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Predominant genotypes and alleles of the Val-9Ala polymorphisms in the mitochondrial targeting sequence leading the manganese superoxide dismutase gene associated with their superoxide dismutase activity and total antioxidant status of healthy Thai subjects

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ABSTRACT

Genotype distributions and allele frequencies of the Val-9Ala polymorphisms in the mitochondrial targeting sequence leading the manganese superoxide dismutase gene as well as associations with their superoxide dismutase activity and total antioxidant status of healthy Thai subjects were determined. The Val-9Ala polymorphisms in MTS-MnSOD gene of 221 healthy Thais including 135 females and 86 males were genotyped by real-time PCR. The SOD activity of whole blood cells and plasma total antioxidant status (TAS) of each subject were assayed. The Val/Val and the Val/Ala genotypes were found to be predominant rather than the Ala/Ala genotype. The Val allele frequencies in healthy Thai subjects, both females and males, were similar to those previously reported in healthy Chinese, Japanese and Korean subjects. The average values of SOD activity and TAS level in healthy Thais were 2,129.±778 U/g Hb and 1.27±0.26 mmol/L, respectively. The association of each genotype with SOD activity as well as TAS levels showed that the average values of SOD activity and TAS in healthy Thais with the Val/Val and the Val/Ala genotypes were not statistically different ($p>0.05$) and not lower than those of the Ala/Ala genotypes. In conclusion, the predominant genotypes were the Val/Val and the Val/Ala and they were not associated with low levels of SOD and TAS.

Keywords: manganese superoxide dismutase (MnSOD), mitochondrial targeting sequence (MTS), Val-9Ala polymorphism, superoxide dismutase (SOD), total antioxidant status (TAS), healthy Thai subjects

INTRODUCTION

Human mitochondria consume more than 95% of cellular oxygen and the mitochondrial electron transport chain is a main source of endogenous reactive oxygen species (ROS) generation. ROS are free radicals that can attack cellular components, causing damage to lipids, proteins and DNA. The ROS can initiate the onset of oxidative damage and apoptosis (Kannan and Jain, 2000). Extensive research in the field of ROS has linked them to a wide range of cancers, chronic and acute diseases. To protect against toxic effects of these free radicals, cells have developed an intrinsically regulated antioxidant system, which regulates and controls the level of ROS at the required physiological concentration. Oxidative stress is resulted from a serious imbalance between the ROS produced and the effective action of the antioxidant system (Halliwell, 1997).

The antioxidant system of human cells is very complex, being composed of three main groups including primary, secondary and tertiary antioxidants. Manganese superoxide dismutase (MnSOD), an important primary antioxidant enzyme, protects cells from ROS-induced oxidative damage by catalyzing dismutation of superoxide (O_2^-) to hydrogen peroxide and oxygen and thus constitutes the first line of defense against ROS in the mitochondria. MnSOD is a ubiquitous metalloenzyme synthesized in the cytoplasm. Its precursor contains 24 amino acids mitochondrial targeting sequence (MTS) at the N-terminus that helps in targeting the mature enzyme across the mitochondrial membrane to the matrix. The MTS has a single nucleotide polymorphism (SNP) in codon 16. A substitution of T for C changes the encoded amino acid at position 9 upstream of the cleavage site from Val (GTT) to Ala (GCT), designated as the Val-9Ala polymorphism. It was predicted that the Val form would encode β-sheet conformation rather than the preferred α-helical structure of MnSOD precursor protein leading to an impaired transport of MnSOD to mitochondria (Shimoda-Matsubayashi *et al*., 1996). The secondary structure alteration of MnSOD was suggested to affect the cellular allocation and transportation of the enzyme into the mitochondrial matrix (Rosenblum *et al*., 1996). The Ala form was targeted into mitochondria, whereas the Val form was partially arrested in the inner mitochondrial membrane within the narrow translocase of the inner mitochondrial membrane and degraded by the proteosome (Sutton *et al*., 2003). The slower mitochondrial import might be associated with decreased mRNA stability and possibly due to impaired cotranslational import (Sutton *et al*., 2005). The Ala form of MnSOD was approximately 40% more efficiently localized to mitochondria than the Val form. The Val-MnSOD was likely to be associated with higher

levels of ROS and thus predisposes to a greater risk of ROS induced diseases.

Many epidemiological studies have shown that low antioxidant levels are associated with increased incidences of over 100 diseases including cancers and aging. The antioxidant defense system has many components. A deficiency in any of these components can cause a reduction in the overall antioxidant status of an individual. SOD activity level can be used as an important screening index to detect individuals with lowered antioxidant defenses, and may be at greater risk of developing ROS-induced diseases. TAS aims to describe the dynamic equilibrium between pro-oxidants and antioxidants in the plasma compartment. It enables assessment of the integrated antioxidant system which encompasses all biological components with antioxidant activity (Polidori, 2003).

