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REVIEW ARTICLE

The MnSOD Ala16Val SNP: Relevance to human diseases and interaction with environmental factors

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Abstract

The relevance of reactive oxygen species (ROS) production relies on the dual role shown by these molecules in aerobes. ROS are known to modulate several physiological phenomena, such as immune response and cell growth and differentiation; on the other hand, uncontrolled ROS production may cause important tissue and cell damage, such as deoxyribonucleic acid oxidation, lipid peroxidation, and protein carbonylation. The manganese superoxide dismutase (MnSOD) antioxidant enzyme affords the major defense against ROS within the mitochondria, which is considered the main ROS production locus in aerobes. Structural and/or functional single nucleotide polymorphisms (SNP) within the MnSOD encoding gene may be relevant for ROS detoxification. Specifically, the MnSOD Ala16Val SNP has been shown to alter the enzyme localization and mitochondrial transportation, affecting the redox status balance. Oxidative stress may contribute to the development of type 2 diabetes, cardiovascular diseases, various inflammatory conditions, or cancer. The Ala16Val MnSOD SNP has been associated with these and other chronic diseases; however, inconsistent findings between studies have made difficult drawing definitive conclusions. Environmental factors, such as dietary antioxidant intake and exercise have been shown to affect ROS metabolism through antioxidant enzyme regulation and may contribute to explain inconsistencies in the literature. Nevertheless, whether environmental factors may be associated to the Ala16Val genotypes in human diseases still needs to be clarified.

Keywords: antioxidant enzymes, oxidative stress, health conditions, nature and nurture

Introduction

Some 2–3 billion years ago, oxygen (O_2) was introduced into the earth's atmosphere through the evolution of the O_2-releasing photosynthetic organisms. Within a few million years, the atmospheric O_2 content increased to approximately 21%. This shift to an O_2-containing environment provided a selective pressure for the evolution of the O_2-requiring organisms. Nowadays O_2 is broadly known to be vital to aerobes, and oxidative metabolism provides an enormous advantage through complete glucose combustion. During oxidation more than 90% of the body's O_2 is consumed by the electron transport chain (ETC) in the mitochondria, giving rise to the superoxide anion ($O_2^{\bullet -}$) and other reactive oxygen species (ROS) as byproducts [1].

ROS may be also generated by oestrogens and their metabolites, by a variety of xenobiotics, and across the xanthine–xanthine oxidase system [2]. However, most of the ROS generation occurs during the ETC in the mitochondria, where great amounts of $O_2^{\bullet -}$ are formed from the oxidative phosphorylation [3]. This triggers a ROS cascade production which is summarized in Figure 1. Due to the high ROS reactivity and their potential role in damaging cell membranes and biomolecules, aerobes have developed antioxidant systems to cope with the harmful effects of excessive ROS production. Non-enzymatic antioxidants include a variety of ROS quenchers, such as vitamins, micronutrients (iron, zinc, cooper, selenium, and manganese), thiols (including glutathione [GSH]), uric acid, bilirubin, and flavonoids [4]. The main antioxidant enzyme pool against ROS includes superoxide dismutase (SOD, EC 1.15.1.1, superoxide:superoxide oxidoreductase), catalase (CAT, EC 1.11.1.6, hydrogen-peroxide:hydrogen-peroxide oxidoreductase), and glutathione peroxidase (GPx, EC 1.11.1.9, glutathione: hydrogen-peroxide oxidoreductase) [5]. SOD dismutates the $O_2^{\bullet -}$ radical into H_2O_2, which serves as substrate to CAT and GPx. The last two enzymes catalyze the conversion of H_2O_2 into H_2O. GPx presents a higher affinity for H_2O_2 and requires providing electrons a supply of GSH which is converted into oxidized glutathione (GSSG) [6].

The relevance of ROS generated from mitochondria and other cellular sources relies on the dual role played in both physiological and pathology-related outcomes.

