may be even lower than the value that is considered normal in a patient without glaucoma [3]. In low tension glaucoma, that is, when the atrophy of the optic nerve head plays a major role and IOP is of secondary importance, it seems that reducing IOP also slows down the progression of the disease [14, 15].

When IOP cannot be well-controlled by medication or when the optic nerve damage progresses even though the IOP has decreased or when the patient is unable to follow/tolerate the drug treatment, ophthalmologists can resort to surgical procedures.

However, the current treatment for POAG (hypotensive therapy) should be complemented by a neuroprotective treatment. The most important feature of glaucoma is the loss of optic nerve fibers. The axons of the retinal ganglion cells (RGC) converge in the optic disc and form the optic nerve. The fibers exit the eye through the lamina cribrosa and the convergence of these axons creates a depression on the disk known as the cup [3]. It is known that retinal ganglion cell die by apoptosis and probably this fact is a consequence of the evolution of glaucomatous disease. However, the survival of RGC could be increased by means of the neuroprotective treatment; even it could be achieved the recovery of these cells. In any case, this neuroprotective treatment should not replace the hypotensive treatment, but both of them must be administered in combination, so as to allow greater control of the disease [16].

The intraocular pressure is not the only factor involved in the etiopathogenesis of POAG. It is a multifactorial disease. There are genetic and environmental factors influencing the onset and progression of this optic neuropathy. Therefore, POAG is determined not only by the individual effect of all these factors, but also by the interactions between all of them (figure 1).

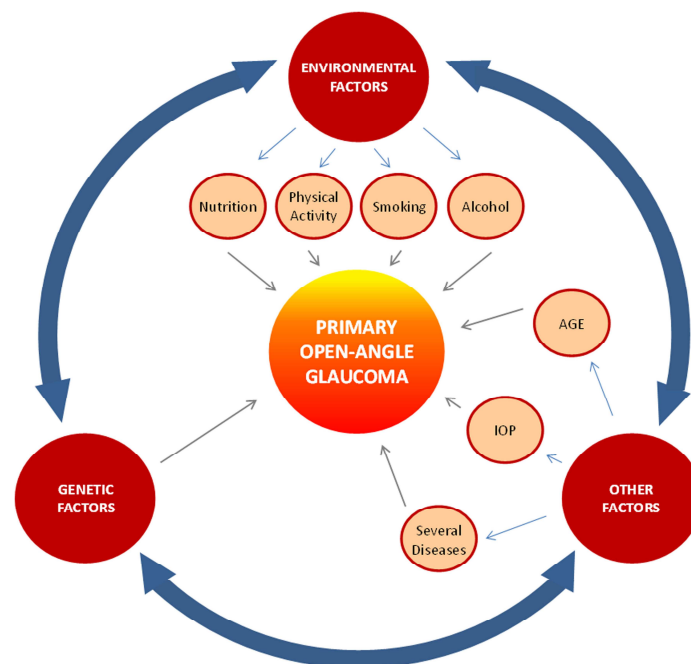


Figure 1. Risk factors for primary open-angle glaucoma.

Different risk factors have been described that increase the risk of developing POAG. In addition to the high IOP, there are three other important factors related to this optic neuropathy: age, race and family history. As explained above, the prevalence of the disease is directly proportional to increasing age and also varies by race being more prevalent in Afro-Caribbean than Caucasian subjects [17]. Family studies have shown that patients with relatives with glaucoma have a 22% risk of developing POAG, while the risk of patients without a family history is 2-3% [18, 19].

Other risk factors are myopia, diabetes mellitus, systemic hypertension or cardiovascular disease. It has been shown that the risk of POAG is 3.6 times higher in hypertensive individuals than in normotensive individuals. This may be because high blood pressure can reduce the blood flow to the optic nerve and cause ischemia. In the long term, this can cause apoptosis of cells of the optic nerve, causing loss of visual field [1].

