Genetic and epigenetic modifications in the pathogenesis of diabetic retinopathy: a molecular link to regulate gene expression

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Abstract

Intensification in the frequency of diabetes and the associated vascular complications has been a root cause of blindness and visual impairment worldwide. One such vascular complication which has been the prominent cause of blindness; retinal vasculature, neuronal and glial abnormalities is diabetic retinopathy (DR), a chronic complicated outcome of Type 1 and Type 2 diabetes. It has also become clear that “genetic” variations in population alone can't explain the development and progression of diabetes and its complications including DR. DR experiences engagement of foremost mediators of diabetes such as hyperglycemia, oxidant stress, and inflammatory factors that lead to the dysregulation of “epigenetic” mechanisms involving histone acetylation and histone and DNA methylation, chromatin remodeling and expression of a complex set of stress-regulated and disease-associated genes. In addition, both elevated glucose concentration and insulin resistance leave a robust effect on epigenetic reprogramming of the endothelial cells too, since endothelium associated with the eye aids in maintaining the vascular homeostasis. Furthermore, several studies conducted on the disease suggest that the modifications of the epigenome might be the fundamental mechanism(s) for the proposed 'metabolic memory' resulting into prolonged gene expression for inflammation and cellular dysfunction even after attaining the glycemic control in diabetics. Henceforth, the present review focuses on the aspects of genetic and epigenetic alterations in genes such as vascular endothelial growth factor and aldose reductase considered being associated with DR. In addition, we discuss briefly the role of the thioredoxin-interacting protein TXNIP, which is strongly induced by high glucose and diabetes, in cellular oxidative stress and mitochondrial dysfunction potentially leading to chromatin remodeling and ocular complications of diabetes. The identification of disease-associated genes and their epigenetic regulations will lead to potential new drugs and gene therapies as well as personalized medicine to prevent or slow down the progression of DR.

Background

Diabetes, obesity and pre-diabetes are increasing enormously around the globe with multiplying of middle class families in developed as well as in developing countries together fueled by modern sedentary work-related physical inactivity and easy access to processed high calorie foods. Nonetheless, the disease initiation and progression of diabetes and its complications can’t be fully explained by known genetic mutation and polymorphisms alone in these diverse populations thereby advocating an environmental factor that influences the disease-associated gene functions. Thus, epigenetics is considered as a phenomenon that is beyond genetics, having its association with development involving interaction of numerous genes with each other and with the environment without changes in DNA sequences. Nonetheless, in the course of 50 years till date, the significance of the term “epigenetics” has itself suffered an evolution that equals our amplified knowledge of the molecular mechanisms essentially regulating the gene expression in eukaryotes [1]. Waddington in the year 1942 coined the term “epigenotype” to initiate the study of fundamental mechanisms like DNA methylation, RNA regulation and histone alterations [2]. These mechanisms alter the regular metabolic processes by heritable gene silencing and also do not cause any changes to nucleotide sequences [3]. In a study by Holliday, cytosine methylation was observed in DNA and consistent suppression of gene expression in higher order organisms [4] due to this epigenetic DNA modification implying its significant influence on tissue-specificity and the process of gene silencing and expression. Likewise, numerous nutritional and environmental studies exemplify the influence of epigenetic modifications in an organism. An African-American study demonstrated epigenetic factors similar to psychological stress and social context that are correlated with swelling and infection in heart diseases and strokes [5]. Previous studies validated the contribution of epigenomics in the therapeutics of breast cancer [6] in which it was observed that several dietary chemo-preventive agents (retinoids/Vitamin A, green tea, Vitamin D, etc.) acted on the miRNA signaling pathways, so as to obstruct the uncontrolled metabolic mechanisms of breast cancer. Certainly, literature available has also concluded that dietary supplements and environmental conditions have contributed to the diverse mechanistic patterns involved in epigenomics during the initial and advanced stage of obesity [7,8] as well.

In the same way, in early 90s Hales and Barker described a vital role of epigenetic modifications in the development of diseases, elucidating
Type 2 diabetes (T2D) as the major breakthrough [9] since T2D has been a foundation of disability and morbidity associated to the vascular complications prevailing the onset and development of neuropathy, retinopathy, ischemic heart disease, nephropathy, and peripheral vasculopathy. This was the major hypothesis, deep-rooted by various epidemiological case studies further investigating the consequences of insulin resistance in postnatal, childhood and adulthood tenure [10-14]. Thus, the studies conducted so far towards an association between genetic and environmental factors in the development of diseases indicate epigenetic alterations [15,16] and have an important role in sustained metabolic changes in diabetes leading to its complications.

Supplementary to the above studies, the stability of DNA is a question of great concern while epigenetic modifications are dynamic and reversible in nature and henceforth are the most potent and promising targets for the pharmacological mediations. According to the statistics of International Federation of Diabetes (IFD) [17,18] more than 382 million people were affected with diabetes in the year 2013, and the remaining were left undiagnosed. It has also being estimated that the number of cases is expected to rise to 592 million till 2035, this will thus suppress the health, life span and productivity of an individual. There will be an enormous burden on health costs across the globe and not only this; it will affect quality of life, socio-economic status, lifestyle and pathological manifestations of an entity. The progressive role of diabetes on macro- and microvascular complications has become a great concern for the scientific community. The hallmarks for diabetes are the defective insulin secretion/resistance, resulting into hyperglycemia. Recently, American Diabetes Association (ADA) had published its guidelines for diabetic care, which is contemplative over the use and exploration on the needs to individualize treatment objectives and plans [19]. Therefore, diagnosis, treatment, prevention on the genetic, phenotypic and clinical manifestations of a particular individual forms the foundation of personalized or precision medicines, with management strategies to combat the disease more effectively.