Previous studies reported that frequencies of Val/Ala allele of the MTS-MnSOD were approximately equal in Caucasian populations (Van Landeghem *et al*., 1999). The Val/Ala allele frequencies as well as different risk assessment in various ROS induced diseases should be studied in different ethnics. There have been no reports on the genotypes as well as the allelic frequencies of the Val-9Ala polymorphisms in the MTS-MnSOD and their associations of SOD activity and TAS in Thai subjects. The population of Thailand in 2011 stood at approximately 64 millions with the ratio of 0.98 males/females, of which 70% between the age of 15 and 64, 24% below 15 and only 6% older than 64. The population is relatively homogenous; however, this is changing due to immigration. Up to 75% of people in Thailand are Thai ethnic, whereas 14% are Chinese, 2% are Malay and Yawi-speaking Muslims. In addition, the rest 9% are other ethnic groups including the Khmer; the Mon, the Vietnamese and mountain-dwelling tribes such as Hmong, Mein and Karen (The CIA-World Factbook-Thailand, 2010)

In this study, three allelic genotypes of the Val-9Ala polymorphisms in the MTS-MnSOD gene of healthy Thai subjects (n=221), including 135 females and 86 males, and their associations with SOD activity of whole blood cells as well as plasma TAS levels of healthy Thai subjects were firstly analysed and reported.

MATERIALS AND METHODS

Human specimen collection

Healthy Thai subjects in this study had applied in a check-up program of the National Cancer Institute, Bangkok, Thailand during January 2009 to January 2011. They were between 20-75 years old with 135 females and 86 males (N=221). These healthy Thais were defined by physical examination, laboratory examination and historical questionnaires. None of them had acute or chronic diseases or cancers. All the presented subjects were accepted to participate after their informed consent. The Ethical Committee of the National Cancer Institute, Bangkok, Thailand approved the research protocol for this study. Blood

samples were collected after a 12-hour fasting period. Heparinized blood samples were used for SOD activity and TAS assays. EDTA blood samples were used for the Val-9Ala MnSOD genotyping. The following serum quantifications were conducted: glucose, urea, creatinine, uric acid, albumin, cholesterol, triglycerides, total protein, total bilirubin, directed bilirubin, high-density lipoproteins (HDL) cholesterol, low-density lipoproteins (LDL), aspartate amino transferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). All these clinical chemistry tests were analysed by an automatic analyzer (Hitachi 917) using commercial diagnostic reagents obtained from Hoffmann-La Roche Ltd, Switzerland. Cut-off points for reference values were determined at Pathology Department, National Cancer Institute, Bangkok, Thailand. These tests were used as screening profile measurements for the diagnosis of the clinically healthy subjects.

DNA extraction

Total nucleic acids of all the subjects were extracted from buffy coat of two milliliters of EDTA-whole blood by modified guanidinium thiocyanate-silica based Boom's method (Boom *et al*., 1990). Total nucleic acids were eluted from the silica with 100 µl of elution buffer (1x TE buffer pH 8.0) and kept at 4 ^{o}C until used.

Amplification of MTS-MnSOD gene

Amplification of the MTS-MnSOD Gene (246 bp) by PCR was carried out in 50 µl reaction mixture containing Tris-HCl, pH 8.4, 1.5 mM $MgCl_2$, 50 mM KCl, 0.2mM of dNTPs, 0.4 pM forward primer (V9A-F: 5'-AGCCCA GCCTGCGTAGAC-3'), 0.4 pM reverse primer (V9A-R: 5'-TACT TCTCCTCGGTGACG-3') and 2.5 U *Taq* DNA polymerase. The reactions were placed in a Perkin Elmer 480 Thermal Cycler. PCR cycle was set at one cycle of initial denaturation at 96 ^{o}C for 6 min, followed by 35 repeated cycles of 95 ^{o}C, 1 min; 60 ^{o}C, 1 min; 72 ^{o}C, 2 min and one cycle of final extension at 72 ^{o}C for 10 min. The PCR product of MTS-MnSOD gene was expected to be a single band of 246 bp.

Identification of the 246 bps of MTS-MnSOD gene

The PCR products of MTS-MnSOD gene (246 bp) were identified by restriction fragment length polymorphisms (RFLPs) analysis using *Bsa*WI endonuclease (New England BioLabs, USA). The reaction was performed in a final volume of 15 µl containing 1X NE buffer, 1 unit of *Bsa*WI and 10 µl of PCR product. The mixture was incubated at 60 ^{o}C for 4 hours. The digested products of the MTS-MnSOD gene analysed by agarose gel electrophoresis were expected to be 246 bp for the Ala-allele, as the non-digested PCR product, and two digested fragments of 164 bp and 82 bp for the Val-allele.

Genotyping Val -9Ala polymorphisms of the MTS-MnSOD gene

The Val-9Ala polymorphisms of MTS-MnSOD gene were genotyped by real-time

PCR using TaqMan allelic discrimination assay (PE Applied Biosystem, Foster city, CA) (Heid *et al*., 1996). It is based on the design of two TaqMan probes specific for the Val allele labeled with VIC fluorophore and the Ala allele labeled with FAM fluorophore. The VIC and FAM reporter dyes were covalently attached to the 5' terminal base of the two probes and the non-fluorescent quencher dye is attached near the 3' ends. All PCR reactions containing 100 ng of DNA, 12.5 µl of TaqMan universal PCR master mix and 1.25 µl of allelic discrimination mix were run in duplicate. Appropriate negative controls were run comparatively. Real-time PCR of all samples were performed in ABI Prism 7000 Sequence Detection System (PE Applied Biosystem,USA) setting at the following conditions: one cycle of 50 $^{\circ}$C for 2 min followed by 95 $^{\circ}$C for 10 min and then 40 cycles of amplification (92 $^{\circ}$C denaturation for 15 seconds, 60 $^{\circ}$C annealing/extension for 1 minute). For each cycle, the SDS software determined the ΔRn, which was the normalized fluorescent signal from the VIC- or FAM-labeled probe.