2.1. Genetic Factors

Through traditional linkage analysis, a region of genes was found in chromosome 1q called GLC1A related to glaucoma. Subsequently, similar studies in other glaucoma pedigrees describe other 13 genes related to the disease (GLC1B to GLC1N) [13, 18, 19]. Three glaucoma-related genes have been identified within that loci, myocilin (GLC1A) optineurin (GLC1E) and WDR36 (GLC1G). Recently a new gene was discovered, neurotrophin-4 (NTF4), identified in European and Chinese population.

In 1997, Stone et al [20] found the first gene linked to POAG, the gene encoding the myocilin (MYOC). A variety of mutations of this gene has been detected in 3-5% of patients with adult-onset POAG around the world. Myocilin associated with glaucoma is transmitted as an autosomal dominant Mendelian trait and its function is unknown. The myocilin is produced in different tissues, including the ciliary body and trabecular meshwork. A decrease in the secretion of this protein in the aqueous humor has been found in patients with glaucoma associated with MYOC mutations. In animal models it was observed that neither the absence of wild-type myocilin nor its over expression increased IOP. In contrast, forms of mutant myocilin were not secreted and remained trapped in the intracellular space by the formation of abnormal associations with exosomes. This accumulation in the intracellular space might be toxic to the trabecular meshwork, altering their function and decreasing the outflow. All this will entail an increase of IOP and, eventually, damage to the optic nerve. The mechanism by which the outflow is decreased is not clear. He Y et al. [21] reported that MYOC produces deregulation in the calcium channels causing depolarization of the mitochondrial membrane in trabecular meshwork, resulting in its contraction, lowering the outflow and increasing IOP. This suggests that preventive measures could be applied for mitochondrial protection in an attempt to delay the onset of the disease. Although much still remains unknown about the function of the protein and how

the increased outflow resistance occurs, the discovery of myocilin has led to a better understanding of the pathobiology of POAG [13, 18, 19].

The second gene related to POAG was optineurin (OPTN for optic neuropathy-inducing protein) [22]. It is located in the GLC1E locus on chromosome 10. It is believed that mutations in this gene may be responsible for 16.7% of hereditary forms of normal stress glaucoma (NTG) and that there is an additional risk factor of 13.6% in both familial and sporadic cases. The E50K variant has been strongly associated with NTG. Patients with this mutation develop a more severe form of glaucoma compared with NTG patients who do not have the mutation. This protein is expressed in human trabecular meshwork, non-pigmented ciliary epithelium, retina, brain, adrenal cortex, liver, fetus, lymphocytes and fibroblast. There is evidence that optineurin has a neuroprotective role reducing retinal ganglion cell susceptibility to apoptosis. In response to an apoptotic signal, OPTN translocates from the Golgi to the nucleus. Over-expression of OPTN blocks the release of cytochrome c from the mitochondria and protects the cells from hydrogen peroxide-induced cell death. The OPTN E50K mutants inhibit the translocation of the protein to the nucleus, so that the ability to respond to oxidative stress is lost, and its over-expression leads to the release of cytochrome c, loss of the potential of the plasma membrane and activation of the mitochondrial pathway in neural cell apoptosis. Other studies have shown that other OPTN variants combined with TNF-alpha variants (pro-inflammatory cytokine) increase the severity of the damage to the optic nerve of the patient and have a worse prognosis. MYOC and OPTN seem to have different roles and contribute to the development of POAG using different mechanisms [18, 19].

The contribution of the other two discovered genes, WDR36 and NTF4, is controversial and has not yet established a role in the pathogenicity of the glaucoma. The WDR36 gene is located on GLC1G locus [23]. Its prevalence has been estimated between 1.6 and 17% of POAG patients. WDR36 is expressed in lens, iris, sclera, ciliary muscles, ciliary body, trabecular meshwork, retina and the optic nerve. Patients with variations in the WDR sequence have been associated with more severe phenotypes of the disease, so they must play a role in disease susceptibility rather than causation. It has also been observed that it is related to both glaucoma with high IOP and low-pressure glaucoma [18, 19].