Over-and-above, ADA explained three subtypes of diabetes [19] viz., Type 1 diabetes (T1D), Type 2 diabetes, and gestational diabetes. Of these, T2D is the prime and prevalent diabetes covering 90% of all cases, thus causing indisposition and mortality in the developed and developing nations [17]. Also, the complications of this emerging disease is dreadful, consequences of which can be identified and diagnosed on endothelial tissues and cells of retina, peripheral neurons, cardiac and renal organs of an individual. The molecular mechanisms and glucose abundant pathways are complex but may include an elevated hexosamine pathway flux, polyol pathway, diacylglycerol PKC pathway, AGE-RAGE pathway and mitochondrial dysfunction, oxidative stress, and bioenergetics failure (Figure 1). In addition, the thioredoxin-interacting protein (TXNIP), which binds to thioredoxin (Trx) and inhibits it's thiol reducing and oxidant scavenging capacity, has recently been shown to involve in cellular oxidative stress, NLRP3 inflammasome activation, inflammation, and apoptosis of pancreatic β cells and other cell types in diabetes suggesting a critical role for TXNIP in diabetes and its complications [20,21].

Moreover, for epigenetic mechanisms there has been various marks; one such major, countable and primary mark is DNA methylation,
which involves the addition of a methyl group to the DNA fragment at nucleotide cytosine [16, 22, 23]. DNA methyltransferases, DNMT1 and DNMT3A/B, use S-adenosylmethionine (SAM) as methyl donors to cytosine. Cytosine methylation, in general, represents repressive DNA via chromatin closing [24-27].

Second still prominent is histone modifications such as arginine methylation; lysine methylation and acetylation [28, 29] and alterations in ncRNAs (miRNAs, piwi RNAs, and long non-coding RNAs). These are the principle components involved in the epigenetic gene regulation of diabetes [30] and its complications. Histone acetyltransferases (HAT) adds an acetyl group to histone lysine using acetyl-coA as a substrate while histone deacetylases (HDACs) remove the acetyl group. Histone acetylation is a marker for chromatin opening and gene transcription.

Conversely, histone lysine or arginine can be alternatively methylated using histone methyltransferases and SAM as substrates. Histone methylation and DNA methylation condenses chromatin, making them inaccessible to transcription factors and co-factors, thereby inhibiting gene transcription or silencing [24]. Consitt et al. [31] and Liu et al. [32] studied interactions amidst epigenetic and environmental factors (lifestyle and principally dietary practices), in the progression of T2D and its complications.

However, diabetes-specific macro- and microvascular diseases in the glomerulus, retina, and vasa nervorum have comparable pathophysiological features. In the initial course of diabetes, intracellular hyperglycemic condition causes anomalies in blood flow thus increasing vascular permeability. This reveals reduced action of vasodilators such as nitric oxide, and amplified activity of vasoconstrictors like angiotensin II and endothelin-1, and amplification of the permeability factors such as vascular endothelial growth factor (VEGF). Similarly, the polymorphic activities in the promoter region of the VEGF gene along with aldose reductase (ALR) 2 gene run parallel with the pathogenesis of diabetic nephropathy [33] and might have its effect on retinopathy as well. In addition, both hypoxia and hyperglycemia enhances VEGF and its receptor expression, because of which elevated VEGF have been demonstrated in diabetic retinas leading to the chronic retinopathy complications [34-38]. Besides VEGF, some of the databases available also provides information that aldose reductase (aka aldehyde reductase) is expressed in most of the permeability factors such as vascular endothelial growth factor (VEGF). Similarly, the polymorphic activities in the promoter region of the VEGF gene along with aldose reductase (ALR) 2 gene run parallel with the pathogenesis of diabetic nephropathy [33] and might have its effect on retinopathy as well. In addition, both hypoxia and hyperglycemia enhances VEGF and its receptor expression, because of which elevated VEGF have been demonstrated in diabetic retinas leading to the chronic retinopathy complications [34-38].

Henceforth, the intent of the review is to provide an overview of genetics and epigenetics involved in the metabolic pathway of diabetes and its complications prominently converging on diabetic retinopathy, leading to the notion of personalized medicines, concentrating over patient centric approach in conclusion. The article also comprises of the discussion of some candidate genes and their pathway connectivity. Previous findings suggest that the two candidate genes VEGF and ALR belong to the families that are closely associated (mutations/alterations/modifications) with diabetes and its complications (retinopathy). In addition, recent finding that TXNIP is strongly induced in pancreatic β cells and other tissues including the retina has proposed to be a potential target for diabetes and its complications. TXNIP has been defined as a pro-oxidative stress, pro-inflammatory and pro-apoptotic protein in diabetes and under hyperglycemic conditions. In short this article revolves around the polymorphic depiction of candidate genes, interaction with environment causing epigenetic changes, and their potent association with the susceptibility of retinopathy [40] and associated complications.

**Insulin sensitivity**

Capability of pancreatic β cells to secrete and produce insulin in response to glucose fluctuations is one of the main features to regulate glycermia in normal entities. Table 1 depicts some epigenetic molecules that aid the treatment and therapeutics of DR either being anti-inflammatory or delaying the onset of nephropathy and retinopathy or initiating or hindering β cell differentiation. Throughout the commencement and growth of DR, the need for insulin increases because of the increased insulin resistance in the body as seen in T2D or lack of insulin in T1D. Henceforward, insulin production, cell viability, and secretion potential are mechanisms that distress the pancreatic β cell and their functions. Studies also anticipated that the altered DNA methylation patterns (genome-wide) of human cells are obtained from the pancreatic islets of the deceased donors [41,42]. Current findings suggest that from a total of 1649 CpG sites corresponding to 853 genes, there have been alterations in the level of DNA methylation patterns in pancreatic islets from diabetic T2D patients versus non-diabetic individuals. Likewise, there were 102 genes presenting distinct DNA methylation that directed towards the conclusion of modified mRNA expression between the non-diabetic and diabetic patients (T2D), signifying epigenetic regulation of transcriptional activity [41,42].

