Association of VEGF *gene polymorphisms with Type 2 Diabetic Retinopathy*

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By

Noha Khalifah Abdelgafar
(M.B., B.Ch)

Supervised by

Prof. Dr.Ola Abdelmonaem Elsesy
Professor of Clinical and Chemical Pathology
Faculty of Medicine, Cairo University

Dr.Hoyida Ahmad Abdelrasool
Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine, Fayoum University

Dr. Marianne Samir Makboul
Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine, Cairo University

Faculty of Medicine
Cairo University
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Abstract

As VEGF is involved in the process of new blood vessel formation, It seems to be a potential candidate gene for DR. the 5' UTR, promoter region of VEGF has been shown to be highly polymorphic. In our study we analyzed the VEGF –634 C/G polymorphism as a potential genetic marker of DR.

There were significant differences in high density lipoprotein (HDL) cholesterol and hemoglobin A1c between diabetic groups.

The CG and GG genotype was significantly higher in the DR group compared with the DWR group, also when compared with healthy control group

The CC genotype was significantly lower in the DR group when compared with the DWR group, also when compared with healthy control group.

The –634 C/G polymorphism might serve as a predictive factor for the development of diabetic retinopathy.

Key words:
Diabetic retinopathy, vascular endothelial growth factor, RFLP

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AGE	Advanced Glycation End Products
bFGF	basic fibroblast growth factor
BFGF	Basic fibroblast growth factor
BUN	Blood urea nitrogen
CVD	Cardiovascular disease
DAG	Diacylglycerol
DM	Diabetes mellitus
DR	Diabetic retinopathy
DWR	Diabetic without Retinopathy
ESRD	End stage renal disease
FABP2	Fatty acid binding protein 2
FFAs	Free fatty acids
HbA1c	Glycosylated hemoglobin
HDL-c	High density lipoprotein-cholesterol
HGF	Hepatocyte growth factor
HIF-1	Hypoxia inducible factor-1
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
IGT	Impaired Glucose Tolerance
IL6	Interleukin 6
KDR/flk-1	kinase domain receptor and Fms-like tyrosine kinase1
LDL-c	High density lipoprotein-cholesterol
MI	Myocardial infarction
MMP9	Matrix metalloproteinase-9
MODY1	Maturity Onset Diabetes of the Young 1
NP-1	Neuropilin-1
NPDR	Non-proliferative diabetic retinopathy
PDR	Proliferative diabetic retinopathy
PEDF	Pigment epithelium derived growth factor
PKC	Protein kinase C
PPAR	Peroxisome proliferator-activated receptor
PTP1B	Protein tyrosine phosphate 1 B
RAGE	Receptors for Advanced Glycated End products
TGF-β	Transformation growth factor
TGs	Triglycerides
TKI	Tyrosine kinase inhibitors
tPA	tissue-type plasminogen activator
uPA	urokinase-type plasminogen activator
VEGF	Vascular endothelial growth factor
VEGFR-2	Vascular endothelial growth factor receptor-2
VPF	Vascular permeability factor

Introduction and Aim of the work

By 2030, it is estimated that 366 million persons worldwide will have diabetes **(Wild et al, 2004).** Type 2 diabetes is the most common form of diabetes. Type 2 diabetes frequently goes undiagnosed for many years because hyperglycemia develops gradually and in the earlier stages is not severe enough to produce the classic symptoms of diabetes; however, such patients are at increased risk of developing macrovascular and microvascular complications. Ocular complications in diabetes are frequent, distressing and destined to become one of the challenging problems of the future. Diabetic retinopathy is a highly specific vascular complication of both type 1 and type 2diabetes, and the duration of diabetes is a significant risk factor for the development of retinopathy **(Rand, 1985).**