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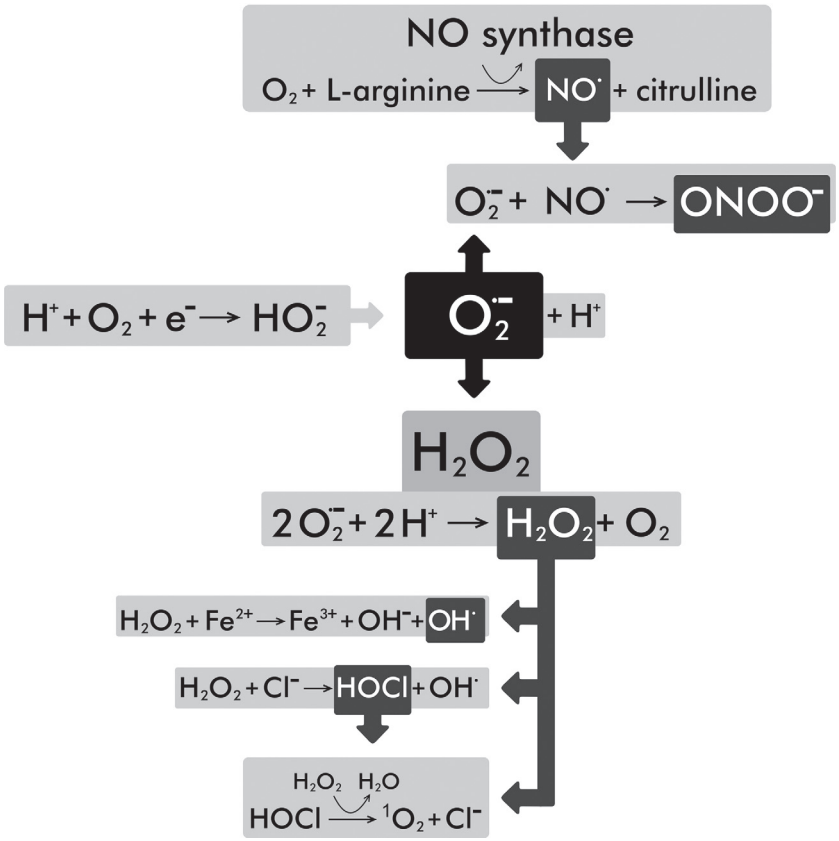

Figure 1. Pathways of ROS production through superoxide generation. In the mitochondria, O_2 is reduced and partially converted into superoxide radical ($O_2^{\bullet -}$). Apart from $O_2^{\bullet -}$ production, a few other ROS are generated through different oxireduction reactions. These reactions may be mediated by enzymes and/or direct combination with specific molecules. $O_2^{\bullet -}$ dismutation by the superoxide dismutases produces hydrogen peroxide (H_2O_2), while its combination with free iron generates the hydroxyl radical ($OH^{\bullet}$). Reaction of nitric oxide ($NO^{\bullet}$) and $O_2^{\bullet -}$ produces the potent and versatile oxidant peroxynitrite ($ONOO^-$). Generation of hypochlorous acid (HOCl) and singlet oxygen ($^1O_2$) are also shown.

Low levels of ROS are required to maintain redox-dependent regulation processes [7], and these molecules are related to signaling transduction pathways, gene expression, or immune system cell activation [3]. Nevertheless, excessive ROS production or insufficient *in vivo* defense mechanisms may result in tissue and cell damage through DNA oxidation, lipid peroxidation, or protein carbonylation, causing the so-called oxidative stress. Of note, oxidative stress prevention and management depends on the proper functioning of the endogenous and exogenous antioxidant defenses, and genetic variations may influence both of them [8].

Superoxide dismutases and gene polymorphisms

The SOD is the first acting antioxidant enzyme and plays a critical role in protecting cells against ROS-induced damage [9]. The SOD family dismutates $O_2^{\bullet -}$ derived from extracellular sources or produced within the mitochondrial matrix as a by-product of O_2 metabolism through the ETC [10]. Three SOD isoforms have been described in mammalian cells: the cytosolic copper/zinc-containing SOD1 (Cu/ZnSOD), the mitochondrial manganese-containing SOD2 (MnSOD), and the extracellular copper/zinc-containing SOD3 (ECSOD) [9–12]. Genetic comparisons indicate similarities between Cu/ZnSOD and ECSOD genes in certain levels of amino acids homology, whereas MnSOD does not share substantial characteristics with the other SODs family [13]. The MnSOD isoform becomes a key antioxidant enzyme in the protection of cells from $O_2^{\bullet -}$ anions due to its unique genetic organization and mitochondria matrix localization [11,12].

Human MnSOD is a homotetrametric molecule of 23 kDa encoded by the MnSOD nuclear gene localized in chromosome 6q25.3 [14,15] (Figure 2). The MnSOD enzyme is synthesized with a mitochondrial targeting sequence (MTS) and is translated in the cytoplasm, transported into the mitochondria, processed, and assembled into an active homotetramer [15,16]. MnSOD is the only known antioxidant enzyme present within the mitochondria [17], and it has been considered a unique tumor suppressor protein presenting a pivotal role in regulating cell death events [12]. However, under certain circumstances, an increased MnSOD activity may lead to cell damage by H_2O_2 overproduction, especially in individuals with a decreased capacity to remove this highly toxic ROS by GPx or CAT [9].