**Diabetic retinopathy**

DR is becoming the foremost reason of blindness among the working individuals in the developed countries and among the elderly individuals in the developing countries. With the worldwide dominance of diabetes being anticipated to intensify to 438 million subjects by the year 2030, DR will undoubtedly pose as one of the major public health concerns [43,44]. The warning signs for the occurrence of DR are increased blood sugar levels, hazy vision, sudden loss of vision, etc. [45]. DR may lead to macular edema when blood and fluid leak into the retina caused by swelling of the central retina [46]. Clinically, the occurrence of DR is manifested by the advent of retinal microvascular lesions.

An initial change include hard exudates, intra-retinal microvascular abnormalities, hemorrhages, cotton wool spots, microaneurysms, and beading in the veins thus illustrating non-proliferative diabetic retinopathy (NPDR). The most severe form of DR is its proliferative form, as proliferative diabetic retinopathy (PDR) that is noticeable by the formation of irregular fragile and friable new blood vessels, which are susceptible to hemorrhage outflow more often as a final point, visual impairment results [44].

With an advent of diabetes and its duration, DR proliferates with various clinical complications, though the initial glycemic control can delay the effect and expansion of DR, it cannot stop the progression of DR [44,47,48]. Thus, the phenomenon of metabolic memory or epigenetic memory has been proposed for the aberrant gene expressions even after normalization of blood glucose once a specific period of hyperglycemic exposure had previously occurred [24-27,49]. To contest this disease therefore candidate gene approach is an essentiality to study the pathogenic mechanisms underlying DR [50-52].

Consistently, numerous genes involved in DR pathways have been
Table 1. Epigenetic molecules of latent interest for diabetes treatment.

| Epigenetic molecules | Activity | Effect | Reference |
|----------------------|----------|--------|-----------|
| Trichostatin A       | HDACi    | Anti-inflammatory | [162] |
|                      |          | Insulin sensitivity restoration | [163] |
|                      |          | Nephropathy onset delay | [164] |
|                      |          | Retinopathy onset delay | [165] |
|                      |          | \(\beta\)-cell differentiation | [166] |
|                      |          | Glucose uptake | [167] |
| Vorinostat (SAHA)    | HDACi    | Anti-inflammatory | [168] |
|                      |          | Nephropathy progression | [169] |
| Givinostat (ITF2357) | HDACi    | Anti-inflammatory | [170] |
|                      |          | Increased insulin secretion | [171] |
| THS-78–5            | HDACi    | Cyto-protective effect | [172] |
| Scriptaid           | HDACi    | Insulin sensitivity restoration | [173] |
| MS275               | HDACi    | Insulin sensitivity restoration | [174] |
| Sodium butyrate     | HDACi    | \(\beta\)-cell differentiation | [175] |
| MC1568              | HDACi    | \(\beta\)-cell differentiation | [176] |
| ANAC                 | HATi     | Glucose uptake | [177] |
| Garcinol            | HATi     | Anti-inflammatory | [178] |
| Curcumin            | HATi     | Decreased ECM proteins diabetic vascular complication | [179] |
| SPV106              | HATi     | Rescue of diabetic phenotype | [180] |
| 5-Aza-cytidine       | DNMT inhibitor | Ngn3 inducer | \(\beta\)-cell differentiation | [181] |
| Indolactam V        | Pdx1 inducer | Ngn3 inducer | \(\beta\)-cell differentiation | [182] |
| Retinoic acid       | Ngn3 inducer | Ngn3 inducer | \(\beta\)-cell differentiation | [183] |
| BRD7552             | Pdx1 inducer | Ngn3 inducer | \(\beta\)-cell differentiation | [184] |
| WS6                 | icB kinase Activator | Ngn3 inducer | \(\beta\)-cell proliferation | [185] |

**Retinopathy and candidate genes**

**Vascular endothelial growth factor gene (Human chromosome 6p12)**

Endothelial growth factors that are involved in the vascular activities function as signaling proteins for both de novo development of the embryonic circulatory system and for the growth of new blood vessels from the already existing vasculature. Secretion of VEGF in the retina of an organism is primarily done from retinal pigmented epithelial cells, Müller glial cells, astrocytes, endothelial cells and pericytes. This growth factor consists of several members such as VEGF-A, VEGF-B, VEGF-C, VEGF-D and PGF (placental growth factor) [64,65]. The numerous polymorphic activities specifically identified and regulated in the promoter regions of the gene also make it a promising gene model to study the vascular complications and the disease associations. These events are indulged in the signaling pathways associated with the metabolic regularities and irregularities of an organism. VEGF being an attractive candidate gene for the study of DR is associated with the development of diabetic macular edema (DME). This event is in association with the polymorphic activity of C-634G which is present in Japanese [66,67] as well as in Indian populations [62,67,68]. Among the referred studies [66], 378 patients with T2D were examined, out of which 203 patients had no retinopathy, 93 had NPDR, and 82 had PDR. The polymorphic study demonstrated that macular edema was present in 16 patients with NPDR and 47 patients with PDR [66]. Other studies had also instigated the role of other VEGF-SNPs in retinopathy initiated with an advent of early diabetes [69,70] leading to the chronic complications. Moreover, at present several clinical trials are exploring and inspecting the effectiveness of anti-VEGF molecules to aid in the treatment of diabetic retinopathy.

