

# Role of Superoxide Dismutase 2 Gene Ala16Val Polymorphism and Total Antioxidant Capacity in Diabetes and its Complications

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## Abstract

Diabetes Mellitus (DM) is a chronic heterogeneous disorder and oxidative stress is a key participant in the development and progression of it and its complications. Antioxidant status can affect vulnerability to oxidative damage, onset and progression of diabetes and diabetes complications. Superoxide dismutase 2 (SOD2) is one of the major antioxidant defense systems against free radicals. SOD2 is encoded by the nuclear SOD2 gene located on the human chromosome 6q25 and the Ala16Val polymorphism has been identified in exon 2 of the human SOD2 gene. Ala16Val (rs4880) is the most commonly studied SOD2 single nucleotide polymorphism (SNP) in SOD2 gene. This SNP changes the amino acid at position 16 from valine (Val) to alanine (Ala), which has been shown to cause a conformational change in the target sequence of manganese superoxide dismutase (MnSOD) and also affects MnSOD activity in mitochondria. Ala16Val SNP and changes in the activity of the SOD2 antioxidant enzyme have been associated with altered progression and risk of different diseases. Association of this SNP with diabetes and some of its complications have been studied in numerous studies. This review evaluated how rs4880, oxidative stress and antioxidant status are associated with diabetes and its complications although some aspects of this line still remain unclear.

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**Keywords:** Diabetes complications, Diabetes mellitus, Polymorphism, Superoxide dismutase 2

## Introduction

Diabetes Mellitus (DM) is a chronic disorder that affects different people of all ages, race and sex. There are several acute and chronic complications related to this disorder<sup>1-3</sup>. Cardiovascular diseases, diabetic nephropathy, neuropathy and retinopathy are major and common complications of diabetes. These complications are affecting the vascular system, kidney, retina and peripheral nerves in diabetic patients and will also bring high cost for both individuals and society<sup>4-6</sup>. Recent evidence introduces oxidative stress as a key participant in development and progression of diabetes and its micro and macro vascular complications<sup>1,6,7</sup>. Antioxidant defense system works against free radicals<sup>4,6</sup>. Each antioxidant reduces the action of free radicals by different mechanisms<sup>8</sup>, including enzymes that degrade Reactive Oxygen Species (ROS)<sup>4,9</sup>. Superoxide dismutase 2 (SOD2) is a key component of antioxidant defense system against mitochondrial superoxide radicals. Mutations or variations in antioxidant genes happen in diabetes and progress in diabetic patients<sup>1,10</sup>.

The valine-to-alanine substitution in MnSOD Ala16Val SNP decreases the transport efficiency of the enzyme into the mitochondria and modifies the antioxidant defense against ROS. Production of a  $\beta$ -sheet secondary structure instead of the expected  $\alpha$ -helix structure results in a reduced MnSOD activity, which in turn increases oxidative stress<sup>11</sup>. This process is an important pathophysiological mechanism in development and progression of diabetes and its complication<sup>1,10,12,13</sup>. Total antioxidant status decreases in diabetic patients<sup>8</sup>. Total Antioxidant Capacity (TAC) modification in plasma during oxidative stress and dietary modulation of plasma redox status can ameliorate oxidative stress and may delay or prevent progression and onset of the disease<sup>3,6,8,14</sup>.

In agreement with these opinions, some studies showed association between SOD2 gene polymorphisms with diabetes mellitus and some of its complications<sup>1,10,13,15,16</sup>. In diabetes type 2 patients, diminishing of total antioxidant capacity and depletion of plas-

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ma antioxidants could be related to the complications of diabetes<sup>17</sup>. Lower plasma TAC and higher serum levels of malondialdehyde (MDA), which indicate increased oxidative stress and compromised antioxidant defenses are reported in type 2 diabetic patients with severe Diabetic Nephropathy (DN) and Chronic Kidney Disease (CKD)<sup>18,19</sup>. In this article, the importance of antioxidant enzyme gene polymorphisms and total antioxidant capacity in diabetes and the complications were reviewed.