Superoxide dismutase activity

The SOD activity of each subject was assayed manually using RANSOD kit (Randox Laboratories, UK) (Ukeda *et al*., 1997). The method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The SOD activity was measured kinetically at 505 nm by the degree of inhibition of the reaction. The SOD concentration of each sample was calculated and expressed as U/gHb.

Total antioxidant status of human plasma

Plasma total antioxidant status (TAS) was measured manually using protocol and all reagents of TAS kit (Randox Laboratories, UK). The TAS assay (Yu *et al*., 1999) is based on the reduction of free radicals ABTS + (2, 2'-azino-di (3- ethylbenzo thiazoline-6-sulfonate) measured as a decrease of absorbance at 600 nm at 3 min by antioxidants. Each heparinized plasma sample or standard or water (20µl) was mixed with 1 ml of chromogen (metmyoglobin and ABTS®), incubated at 37°C and read initial absorbance (A1) at 600 nm. Hydrogen peroxide (200µl)was added as the substrate immediately into each reaction tube and read absorbance after exactly 3 minutes (A2). The Kinetic was measured at 600 nm. The antioxidant capacity was calculated and expressed as mmol/L.

Statistical analysis

Allele frequencies were calculated by the gene-counting method. The chi-square (χ^2) test was used to determine the observed genotype frequencies deviated from Hardy-Weinberg equilibrium expectations. Statistical analyses were performed using SPSS16.0 (SPSS Inc. Michigan, IL, USA). All presented values were presented as means ± standard deviation (SD). The results were considered as statistically significant at a p-value < 0.05.

RESULTS

All biochemical characteristics including glucose, BUN, creatinine, total protein, albumin, total bilirubin, direct bilirubin, AST, ALT, ALP, cholesterol, uric acids, HDL and triglyceride (Table 1) as well as chest X-ray films and physical examinations (data not shown) of these Thai subjects showed normal results for the diagnosis of the clinically healthy subjects.

The PCR product showed a single band of 246 bp (Fig. 1). According to the RFLPs digested with *Bsa*WI endonuclease, it was confirmed to be the specific PCR product of the MTS-MnSOD gene for genotyping Val-9Ala polymorphims since the non-digested PCR product for the Ala allele was expected to be 246 bp and the two digested fragments for the Val allele should be around 164 bp and 82 bp (Fig. 1). The 246 bp fragment amplified from each genomic DNA sample of these healthy Thais was used as the template for genotyping Val-9Ala polymorphisms of MTS-MnSOD gene by Realtime PCR using Taqman allelic discrimination assay. The percentage of each MTS-MnSOD genotype of the total subjects, females only, and males only were 60.2%, 62.2% and 57.0%, respectively for the Val/Val genotype, 36.6%, 35.6% and 38.4%, respectively for the Val/Ala genotype and 3.2%, 2.2% and 4.7%, respectively for the Ala/Ala genotype (Fig.2, 3 and Table 2). Similarly, the allele frequencies estimated by Hardy-Weinberg Equilibrium were 0.79, 0.80, 0.76 for the Val allele and 0.21, 0.20, 0.24 for the Ala allele (Table 3).

The SOD activities in whole blood cells (Table 4 and Fig 4) and plasma TAS levels (Table 4 and Fig. 5) were compared among the three allelic Val-9Ala MnSOD genotypes. The average of the SOD activities (U/g Hb) was 2,129±778 for total subjects, 1,929±815 for females and 2,174±699 for males. The average

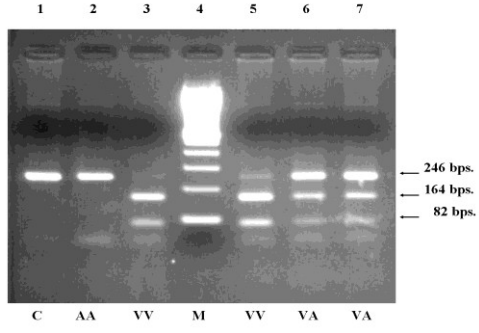

Figure 1 PCR-RFLPs of Val-9Ala MTS-MnSOD gene polymorphisms. PCR products were digested using *Bsa*WI and analysed by agarose gel electrophoresis. Lane 1, control (C) or undigested PCR product; lane 2, digested fragment of 246 bp for the homozygous Ala/Ala; lane 3 and 5, digested fragments of 82 and 164 bp for the homozygous Val/Val; lane 4, 100 bps DNA markers, lane 6 and 7, fragments 246, 82 and 164 bp for the heterozygous Val/Ala.