**History of VEGF molecule:** In 1948, Michaelson discussed a vital event in his studies that "in the pathological angiogenesis, there has been observed a secretion and synthesis of a diffusable factor known as "Factor X" by dint of the ischemic retina" [71]. Later in 1971 studies conducted by Folkman, demonstrated the inhibition of angiogenesis for the treatment of cancer, that paved the way to unearthing the anti-angiogenic factors [72]. Outlying studies in 1983 by Senger et al. discovered that a protein mediator is being secreted from the guinea pig tumor cell line that has its active involvement in angiogenesis. This protein has the efficiency to persuade vascular leakage which is why it has been named as Vascular Permeability Factor (VPF) [73].

Similarly in 1989, Ferrara and Henzel acknowledged a molecule existing in bovine pituitary follicular cells and termed it as Vascular Endothelial Growth Factor [74]. Consequently, via cloning of VEGF and VPF confirmed that the two factors have the same tendency and are actually the same proteins [65,75,76].

Substantiations from the previous clinical studies had supported the acute role of VEGF in ophthalmic neovascularization. Further, it was also concluded that the reason behind the elevation of VEGF level in vitreous samples of patients was active proliferative diabetic retinopathies [77] and its associated complications.

**VEGF action:** Transphosphorylation is a mechanism that activates dimers of tyrosine kinase receptors. These receptors are present on the endothelial cell surface and binds to the VEGF members in turn stimulating the cellular responses. The first member of the VEGF family: VEGF-A is having 2 type of receptors namely, VEGF receptor 1 (VEGFR-1) and 2 (VEGFR-2). VEGFR-1 is a protein present in humans that is encoded by Flt-1 gene [78] and VEGFR-2 is a receptor...
that has the kinase insert domain which is encoded by KDR [79].

Primary receptor that facilitates the cellular responses to VEGF-A is VEGFR-2. Almost all the receptors are compiled of three parts:

1. An extracellular portion, be made up of seven immunoglobulin-like domains (similar)
2. A transmembrane hydrophobic spanning region (single)
3. An intracellular portion comprising of a tyrosine kinase domain (split) [80].

A cellular signal is transduced when the molecule (VEGF) binds to the extracellular portion of the receptors that is having the immunoglobulin-like domains; this causes the intracellular portion to process phosphorylation of the tyrosine residues, this in turn causes a cascading effect in signaling pathways [81].

**VEGF in DR:** VEGF plays a dynamic role in the neovascularization in PDR and also in the collapse of blood-retinal barrier, during the emergence of macular edema in diabetic patients [67], in turn altering the permeability of retinal capillaries by enhancing the content of phosphorylation of proteins indulged in the tight-junctions like zonula occludens [82]. Significantly, elevated vitreous levels of VEGF molecules had been a major setback reported in the patients suffering from DR [67,83]. Induction of VEGF molecules activates mitogen-activated proteins, causing the proliferation of endothelial cell. This signaling cascade overlaps with the stimulation of phosphatidylinositol 3-kinase pathway after the induction of VEGFR-2 [84].

Another VEGF molecule is VEGF-A which initiates endothelial cells to discharge matrix metallo-proteinases and urokinase-plasminogen activator that results in the degradation of membranes including the development and penetrability of the vasculatures. This in turn causes a counter-attack for the disease [87].

**Inhibitors of VEGF receptor expression:** Aflibercept (Regeneron Pharmaceuticals Inc. and the Sanofi-aventis Inc.) is a complete human fusion protein (recombinant) that muddles with all VEGF-A molecules [88]. This drug has been developed for its utilization in ocular diseases by Regeneron and Bayer Inc. (Leverkusen, Germany) companies. Further, the DA VINCI approach exhibited promising conclusions as compared to laser photoocoagulation in cases of DME [89,90].

**Anti-VEGF antibodies:** Bevacizumab (Avastin, Genentech Inc.) is a humanized monoclonal antibody contrary to all VEGF-A molecules, thus preventing the receptor binding. This monoclonal antibody effectively aid in obstructing neovascularization leading to various retinal diseases such as PDR, DME, macular edema and neovascular glaucoma [91]. Furthermore, Ranibizumab (Lucentis, Genentech Inc.) is also a humanized monoclonal antibody (mab) fragment recovered from the parent molecule of bevacizumab in contrast to VEGF-A [92]. This mab was aimed for improved intra-ocular penetration into the retina [93].

**Inhibitors of extracellular VEGF:** A RNA aptamer, Pegaptanib sodium (Macugen, Eyetech Pharmaceuticals Inc. and Pfizer Inc.) has been considered as the earliest anti-VEGF drug that has been approved for the treatment of neovascular diseases by binding and blocking the isoform 165 of VEGF family [94].

**Aldose reductase gene (ALR/ALR2/ AKR1B1, Human Chromosome 7q35):** Aldose reductase (EC 1.1.1.21) is the leading enzyme in polyol pathway. ALR is a cytosolic, oxidoreductase (monomer) that performs the catalysis of various carbonyl compounds via NADPH-dependent reduction, including its prime target glucose. It has a crystal structure, single domain, 8-stranded comparable via NADPH-dependent reduction, including its prime target glucose. It has a crystal structure, single domain, 8-stranded comparable domain, 8-stranded comparable like domains (similar)

**CELLULAR GLUCOSE UTILIZATION IN HUMAN BLOOD:** The major metabolic pathway is polyol pathway connecting hyperglycemia to mellitus tissue complications and aldose reductase enzyme. In this ALR acts as the primary and the rate limiting biocatalyst [50,67,108] in which glucose in the presence of nicotinamide adenine dinucleotide phosphate is reduced to molecules of sorbitol by the action of ALR, which is then converted to fructose by the enzyme sorbitol dehydrogenase and nicotinamide riboside acting as a cofactor [109,110]. It may also be speculated that intracellular fructose can be phosphorylated (Fructose-6-phosphate) and flux through the hexosamine pathway thus experiencing the increase in the UDP-GlcNAc level and protein Ser/Thr-O-GlcNAcylation of histones, kinases and transcription factors [111].