The most common genetic mutation studied in humans is the single nucleotide polymorphism (SNP), which

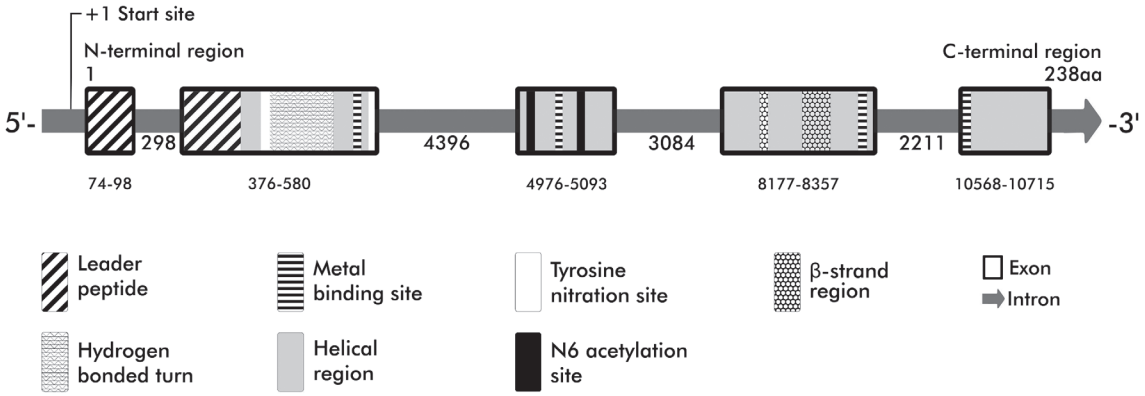

Figure 2. Genomic organization of the human MnSOD gene. MnSOD is a single-copy gene consisting of five exons interrupted by four introns with typical splice junctions. Location and size of each exon in terms of DNA base pairs is shown by using the start site as + 1. Exon positions are aligned showing the domain organization of the gene. Adapted from Dhar and St Clair [112].

occurs when gene single bases are changed or deleted, resulting in amino acids modification at specific positions and thus in altered phenotypes [17]. A few SNPs are "silent", while others may give rise to altered phenotypes across protein modulation or function, and possibly affect homeostasis [18]. These variations in the DNA sequence may alter the individual response to diseases, bacteria, viruses, xenobiotics, etc [19]. SNPs have been described for the genes encoding the main antioxidant enzymes. Thus, a SNP in the GPx1 gene (Pro198Leu rs1050450) has been reported to modulate GPx activity in erythrocytes of breast cancer patients [20], while the CAT C-262T SNP alters the transcription factor binding and basal CAT activity in red blood cells [21].

In humans at least 111 SNPs have been identified for Cu/ZnSOD, 190 for MnSOD, and 100 for ECSOD [17]. Considering the relevance of MnSOD as the first line defense to ROS production, structural and/or functional SNPs of the MnSOD encoding gene are of high importance in the maintenance of ROS cell levels [14]. Two main MnSOD SNPs have been described in the literature. The Ile58Thr SNP is characterized by the presence of isoleucine (Ile) or threonine (Thr) in the 58th position of the amino acid sequence [22]. The MnSOD Ile^{58} form is a better tumor suppressor in human breast cancer cells when compared to the Thr^{58} form [23]. However, the most commonly studied MnSOD SNP is the Ala16Val, characterized by a structural mutation substituting a thiamine (T) for a cytosine (C) in the exon 2. The substitution affects the codon 16, translating the valine amino acid (GTT) into alanine (GCT) (Ala16Val) [14,24]. The signal peptide is removed during the MnSOD processing to a mature enzyme and plays a key role in targeting the enzyme into the mitochondria [24]. The valine-to-alanine substitution produces a β-sheet secondary structure instead of the expected α-helix structure, which may decrease the transport efficiency of the enzyme into the mitochondria, modifying the antioxidant defense against ROS [24]. The Ala variant is able to quickly transverse both mitochondrial membranes to reach the matrix, while most of the Val variant is embedded within the inner membrane [25]. This may be due to the α-helical structure of the Ala-containing precursor which results in an enhanced MnSOD transport, and thus a higher mitochondrial fraction for the 16th amino acid positions in the signal peptide [26]. The Ala-MnSOD precursor generates 30–40% more of the active, matricial, processed MnSOD homotetramer in comparison with the Val-MnSOD precursor [27]. Therefore, according to the Ala16Val homozygous genotypes, a differential MnSOD availability in the mitochondria may occur (Figure 3).