NTF4 gene variants have been found in 1.7% of patients of European origin POAG [24], less than 1% of patients in Chinese population [25] and recently, it has been observed that variations in the NTF4 gene are associated with an increased risk in the United States Caucasian population [26]. NTF4 variants have effect in neuronal survival and that cause a disruption in the signal of the tyrosine kinase-B receptor which has a protective role against high IOP, ischemia and release of cytotoxins.

However, the POAG is a complex pathology where

different variations in several genes may be involved. Recent genome-wide association studies (GWA), a powerful tool for the identification of genetic risk factors for complex diseases, have been used to identify novel genetic variants related to POAG. The first GWA performed to detect variants associated with POAG was held in 2009 in Japanese population, carrying out a study in two stages: first, a proportion of the available samples were genotyped on genome-wide SNP arrays and then, after selecting the most relevant SNPs, they were analyzed in the rest of the sample. This study identified three loci on chromosomes 1, 10 and 12, but did not reach statistical significance [27], and the results have not yet been replicated in other studies [28]. Subsequently, another GWA study was published in 2010 carried out in Japanese population, but limited to patients with normal tension glaucoma (NTG). In this study, authors detect the presence of one SNP in an intron of the *SRBD1* gene with genome-wide significance [29]. Other studies conducted in Japanese patients with NTG and high-tension glaucoma (HTG)[30] and in American population with POAG [31] replicated the results of the study of Meguro[29]. Regarding these results indicated that the gene *SRBD1* is expressed in the retina of neonatal mice, and that in peripheral whole blood of human participants, the expression of the *SRBD1* was higher in carriers of the risk allele of the SNP identified was associated with NTG compared with expression in participants who did not have this allele. Although the mechanisms by which variants in the *SRBD1* gene is not yet known may increase the risk of NTG, it has been suggested that *SRBD1*'s binding properties can influence the synthesis, growth and apoptosis of retinal ganglion cells, promoting the development of NTG [29].

Thorleifsson carried out another GWA in POAG patients from Iceland [32]. This study identified a SNP between the genes caveolin 1 (*CAV1*) and caveolin 2 (*CAV2*) significantly associated with POAG. Caveolins are proteins involved in the generation and function of caveolae, which are small invaginations of the cell membrane, whose function is related to cell signaling as well as endocytosis. In this regard, authors suggest that variations in both caveolins, 1 and 2, may alter TGF- β nitric oxide signalling.

Another GWA conducted in 2011 to detect new variants associated with POAG in an Australian cohort identified two susceptible genes related to this pathology, reaching statistical significance [33]. Authors identified a SNP located downstream of the transmembrane and coiled coil domains 1 (*TMCO1*) gene. Sharma et al studied how the presence of some variation in this gene was linked with a diagnosis of glaucoma in younger individuals [34]. Variants in the *TMCO1* gene have been associated with IOP [35, 36]. The *TMCO1* function is currently unknown but it is highly expressed in several ocular tissues, including the ciliary body, trabecular meshwork and retina [37].

In addition to the SNP in the *TMCO1* gene, Burdon et al also detected association with POAG and cyclin-dependent kinase inhibitor 2B antisense RNA 1 (*CDKN2B-AS1*). The same association has been detected in several subsequent

studies of GWAs conducted both in Caucasian and Japanese populations[38-41]. This *CDKN2B-AS1* is a long antisense non-coding gene involved in regulating the expression of other genes, including the tumor suppressor genes *CDKN2A/B* [42]. Furthermore it has been observed in an animal model of glaucoma that *CDKN2A/B* is upregulated due to the increase of IOP [43]. These findings have led to propose that variations in the IOP may be related to altered expression levels of these genes. These changes in gene expression might be involved in apoptosis of retinal ganglion cells, which could result in glaucomatous visual field loss.

