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# A Epigenetic Analysis of supervillin. An agent Plays an Active Role in Neovascularization in Case of Type 2 Diabetic Retinopathy

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**ABSTRACT:** Endothelial cell is a key component of blood vessels and related structures. Retinal endothelial cells line that forms the arborizing microvasculature structure across vital organs supplies and drains the neural retina. The anatomical and physiological characteristics of these endothelial cells are committed to task related to nutritional requirements and protection of a tissue critical to vision. The retinal endothelial cell is a key player in retinal ischemic vasculopathies that include diabetic retinopathy and retinopathy of prematurity and retinal inflammation as occurs in posterior uveitis. Neovascularization is the key process that causes vasculopathy and characterizing a molecule which help in neovascularization may be helpful in sorting of the problem. Using gene expression and transcriptome analysis, it has been possible to explore the molecular phenotype of the human retinal endothelial cell and provide a way for understanding of the pathogenesis of Type II Diabetic Retinopathy. In addition to providing assistance for the involvement of well-characterized endothelial molecules, profiling has the power to identify new participant in retinal pathologies.

**KEYWORDS:** Supervillin, Epigenetic, Methylation, Neovascularization, Type II Diabetic Retinopathy.

### I. INTRODUCTION

Endothelial cell is a key component of blood vessels and related structures. Human Retinal endothelial cells line that form the arborizing microvasculature structure across vital organs, supplies and drains the neural retina. The anatomical and physiological characteristics of these endothelial cells are committed to task related to nutritional requirements and protection of a tissue critical to vision. On the one hand, the endothelium must ensure the supply of oxygen and other nutrients to the metabolically active retina, and allow access to circulating cells that maintain the vasculature or survey the retina for the presence of potential pathogens. On the other hand, the endothelium contributes to the blood retinal barrier that protects the retina by excluding circulating molecular toxins, microorganisms, and pro-inflammatory leukocytes. Retinal microvessels are complex structures, to which multiple cell populations contribute. Microvascular dysfunction is associated with retinal ischemia and neovascularization in Type II Diabetic Retinopathy and retinopathy of prematurity, and leukocyte or microbial trafficking and potentiation of retinal inflammation in posterior uveitis. (1). Diseases involving the retinal vasculature, comprises two ischemic vasculopathies, Diabetic Retinopathy and Retinopathy of Prematurity and various posterior forms of Uveitis, are important reasons of blindness in both industrialized countries and developing nations. Type II Diabetic Retinopathy affects approximately one-third of all persons who suffer from diabetes mellitus (2), a disease that is expected to affect 300 million people worldwide by 2025 (3). Retinopathy of prematurity causes for up to one third of childhood blindness, particularly in countries with intermediate infant mortality rates (4). Uveitis is a comparably uncommon disease, but due to an often massive impact

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on vision at a relatively earlier age, its socioeconomic impact is roughly equivalent to that of diabetic retinopathy (5). Type II Diabetic Retinopathy is the most widespread microvascular complication of diabetes and a major cause of vision loss worldwide. All over the world, there are approximately 93 million people who are suffering from Type II Diabetic Retinopathy, 17 million with proliferative Type II Diabetic Retinopathy, 21 million with diabetic macular edema, and 28 million with VTDR (6).

## DIABETIC RETINOPATHY

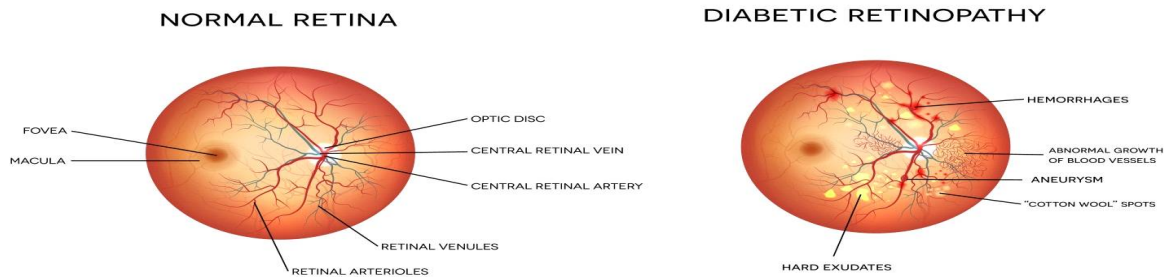


Fig1:- Difference between normal retina and diabetic retina  
<http://www.eyecarevisioncenter.com/eye-health/diabetic-retinopathy/>