Due to the cellular toxicity of hyperglycemic patients, polyol pathway is held responsible, at least in part, for the development of chronic complications of diabetes. This pathway becomes active primarily when there has been an increment in the level of intracellular glucose [110,112-113]. The biocatalysts of this polyol pathway
are present in the tissues of human suffering from diabetes and its complications [114,115].

Discussing the flux mechanism of polyol pathway, the sorbitol present in the organism does not diffuse across the cell membranes easily, which has paved the way for osmotic damage to microvascular cells [96]. During this course of non-diffusive behavior of sorbitol, intra-cellular accumulation of sorbitol initiates, leading to occurrences of osmotic stress [67,116]. Further, with the exhaustive analysis of early studies it was revealed that sorbitol is converted to fructose via sorbitol dehydrogenase, with NAD+ getting reduced to NADH. Detrimental effects of this pathway are: sorbitol-induced osmotic stress, decreased sodium/potassium (ATPase) activity, an upsurge in cytosolic NADH and a drop in cytosolic NADPH [96]. Supplementary to this is the formation of microneurysm in animal models, with pericyte loss and basement membrane thickening [50]. There has been three ALR SNPs that are associated with DR: SNP rs739853, the (CA)n microsatellite polymorphism, and SNP rs9640883 [38,117]. Additionally, considering the facts and results of past studies it can be viewed that the influence of polyol pathway to diabetic hitches may be site specific, tissue and species dependent [96,107].

**ALR as a therapeutic target:** Inhibition of polyol pathway in **in vivo** studies brought forth uneven results. In a five-year study conducted on canine species, it was observed that inhibition of ALR gene prevented diabetic symptoms and complications up to an extent specifically in neuropathy, but was unsuccessful in case of proliferative diabetic retinopathy [96,118]. Also, this positive effect of ALR inhibition on neuropathy had given rise to an effective and promising inhibitor Zenarestat against the mechanistic action of ALR [119].

In addition, synthetic ALR inhibitors are carboxylic acid inhibitors, for example Ponalrestat, Tolerestat and Zopolrestat. The former shows the low target permeability and are not effective in **in vivo** studies and the latter, besides having the enhanced target penetrating capability but, has depicted skin reaction and toxicity in the liver [110, 120-122]. Still the clinical use of ALR inhibitors is yet to be established.

**Genome Wide Association Studies (GWAS)**

Interpretation and explanation of genetics of DR has been in an infancy stage the reason being an individual’s predisposition to diabetes is not entirely explored and the inheritance of genetic risk variants and their vulnerability to environmental factors are one of the key factors to understand the mechanism of this up-surring disease. A genome wide association study entails high density sampling of common human gene variation. Large-scale GWAS analyses in case of familial inheritance have facilitated for the identification of numerous genetic variants conferring threat to diabetes [18]. Hence, GWAS studies are boundless having ample facts of particular genes and the prospective to ascertain biological effects of genes [123-125].

Besides GWAS, linkage studies tend to focus on the transmission of causative genes in families; while the GWAS identify genetic variants in the diseased population versus healthy individuals. In case of T2D leading to DR, the implications have been insightful as the GWAS namely KCNJ11, PPARG, HNF1B, IRS1, HNF1A, and HNF4A. Most of the disease-causing variants are associated with defective working of pancreatic β-cells, involved as a major factor in the pathology of T2D [128] and its complications. So far, vital genes associated with GWAS of different populations include IGF2BP2, SLCO3A8, HHEX, KCNQ11, CDKN2A/B, HMGAA2 and NOTCH2-ADAM30 [129].

A directory of all major GWAS studies has been maintained via National Human Genome Research Institute and can be gained access through their website (https://www.genome.gov/) for further exploration of research and development.

**Diabetes, mitochondrial stress and epigenetics**

Mitochondria are the powerhouse of the cell involved in oxidative phosphorylation and bioenergetics, i.e., the production of adenosine triphosphate (ATP) via its electron transport chain (ETC), which also generates reactive oxygen species. In addition, the mitochondrion also involves in the production epigenetic substrates such as acetyl-coA and betaine as methyl donor in the methionine cycle and S-adenosylmethionine (SAM) biosynthesis. These mitochondrial metabolites such as ATP, acetyl-coA and SAM are known epigenetic substrates. Recently it has been shown that Ser/Thr-O-GlcNAcylation of histones represents potential epigenetic histone codes [111]. Furthermore, mitochondrial tricarboxylic acid (TCA) cycle metabolites and NAD+ have strong influence on histone remodeling via modification of histone acetyltransferases and deacetylases. We and others have shown that under hyperglycemia and diabetes, TXNIP is strongly induced in pancreatic β cells as well as in the retina [20,130]. TXNIP binds to thioredoxin (Trx1 in the cytosol and nucleus and Trx2 in mitochondria) and inhibits its thiol reducing and oxidant scavenging activity thereby causing cellular oxidative/nitrosative stress, NLRP3 inflammasome activation, inflammation, and apoptosis [131]. We also have shown that TXNIP is involved in epigenetic histone modification of pro-inflammatory genes in retinal endothelial cells under hyperglycemia [132]. Furthermore, the TXNIP promoter is under the control histone acetylation (H4K8Ac) and TXNIP expression is highly induced by trichostatin A, a histone deacetylase inhibitor, in retinal endothelial cells, but 5-azacytidine, a DNA methyltransferase inhibitor, was without an effect [130,132].