#### **Diabetes mellitus and its complications**

DM is recognized as a heterogeneous condition and also a multifactorial syndrome<sup>1,2</sup>. Chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism due to deficiencies in insulin secretion and/or insulin action are characteristics of diabetes mellitus<sup>1,8</sup>. DM is certainly a strong contributor to deaths from other causes such as ischemic heart disease, cerebrovascular disease and CKD<sup>1,2,17</sup>.

Type-1 diabetes mellitus (T1DM) is characterized by a near absolute deficiency of insulin secretion. Classical type-2 diabetes mellitus (T2DM) is attributed to insulin resistance and deficient beta-cell function. It is the major form of diabetes worldwide and accounts for nearly 90-95% of those with diabetes. Some risk factors for T2DM are obesity, physical inactivity, hypertension, certain ethnicities (e.g. Middle Eastern, South Asian and Hispanic) and dyslipidemia. Furthermore, family history of T2DM and several genetic risk markers have been shown to be important in relation to diabetes<sup>17,20</sup>.

Based on a recent report by the International Diabetes Federation (IDF), cases are increasing everywhere. There are an estimated 387 million adults having diabetes and by 2035 this will rise to 592 million. In addition, IDF reported the prevalence of diabetes in Iran as 8.6% in 2014 in the Iranian population aged 20 to 79 years<sup>21</sup>. From 2005 to 2011, Iranian trend analysis revealed 35.1% increase in DM prevalence<sup>22</sup>.

DM chronic complications affect different organs and tissues, including eyes, kidneys, heart, blood vessels and peripheral nerves. Diabetic patients are at high risk for micro vascular complications (e.g., nephropathy, retinopathy and neuropathy) and macro vascular complications (e.g., peripheral vascular disease, cerebrovascular disease and cardiovascular disease)<sup>1,3,20</sup>. Diabetes is the most common cause of CKD, which is a worldwide problem with high and rising prevalence affecting 7.2% of the global adult population. The prevalence of some degree of CKD among adults with type 2 diabetes is 40%. CKD and diabetes are considered as important risk factors for cardiovascular disease and all three conditions are key components of multiple morbidities<sup>23-25</sup>.

#### **Antioxidant system, oxidative stress and diabetes**

Humans have evolved highly complex antioxidant systems (enzymatic and non-enzymatic). Antioxidants

can be produced endogenously or be obtained from exogenous sources e.g., as a part of a diet or dietary supplement. Common antioxidants include the vitamins A, C, E, glutathione, and the enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase<sup>6,8</sup>. Antioxidants counter the action of free radicals by several mechanisms. These mechanisms include: 1. enzymes that degrade free radicals, 2. proteins such as transferrin that can bind metals which stimulate the production of free radicals, and 3. antioxidants such as vitamins C and E that act as free radical scavengers<sup>4</sup>. Antioxidant defense systems work in synergy with each other to eliminate excess Reactive Oxygen Species (ROS) and maintain balance between oxidation and antioxidation in normal conditions in order to protect cells and organ systems against free radical induced damage<sup>6,13</sup>.

Oxidative stress is defined as excess formation and/or insufficient removal of highly reactive molecules such as ROS<sup>1</sup>. Excessive generation of ROS is a deleterious factor that leads to pathological consequences including damages to proteins, lipids and DNA<sup>6,8</sup>. Oxidative stress is induced by elevations in glucose and Free Fatty Acid (FFA) levels and has a key role in the pathogenesis of both types of diabetes mellitus. Disruption of antioxidant defense in diabetic subjects (types 1 and 2) as well as increased formation of free radicals reported in many studies lead to oxidative damage of cell components in several tissues, including the kidney, eye, and nervous system<sup>4,26,27</sup>.