Osman [39] and Wiggs [41] have also shown the association between POAG and the intergenic region between *SIX1* and *SIX6* locus. Dimasi et al found one variation adjacent to the *SIX1* gene significantly associated with POAG, suggesting that this gene may be involved in the developmental pathways that increase susceptibility to glaucomatous optic neuropathy[43]. The *SIX1* and *SIX6* genes encoding a number of conserved proteins that belong to the *sine oculis/homeobox* family of transcription factors, whose role has been related to the development of different organs and tissues, among which are those from the retina [44].

Given the multifactorial nature of POAG, in addition to genetic factors, environmental factors are also associated with this disease. Different lifestyle or environmental factors, such as exercise, alcohol consumption or cigarette smoking may be associated with the risk of developing POAG [5, 17, 45]. Among these environmental factors, nutrition has been studied in relation to ocular diseases since long time ago [46-48]. Eyes are particularly sensitive to oxidative stress owing to the high light exposures, ultraviolet radiation and environmental pollutants, and oxidative stress has been linked to several eye diseases, such as POAG [49]. Thus, some studies have examined the effect of dietary antioxidants (ie. vitamin E, C and carotenoids) in the prevention of ocular pathologies [50, 51]. The lack of fatty acids has also been linked to POAG risk. The disc membranes of the outer segments of retinal photoreceptors have a high concentration of omega-3 and omega-6, particularly docosahexaenoic acid (DHA), which protects the retina from oxidative damage [52].

However, as we have mentioned above, we must consider that the development of glaucomatous disease is determined not only by the individual effect of each of these factors, but also by the combined effect of the interaction between them [53]. Therefore, it is necessary to deepen in this fact. We have to analyse not only the study of environmental and genetic factors separately, but also we have to analyse the possible interaction between all these factors and their association with the risk for POAG, as well as other diseases with multifactorial origin.

2.2. Nutritional Factors

It is quite impossible to know all factors involved in the etiopathogenic of glaucomatous optic neuropathy and,

therefore, it is a very hard task to study the interactions between all of them. However, by means of Nutritional Genomics, we can study the relationship between genetic and nutritional factors, and how the interaction between genes and diet determines the individual genetic susceptibility of POAG.

This new scientific discipline combines the investigation of the effect of nutrition and the genome, how different individual genomic variations influence on the effect of nutrients and their association with disease.

Nutritional genomics studies the gene-nutrient relationship from two different points of view, so that we can distinguish two branches in this scientific discipline: Nutrigenomics and Nutrigenetics. The first one, nutrigenomics, aims to study the effect of nutrient intake on gene expression (ie. alterations in the synthesis and/or function of proteins). The second one, nutrigenetics, study the response of different genotypes to the nutrient intake, thus determining the individual susceptibility to diseases related to diet [54].

It has been shown that people could respond differently to the same diet, and that this differential response is influenced by genotype. For this reason, we must take into account not only the physiological and metabolic characteristics of individuals but also the genetic characteristics, so that we can design personalized diets for each subject. This is the main goal of the nutritional genomics.

Therefore, Nutritional Genomics has great potential interest because nutritional intervention based on knowledge of the genome and the interaction between the genes and nutrients could be used in order to develop personalized diets to help prevent or treat multifactorial diseases.

Since the cause of glaucoma is not yet known and all what ophthalmologists can do nowadays is trying to slow the progression of the disease, which is achieved only in some

cases, studies of nutritional genomics applied to this optic neuropathy represent a new tool in the fight against glaucomatous bilateral blindness. The knowledge about gene-nutrient interaction in relation to POAG will allow designing personalized diets which will have a beneficial effect on eye health.

It has been shown that omega 3 deficiency results in RGC dysfunction and the combination of this deficiency and high IOP has a cumulative effect [55]. In addition, it is also important the omega 3:6 ratio. Pérez de Arcelus et al. demonstrated that patients with high omega 3:6 ratio intake had a significantly higher risk for glaucoma compared with those with low omega 3:6 ratio intake [56]. It is also known the beneficial effect of antioxidant intake on risk for POAG [57, 58].