Table 1 Clinical chemistry characteristics using as screening profile measurements for the diagnosis of the clinically healthy subjects of all 221 healthy Thais including 135 females and 86 males.

Biochemical profile	Normal range (Thai population)	Total (N=221)	Females (N=135)	Males (N=86)
Glucose (mg/dl)	70-110	90 ± 8	88 ± 8	94 ± 6.
BUN (mg/dl)	5-20	12.4 ± 4.1	10.7 ± 2.8	15.3 ± 4.4
Uric acid (mg/dl)	2.0-7.5	5.3 ± 1.6	4.6 ± 1.2	6.7 ± 1.6
Creatinine (mg/dl)	0.5-1.5	1.0 ± 0.2	1.2 ± 0.2	1.2 ± 0.2
Total protein (g/dl)	6.5-8.5	7.8 ± 0.4	7.8 ± 0.4	7.8 ± 0.5
Total bilirubin (mg/dl)	0.1-1.2	0.7 ± 0.3	0.7 ± 0.3	0.8 ± 0.4
Direct bilirubin (mg/dl)	0.00-0.50	0.16 ± 0.09	0.15 ± 0.08	0.18 ± 0.12
Albumin (g/dl)	3.4-5.0	4.4 ± 0.2	4.4 ± 0.2	4.4 ± 0.3
Cholesterol (mg/dl)	140-250	198 ± 35	198 ± 33	199 ± 42
LDL (mg/dl)	62-185	141 ± 37	145 ± 48	140 ± 36
Triglyceride (mg/dl)	35-200	135 ± 74	114 ± 61	175 ± 79
HDL (mg/dl)	30-80	51± 13	53 ± 14	46 ± 13
AST (U/L)	2-40	22 ± 10	19 ± 6	27 ± 13
ALT (U/L)	2-40	23 ± 16	20 ± 13	29 ± 20
ALP (U/L)	35-110	76 ± 25	75 ± 20	78 ± 31

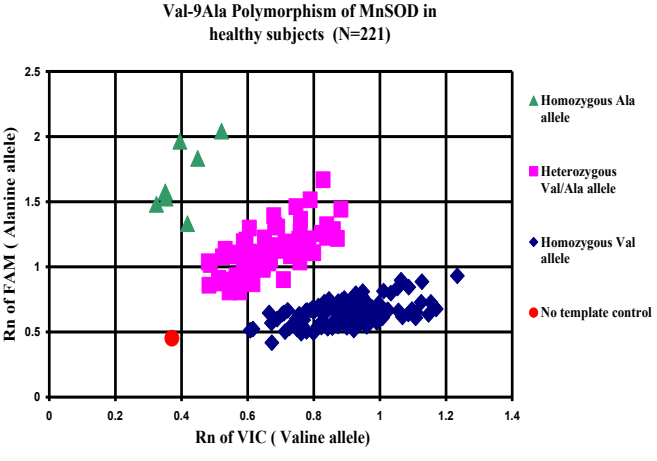

Figure 2 Three allelic MTS-MnSOD genotypes of all 221 healthy Thais including 135 females and 86 males analysed by a real-time PCR using TaqMan allelic discrimination assay (PE Applied Biosystem, Foster city, CA). Two TaqMan probes were used for the Val allele labeled with VIC fluorophore and the Ala allele labeled with FAM fluorophore.

Table 2 Frequencies of three allelic MTS-MnSOD genotypes as the homozygous Val/Val, heterozygous Val/Ala and the homozygous Ala/Ala of 221healthy Thais including 135 females and 86 males.

MTS-MnSOD genotypes	All (N=221)		Females (N=135)		Males (N=86)	
	N	%	N	%	N	%
Valine/Valine	133	60.2	84	62.2	49	57.0
Valine/Alanine	81	36.6	48	35.6	33	38.4
Alanine/Alanine	7	3.2	3	2.2	4	4.7

Table 3 Frequencies of the Val allele and the Ala allele of the MTS-MnSOD gene of healthy Thais including 135 females and 86 males.

MTS-MnSOD alleles	Allele frequencies of Thai subjects			p-values
	All (N=221)	Females (N=135)	Males (N=86)	
Val allele	0.79	0.80	0.76	p>0.05
Ala allele	0.21	0.20	0.24	p>0.05

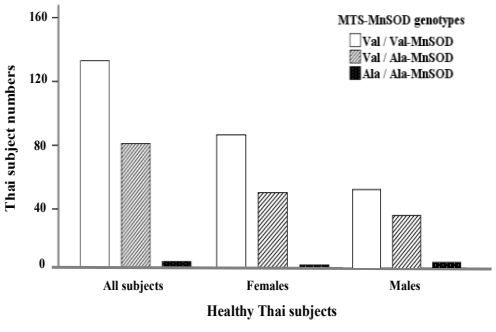

Figure 3 Frequencies of three allelic MTS-MnSOD genotypes as the homozygous Val/Val, heterozygous Val/Ala and the homozygous Ala/Ala of 221healthy Thais including 135 females and 86 males estimated by Hardy-Weinberg Equilibrium.