Epigenetics, as described before, has been distinguished as heritable alterations in gene function that come about without a change in the nucleotide sequence (ref. 24 and Figure 2) by modifying histones (post-translational modification by acetylation, methylation, phosphorylation and others as epigenetic histone marks) and by alterations in DNA methylation patterns [133] and chromatin remodeling [24,26]. These histone and DNA modifications are achieved by different enzyme epigenetic writers (add marks), erasers (remove) and readers (binding proteins). Therefore, these changes are possibly reversible and controlled by the cell environment such as toxins, dietary habits, chronic hyperglycemia (diabetes) or pharmacological medications [134]. Studies also have shown that non-coding RNA sequences, including microRNAs, piwi-RNA and long non-coding RNAs [135], participate in the epigenetic gene expression modulation.

Dysfunctional or depolarized mitochondria produces less ATP but generate more ROS causing mitochondrial protein, mtDNA and lipid damage [26,136]. Under these conditions, defective mitochondrial metabolism may have a greater influence on epigenetic substrate generation and therefore nuclear as well as mitochondrial epigenome
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Figure 2. Hyperglycemia-induced TXNIP upregulation, inhibition of Trx1/Trx2 and mitochondrial stress may alter epigenome regulation by changing histone and DNA epigenetic substrates in DR [24]. Some histone H3 and promoter DNA modification examples are shown. In general, histone acetylation and phosphorylation are activation marks while lysine methylations are repressive marks, although H3K4Me is an activation modification. Cytosine methylation at the CpG islands in proximal promoters is a repressive DNA transcriptional mark. Environmental factors such as diet, exercise, and sedentary lifestyle can influence epigenetics and gene expression in aging-related disorders including diabetes and neurodegenerative diseases.
molecular and cellular biology of the diabetic complications. First, the foremost is the phenomenon of metabolic or epigenetic memory, which refers to the remembrance of hyperglycemic episodes inducing microvascular modifications during the normal homeostasis or after glucose normalization [23,26,27]. Second, another aspect is the genetic determinants of susceptibility to macro- and microvascular complications involved in diabetic patients. Thus, to concentrate on this issue, gene mapping studies should be designed to identify predisposition to complications as well as interactions of these genes with the metabolic factors [96,144].

Third, next generation DNA/RNA sequencing (NGS) may provide assistance by identifying exceptional genetic variants having the significant effects on T1D and T2D sufferers that could aid in the early detection of diabetic complications [18]. Epigenomics, transcriptomics, proteomics, metabolomics and systems biology are also rapidly developing techniques that are fitted inside the personalized or precision medicine toolbox. In addition, restricting the overproduction of superoxides by mitochondrial electron-transport pathway, together with activation of NADPH and xanthine oxidases, would put on an equal footing as controlling the polyl pathway, hexosamine flux, PKC activation, AGE formation, and NF-κB activation, inflammation and overall glycemic control [96,145]. Functional studies are also in need to progress at a rapid pace so as to translate these findings into clinical practice. Thus, mitochondrial cell permeable antioxidant therapies may prove to be critical in maintaining mitochondrial bioenergetics, metabolism and epigenetics in diabetes and preventing its complications [146-148]. Finally, the newly acquired CRISPR-Cas9 or dCas9-mediated genome editing approaches [149,150] will also prove to be powerful approaches to correct epigenetic and genetic aberrations in diseases that involve metabolic or epigenetic memory by targeting histone and DNA modifying enzymes and their binding proteins, especially in treating diabetic ocular complications. The retina is a relatively immune privileged and confined organ therefore gene therapy approaches are most suitable via an intravitreal delivery method. Thus, it is an exciting time for epigenetic exploration and ocular gene therapy in DR, which is just beginning to scratch the proverbial tip of the iceberg.

Therapeutic opportunities

The upsurge of diabetic complications demands for novel therapeutic approaches and development of evidence-based and stage-specific drugs. Current drug library available to hit the diabetic complications specifically T2D, involve numerous mechanisms like: blocking the carb digestion; hindering the hepatic glucose production; stimulation in the secretion of pancreatic insulin, etc. Some of the anti-diabetic drugs and their functions are given in Table 2. Nonetheless, the extent to which these drugs work at the epigenetic level is yet to be determined.

Prior studies have also pointed out the causal relations between diabetes and epigenetic modifications [26,151-154]. These include a large variety of molecular inhibitors and/or activators of the enzymatic machinery, signaling factors and involvement of growth factors and their SNPs as well, that could slow down the early or late onset of diabetes and its related chronic complications [155,156].

**Personalized medicines**

Personalized medicines have vital characteristics so as to tailor the best fit therapies for an individual to treat. Since patients are of diverse subsets, they have varying clinical considerations and features. Factors influencing the treatment goals and strategies include age, gender, diabetes duration, epigenetics, diabetic complications and the presence of comorbidities (cardiovascular diseases, obesity, etc.). For instance, treatment varies from individual to individual, the treatment plan for an individual with an early onset of diabetic complications, who is actually at an increased risk will vary from the patient having late onset of the disease due to prolonged exposure to hyperglycemic conditions [18,157].

Although there has been a limited scope for disease diagnosis, the genetic information of an individual might help to identify the risk involved and aid in differentiating among individuals benefiting with a certain treatment or not. For example, sulfonylureas show enhanced activities over the insulin therapy for the individuals having KCN11 mutations (ATP-sensitive potassium channel Kir6.2), that is causing diabetes of neonatal [158], while those individuals with glucokinase (GCK) mutation, persist unresponsiveness with anti-diabetic agents for the control of glycemia [159].

Considering the case of T2D, lifestyle modifications of the individuals at risk can aid in preventing or delaying the growth of T2D [160]. This could also correlate well with the severe diabetic complications and issues such as retinopathy and cardiovascular disease [161]. The degree and extent of epigenetic alterations and marks (histone and/or DNA modifications) may also be different from individual to individual as its marks will be dependent both on genetics and personal life style maintenance – particularly diet and physical activity. Hence, epigenetic studies complementation with GWAS, proteogenomics and metabolomics will add in achieving precision or personalized medicine tailored to suit individual epigenetic and metabolomics profiles.