Type II Diabetic Retinopathy, a disease of the retina, is the leading cause of acquired blindness in working adults. The microvasculature of the retina is injured, the blood vessels swell and leak fluid, and if not prohibited, new vessels start to grow, and ultimately lead to the detachment of the retina (7, 8). It is a duration-dependent disease that grows in stages; the incidence of retinopathy is rarely detected in the first few years of diabetes, but the incidence increases to 50% by 10 years, and to 90% by 25 years of diabetes. The prevalence of diabetic retinopathy is growing due to prolonged survival of diabetic patients (9). Type II Diabetic Retinopathy can be divided into two types: background Type II Diabetic Retinopathy and proliferative Type II Diabetic Retinopathy. Proliferative Type II Diabetic Retinopathy refers to retinal neovascularization following ischemia whereas background Type II Diabetic Retinopathy changes occur before the onset of neovascularization (10, 11). Type II Diabetic Retinopathy is one of the leading causes of blindness in the industrialized world (12) and is a result of multiple pathogenetic processes caused by hyperglycemia and abnormalities of insulin signaling pathways (13, 14) leading to retinal microvascular defects (15) and neuroretinal dysfunction and degeneration.

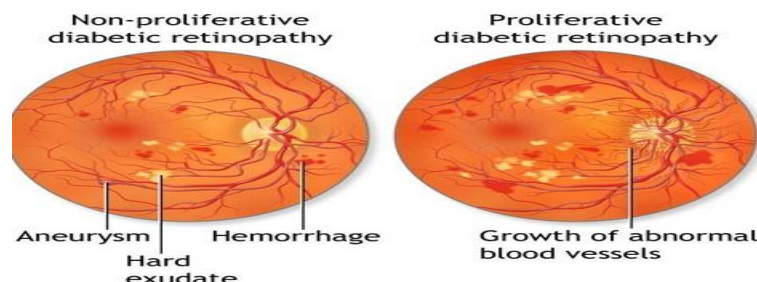


Fig2:- Image of proliferative and non-proliferative cells of diabetic retinas  
<http://www.newhealthguide.org/Pdr.html>

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Supervillin is a 205 kDa member of the gelsolin superfamily of actin-binding proteins (16). A muscle-specific isoform, termed archvillin, is a closely related 250 KDa protein (17). Supervillin comes as a novel molecule that can target as an agent for the pathogenesis of Type II Diabetic Retinopathy disease. Previous study can be concluded that supervillin might be responsible for the neovascularization which is a key process for various forms of retinal vasculopathies. Epigenetic is now emerging as one of the important factors in many diseases as it can regulate the complex interplay between genes and the environment, and these heritable changes can occur without any change in the DNA sequence. Epigenetic change is a regular and natural phenomenon, and can also be influenced by several factors including age, the environment, lifestyle and disease state (18). Epigenetic modifications can act like switches helping to control gene activity and allowing alternations in genome function without altering the gene sequences. At least three major epigenetic modifications including DNA methylation, histone modification and non-coding RNA are considered to initiate and sustain changes in gene regulation (19, 20). Methylation of cytosine to 5-methyl cytosine (5mC) is considered as one of the major epigenetic modification; methylation of the CpG islands, a CG rich region in the promoter of many genes, changes protein-DNA interactions leading to alterations in chromatin structure, and this interferes with the binding of transcriptional machinery, resulting in gene suppression (21, 22).

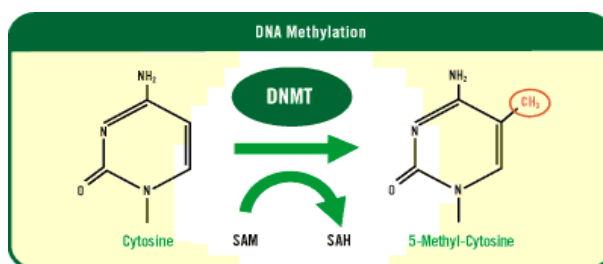


Fig3:- Conversion of Cytosine to 5-Methyl- Cytosine.