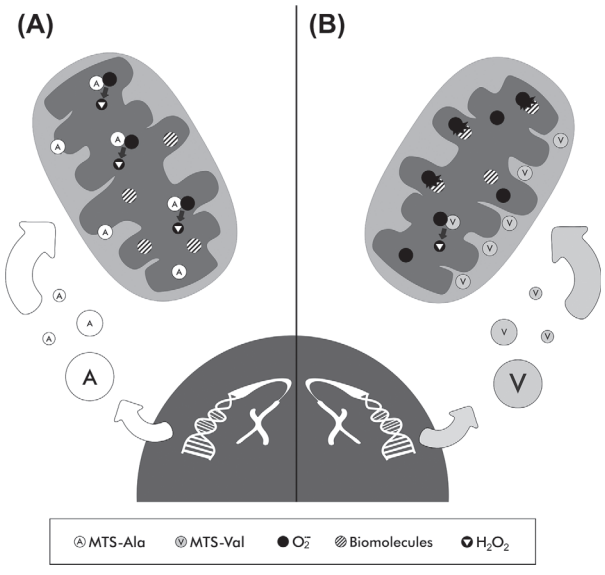

Figure 3. Differential MnSOD availability in the mitochondria according to the Ala16Val homozygous genotypes. The MTS-Ala MnSOD precursor (A) is correctly transported through both mitochondrial membranes, increasing superoxide radical ($O_2^{\bullet -}$) dismutation. Due to membrane permeability H_2O_2 easily reaches the cytosol and thus ROS-mediated toxicity in the mitochondria decreases. The MTS-Val MnSOD precursor (B) instead is partially arrested in the mitochondria inner membrane. This results in decreased antioxidant defenses within mitochondria, and higher amounts of $O_2^{\bullet -}$ which may react with different molecules, enhancing tissue oxidation.

MnSOD, human diseases, and SNPs

The damaging effect of ROS was first recognized by Harman, who proposed that aging is a process caused, at least in part, by ROS insult to cells and tissues [28]. Nowadays ROS are frequently related to pathophysiological mechanisms behind different diseases, and an increasing number of pathologies appear to have ROS damage as an ethiological component or as a contributing factor in the development of complications [29,30]. Moreover, oxidative stress may be linked to chronic diseases development, such as cardiovascular diseases, type 2 diabetes, and cancer [30]. In this sense, intrinsic and/or extrinsic factors that may modulate the antioxidant response to different stimuli have been described. The relationship between gene mutations of encoding antioxidant enzymes and oxidative stress-related diseases has raised a growing interest on how these SNPs may be helpful in the biomedical scenario [17]. SNPs in genes encoding for antioxidant enzymes or uptake and usage of dietary proteins may directly impact on the oxidative stress modulation, preventing subsequent disease development [8].

Numerous studies have shown that MnSOD may be induced to protect against neurotoxic conditions [25], certain tumors [11], cardiovascular diseases [31,32], and diabetic-induced abnormalities [33,34]. Furthermore, MnSOD is essential for life as dramatically illustrated by the neonatal lethality in MnSOD-deficient mice [35,36]. In addition, mice expressing only 50% of the normal MnSOD content demonstrate increased susceptibility to oxidative stress and severe mitochondrial dysfunction resulting from elevated ROS production [37]. Therefore, considering its remarkable role in the O_2 metabolism, an ever increasing number of studies have investigated the relationship of the MnSOD Ala16Val SNP with human diseases, and a few representative reports are here presented to illustrate the actual landscape.

Diabetes-related diseases

Excessive oxidative stress has been considered a major factor in the onset of diabetes, and mitochondrial $O_2^{\bullet -}$ overproduction plays an important role in the development of diabetes complications [38,39]. Val/Val carriers presented higher risk for diabetes development in comparison to Ala carriers after adjustment for age, gender, systolic blood pressure, total cholesterol, and body mass index, and insufficient ROS scavenging related to the MnSOD gene genotype may be associated with susceptibility to glucose intolerance [40] (Table I). The Ala16Val SNP is associated with nephropathy in Japanese type 2 diabetic patients [41], a strong association between the Val allele and diabetic nephropathy has been found in a follow-up study with Danish patients [42], and in Finnish and Danish patients a Val/Val genotype association with increased risk of diabetic nephropathy has been reported when controlling for age onset, diabetes duration, smoking, and gender [43]. The Ala16Val substitution of the MnSOD gene also associates to neuropathy in diabetic patients, with a higher risk of neuropathy development for the Val allele and the homozygous Val/Val genotype [44]. In the same line, the Val/Val genotype is associated with diabetic retinopathy in Slovenian patients [45], and a higher Ala allele and Ala/Ala genotype frequency has been found in Finnish patients with retinopathy [46]. Significantly, different allele and genotype frequencies of this MnSOD SNP have been reported among diabetic patients with and without macroangiopathy, being diabetes control poorer in patients presenting the Val/Val genotype [47]. Very recently, it has been shown that polymorphic variations in MnSOD contribute to elevated plasma triglyceride levels in Chinese patients with type 2 diabetes or diabetic cardiovascular disease [48].