Insulin Resistance (IR),  $\beta$ -cell dysfunction, Impaired Glucose Tolerance (IGT) and ultimately T2DM, all occur by oxidative stress through activating stress pathways (e.g., increased production of Advanced Glycosylated End (AGE) products, sorbitol, cytokines, activation of nuclear factor kappa-light-chain-enhancer of activated cells (NF- $\kappa$ B), and p38 class of mitogen activated protein kinases (P38MAPK))<sup>1,8</sup>. Activation of these stress-related signaling pathways by reactive metabolites results in Vascular Smooth Muscle Cell (VSMC) migration and proliferation, a decrease in endothelial production of nitric oxide, insulin action alteration in glucose uptake and insulin clearance or insulin secretion, which ultimately cause insulin resistance<sup>28-30</sup>. On the other hand, oxidative stress results in activation of multiple stress-sensitive kinase signaling cascades such as inhibitor kinase beta (IKK- $\beta$ ) and some isozymes of protein kinase C (PKC). Once activated, these kinases are able to phosphorylate multiple targets, such as the insulin receptor substrate (IRS) proteins (including IRS-1 and IRS-2). Increased phosphorylation of them decreases downstream signaling molecules (e.g., phosphatidylinositol 3-kinase [PI3K]), resulting in reduced insulin action<sup>31</sup>.

Furthermore, many studies demonstrated that increased oxidative stress can cause insulin resistance by inhibition of insulin signals and adipokines deregulation<sup>1,6,8,20</sup>. In pathology of T1DM, oxidative stress has

also been suggested to play a role as a destructive factor of pancreatic  $\beta$ -cells in the Islets of Langerhans<sup>32</sup>.

Oxidative stress can also participate in development and progression of diabetes complications<sup>6,20</sup>. Preventing cardiomyopathy, retinopathy, nephropathy and neuropathy in patients with DM through neutralization of reactive molecules has been demonstrated<sup>33</sup>. Mechanisms of oxidative stress in diabetes complications are partly known and they include activation of transcription factors, AGEs products and protein kinase C<sup>6</sup>. Oxidative stress is an important participant in pathogenesis and progression of CKD as well. Oxidative stress is increased in these patients as a result of mitochondrial respiratory system impairment. The increased production of ROS and impaired antioxidant defense mechanisms in patients with CKD can inhibit normal cell function by damaging cells biomolecules. A significant increase in the generation of ROS causes progression of CKD to advanced stages<sup>34,35</sup>. Increasing generation of ROS has been identified as a potentially major contributor to pathogenesis of diabetic kidney disease. Responsible pathways are still under investigation<sup>36-38</sup>. ROS overproduction and increased oxidative stress can also cause vascular endothelial and smooth muscle dysfunction. This can lead to pathogenesis of diabetic vascular diseases including diabetic neuropathy, retinopathy and nephropathy<sup>39-41</sup>. There are extensive investigations on evaluation of the ability of antioxidants in management of diabetes and amelioration of its complications. Animal studies have shown that primary antioxidants or genetic manipulation of antioxidant defenses can at least partially ameliorate oxidative stress and consequentially, reduce severity of diabetes complications but data from humans is less clear and more studies are required<sup>9,26,27</sup>.

#### Antioxidant capacity

The concept of TAC was introduced to consider the cumulative antioxidant capacity of all the antioxidants present in foods (dietary total antioxidant capacity), plasma/serum and other body fluids<sup>15,42,43</sup>. Total antioxidant capacity provides an integrated parameter rather than the simple sum of measurable antioxidants. The capacity of known and unknown antioxidants and their synergistic interaction is therefore assessed, thus giving an insight into the delicate balance *in vivo* between oxidants and antioxidants<sup>14</sup>. Coexistence of decreased antioxidant status with diabetic oxidative stress can further increase the deleterious effects of free radicals. Reduction in antioxidant potential of plasma increases complications of diabetes including cardiovascular disease, nerve damage, blindness and nephropathy. Thus, the increasing incidence of diabetes is a significant health concern beyond the disease itself<sup>5,44</sup>.

Some studies have reported dietary TAC as a good indicator of diet quality and plasma antioxidant status in different populations<sup>42,45</sup>. Dietary TAC can be calculated by using TAC food databases that have been constructed by using a number of assays for measuring

TAC in commonly consumed foods, including the Oxygen Radical Absorbance Capacity (ORAC), the Total Radical-trapping Antioxidant Parameters (TRAP), and the Ferric-Reducing Antioxidant Power (FRAP) assays. The ORAC, TRAP, and FRAP assays can also be used for analyzing TAC in blood plasma<sup>42,46,47</sup>.