<http://www.vidaza.net/mds/about-vidaza/epigenetics-vidaza/>

## II. MOLECULAR AND BIOCHEMICAL MECHANISMS OF DIABETIC RETINOPATHY AND ITS PATHOGENESIS

A number of studies have defined the biochemical mechanisms in the growth and evolution of diabetic retinopathy; however, no mechanism can be observed as established. Each type of diabetes is considered by hyperglycemia, insulin resistance, relative or absolute lack of insulin action, and the development of diabetes-specific pathology in the retina (23). Type II Diabetic Retinopathy has been one of the main factors of vision impairment in the world. The basic hallmarks of this disease include loss of pericytes, basement membrane thickening, microaneurysms, neovascularization, and blood-retinal barrier breakdown (24). Molecular and biochemical mechanisms that have been implicated in diabetic retinopathy have increased the flux of glucose through the polyol and hexosamine pathways, activation of protein kinase C, and increased advanced glycation end product formation (25).

## III. ANATOMY OF THE RETINAL MICROVASCULATURE

The microvasculature which supplies and sanitations the inner retina is well defined in the classic anatomical text, Gray's Anatomy (26). The artery of the central retinal are originates from the ophthalmic branch of the internal carotid artery, entering the optic nerve within the orbit approximately 12 mm behind the globe and subsequently coursing through the lamina cribrosa to contact the retina. On the inner surface of the retina, superior and inferior branches directly give rise to temporal and nasal arcades, which supply the 4 quadrants of the retina. Corresponding retinal veins drain these quadrants and meet at the optic nerve head as the central retinal vein, which drains into the cavernous sinus both directly and through the superior ophthalmic vein. The other intraocular circulations of the iris and choroid also derive from the ophthalmic artery, but via ciliary arteries, which branch off the main trunk within the orbit subsequent to the central retinal artery.

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## IV. ANGIOGENESIS IN THE RETINA

Retinal angiogenesis is part of a normal vascular development and an essential of retinal neovascularization in ischemic vasculopathy. In both circumstances, retinal hypoxia stimulates the synthesis of endothelial growth factors by various retinal cells. Vascular endothelial growth factor (VEGF) was the first angiogenic factor recognized in retinal ischemic vasculopathy (27) and almost simultaneously in retinal vascular development (28), and while other hypoxia-induced and hypoxia-independent factors have since been identified, it is clear that VEGF—or VEGFA—plays a critical role in normal and pathological angiogenesis (29, 30). Angiogenesis involves specialization of endothelial cells into “tip cells” and “stalk cells” (31, 32). The migrating tip cells extend filopodia in association with astrocyte processes. Behind each tip cell, stalk cells proliferate to grow the endothelial sprout that initiates vessel formation. VEGF acts through at least 2 receptors, VEGFR-1 and VEGFR-2, to direct endothelial tip cell migration and stalk cell proliferation.

## V. GENETICS AND TYPE II DIABETIC RETINOPATHY

Pathogenesis of any type of disease is also influenced by genetic factors, and due to variability in the severity of retinopathy among diabetic patients with similar risk factors, the role of genetic factors in diabetic retinopathy should be slightly predictable. However, such genetic associations are not yet clearly established. Landmark “Genome-Wide Association Studies” have identified number of genetic variants that could explain some of the inter individual variations in the susceptibility of diabetes (33). A meta- analysis study is a type of study which used to investigate possible genetic associations with Type II Diabetic Retinopathy, which incorporated reports published between January 1990 and August 2008, has exposed a significant dissimilarity in the AKR1B1 gene. This gene encodes aldo-keto reductase family 1 member B1, which is the rate limiting enzyme of the polyol pathway one of the pathways implicated in the development of diabetic retinopathy (34-36). Identifying the gene or genes that donate to the pathogenesis of diabetic retinopathy has been challenging despite being a major focus of research over the past few decades. A large number of putative genes and genetic variants have been reported in the literature but few of these have been consistently replicated. As a result no genes have attained widespread receiving as conferring high risk of diabetic retinopathy, in contrast to the situation with CFH polymorphisms and risk of age-related macular degeneration (37). In part, this may be because identifying genes for diabetic retinopathy is more challenging due to the greater complexity of the disease which may have more multifactorial, polygenic and environmental influences than age-related macular degeneration.

## VI. SUPERVILLIN : A RETINAL ENDOTHELIAL PROTEIN

Supervillin is a member of the gelsolin superfamily of actin-binding proteins (205 kDa) (38). A muscle-specific isoform, termed archvillin, is a closely related 250 KDa protein (39). Supervillin comprises 6 gelsolin-related repeating units, plus an actin-binding domain/nuclear localization signal (40). Supervillin (SV) one of the largest members of the villin/gelsolin family of actin-organizing proteins, which have both overlapping and distinct functions (41-43) Villin is related with precancerous morphological changes in epithelia, and gelsolin is required for the formation and degradative activity of osteoclast podosomes; gelsolin also promotes cancer cell invasion and motility (44-48).