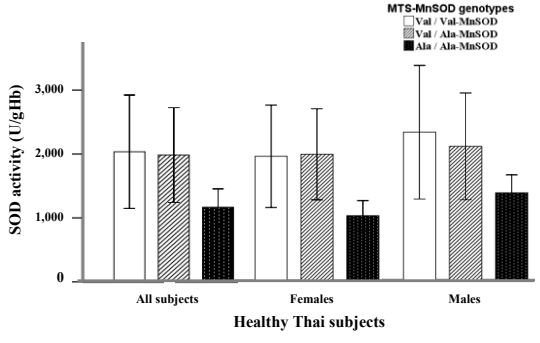

Figure 4 SOD levels of healthy Thais both females and males compared among three allelic MTS-MnSOD genotypes. The SOD activity of each subject was assayed manually using RANSOD kit (Randox Laboratories, UK). The results were plotted and compared among the homozygous Val/Val, the heterozygous Val/Ala and the homozygous Ala/Ala.

Table 4 Whole blood SOD activity and plasma total antioxidant status (TAS) of all 221 healthy Thai subjects including 135 females and 86 males compared among the subjects with the homozygous Val/Val, the heterozygous Val/Ala and the homozygous Ala/Ala forms of the MTS-MnSOD gene.

MTS-MnSOD genotypes	SOD activity (U / gHb) (Mean ± SD)			Total antioxidant status (mmol / L) (Mean ± SD)		
	All (N=221)	Females (N=135)	Males (N=86)	All (N=221)	Females (N=135)	Males (N=86)
Val / Val	2,183 ±815	1,902±783	2,268±1,024	1.24 ± 0.22	1.21 ± 0.23	1.34 ± 0.20
Val / Ala	2,341 ± 724	1,934±695	2,050±819	1.32 ± 0.32	1.31 ± 0.37	1.32 ± 0.24
Ala / Ala	1,346 ± 264	997±231	1,339±275	1.36 ± 0.22	1.10 ± 0.13	1.30 ± 0.08
Total	2,129±778	1,929±815	2,174±699	1.27± 0.26	1.25±0.29	1.32±0.21

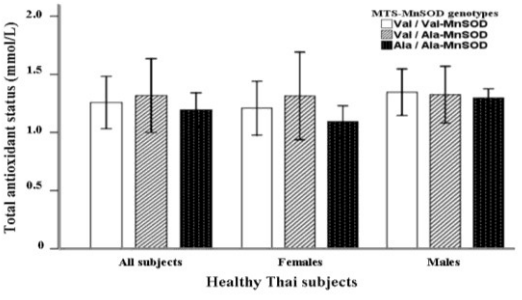

Figure 5 The total antioxidant status (TAS) of healthy Thai subjects compared among three allelic MTS-MnSOD genotypes. Plasma total antioxidant status (TAS) was measured manually using protocol and all reagents of a commercial kit (Randox Laboratories,UK). Plasma TAS of all 221 healthy Thai subjects including 135 females and 86 males were plotted and compared among the homozygous Val/Val, the heterozygous Val/Ala and the homozygous Ala/Ala.

TAS levels (mmol/L) was 1.27± 0.26 for total subjects, 1.25±0.29 for females, and 1.32±0.21 for males. By statistical analysis using standard software SPSS16.0 (SPSS Inc. Michigan, IL, USA), the SOD activity of whole blood cells as well as the plasma TAS levels between males and females were not significantly different at $p>0.05$. The SOD activities of both female and male subjects with the Val/Val and the Val/Ala genotypes were not significantly different at $p>0.05$. Although the present data showed the SOD activities of 3 females and 4 males with the Ala/Ala genotype lower than those of the other two genotypes, the association of the SOD activity with the Ala/Ala genotype could not be statistically analysed because their sample sizes were too small for the statistical requirement. The TAS levels of the subjects with the Val/Val genotype, the Val/Ala genotype and the Ala/Ala genotype were not significantly different at $p>0.05$. The SOD activities and the TAS levels of both female and male subjects with the Val/Val genotype and the Val/Ala genotype were not lower than those of the subjects with the Ala/Ala genotype (Fig. 4, 5 and Table 4).

DISCUSSION

MnSOD, being an important primary antioxidant enzyme in the detoxification pathway of mitochondrial ROS, has been discussed very often for its association with many ROS induced diseases and cancers (Polidori, 2003). The targeting and assembly of nuclear-encoded MnSOD protein are essential processes for the proper functioning of mitochondria. The Val-MnSOD prefers a β-sheet conformation rather than an α-helical structure precursor protein that leads to an impaired transport of MnSOD to mitochondria (Shimoda-Matsubayashi *et al*., 1996). It has been proposed that the Ala-MnSOD is targeted into the mitochondria, whereas the Val-MnSOD is partially arrested in the inner mitochondrial membrane (Sutton *et al*., 2003*)*. Defective import of MnSOD resulted from Val-9Ala polymorphisms in the MTS revealed that the Ala-MnSOD was approximately 30% to 40% more efficiently localized to mitochondria than the Val-MnSOD (Sutton *et al*., 2003; 2005). In view of these previous findings, low activity of Val-MnSOD was predicted to be associated with higher levels of ROS and thus predisposed to a greater risk of ROS induced diseases and cancers.