Cardiovascular diseases

Oxidative stress has been demonstrated in peripheral blood vessels during hypertension [49], and vascular $O_2^{\bullet -}$ production has been related to blood pressure increases in different forms of hypertension [50,51] and atherosclerosis [52,53]. Interestingly, MnSOD overexpression has been proven to inhibit hypertension and atherosclerosis outcomes [54,55]. An association between the Val allele and a high intima-media thickness, as well as a significant interaction with plasma levels of LDL cholesterol has been described in middle-age hypertensive women [56] (Table II). The Ala16Val SNP was found to be associated to the risk of hypertension regardless of arsenic exposure in an arsenic-related hypertension risk population, with the Ala allele carriers presenting a significantly higher risk [57]. Cardiomyopathy prevalence was higher in Val allele carriers with unrelated hemochromatosis independent of gender, age, alcohol misuse, diabetes, and iron overload [58]. In another study, Ala/Val and Val/Val subjects presented higher ox-LDL level in comparison with Ala/Ala participants, and multivariate analysis showed this MnSOD SNP to be an independent factor associated to high ox-LDL levels in Val carries [59]. In the same line, it has been shown that the Ala variant increases MnSOD activity and protects macrophages against ox-LDL-induced apoptosis, thus reducing risk of coronary artery disease and acute myocardial infarction in healthy subjects [60]. The Val allele was also closely associated with vasospastic angina pectoris patients, and logistic regression analysis revealed Val/Val genotype to be an independent risk factor for its development [61]. Finally, the Val-encoding MnSOD allele significantly correlates with severity and prognosis in cardiogenic shock due to dilated cardiopathy [62].

Liver-related diseases

Increase in ROS production has also been implicated in liver-related diseases, such as alcohol intoxication, hemochromatosis, and chronic hepatitis C virus infection [63–66]. ROS formation in different cell compartments, including mitochondria, leads to oxidative stress and mitochondrial damage [67], causing hepatocyte apoptosis and

Table I. Characteristics of studies on MnSOD Ala16Val SNP and diabetes-related diseases.

First author	Condition	Country	Controls	Case/Control	Gender	Cases (%)			Controls (%)		
						Ala/Ala	Ala/Val	Val/Val	Ala/Ala	Ala/Val	Val/Val
Nomiyama et al. (2003)	Nephropathy	Japan	Healthy nondiabetic	478/261	M/F	76.2	23.2	0.6	72.8	25.7	1.5
Strokov et al. (2003)	Polyneuropathy	Russia	Diabetic patients without diabetic polyneuropahty	54/54	NS	8(*n*)	35(*n*)	11(*n*)	17(*n*)	34(*n*)	3(*n*)
Möllsten et al. (2007)	Nephropathy	Sweden/ Finland	T1DM patients without albuminuria and without antihypertensive treatment	46–181/197–358	M/F	28.0/24.6 (S/F)	43.3/48.9 (S/F)	28.7/26.5 (S/F)	28.6/23.5 (S/F)	51.5/51.1 (S/F)	19.9/25.4 (S/F)
Nakanishi et al. (2008)	T2DM	USA/Japan	none	523/none	M/F	1.7	22.0	76.3	None	None	none
Flekac et al. (2008)	Vascular complications	Czech Republic	Healthy subjects	120(T1DM)-306(T2DM/140	M/F	4/2	30/26	66/72	38	90	52
Hovnik et al. (2009)	Retinopathy/ Nephropathy	Slovenia	Diabetic patients without complications	62/62	M/F	18.8/18.9 (r/n)	46.9/62.2 (r/n)	34.4/18.9 (r/n)	28.3/28.7 (r/n)	54.3/48.3 (r/n)	17.4/23.0 (r/n)
Möllsten et al. (2009)	Nephropathy	Denmark	Diabetic patients without nephropathy	441/314	NS	30	48	22	25	50	26
Chen et al. (2012)	Diabetic CVD	China	Non-CVD patients	85/83	NS	2.3	21.2	76.5	0.0	20.5	79.5
Kangas-Kontio et al. (2012)	Diabetic control/ Diabetic Retinopathy	Finland	Healthy subjects	131(diabetic without retinopathy) 98(diabetic with retinopathy)—/ 526	M/F	19/32	51/44	30/24	23	50	27

MnSOD, manganese superoxide dismutase; SNP, single nucleotide polymorphism; NS, not specified; *n,* total number of subjects*;* T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; S, Sweden; F, Finland; CVD, cardiovascular disease; r, retinopathy; n, nephropathy.

Table II. Characteristics of studies on MnSOD Ala16Val SNP and cardiovascular diseases.