## VII. SVIL GENE STRUCTURE AND LOCATION

SVIL gene encoded supervillin protein which is located at short arm (p) of chromosome 10(10p11.23) at position 11.23 More precisely; the SVIL gene is located from base pair 29,457,338 to base pair 29,737001 on the chromosome 10.

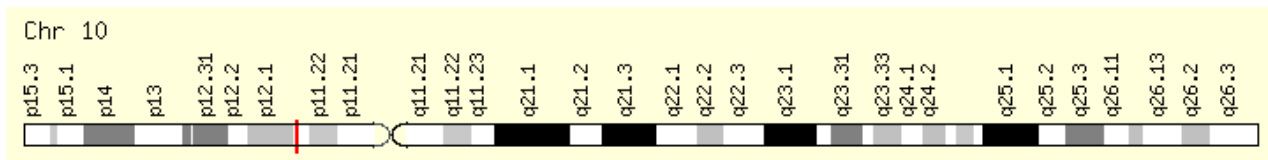


Figure 1:- Location of *SVIL* gene on chromosome 100  
(<http://www.genecards.org/cgi-bin/carddisp.pl?gene=SVIL>)

### VIII. EXPRESSION AND FUNCTION OF SUPERVILLINS

Supervillin protein is expressed in human and also in mouse, and the structure is well conserved across species (49, 50). Expression is widespread, but varies considerably between tissues; supervillin is expressed at very low stages in neural tissue (51). As well as actin, supervillin binds myosin and filamin (52), and has been associated in cytoarchitecture at the plasma membrane and within the nucleus (53, 54). Supervillin promotes disassembly of cell-substrate focal adhesions, inhibits cell spreading, and promotes invadopodial function (55, 56, 57, 58). The organization of motor function with membrane dynamics is of fundamental importance for cell motility and membrane trafficking in interphase cells, as well as for the proper segregation of chromosomes and cytoplasm during cell division (59, 60, 61, 62, 63). The actin motor, myosin II, that is required for cell adhesion and migration, and also regulates membrane trafficking and microtubule dynamics (64, 65, 66, 67). In addition, studies in HeLa cells indicate that supervillin promotes cell motility (68). While the gelsolin family proteins control actin organization, various members have other unrelated functions in cell processes, from controlling apoptosis to regulating phagocytosis (69). Platelets play a very important role in ischemic arterial vascular disease, and antiplatelet therapies are mainstays of treatment. The results in this study identify a novel platelet protein, supervillin, which functions to dampen the early formation of platelet thrombi under high shear stress. Although these results will not alter current management of vascular disease, there are potential clinical implications. Supervillin is an interaction hub for many proteins that control cell adhesion and contractility. The shear requirement of the supervillin effect presents an prospect to develop therapies that differentially affect arterial and venous thrombosis by inhibiting platelet thrombus formation under high shear settings (eg, acute coronary syndromes or percutaneous coronary intervention) without altering the normal hemostatic function of platelets in low-shear veins or microcirculation. (70). Supervillin plays an important role in cell motility suggested a potential role in retinal vessel formation. Supervillin has its function in cell motility and play a significant role in cell proliferation which suspects its role in Type II Diabetic Retinopathy. When endothelial cells were stimulated with human VEGF for up to 24 hours, there is upregulation of supervillin. Knockdown of supervillin gene resulted in significantly less proliferation of retinal endothelial cell in comparison to the non-targeted supervillin containing retinal endothelial cell containing culture. Enormous work has already been done in understanding the mechanism of pathogenicity of Type II Diabetic Retinopathy such as polyol pathway, eNOS pathway and role of certain genes (AKR1B1, VEGF), etc. Identifying the gene or genes that contribute to the pathogenesis of diabetic retinopathy has been challenging despite being a major focus of research over the past few decades. Nonetheless, a role for supervillin in endothelial cell function has not yet been investigated, although CapG, another gelsolin family protein, is known to promote endothelial cell motility (71). Therefore a valid reason for suspecting supervillin its role in neovascularization, a process responsible for causing Type II Diabetic Retinopathy has been found. VEGF is a multiple function associated cytokine that promotes the process of angiogenesis and is a potent mediator for the micro vascular permeability. Microvascular changes in the retina lead to hypoxia, a stimulator of VEGF production (72). In relation to diabetic retinopathy, the VEGF gene had the largest number of individual SNPs. Of these six polymorphisms, the G variant of the rs2010963 polymorphism has been found to significantly protect against the development of NPDR in Type II Diabetes but not with overall diabetic retinopathy development. Raising the antibodies against VEGF is not as much effective as it may cause several other complications. As per the avail literature, various studies are carried out for gene susceptible for the disease but the possible mechanism for the incidence of disease was not well understood till date. Therefore, the need of an our is to develop a new potential target for understanding the mechanism of Type II

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Diabetic Retinopathy as well as for the treatment of the disease and move one step forward in the field of Personalized Medicine.