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Conflict of interest
All authors declare no conflict of interest.

References
1. Choudhuri S (2011) From Waddington’s epigenetic landscape to small noncoding RNA: some important milestones in the history of epigenetics research. Toxicol Mech Methods 21: 252-274.
2. Waddington CH (2012) The Epigenotype. Endeavour. 1942. Int J Epidemiol 41: 10-13. [Crossref]
3. Egger G, Liang G, Aparicio A, Jones PA (2004) Epigenetics in human disease and prospects for epitogen therapy. Nature 429: 457-463. [Crossref]
4. Holliday R (1987) The inheritance of epigenetic defects. Science 238: 163-170. [Crossref]
5. Saban KL, Mathews HL, DeV on HA, Janusek LW (2014) Epigenetics and social conflict of interest.
6. Egger G, Liang G, Aparicio A, Jones PA (2004) Epigenetics in human disease and implications for disparity in cardiovascular disease. Aging Dis 5: 346-355. [Crossref]
7. Xu F, Zhou X, Shen F, Pang R, Liu S (2012) Decreased peripheral blood mitochondrial DNA content is related to HbA1c, fasting plasma glucose level and age of onset in type 2 diabetes mellitus. Diabetic Medicine 29: e47-e54. [Crossref]
8. Ritzov VB, Menshikova EV, He J, Ferrell RE, Goodpaster BH, et al. (2005) Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. Diabetes 54: 8-14. [Crossref]
9. Irin AK, Kodamullil AT, Gündel M, Hofmann-Apitius M (2015) Computational Modelling Approaches on Epigenetic Factors in Neurodegenerative and Autoimmune Diseases and Their Mechanistic Analysis. J Immunol Res 2015: 737168.
10. Hales CN, Barker DJ (1992) Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 35: 595-601. [Crossref]
11. Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, et al. (1991) Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 303: 1019-1022. [Crossref]
12. Barker DJ, Hales CN, Fall C, Osmond C, Platts K, et al. (1993) Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36: 62-67. [Crossref]
13. Lithell HO, McKeigue PM, Berglund L, Mohns R, Lithell UB, et al. (1996) Relation of size at birth to non-insulin dependent diabetes and insulin concentrations in men aged 50-60 years. BMJ 312: 406-410. [Crossref]
14. Rönn T, Ling C (2015) DNA methylation as a diagnostic and therapeutic target in the battle against Type 2 diabetes. Epigenomics 7: 451-460. [Crossref]
15. Eriksson J, Forsen T, Tuomilehto J, Jaddoe V, Osmond C, et al. (2002) Effects of size at birth and childhood growth on the insulin resistance syndrome in elderly individuals. Diabetologia 45: 342-348. [Crossref]
16. Yamada L, Chong S (2016) Epigenetic studies in Developmental Origins of Health and Disease: pitfalls and key considerations for study design and interpretation. J Dev Orig Health Dis. [Crossref]
17. Ling C, Group L (2009) Epigenetics: a molecular link between environmental factors and type 2 diabetes. Diabetes 58: 2718-2725. [Crossref]
18. Cho NH, Whiting D, Guariguata L, Montaya J, Roger HR, et al. (2013) IDF diabetes atlas. Brussels, Belgium: International Diabetes Federation.
19. Siddiqui K, Tyagi S (2015) Genetics, genomics and personalized medicine in Type 2 diabetes: a perspective on the Arab region. Personalized Medicine 12: 417-431. [Crossref]
20. American Diabetes Association (2014) Standards of medical care in diabetes–2014. Diabetes Care 37 Suppl 1: S14-S80. [Crossref]
21. Shoaleh A (2014) Mintreview: Thioredoxin-interacting protein: regulation and function in the pancreatic β-cell. Mol Endocrinol 28: 1211-1220. [Crossref]
22. Singh LP (2013) Thioredoxin Interacting Protein (TXNIP) and Progression of Diabetic Retinopathy. J Clin Exp Ophthalmol 4: 1-29. [Crossref]
23. Kreuz S, Fischle W (2016) Oxidative stress signaling to chromatin in health and disease. Epigenomics 8: 843-862. [Crossref]
24. Campbell SA, Hoffman BG (2016) Chromatin Regulators in Pancreas Development and Diabetes. Trends Endocrinol Metab 27: 142-152. [Crossref]
25. Perrone L, Matrone C, Singh LP (2014) Epigenetic modifications and potential new treatment targets in diabetic retinopathy. J Ophthalmol 2014: 789120. [Crossref]
26. Reddy MA, Zhang E, Natarajan R (2015) Epigenetic mechanisms in diabetic complications and metabolic memory. Diabetologia 58: 443-455. [Crossref]
27. Chen Z, Xiao F, Paterson AD, Lachin JM, Zhang L, et al. (2016) Epigenomic profiling reveals an association between presence of DNA methylation and metabolic memory in the DCCT/EDIC type 1 diabetes cohort. Proc Natl Acad Sci USA 113: E3002-3011. [Crossref]
28. Cheng X, Blumenthal RM (2010) Coordinated chromatin control: structural and functional linkage of DNA and histone methylation. Biochemistry 49: 2999-3008. [Crossref]
29. Hashimoto H, Vertino PM, Cheng X (2010) Molecular coupling of DNA methylation and histone methylation. Epigenomics 2: 657-669. [Crossref]
30. Muhonen P, Holthofer H (2009) Epigenetic and microRNA-mediated regulation in diabetes. Nephrol Dial Transplant 24: 1088-1096. [Crossref]
31. Consiti LA, Bell JA, Koves TR, Musio DM, Hulver MW, et al. (2010) Peroxisome Proliferator–Activated Receptor-γ Coactivator-1α Overexpression Increases Lipid Oxidation in Myocytes From Extremely Obese Individuals. Diabetes 59: 1407-1415. [Crossref]
32. Liu MM, Chan CC, Tuo J (2013) Epigenetics in ocular diseases. Curr Genomics 14: 166-172. [Crossref]
33. Yang B, Cross DF, Ollerszenshaw M, Millward BA, Demaine AG (2003) Polymorphisms of the vascular endothelial growth factor and susceptibility to diabetic microvascular complications in patients with type 1 diabetes mellitus. J Diabetes Complications 17: 1-6. [Crossref]
34. Ishida S, Shinoda K, Kawashima S, Oguchi Y, Okada Y, et al. (2006) Coexpression of VEGF receptors VEGF-R2 and neuropilin-1 in proliferative diabetic retinopathy. Invest Ophthalmol Vis Sci 41: 1649-1656. [Crossref]
35. Simó R, Carrasco E, García-Ramírez M, Hernández C (2006) Angiogenic and antiangiogenic factors in proliferative diabetic retinopathy. Curr Diabetes Rev 2: 71-98. [Crossref]
36. Penn JS, Madan A, Caldwell RB, Bartoli M, Caldwell RW, et al. (2008) Vascular endothelial growth factor in eye disease. Prog Retin Eye Res 27: 331-371. [Crossref]
37. Więröszo B, Wong TY, Simó R (2008) Vascular endothelial growth factor and diabetic complications. Prog Retin Eye Res 27: 608-621. [Crossref]
38. Simó-Servat O, Hernández C, Simó R (2013) Genetics in diabetic retinopathy: current concepts and new insights. Curr Genomics 14: 289-299. [Crossref]
39. Safran M, Dalah I, Alexander J, Rosen N, Ivey Stein T, et al. (2010) GeneCards Version 3: the human gene integrator. Nucleic Acids Res 38: 1-6. [Crossref]
40. Safran M, Dalah I, Alexander J, Rosen N, Ivey Stein T, et al. (2010) GeneCards Version 3: the human gene integrator. Database (Oxford) 2010: baq620. [Crossref]
41. Demainé A, Cross D, Millward A (2009) Polymorphisms of the aldose reductase gene and susceptibility to retinopathy in type 1 diabetes mellitus. Invest Ophthalmol Vis Sci 41: 4064-4068. [Crossref]
42. Dayeh T, Volkov P, Saílo S, Hall E, Nilsson E, et al. (2014) Genome-wide DNA methylation analysis of human pancreatic islets from type 2 diabetic and non-diabetic donors identifies candidate genes that influence insulin secretion. PLoS Genet 10: e1004160. [Crossref]
43. Rönn T, Ling C (2015) DNA methylation as a diagnostic and therapeutic target in the battle against Type 2 diabetes. Epigenomics 7: 451-460. [Crossref]
44. Atlas D (2000) International diabetes federation. Hallado en: http://www.idf.org/diabetesatlas/5e/es/prologo.
45. Ng DP (2010) Human genetics of diabetic retinopathy: current perspectives. J Ophthalmol 2010: 7911-916. [Crossref]
46. Robinson R, Barathi VA, Chaurasia SS, Wong TY, et al. (2012) Update on animal models of diabetic retinopathy: from molecular approaches to mice and higher mammals. Dis Model Mech 5: 444-456. [Crossref]
47. [No authors listed] (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N Engl J Med 328: 977-986. [Crossref]
Pradhan P (2016) Genetic and epigenetic modifications in the pathogenesis of diabetic retinopathy: a molecular link to regulate gene expression
Pradhan P (2016) Genetic and epigenetic modifications in the pathogenesis of diabetic retinopathy: a molecular link to regulate gene expression.
179. Chen S, Feng B, George B, Chakrabarti R, Chen M, et al. (2010) Transcriptional coactivator p300 regulates glucose-induced gene expression in endothelial cells. *Am J Physiol Endocrinol Metab* 298: E127-E137. [Crossref]