Ongoing research efforts continue to hold promise that diabetic retinopathy eventually will be curable or preventable. Presently, however, clinical goals must concentrate on identifying eyes at risk of visual loss and ensuring that appropriate and timely laser surgery is offered. If patients with diabetes receive currently recommended care, remarkable preservation of vision can be achieved. Clinical trials in the United States **(DCCT/EDICRG,2000)** , the United Kingdom **(Kohner et al,1998)** and Japan **(Ohkubo et al, 1995**) have demonstrated that intensive control of diabetes, as measured by glycosylated hemoglobin (HbA1c) levels, can reduce the risk of onset and progression of diabetic retinopathy. Because diabetic retinopathy is often asymptomatic in its most treatable stages, early detection of diabetic retinopathy through regularly scheduled ocular examination is critical. Collateral health and medical problems present a significant risk for the development and progression of diabetic retinopathy **(Aiello et al, 2001**). These factors include pregnancy **(Phelps et al, 1986**), chronic hyperglycemia**(Brinchmann-Hansen et al, 1985),**hypertension **(Krolewski et al, 1988),** renal disease **(Chase et al,1989),** and hyperlipidemia **(Chew et al,1996).**

Patients with these conditions require careful medical evaluation and follow-up for the progression of diabetic retinopathy and optimization of their medical status. Several angiogenic growth factors have been isolated from eyes with diabetic retinopathy, including insulin-

like growth factors, basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF) **(Nishimura et al.1999).** These factors promote the development of new vessel growth and retinal vascular permeability **(Aiello & Hata, 1999)**. Indeed, inhibition of molecules such as VEGF and their signaling pathways can suppress the development of retinal neovascularization and retinal vascular permeability **(Ozaki et al., 2000).**

Vascular endothelial gowth factor (VEGF) plays an important role in the pathogenesis of diabetic microvascular complications. Diabetic retinopathy is characterized by increased vascular permeability, tissue ischemia and neovascularization. VEGF can stimulate angiogenesis and increases the permeability of the microvasculature **(Witmer et al,2003).**

The VEGF gene was previously found to be implicated in the development of diabetic retinopathy. In previous studies involving type 2 diabetic subjects, the C(–634)G polymorphism in the 5'UTR of the VEGF gene was strongly associated with an increased risk of retinopathy**(Awata et al,2002),** while other studies showed the opposite finding **(Petrovic et al, 2008**). The association of VEGF gene polymorphism and the increased risk of retinopathy still remain controversial.

This work aimed to investigate the impact of genetic polymorphisms of *VEGF* gene on diabetic retinopathy (DR) with type 2 diabetes, we searched for the association between the -634 C/G *VEGF* polymorphism and DR in subjects with type 2diabetes.

Diabetes Mellitus

Definition:

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetics and environmental factors. Depending on the etiology of the DM, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system **(Harrison's, 2008).**

Epidemiology:

Diabetes is worldwide in distribution .The incidence of both types of diabetes (type 1 and type 2) is rising, however the prevalence of both varies considerably in different parts of the world .This seems to be due to differences in both genetic and environmental factors .In type 1 diabetes mellitus, the incidence is markedly age dependent, increasing during the early months of life to a peak coincident with pubertal development. The disease can occur at any age with a small mid-life peak in incidence. **(Fujimoto et al, 2000)**.

Type 2 diabetes mellitus is common in middle aged and elderly individuals and it is responsible for approximately 85% of all cases of diabetes mellitus in developed countries. Diabetes is more prevalent in urban areas than in rural areas. Numerous epidemiological studies have demonstrated an increase in prevalence of insulin resistance and type 2 DM in various ethnic populations that have migrated from their native lands to more urbanized and westernized regions of the world. Type 2 DM has already reached epidemic proportions worldwide. **(Fujimoto et al, 2000)**.

Classification and criteria of diabetes mellitus:

1- Type1 Diabetes Mellitus:

It is characterized by beta cell destruction caused usually by an autoimmune process leading to absolute insulin deficiency. The onset is usually acute, developing over a period of a few days to weeks. Over

95% of persons with Type 1 diabetes mellitus develop the disease before the age of 25 years, with an equal incidence in both sexes and increased prevalence in white population. A family history of type1 diabetes mellitus, gluten enteropathy (coeliac disease) or other endocrine disease is often found. Most of these patients have the (immune mediated form) of type 1 diabetes mellitus with islet cell antibodies and often have other auto immune disorders such as Hashimoto's thyroiditis, Addison's disease, vitiligo or pernicious anemia. Few patients usually those of African or Asian origin have no antibodies but have a similar clinical presentation, consequently, they are included in this classification and their disease is called (the idiopathic form of type 1 diabetes mellitus). Viruses, such as rubella and mumps may be another cause **(Fujimoto et al, 2000)**