Three genotypes of MTS-MnSOD in 221 healthy Thais including 135 females and 86 males were firstly reported herein as 57.0-62.2% for the Val/Val genotype, 35.6-38.4% for the Val/Ala genotype and 2.2-4.7% for the Ala/Ala genotype (Fig. 3, Table 2). In addition, the frequency was 0.76-0.80 for the Val allele and 0.20-0.24 for the Ala allele. The presented data were similar to previous reports of a few Asian ethnics in China, Japan and Korea (Table 5). In contrast, frequencies of the Val and the Ala allele were approximately equal in those of Caucasian ethnics in the USA, United Kingdom, Germany, Ireland, Italy, Finland, Russia, Turkey, South Africa-Xhosa, Moroccan and Australia (Table 5). Sutton *et al*. (2003, 2005) previously predicted that the Ala form (not the Val form of MnSOD) was targeted into the mitochondria and the Val form was associated with higher cancer risk. However, very few previous studies documented the Val-MnSOD with higher cancer risk against a large data for the Ala-MnSOD as a risk factor not only for cancer but also for other diseases (Bag and Bag, 2008). It would be a premature conclusion to remark that this polymorphism had no role in cancer development due to the fact that MnSOD constitutes a first-line defense against ROS. Some polymorphisms might be associated with cancer risk for some ethnic communities only not for other ones (Mao *et al*., 2009). All previous studies of various ethnics suggested that the genotype of the Val-9Ala polymorphisms in the MTS of MnSOD was likely to be a genetic marker for differentiating Asian ethnic from other ethnics such as European ethnics, African ethnics, Australian ethnics and American ethnics.

Association studies linking three allelic genotypes of MTS-MnSOD to SOD activity of whole blood cells as well as the plasma TAS of all 221 healthy Thai subjects both females and males reveal contradictory results to some

Table 5 Allelic Val/Ala frequency of MTS-MnSOD gene of Thai ethnics compared to various Asian ethnics and others such as European ethnics, African ethnics, Australian ethnics and American ethnics previously reported.

Ethnics	Country	Total N	Allele frequency		References
			Valine	Alanine	
Thai	Thailand	221	0.79	0.21	This work
Chinese	China	103	0.86	0.14	Liu *et al.,* 2009
Chinese	Taiwan	115	0.86	0.14	Huang *et al.,* 2007
Chinese	Hongkong	239	0.86	0.14	Ho *et al.,* 2006
Japanese	Japan	627	0.83	0.17	Fujimoto *et al.,* 2008
Korean	Korea	262	0.82	0.18	Pae *et al.,* 2007
American men	USA	652	0.50	0.50	Mikhak *et al.,* 2008
American	USA	833	0.49	0.51	Han *et al.,* 2007
American	USA	1,382	0.52	0.48	Kang *et al.,* 2007
English	United Kingdom	100	0.58	0.42	Elsakka *et al.,* 2007
German	Germany	160	0.48	0.52	Osterreicher *et al.,* 2007
Irish	Ireland	223	0.52	0.47	Murphy *et al.,*2007
Italian	Italy	257	0.50	0.50	Ventriglia *et al.,* 2006
Finnish	Finland	989	0.52	0.48	Kakko *et al.,*2003
Russian	Russia	88	0.49	0.51	Chistyakov *et al.,* 2001
Turkish	Turkey	50	0.44	0.56	Zejnilovic *et al,* 2009
Xhosa	South Africa	243	0.57	0.43	Hitzeroth *et al.,* 2007
Moroccan	Morocco	222	0.59	0.41	Ezzikouri *et al.,* 2008
Australian women	Australia	1,130	0.48	0.52	Johnatty *et al.,* 2007

previous studies (Sutton *et al*., 2003; 2005). Most studies predicted that low SOD activities and higher levels of ROS were associated with the Val-MnSOD and tend to be a greater risk of ROS-mediated diseases and cancers. The present data showed no overall association between the SOD activity as well as the TAS and different genotypes. Individuals who were Ala homozygotes have no advantage over the Val homozygotes or the Val/Ala heterozygotes. In addition, the SOD activity of healthy Thai subjects who were Val homozygotes and Val/Ala heterozygotes were not lower than those who were Ala homozygotes (Fig. 4, Table 4). On the other hand, 7 subjects who were Ala homozygotes showed much lower SOD activity than those of the Val homozygotes and the Val/Ala heterozygotes (Fig. 4, Table 4). However,

lower SOD activity of the Ala homozygotes could not be statistically analyzed since their sample sizes were not large enough for the statistical requirement. Moreover, plasma TAS levels were not significantly different among these three genotypes at $p < 0.05$ (Fig. 5, Table 5). Dasgupta *et al*. (2006) reported very interesting results about ROS-mediated apoptosis. If MnSOD was not allowed to enter the mitochondrial matrix, as in the case of the Val form, superoxides could not be dismutated to H_2O_2. A steady-state increase in the intracellular H_2O_2 production by MnSOD could block the activation of key processes involving induction of programmed cell death. An increased H_2O_2 level was associated with decreased sensitivity to tumor necrosis factor-a-mediated apoptosis and this might increase the rate of programmed cell death. Hence, although there would be an enormous amount of cellular damage by superoxide free radicals, at the same time death of the handicapped cells might check cancer. However, much more exhaustive studies involving a large sample size and other variables such as polymorphisms in linkage disequilibrium, gene-gene interactions and environmental exposures are required to acquire an overall knowledge on the link between MnSOD polymorphisms and cancer risks.