180. Vecellio M, Spalotta F, Nanni S, Colussi C, Cencioni C, et al. (2014) The histone acetylase activator pentadecylidenemalonate 1b rescues proliferation and differentiation in the human cardiac mesenchymal cells of type 2 diabetic patients. *Diabetes* 63: 2132-2147. [Crossref]

181. Lefebvre B, Belaich S, Longue J, Vandewalle B, Oberholzer J, et al. (2010) 5’-AZA induces Ngn3 expression and endocrine differentiation in the PANC-1 human ductal cell line. *Biochem Biophys Res Commun* 391: 305-309. [Crossref]

182. Chen S, Borowiak M, Fox JL, Maehr R, Osafune K, et al. (2009) A small molecule that directs differentiation of human ESCs into the pancreatic lineage. *Nat Chem Biol* 5: 258-265. [Crossref]

183. Öström M, Loffler KA, Edfalk S, Selander L, Dahl U, et al. (2008) Retinoic acid promotes the generation of pancreatic endocrine progenitor cells and their further differentiation into β-cells. *PLoS One* 3: e2841. [Crossref]

184. Yuan Y, Hartland K, Boskovic Z, Wang Y, Walpita D, et al. (2013) A small-molecule inducer of PDX1 expression identified by high-throughput screening. *Chem Biol* 20: 1513-1522. [Crossref]

185. Shen W, Tremblay MS, Deshmukh VA, Wang W, Filippi CM, et al. (2013) Small-molecule inducer of β cell proliferation identified by high-throughput screening. *J Am Chem Soc* 135: 1669-1672. [Crossref]