2- Type 2 Diabetes Mellitus:

Type 2 diabetes mellitus is a major global health problem that affects over 200 million individuals worldwide **(Quinn, 2001**). It is characterized by insulin resistance in peripheral tissue and an insulin secretory defect of the beta cell **(Groop, 2000).** Insulin resistance is a major contributor to the pathogenesis of type 2 diabetes mellitus and plays a key role in associated metabolic, abnormalities, such as dyslipidemia and hypertension **(Gold stein, 2002)**.

3-Gestational Diabetes Mellitus:

This term should be applied to patients developing hyperglycemia compatible with diagnostic criteria during pregnancy. Anyhow, a variable proportion may be type 1 or type 2. This can only be predicted from the natural history as most cases of proper gestational diabetes revert to normal glucose tolerance after parturition. A small percent develops permanent diabetes within 15 years after delivery or termination of pregnancy. The national diabetes data group in 1990 concluded that gestational diabetes will develop in about 1-2% of all pregnancies. Also there is highly significant and consistent correlation between HbAlc values above 6.6 %and adverse fetal outcome **(Pratley &Weger, 2002)**.

4- Other types of Diabetes (Groop, 2000):

A- Genetic defects of beta-cell function:

- Chromosome 20 HNF-4-a (maturity Onset Diabetes of the Young I) (MODY l)

- Chromosome 7, glucokinase (MODY 2)
- Chromosome 12 HNF-1-a (MODY 3)
- Chromosome 13 IPF-l (MODY 4)
- Mitochondrial mutation
- Others.

B- Genetic defects in insulin action:

- Type A insulin resistance.
- Rabson-Mendelhall syndrome.
- Others.

C. Diseases of the exocrine pancreas:

- Pancreatitis.
- Trauma & pancreatectomy.
- Neoplasia
- Cystic fibrosis.
- Fibrocalculous pancreatopathy.

D. Endocrinopathies:

- Cushing syndrome
- Acromegally
- Pheochromocytoma
- Somatostatinoma
- Glucagonoma
- Hyperthyroidism
- Others.

E. Drug-or chemical-induced diabetes:

- Nicotinic acid
- glucocorticoids
- Thyroid hormones.
- α-Adrenergic agonists.
- B-adrenergic agonist.
- Dilantin.
- Thiazides.
- pentamide
- Interferon therapy.
- Others.

F.viral infections:

- Congenital Rubella.
- Cytomegalovirus.
- Others.

G.Uncommon forms of immune-mediated diabetes:

- Insulin auto immune syndrome
- Stiffman syndrome
- Insulin receptor antibodies

Impaired Glucose Tolerance (IGT):

The term was applied to person with glucose values between normal and diabetic to avoid the psychological and socio-economic stigmata of diabetes diagnosis. IGT exists if the fasting plasma glucose level 100-126 mg/dl (5.6-7mmol/l) and after 2 hours between 140 and 200 mg/dl (7.8 and 11.1 mmol/l), IGT was found in both obese and non obese persons and when followed for 10 years, about 10 to50% become diabetics, while mainly remained the same or reverted to normal. IGT(impaired glucose tolerance) is a common condition that greatly increases risk for the subsequent development of type 2 DM. Individuals with IGT manifest abnormalities in both insulin action and early insulin secretion similar to those seen in patients with type 2 DM. Furthermore, the progression from IGT to diabetes is characterized by a dramatic decline in early insulin secretion **(prailey & Weger, 2002)**.