Our research group conducted a case-control study in which we aimed to investigate the possible association of several genetic variations with risk for POAG, the plasma level of several vitamins in relation to POAG risk and the effect of gene-nutrient interaction on risk for POAG [59].

We found several genetic polymorphisms associated to high risk for POAG. For example, the rs1279683 polymorphism in the SLC23A2 gene, a gene related to vitamin C metabolism, was associated with high risk for this optic neuropathy (OR=1.70, 95%CI=[1.17-2.47]). The rs737723 polymorphism in the SEC14L2 gene, a gene involved in the tocopherol metabolism, was also associated with high risk for this disease (OR=1.78, 95%CI=[1.18 - 2.69]). However, the risk for POAG increased more than 10 times in subjects carrying the variant alleles for these two polymorphisms (OR=14.8, 95%CI=[1.9-113.7]). In this study, we also determined the plasma concentration of several nutrition factors, such as vitamin C and vitamin E which were significantly decreased in POAG group comparing with healthy controls (figure 2).

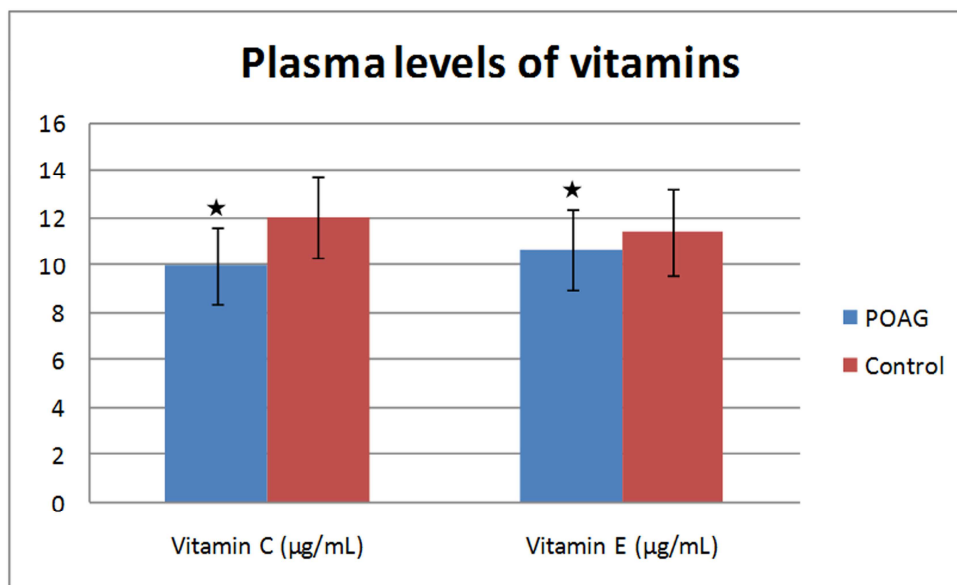


Figure 2. Plasma levels of vitamin C and vitamin E in POAG patients and healthy subjects.

The rs1279683 polymorphism in the SLC23A2 gene was associated to lower levels of vitamin C both in POAG

patients as healthy controls. Another SNP related to vitamin E metabolism, rs6994076 in the TTPA gene, was associated with lower levels of vitamin E in both groups. At the same time, we conducted a study of the adherence to Mediterranean diet in a representative sample of both groups, by means a survey that included 14 items. The data obtained in this study were processed and we observed that subjects

with higher adherence to Mediterranean diet have higher plasma levels of vitamins and less risk of POAG. These results demonstrate the relationship between genes and nutrients in this optic neuropathy, suggesting that it would be possible to help prevent glaucomatous blindness by means of personalized diets (figure 3).