First author	Condition	Country	Controls	Case/Control	Gender	Cases (%)			Controls (%)		
						Ala/Ala	Ala/Val	Val/Val	Ala/Ala	Ala/Val	Val/Val
Kakko et al. (2003)	Carotid atherosclerosis	Finland	Healthy subjects	261-258/259–267	M/F	61/58 (M/F)	117/134 (M/F)	72/62 (M/F)	54/59 (M/F)	134/107 (M/F)	64/57 (M/F)
Valenti et al. (2004)	Hemochromatosis	Italy	Healthy blood donors	217/212	M/F	23	60	17	20	56	24
Hsueh et al. (2005)	Arsenic-related hypertension	Taiwan	Healthy subjects	79/213	M/F	1.4	40.5	57	1.4	31.8	66.8
Gottlieb et al. (2005)	Cardiovascular risk	Brazil	Ox-LDL < 0.5	82/170	M/F	8.1	32.4	59.5	24.6	26.2	49.2
Fujimoto et al. (2008)	CAD/AMI	Japan	Healthy subjects	498-87/627	NS	1.2/0.0	20.7/75.0	78.1/85.3	2.2	29.0	68.8
Fujimoto et al. (2010)	vasospastic angina pectoris	Japan	Healthy subjects	228/618	M/F	1.3	19.3	79.4	1.9	28.3	69.8
Charniot et al. (2011)	Cardiogenic shock	France	None	24	M/F	23.5	29.5	47	None	None	None

MnSOD, manganese superoxide dismutase; SNP, single nucleotide polymorphism; NS, not specified; Ox-LDL, oxidized low-density lipoprotein; CAD, coronary artery disease; AMI, acute myocardial infarction.

liver injury [68,69]. Since a differential codification of MnSOD precursors according to different MnSOD Ala16-Val genotypes was first described in rat liver mitochondria [27], studies aimed at identifying relationships between liver diseases and this SNP have been carried out (Table III). Although research is still incipient in this field, a few investigations have brought to light the role of the Ala16Val SNP in liver MnSOD modulation. In this line, the association of the Ala16Val SNP in alcohol-induced oxidative stress has been investigated in patients with advanced alcohol-induced liver disease (ALD) and patients without ALD; the genotype distribution among patients and controls was not different, and authors concluded that the Ala16Val SNP did not influence alcohol-induced oxidative stress and ALD [70]. Equally, no association was found between the MnSOD Ala16Val SNP and ALD in patients with hepatic decompensation from a population of heavy drinkers [71]. In the same line, no influence was seen for the Ala16Val genotypes in patients with hemochromatosis and chronic hepatitis C virus infection [72]. On the contrary, the Val allele combined with the Pro allele of the Pro198Leu-GPx1 has been found to be associated with lower incidence of hepatocellular carcinoma (HCC) in patients with alcohol-induced cirrhosis [73]. Another study on patients with alcoholic cirrhosis has shown the Ala allele to be associated with higher liver iron scores and increased HCC development and mortality rate [74]. The same authors pointed out that the MnSOD Ala16Val SNP did not modulate the risk of HCC development in hepatitis C virus patients [75]. An association between the MnSOD Ala16Val SNP and non-alcoholic steatohepatitis (NASH) has been reported in French patients showing an increased Val genotype prevalence [76]. The MnSOD Ala16Val SNP has also been associated with NASH in overweight and obese Egyptian children, with biopsy-proven NASH patients presenting 100% prevalence of the Val/Val genotype [69].

Other pathologies and disease-related outcomes

The MnSOD Ala16Val SNP might be also involved with other metabolic diseases. An association of the Val/Val genotype with obesity, independent of sex, age, diabetes, dyslipidemia, hypertension, and metabolic syndrome, has been reported in a free living Brazilian community [77]. In the same line, an association has been suggested between the genotypes of this MnSOD SNP, hypercholesterolemia, and oxidative stress biomarkers, with a pro-oxidative status associated with Val/Val genotype in hypercholesterolemic patients [78]. The MnSOD Ala16Val SNP also influences the harmful effects produced by lymphocyte ultraviolet (UV) light exposition, having been shown that Ala/Ala cell cultures present a higher viability and mitotic index, combined with decreased levels of TBARS when compared to the other two genotypes after UV exposure [79]. The MnSOD SNP could also play a role in inflammatory conditions. Thus, the Ala/Ala genotype is much more frequent in

Table III. Characteristics of studies on MnSOD Ala16Val SNP and liver-related diseases.

First author	Condition	Country	Controls	Case/Control	Gender	Cases (%)			Controls (%)		
						Ala/Ala	Ala/Val	Val/Val	Ala/Ala	Ala/Val	Val/Val
Stewart et al. (2002)	Alcohol-induced oxidative stress/liver fibrosis	United Kingdom	Healthy subjects	281(AALD)-218(NALD)/244	NS	53/51	156/109	72/58	55	125	64
Namikawa et al. (2004)	NASH	Japan	Healthy subjects	63/150	M/F	5	11	84	3	29	68
Martins et al. (2005)	ALD	Portugal	NLD	100/76	M/F	22	55	23	19	37	19
Stickel et al. (2005)	HHC/CHC	Germany	Healthy subjects	285(HHC) 157(CHC)/160	M/F	19.7/28.1	59.9/47.7	20.4/24.2	26.9	50.6	22.5
Sutton et al. (2006)	HCC	France	None	162	M/F	24	51	25	None	None	None
Nahon et al. (2009)	HCC and death	France	None	190	M/F	24	51	25	None	None	None
El-Koofy et al. (2011)	NASH in obese	Egypt	Healthy subjects	76/20	M/F	0	0	7	NS	NS	9
Nahon et al. (2012)	HCC patients	France	Patients	84/205	M/F	29.3	41.9	28.8	44	34.8	46.6