Pathogenesis of type 2 Diabetes Mellitus:

Through the past decade, it increasingly has become clear that resistance to insulin mediated glucose disposal at muscle, liver and adipose tissue, along with defective insulin secretion from the pancreatic B-cell characterize type 2 DM .There are marked differences in the phenotypic expression of the type 2 diabetes mellitus with affected individuals exhibiting varying levels of insulin resistance and impairment in insulin secretion. This range of abnormalities includes metabolic derangements characterized by predominant defects in insulin sensitivity with relative B-cell dysfunction to metabolic derangements characterized by severe B-cell dysfunction accompanied by mild insulin resistance. Although, the pathophysiological causes of insulin resistance and B-cell dysfunction are unknown, it increasingly has been clear that there are

genetic and environmental factors leading to development of each of these abnormalities **(Bouchard, 1996).**

It is likely that the interplay between these factors leads to the overt expression of type 2 diabetes mellitus. For example, it is known that obesity and physiological inactivity are associated with the development of insulin resistance and presence of insulin resistance in an individual who has a genetically predisposed defect in B-cell function is likely to progress to impaired glucose tolerance (IGT) and type 2 diabetes mellitus **(Quinn, 2001).**

1-Genetics:

Abundant evidence supports a genetic predisposition of both type 2 diabetes mellitus and the traits that precede diabetes. Unusual causes of diabetes have been identified, including autosomal dominant single gene forms due to mutations of glucokinase, the hepatocyte unclear factors, and insulin promotor factor 1. Mitochondrial mutations also may cause type 2 diabetes, but together these causes explain only a small fraction of type 2 diabetes **(Elbein, 2000)**.

A type 2 diabetic phenotype can develop in individuals with abnormal insulin sensitivity who have a monogenic defect that impairs beta cell function or in individuals who have anyone of many polygenic disorders in which obesity; insulin resistance and impaired beta cell insulin secretory function are part of the altered metabolic state **(Harolds, 1999).**

It has been suggested that this cluster of condition is a specific entity called "syndrome X", or "insulin resistance syndrome" reduced activity and obesity also make a major contribution. Although so far no specific genetic variation has been shown to be consistently linked with type 2 diabetes mellitus, a mutation of the glucokinase gene located on the short arm of chromosome 7 is associated with some cases of the uncommon syndrome of maturity onset diabetes in the young (MODY syndrome) **(Chrisholm et al, 1997).**

A defective fatty acid binding protein 2 (FABP2) gene may result in higher levels of unhealthy fat molecules (particularly triglycerides) which may be critical in the link between obesity and insulin resistance in some people with type 2 diabetes mellitus. Alterations in five genes that beta cell and pancreas function have been identified that may play an important role in inherited cases of type 2 diabetes mellitus. Variation in

a gene that regulates a protein called calpain-l0 is proving to affect insulin secretion and action and may play a role in type 2 diabetes mellitus in certain populations. Calpains are enzymes that play a wide role in many essential cellular functions. Evidence is strongly suggesting that genetic activation of these enzymes may be important in many aging-related diseases. Defective genes that regulate a molecule called peroxisome proliferator-activated receptor (PPAR) gamma may contribute to both type 2 diabetes mellitus and high blood pressure in some patients. A defective gene has been, detected that reduces activity of a protective substance called beta3-adrenergic receptor, which is found in visceral fat cells (those occurring around the abdominal region). The result is a slowdown in metabolism and an increase in obesity **(Chrisholm et al, 1997)**.

2-Environmental factors:

i. Lifestyle:

Overeating, especially when combined with obesity and under activity is associated with the development of type 2 diabetes. Obesity probably acts as a diabetogenic factor through increasing resistance to the action of insulin in those genetically predisposed to develop type 2diabetes **(Grimm, 1999).**

In individuals with type 2 DM, obesity is a big problem. In fact, according to the reference quoted, 80% of type2 DM are obese. The link between weight and CVD (cardiovascular disease) is obvious. It is clear that CVD mortality is 2 to 4 times higher in diabetic patients than non diabetic patients. Myocardial infarction (MI) -related mortality shows a 2-fold excess. Atherosclerosis is greatly increased in people with type 2 DM. Hypertension, of course, is elevated and is prevalent in two thirds of people with type 2 DM. Stroke rate is also much higher ..Thus, there is a tremendous link between weight, metabolic dysfunction and CVD. It is well known that weight loss, even mild weight loss, is very beneficial with regard to HbA1c (glucose control), blood pressure, total cholesterol, HDL and TG. In relation to fat content, there is the visceral compartment and the subcutaneous compartment. As we get more obese, the visceral compartment fills first, and then, we get hepatic insulin resistance. The visceral fat compartment is very much related to insulin resistance of liver. As we become more obese, we then begin to fill the peripheral