#### Total antioxidant capacity and diabetes

Dietary total antioxidant capacity has been shown to be inversely associated with risks of chronic diseases<sup>43</sup>. Several studies have shown that the total plasma antioxidant status is significantly lower in diabetic patients rather than normal subjects. A lower risk of type 2 diabetes mellitus in individuals with higher levels of serum antioxidants was also reported<sup>4,8,48</sup>. Decreased aqueous humor TAC level in diabetic patients and its correlation with retinopathy progression was also reported<sup>49</sup>. The blood TAC was significantly depleted in the diabetic group but wasn't correlated with the serum glucose level, HbA1c and the duration of DM. Furthermore, there was no significant correlation between TAC and peripheral nerve conduction parameters<sup>50</sup>. Diminishing of the total antioxidant capacity noted in diabetes type 2 patients, especially inadequately metabolically controlled may constitute the essential pathogenic factor of vascular complication in diabetes. Low TAC level accompanied by high blood glucose in poor metabolically controlled condition can increase and exacerbate oxidative stress in diabetic patients. Activation of stress pathways through oxidative stress and hyperglycemia in these patients and also elevation in radical superoxide under this condition all can cause vascular complication progression by increasing lipid oxidation, accelerating atherogenesis and decreasing Nitric Oxide (NO) bioavailability<sup>51</sup>. Presence of Coronary Artery Calcification (CAC) in type 1 diabetes is significantly associated with serum Total Antioxidant Status (TAS) reduction. The reduction in Total Antioxidant Status (TAS) in type 1 diabetic subjects was associated with increasing HbA1c and duration of diabetes in Valabhji *et al*'s study, although it suggested that the effect and contribution of oxidative stress to the higher coronary risk in diabetic patients isn't mediated solely by hyperglycemia<sup>52</sup>. This decrease in TAC of diabetic patients reflects the action of antioxidants against oxidative stress in order to control its related damages<sup>8</sup>. In contrast to most studies in this area, some studies demonstrated that the total plasma antioxidant status was significantly higher in patients with diabetes compared with the control subjects. These studies concluded that plasma TAC increased in order to provide greater protection against free radical aggression. Because the results are controversial and the exact mechanisms underlying this effect are not clear yet, further studies are required<sup>53-55</sup>.

#### Superoxide dismutase (SOD)

Regarding superoxide dismutase [SOD catalyzes the dismutation of the superoxide ( $O_2^-$ ) radical into either



oxygen molecule ( $O_2$ ) or hydrogen peroxide ( $H_2O_2$ )] it plays important protective roles against cellular and histological damages that are caused by ROS. In comparison with other antioxidant enzymes, SOD has a high turnover (speed of reaction with its substrate) and the reaction is limited only by the frequency of collision between it and  $O_2^-$ . Therefore, SOD catalyzes dangerous reaction of  $O_2^-$ , protecting cells from the toxicity of this anion. SOD family of antioxidant enzymes includes intracellular (Cu Zn-SOD), mitochondrial (MnSOD), and extracellular (EC-SOD) enzymes also referred to as SOD types 1, 2 and 3, respectively <sup>1,3,5</sup>. Serious illnesses in mice lacking these enzymes evidence the physiological significance of SODs <sup>3</sup>. Many studies have shown SOD levels in diabetic tissue and blood decline <sup>5,56,57</sup>. There is compelling evidence that superoxide excess induced by diabetic hyperglycemia plays a central role in tissue damage and diabetic vascular complications. Alteration of SOD activity in diabetic animals in various tissues, red blood cells and plasma are surveyed in many studies <sup>6,58-60</sup>. SOD activity in peripheral blood cells is reduced in diabetic patients with DN as compared with those without diabetes complication <sup>61</sup>. Death of mice lacking SOD2 due to the strong oxidative stress, cardiomyopathy and lipid accumulation in the liver and skeletal muscles has also been shown <sup>3</sup>.