## IX. DNA METHYLATION PROFILING

DNA methylation in vertebrates is categorized by the addition of a methyl or hydroxymethyl group to the C5 position of cytosine, which arises mainly in the context of CG dinucleotides. Non-CpG methylation in a CHH and CHG context (where H = A, C or T) exist in embryonic stem cells and in plants. It can be done in various suitable ways, few are mentioned below:

## X. METHYLATION-SPECIFIC PCR

Another classical method that uses bisulfite-converted DNA is methylation-specific PCR (73). To perform it, two pairs of primers are designed; one pair that favours amplification of methylated and another one of unmethylated DNA. Two qPCR reactions are complete for each sample, and relative methylation calculation based on the difference of their Ct values. It is a rapid method, which might be used for simultaneously profiling of multiple samples. The obvious drawback is that methylation status of only one or two CpG sites is assessed at a time.

## XI. PCR WITH HIGH RESOLUTION MELTING

The amplified PCR product could be analysed using high resolution melting. Most of the qPCR instruments have such a capability or special instruments could be used. Intercalating dye, e.g., Eva green or SYBR, when bound to double-stranded DNA is highly fluorescent, but as the temperature rises, the DNA strands dissociates, the dye goes back to solution and the fluorescence drops. DNA that was originally methylated retains cytosines and has a higher melting temperature compared to an unmethylated one. Level of methylation is correlated with the melting profile of the product. The drawback of the method is the necessity to obtain a pure PCR product, which is difficult in many cases. However, if a pure PCR product is obtained, then even small differences (5%–10%) in DNA methylation or the contribution of methylated DNA from subpopulations of cells within heterogeneous populations, such as cancer sample, could be detected (74).

## XII. COLD-PCR FOR THE DETECTION OF UNMETHYLATED ISLANDS

COLD-PCR is able to detect rare unmethylated sequences among an excess of methylated copies (75). It is a very delicate method used for diagnostic purposes. CpG islands heavily methylated in normal tissues often become hypomethylated in cancer, and the ability to detect them is important in clinical practice. The first step is bisulfite conversion. Principle of the COLD-PCR is simple: the denaturation temperature of the PCR is lowered to allow only unmethylated (and therefore less GC-rich) fragments to be efficiently amplified. Obviously, to be efficient, target sequences should contain several CpG sites.

## XIII. OTHER METHOD

Deletion of methylated DNA is possible with anti-methylcytosine binding proteins (MBD) or antibodies against 5 mC (MeDIP). Once it is obtained from different samples, methylated fractions of DNA could be directly compared using microarray hybridization or used for NGS. An approach that combined MeDIP and bisulfite conversion has been recently published; it has an improved level of specificity and provided single-CpG resolution (76).

As per the available literature, epigenetic signature is not searched in case of supervillin suspecting its role in neovascularization and diabetic retinopathy.

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## XIV. CONCLUSION

The intent of this review is to provide better understanding of the complex molecular mechanisms and treatment modalities. There is no doubt that our vascular system is extremely diverse in structure, architecture, and physiology. The branching architectures, histological structures, and hemodynamic characteristics are clearly specified and regulated in great detail and with exquisite anatomical specificity, during development and in physiological adaptation. Diabetic retinopathy remains an important cause of blindness and treatment options have limitations. Several lines of evidence point to a considerable genetic influence in susceptibility to diabetic retinopathy and there has been intensive research to uncover the genes responsible. Epigenetic studies and candidate gene approaches have suggested many potential genetic variants which may underlie the disease but replication of these results has often been inconsistent. Epigenetic signature in supervillin gene may unfold new source of information regarding its participation in retinal vasculopathies.

Newer therapeutic mechanisms are under investigation including PKC pathway inhibitors, VEGF antagonists, AGE pathway inhibitors, and supervillin inhibitors and Antagonists. Therefore, within the near future pharmacologic treatment strategies may include multiple pharmacological agents, targeted simultaneously to block multiple pathways. This will probably be available for treating and preventing the progression of DR after further potential clinical trials.

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