MnSOD, manganese superoxide dismutase; SNP, single nucleotide polymorphism; NS, not specified; AALD, advanced alcohol disease; NALD, non-advanced liver disease; NASH, non-alcoholic steatohepatitis; ALD, alcohol liver disease; NLD, non-liver disease; HHC, hereditary hemochromatosis; CHC, chronic hepatitis C virus infection; HCC, hepatocellular carcinoma.

patients with age-related macular degeneration than in healthy subjects [80], the MnSOD Ala16Val SNP influences IL-6 production in open heart surgery [81], and it has been reported that glucose and insulin may trigger proinflammatory cytokines in Val/Val peripheral blood mononuclear cells (PBMCs) [82].

It is also important to mention that the MnSOD Ala16-Val SNP has been broadly related to different cancer types. In this scenario, Val genotype has been shown to be associated with breast cancer in patients with axillary lymph node metastasis [83], while both breast and prostate cancer risks are elevated in male and female patients with the Ala/Ala genotype [84]. A possible synergistic effect of Ala/Ala and Leu/Leu (Pro198Leu-GPx1) genotypes on bladder cancer risk has been found in a Turkish population [85]. On the other hand, the Val/Val genotype is associated with increased lung cancer risk in the same ethnic population [86]. Increased risk of pancreatic cancer has been reported in Val/Val carriers with confirmed pancreatic adenocarcinoma patients [87]. Moreover, the Val/Val genotype increases the risk of nonsmall cell lung carcinoma in Caucasian patients when combined with other SNPs (Arg72Pro-p53 and Arg399Gln-XRCC1) [88]. Conversely, the Val allele was associated with survival advantage in Finish acute myeloid leukemia patients [89]. For further information concerning cancer and MnSOD Ala16Val SNP association please refer to recent reviews on the topic [14,17].

Environmental factors and MnSOD modulation

Inconsistent findings between studies in the literature may be partially explained by SNPs interactions with environmental factors [8]. ROS generation and effects are closely associated to the diet, physical activity patterns, and other environmental factors, which interfere in the production and catalysis of these molecules. Therefore, the interaction between the MnSOD Ala16-Val SNP and environmental factors could exert positive or negative influences on the redox balance. Despite of the number of epidemiologic-based studies carried out, results concerning this interaction are still far from a conclusive standpoint. Different studies have analyzed the combined effect of the MnSOD SNP with factors that may increase ROS production, such as smoking and alcohol intake. In general, research has shown an influence of the Ala16Val SNP in the response to these prooxidant factors, which could increase the risk of developing diseases or dysfunctions [74,90]. Thus, a combined effect for Val/Val genotype with smoking has been found in lung cancer patients [86], female Ala carries who consume 19 g alcohol/day or more present increased risk of breast cancer [91], and there is an association between the Ala allele and increased prostate cancer risk in smokers with low vitamin intake [92]. The open question is still whether health-related environmental factors such as diet and exercise could reduce the risk of the Ala16Val SNP-associated diseases.

MnSOD Ala16Val SNP and diet

Recently, several studies have been carried out to understand the interactions between diet and SNPs of antioxidant enzymes implicated in oxidative stress-associated diseases, in particular cancer [8]. This is due to the fact that diets rich in fruits and vegetables are associated with lower risk of cancer, probably partially conferred to the antioxidant properties of these foods. However, antioxidant supplementation or increased consumption of antioxidant-rich foods has reported inconsistent effects in cancer patients, which could be related to diet–gene interactions [8]. Unfortunately, research verifying the potential interaction between the MnSOD Ala16Val SNP and dietary factors is conflicting. In the first study considering such interaction, it was found that Ala homozygous women with lower antioxidant intake presented a higher risk for breast cancer development, pointing out to the remarkable role of the environmental factors combined with genetics in oxidative stress modulation [93]. A meta-analysis which included 15320 cancer cases and 19534 controls from 34 published case-control studies showed no significant independent effect for the MnSOD Ala16-Val SNP on cancer risk [16]; however, authors found an association between premenopausal Ala allele carriers who had low antioxidants consumption (such as vitamin C, vitamin E, and carotenoids) and breast cancer risk. An association of the MnSOD Ala16Val SNP with plasma carotenoid concentrations has also been reported for prostate cancer risk and aggressiveness, with a significant higher risk from the combination between low antioxidant status and the Ala/Ala genotype [94]. The potential interaction between this MnSOD SNP and antioxidant status on cancer risk has also been described for other cancer types, such as cervical cancer [95]. The Ala allele association with cancer mediated by an antioxidant diet may be related to the potential imbalance in the H_2O_2 production. The overproduced H_2O_2 may react with metal ions yielding highly carcinogenic ROS, such as the hydroxyl radical ($HO^{\bullet}$). In fact, it has been observed that higher iron intake was associated with greater than 2-fold increase in risk on aggressive prostate cancer cases presenting Val/Val genotype [96]. Therefore, despite the lack of consistent data, there is mechanistic support for an interaction between MnSOD Ala16Val genotypes and iron on cancer risk.