#### **SOD2 gene polymorphisms**

MnSOD (SOD2) gene is located on chromosome 6q25. It is the only known antioxidant enzyme present within the mitochondria. Considering the relevance of MnSOD as the first line of defense to ROS production, structural and/or functional SNP of the MnSOD encoding gene are of high importance in the maintenance of cellular ROS levels. In humans, at least 190 SNPs have been identified for MnSOD <sup>11</sup>. Ala16Val (rs4880) is a functional and most studied MnSOD SNP polymorphism in exon 2 of SOD2 gene. A functional polymorphism in exon 2 of SOD2 gene Ala16Val (rs4880) was identified that resulted in structural alterations in the mitochondrial targeting domain, implicating its decreased antioxidant potential to limited post-transcriptional transport <sup>1</sup>. This substitution of C to T (GCT to GTT), that is alanine to valine, results in structural alterations in the mitochondrial targeting domain from  $\beta$ -sheet to  $\alpha$ -helix, which induces a 30-40% increase in MnSOD activity in mitochondria <sup>1,11,62</sup>.

#### **SOD2 Ala16Val SNP and antioxidant status**

Conflicting interaction between Ala16Val SNP and antioxidant status was first found in Ala homozygous women who had lower antioxidant intake that showed higher risk for breast cancer development <sup>11</sup>. Shanghai Breast Cancer Study provided some evidence that genetic polymorphism in the MnSOD gene may be associated with increased risk of breast cancer among Chinese women with high levels of oxidative stress or low intake of antioxidants <sup>63</sup>. Greatest risk of breast cancer

among women who consumed lower amounts of dietary antioxidants rather than high consumers indicates that a diet rich in sources of antioxidants may minimize the deleterious effects of the MnSOD polymorphism <sup>64</sup>. Modulating effects of serum antioxidant nutrient status (beta-carotene, lycopene, zeaxanthin/lutein, retinol, alpha-tocopherol and gamma-tocopherol) on interaction between MnSOD rs4880 polymorphism and cervical carcinogenesis risk was suggested according to findings of Tong *et al*'s study <sup>65</sup>. These conflicting results in investigation of Ala16Val SNP, antioxidant status and cancer may be due to the complex cancer etiology, methodological limitations and differences in study designs, including the nature and duration of intervention, age, sex, health status and lifestyle characteristics of the study populations <sup>11</sup>. Despite inconsistencies, the overall results suggest that the MnSOD Ala16Val SNP can be modulated by dietary factors. However, future studies are needed to be performed to clarify the nature of this association <sup>11</sup>.

#### **SOD2 Ala16Val SNP and diabetes**

Impact of SNPs in genes encoding for antioxidant enzymes on oxidative stress modulation and preventing subsequent disease development in recent studies has raised a growing interest. Protecting effect of MnSOD against diabetes has been also shown by numerous studies <sup>12,66,67</sup>. Protective role of "CC" (Ala/Ala) genotype was first reported in T1DM cases and control subjects with A16V polymorphism (rs4880) <sup>62</sup>. Mutations or variations in antioxidantase (Ala16Val polymorphism) can decrease its activity, which in turn increases oxidative stress. This process is an important pathophysiological mechanism in the development and progression of diabetes and its vascular complication. As Ala16Val polymorphism can decrease MnSOD activity, it makes Val carriers less resistance to oxidative stress because of limited antioxidant potential and consequently stressful conditions like hyperglycemia itself and activation of other stress-sensitive signaling pathways by Reactive Metabolites (RMs) resulting in further damages rather than Ala/Ala and Ala/Val carriers. Through such hypothesis that presented in relation to A16V SNP, some studies investigated whether this polymorphism is related to etiology of type 2 diabetes or diabetes complication in a sample of population <sup>1,13</sup>. The Ala16Val polymorphism of SOD2 might be a risk factor for diabetes among Japanese Americans. Results of this study suggest that an insufficiency of ROS scavenging associated with the lack of Ala allele may lead to glucose intolerance <sup>68</sup>. Another study showed that TT genotype (Val/Val) was most common in both T1DM and T2DM patients; CT genotype was common in healthy subjects whereas the CC genotype (Ala/Ala) was rare in all groups <sup>69</sup>.