CONCLUSIONS

The Val allele as well as the Val/Val and the Val/Ala genotypes were found predominantly rather than the Ala or the Ala/Ala genotype in the MTS leading the MnSOD gene of both healthy Thai males and females. The SOD activities and the total antioxidant status of healthy Thais with the Val/Val and the Val/Ala genotypes were not statistically different from ($p>0.05$) and were not lower than those with the Ala/Ala genotype.

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REFERENCES

Bag, A.and Bag, N. 2008. Target sequence polymorphism of human manganese superoxide dismutase gene and its association with cancer risk: a review. *Cancer Epidemiol Biomarkers Prev* 17: 3298–3305.

Boom, R., Sol, C.J., Salimans, M.M., Jansen, C.L., Wertheim-van Dillen, P.M. and van der Noordaa, J. 1990. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 28: 495–503.

Chistyakov, D.A., Savostanov, K.V., Zotova, E.V. and Nosikov, V.V. 2001. Polymorphisms in the MnSOD and EC-SOD genes and their relationship to diabetic neuropathy in type I diabetes mellitus. *BMC Med Gen* 2: 4–8.

Dasgupta, J., Subbaram, S., Connor, K.M., Rodriguez, A.M. and Tirosh. O. 2006. Manganese superoxide dismutase protects from TNF-a-induced apoptosis by increasing the steady-state production of H_2O_2. *Antioxid Redox Signal* 8: 1295–1305.

Elsakka, N.E., Webster, N.R. and Galley, H.F. 2007. Polymorphism in the manganese superoxide dismutase gene. *Free Rad Res* 41: 770–778.

Ezzikouri, S., El Feydi, A.E., Chafik, A., Afifi, R., El Kihal, L., Benazzouz, M., Hassar, M., Pineau, P. and Benjelloun, S. 2008. Genetic polymorphism in the manganese superoxide dismutase gene is associated with an increased risk for hepatocellular carcinoma in HCV-infected Moroccan patients. *Mutat Res* 649: 1–6.

Fujimoto, H., Taguchi, J., Imai, Y., Ayabe, S., Hashimoto, H., Kobayashi, H., Ogasawara, K., Aizawa, T., Yamakado, M., Nagai, R. and Ohno, M. 2008. Manganese superoxide dismutase polymorphism affects the oxidized low-density lipoprotein-induced apoptosis of macrophages and coronary artery disease. *Eur Heart J* 29: 1267–1274.

Halliwell, B. 1997. Antioxidants and human disease: A general introduction. Nutr Rev., 55: 44–49.

Han, J., Colditz, G.A. and Hunter, D.J. 2007. Manganese superoxide dismutase polymorphism and risk of skin cancer (United States). *Cancer Causes Control* 18: 79–89.

Heid, C.A, Stevens, J., Livak, K.J. and Williams, P.M. 1996. Real time quantitive PCR, *Genome Res* 6: 986–994.

Hitzeroth, A., Niehaus, D.J., Koen, L., Botes, W.C., Deleuze, J.F. and Warnich, L. 2007. Association between the MnSOD Ala-9Val polymorphism and development of schizophrenia and abnormal involuntary movements in the Xhosa population. *Prog Neuropsychopharmacol Biol Psychiatry* 31: 664–672.

Ho, J.C., Mak, J.C., Ho, S.P., Tsang, K.W., Lam, W.K. and Chan-Yeung M. 2006. Manganese superoxide dismutase and catalase genetic polymorphisms, activity levels, and lung cancer risk in Chinese in Hong Kong. *J Thorac Oncol* 1: 648–653.

Huang, Y.S., Su, W.J., Huang, Y.H., Chen, C.Y., Chang, F.Y., Lin, H.C. and Lee, S.D. 2007. Geneticpolymorphisms of manganese superoxide dismutase, NAD(P)H: quinine oxidoreductase, glutathione S-transferase M1 and T1, and the susceptibility to drug-induced liver injury. *J Hepatol* 47: 128–134.

Johnatty, S.E., Nagle, C.M., Spurdle, A.B.and Chen, X. 2007. Australian Breast Cancer Family Study, Webb PM, Chenevix-Trench G. The MnSOD Val9Ala polymorphism, dietary antioxidant intake, risk and survival

in ovarian cancer (Australia). *Gynecol Onco* 107: 388–391.

Kakko, S., Päivänsalo, M., Koistinen, P., Kesäniemi, Y.A., Kinnula, V.L. and Savolainen, M.J. 2003. The signal sequence polymorphism of the MnSOD gene is associated with the degree of carotid atherosclerosis. *Atherosclerosis* 168: 147–152.