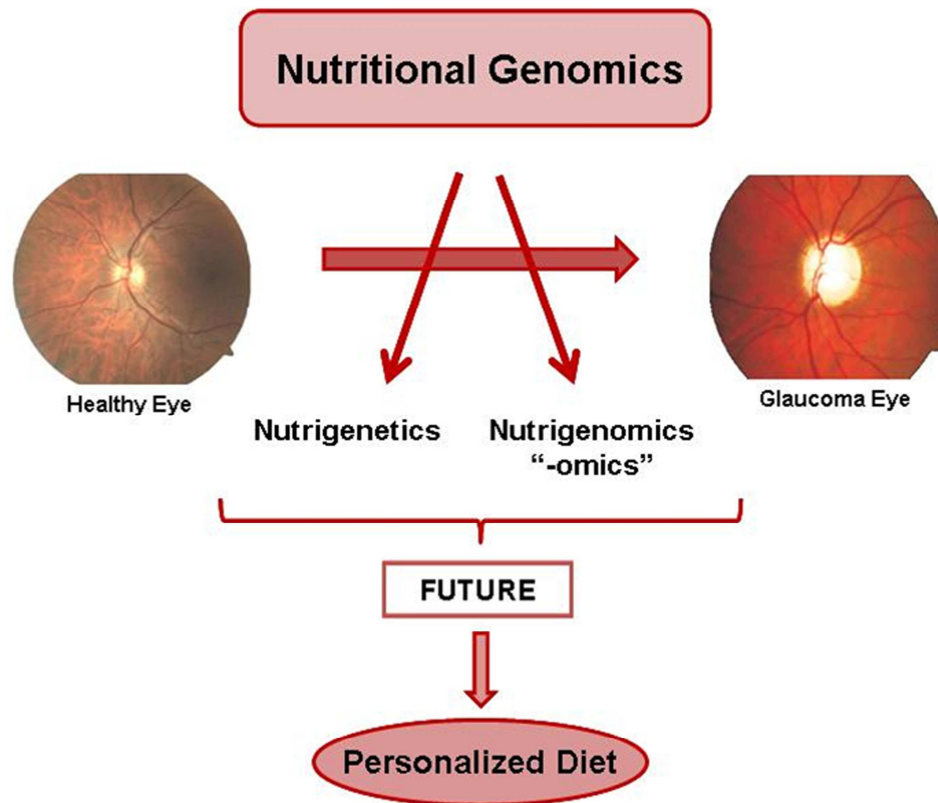


Figure 3. Nutritional Genomics is expected to help prevent glaucomatous blindness by means of personalized diets.

Of course, there is still much to be done, since we only know a small part of the gene-gene and gene-environment interactions that influence the onset and development of this disease. And we need to take into account that also epigenetic modifications are involved in the glaucomatous pathogenesis [60-63]. In fact, changes in gene expression are due, in part, to epigenetic modifications, heritable alterations in DNA activity without change in DNA sequence [64]. Epigenetics describes the mechanisms that allow cells to respond quickly to changes in the environment, thus establishing a link between genes and the environment [65]. Although there are a large number of studies emerging in this field, the specific mechanisms involved in these epigenetic changes are not yet accurately known. However, it is becoming more accepted the idea that the environmental factors, including nutrient intake, could be responsible for these variations[66].

Given this change in the expression of genes, there is increasing evidence that the epigenetic alterations affect the risk of many complex conditions, such as ocular neurodegeneration diseases. In this regard, it has been shown that retinal ganglion cell death in a mouse model is closely

related to the silencing of *Fem1cR* gene and deacetylation of histone H4 [67]. Although the epigenetic modifications in relation to ocular pathologies are little studied, Jünemann observed an increase of the DNA methylation degree of peripheral mononuclear cells in patients with POAG compared to control subjects, suggesting that these results may have implications for a possible epigenetic control in patients with POAG [68].

3. Concluding Remarks

Considering all the explanations and comments above, it is necessary to continue these studies, taking into account all the factors involved in the etiopathogenesis of glaucoma (environmental, genetics and epigenetics) and their interactions in order to deepen the molecular and genetic basis of glaucoma and the knowledge of the etiology of this complex eye disease, which will allow us to prevent glaucomatous blindness and develop new treatments for this optic neuropathy

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