#### **SOD2 SNP and diabetes complications**

**Renal complications:** DN is one of the important microvascular complications of DM, which is the lead-

ing cause of CKD<sup>33,70,71</sup>. Risk of cardiovascular diseases, progression to End-Stage Renal Disease (ESRD) and all-cause mortality increased in these patients<sup>70,71</sup>. Association of functional impairment of the MnSOD gene with an increased risk of DN was studied and results showed that V allele of the SOD2 rs4880 polymorphism in patients with T1DM increased the risk of DN<sup>10</sup>. This association was also studied in Chinese patients with type 2 diabetes which indicated protective effects of Ala allele for the development of DN<sup>13</sup>. The VV type showed a significantly higher frequency in Japanese diabetic patients with nephropathy than AA or VA<sup>16</sup>. In agreement with a role for SOD2 in the protection against oxidative stress and kidney disease in type 1 diabetes, the results of Mohammedi *et al*'s study showed that V-allele of rs4880 (V16A) was associated with the incidence and the progression of diabetic nephropathy, with a faster decline in estimated Glomerular Filtration Rate (eGFR)<sup>70</sup>. Results of another study on Korean type 2 diabetic patients suggested that V16A polymorphism of MnSOD gene is not related to pathogenesis of diabetes but is associated with stages of albuminuria. Also, patients with nephropathy, micro and macro groups, had significantly lower A allele frequency than those patients without nephropathy<sup>72</sup>. Homozygosity for the MnSOD Val allele contributed to development of DN in Finnish and Swedish patients with type 1 diabetes<sup>73</sup>. Results from 8 years of type 1 Danish diabetic patients follow up, showed that VV genotype patients had double risk for DN compared with those with AA genotype<sup>74</sup>. The frequency of the V allele was higher in the Mexican type 2 diabetes patients with macro albuminuria than in the norm albuminuria group (41.6 vs. 32.9%). Data of this study showed that the SOD2 Ala16Val A allele, which codes for the amino acid alanine, confers a protective effect against macro albuminuria<sup>10</sup>. It is now clear that susceptibility to DN could be affected by genetic factors<sup>70</sup>. The role of MnSOD genetic variants in protection against diabetes kidney complication is supported by previous studies. These studies are important in order to help us clarify the effect of oxidative stress related genes SNPs on diabetes complication<sup>10,70</sup>.

**Neuropathy:** Increased oxidative stress, which is induced by DM, has been implicated in the etiology and development of diabetic neuropathy<sup>33,75,76</sup>. Oxidative stress can cause damage in neurons via nerve lipid peroxidation, the breakdown of mitochondrial DNA and inhibition of the respiratory chain, and the cross-linking of the neurofilament protein<sup>75</sup>. Rapid changes of glia cells may also occur through oxidative disorders<sup>33</sup>. Severe pain, suffering, disability, cardiac death and silent myocardial ischemia are some of the important consequences of diabetic neuropathy<sup>40</sup>. Antioxidant enzymes may protect against the rapid onset and progression of diabetic neuropathy. Chistyakov *et al* showed that the frequencies of the Val allele (49.4 vs. 31.5%) of the MnSOD gene and the Val/Val genotype

(15.9 vs. 2.4%) were significantly more in the DN patients than in the control group<sup>75</sup>. Significantly higher frequencies of the Val allele and the homozygous Val/Val genotype in patients with diabetic neuropathy than diabetics without neuropathy were also shown in Egyptian type 1 diabetic patients. But alanine to valine substitution in MnSOD gene was not a significant factor in Egyptian diabetic patients with nephropathy<sup>76</sup>. According to the research findings, oxidative stress is an important linkage between hyperglycemia and development and progression of diabetic neuropathy. Much more studies are needed in order to investigate the role of genetic factors directly related to oxidative stress in DN formation and progression and to compare their results with each other<sup>40,75</sup>.