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 may explain conflicting results when the relationship between cancer, MnSOD Ala16Val SNP, and diet is investigated. For these reasons, complementary studies in healthy individuals or controlled *in vitro* assays could help us to understand the interactions between the MnSOD Ala16-Val SNP and diet. Thus, when the extent of interindividual variation in lymphocyte DNA damage is analyzed in healthy volunteers, results obtained indicate that endogenous DNA damage before antioxidant supplementation is lower in the Val/Val genotype, but 6 weeks of antioxidant supplementation decrease the DNA damage of the Ala allele carriers to levels found in the Val/Val genotype without supplementation [97].

Despite inconsistencies, the overall results suggest that the MnSOD Ala16Val SNP can be modulated by dietary factors. However, future *in vivo* and *in vitro* controlled studies need to be performed to clarify the nature of this association, and to better understand whether these results have epidemiological and clinical applications.

MnSOD Ala16Val SNP and exercise

Generally, the body has adequate antioxidant defenses to cope with the production of ROS under physiological conditions to maintain homeostasis for cell function during rest and mild exercise [98,99]. However, intense or unaccustomed exercise increases O_2 consumption leading to increased ROS production, and an imbalance between ROS and antioxidants [100,101]. On the same line, SNPs result in changes in the activities of antioxidant enzymes which can suppose a reduced protection against oxidative stress induced by exercise. A possible association between the Ala16Val MnSOD SNP and exercise-induced damage has been reported in runners, being DNA damage higher for individuals carrying the Ala/Ala genotype than for other MnSOD genotypes [102]. We have recently studied the differential modulation of exercise-induced oxidative stress by the Ala16Val SNP in a group of subjects who performed a bout of intense exercise. The Ala/Ala genotype participants showed increased post-exercise MnSOD mRNA expression and enzyme activity in PBMCs. Conversely, MnSOD mRNA expression did not change, but protein thiol content decreased significantly after the bout of exercise in Val/Val carriers, and a comparison of the genotypes showed that the Ala/Ala genotype presented a higher MnSOD protein content than Val/Val volunteers after exercise; moreover, a dose-effect for the Ala allele was found for enzyme activity [103]. Another investigation concerning the MnSOD Ala16Val SNP has analyzed the interaction between antioxidant intake and acute exercise, showing that heterozygous genotype was related to less DNA damage and lipid peroxidation following an outdoor race, with a better response to a carotenoid-enriched oil against-exercise-induced damage [104]. These studies provide evidence that environmental factors such as exercise and diet may play an important role in the interaction between genetic variations and oxidative stress.

An increasing number of studies show that moderate exercise training is beneficial for many chronic conditions, such as diabetes, cardiovascular diseases, and cancer [105–107], and there is considerable evidence to suggest that exercise training may result in positive MnSOD modulation though redox sensitive pathways [108–110]. Leukocytes of healthy and trained subjects showed differences in

DNA damage according to different Ala16Val genotypes when exposed to H_2O_2 after an outdoor race protocol [111]. However, studies aiming to assess long-term moderate exercise effects on the MnSOD Ala16Val SNP modulation are still necessary. An open question is whether moderate exercise training may also prevent disease-associated risks in subjects carrying different Ala16-Val genotypes.

Concluding remarks

Superoxide dismutases play a key role as the first-line antioxidant enzyme in the aerobic organisms as its mitochondrially localized isoform MnSOD detoxifies the first ROS produced during aerobic metabolism. The most widely investigated MnSOD SNP is the Ala16Val, which alters the secondary structure of the mature protein and has been proven to affect the enzyme processing into the mitochondria. Therefore, it has been hypothesized that this SNP may influence the defenses against oxidative stress in several human conditions. Numerous studies have shown that genetic variation in the Ala16-Val MnSOD SNP significantly affects risk of diabetes, cardiovascular diseases, liver diseases, or cancer. However, inconsistencies between studies are leading to the investigation of potential environmental factors, such as diet or exercise, which can significantly modify the relationship between this MnSOD SNP and disease development. The understanding of this association may bring to light important knowledge to support the relevance of environmental factors on metabolic-related diseases treatment.

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Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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