Kang, D., Lee, K.M., Park, S.K., Berndt, S.I., Peters, U., Reding, D., Chatterjee, N., Welch, R., Chanock, S., Huang, W.Y.and Hayes, R.B. 2007. Functional variant of manganese superoxide dismutase (SOD2 V16A) polymorphism is associated with prostate cancer risk in the prostate, lung, colorectal, and ovarian cancer study. *Cancer Epidemiol Biomarkers Prev* 16: 1581–1586.

Kannan, K. and Jain, S.K. 2000. Oxidative stress and apoptosis. *Pathophysiology* 7: 153–163.

Liu, L., Zheng, T., Wang, N., Wang, F. and Li, M. 2009. The manganese superoxide dismutase Val16Ala polymorphism is associated with decreased risk of diabetic nephropathy in Chinese patients with type 2 diabetes. *Mol Cell Biochem* 322: 87–91.

Mao, C., Qiu, L.X., Zhan, P., Xue, K. and Ding H. 2009. MnSOD Val (16) Ala polymorphism and prostate cancer susceptibility: A meta-analysis involving 8,962 subjects. *J Cancer Res Clin Onco* 136: 975–979.

Mikhak, B., Hunter, D.J., Spiegelman, D., Platz, E.A., Wu, K., Erdman, J.W. Jr., and Giovannucci, E. 2008. Manganese superoxide dismutase (MnSOD) gene polymorphism, interactions with carotenoid levels, and prostate cancer risk. *Carcinogenesis* 29: 2335–2340.

Murphy, S.J., Hughes, A.E., Patterson, C.C., Anderson, L.A., Watson, R.G., Johnston, B.T., Comber, H., McGuigan, J., Reynolds, J.V. and Murray, L.J. 2007. A population-based association study of SNPs of GSTP1, MnSOD, GPX2 and Barrett's esophagus and esophageal adenocarcinoma. *Carcinogenesis* 28: 1323–1328.

Osterreicher, C.H., Schultheiss, J., Wehler, M., Homann, N.,Hellerbrand, C., Künzli, B., Friess, H., Seitz, H.K.and Stickel, F. 2007. Genetic polymorphisms of manganese-superoxide dismutase and glutathione-S-transferase in chronic alcoholic pancreatitis. *Mutagenesis* 22: 305–310.

Pae, C.U., Kim, T.S., Patkar, A.A., Kim, J.J. and Lee, C.U. 2007. Manganese superoxide dismutase (MnSOD: Ala-9Val) gene polymorphism may not be associated with schizophrenia and tardive dyskinesia. *Psychiatry Res* 153: 77–81.

Polidori, M.C. 2003. Antioxidant micronutrients in the prevention of age-related diseases. *J Postgrad Med* 49: 229–235.

Rosenblum, J.S., Gilula, N.B. and Lerner, R.A. 1996. On signal sequence polymorphism and disease of distribution. *Proc Natl Acad Sci USA* 93: 4471–4473.

Shimoda-Matsubayashi, S., Matsumine, H., Kobayashi, T., Nakagawa-Hattori, Y., Shimizu,

Y. and Mizuno, T. 1996. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. *Biochem Biophys Res Commun* 226: 561–565.

Sutton, A., Imbert, A., Igoudjil, A., Descatoire, V., Cazanave, S., Pessayre, D. and Degoul, F. 2005. The manganese superoxide dismutase Ala16Val dimorphism modulates both mitochondrial import and mRNA stability. *Pharmacogenet Genomics* 15: 311–319.

Sutton, A., Khoury, H., Prip-Buus, C., Cepanec, C., Pessayre, D. and Degoul, F. 2003. The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. *Pharmacogenetics* 13: 145–157.

The CIA-World Factbook-Thailand 2010. *The World Factbook*. Washington, DC: Central Intelligence Agency.

Ukeda, H., Maeda, S., Ishii, T.and Sawamura, M. 1997. Spectrophotometric assay for superoxide dismutase based on tetrazolium salt 3´-{1-[(phenyl-amino)-carbonyl] -3,4-tetrazolium} -bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate reduction by xanthine-xanthine oxidase. *Anal Biochem* 251: 206–209.

Van Landeghem, G.F., Tabatabaie, P., Kucinskas, V., Saha, N. and Beckman, G. 1999. Ethnic variation in the mitochondrial MnSOD. *Hum Hered* 49: 190–193.

Ventriglia, M., Scassellati, C., Bonvicini, C., Squitti, R., Bevacqua, M.G., Foresti, G., Tura, G.B.and Gennarelli, M. 2006. No association between Ala9Val functional polymorphism of MnSOD gene and schizophrenia in a representative Italian sample. *Neurosci Lett* 410: 208–211.

Yu. T-W., and Ong, C. 1999. Lag-time measurement of antioxidant capacity using myoglobin and 2,2′-azino-bis (3-ethyl-benzthiazoline-6-sulfonic acid): rationale, application and limitation. *Anal Biochem* 275: 217–223.

Zejnilovic, J., Akev, N., Yilmaz, H. and Isbir, T. 2009. Association between manganese superoxide dismutase polymorphism and risk of lung cancer. *Cancer Genetics Cytogenetic*s 189: 1–4.