**Retinopathy:** The other complication of DM is retinopathy<sup>33</sup>. The significant role of oxidative stress in the development of diabetic retinopathy has been indicated in some studies<sup>77,78</sup>. Oxidative stress can cause damage to the vascular endothelium in diabetic retinopathy<sup>78</sup>. High consumption of oxygen, high proportion of polyunsaturated fatty acids, and exposure to visible light are some reasons for high susceptibility of retina to oxidative stress<sup>77</sup>. Study on Slovene population (Caucasians) with type 2 diabetes, provides evidence that the VV genotype of the V16A polymorphism of the MnSOD gene might be a risk factor for diabetic retinopathy<sup>77</sup>. Contribution of the V allele to the development of diabetic retinopathy was shown in Chinese type 2 diabetic patients, too<sup>79</sup>. Study on the results of Korean type 2 diabetic patients suggests that V16A polymorphism of the MnSOD gene is not related to the development of diabetes and progression of Diabetic Retinopathy (DR), but is associated with Diabetic Macular Edema (DME)<sup>80</sup>. Further studies, with larger sample size from different populations are needed to better understand the genes associated with diabetic retinopathy. In this way, the biochemical mechanisms of the disease can be identified that help to develop new tools for identification of patients at risk<sup>77,78</sup>.

**Cardiovascular disease:** Cardiovascular disease is one of the major diabetes complications<sup>34</sup>. Oxidative stress is implicated in the onset and progression of cardiovascular disease by its possible important role in atherogenesis and LDL oxidation<sup>33,81</sup>. Genotypic distribution or allelic frequency of the MnSOD Ala16Val polymorphisms do not individually contribute to the etiology of diabetic Cardio Vascular Disease (CVD) in Chinese type 2 diabetes patients but may contribute to hypertriglyceridemia<sup>81</sup>. A meta-analysis of the Ala16Val polymorphism in SOD2 gene suggested that A allele of it has reduced the risk of diabetes mellitus, including type 1 and type 2 diabetes, and Diabetic Micro vascular Complications (DMI) including diabetic nephropathy, diabetic retinopathy and diabetic polyneuropathy<sup>82</sup>. A cohort study in Denmark concluded that MnSOD V16A polymorphism is the independent predictable

risk factor of CVD. Increased risk of developing CVD in VV and AV genotypes compared with AA genotype was also shown in this study<sup>74</sup>. Because of inconsistency between results of these studies, further study will be required to elucidate the association between MnSOD Ala16Val genotype and CVD in diabetic patients<sup>81</sup>.

### Conclusion

Oxidative stress, largely caused by excess generation of highly reactive free radicals due to hyperglycemia, not only has a potential role in diabetogenesis and development of diabetic complications but also can exacerbate progression of them. Antioxidant enzymes play crucial role in regulating oxidative status, which is affecting diabetes and its related complications. MnSOD antioxidant enzyme plays a major role against ROS within the mitochondria of aerobic organisms. SNP in gene encoding for MnSOD antioxidant enzyme may directly impact on the oxidative stress modulation and protection against diabetes and its induced abnormalities. MnSOD Ala16Val SNP as the most studied structural SNP within the MnSOD encoding gene has been shown to alter the enzyme localization and mitochondrial transportation, affecting the redox status balance. Patients with mutant MnSOD demonstrate increased susceptibility to oxidative stress and severe mitochondrial dysfunction resulting from elevated ROS production. Insufficient ROS scavenging related to the MnSOD gene genotype may be associated with susceptibility to glucose intolerance. Many studies have investigated the association of genetic variation of rs4880 SNP with diabetes and some of its complications. Significant association between this SNP and diabetes complications like cardiovascular disease, nephropathy, neuropathy and retinopathy were also shown in different studies. Because of inconsistencies between studies, many environmental factors such as dietary and plasma antioxidant capacity as significant modifying factors have been studied in relation to diabetes and some of its complications. Study results have shown that these antioxidant statuses can mediate oxidative stress consequences in patients<sup>4,83,84</sup>. The interaction of antioxidant status in association with MnSOD A16V SNP and some chronic diseases like cancers have been studied in different population. However, these probable dietary and plasma antioxidant capacity interactions with rs4880 SNP in relation to diabetes and its complications have not been investigated yet. Association of antioxidant status, as an environmental factor, with the Ala16Val genotypes in diabetes and its related diseases still needs to be clarified.

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### Conflict of Interest

All authors have seen and agreed with the contents of the manuscript and there is no conflict of interest to report.

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