

ORIGINAL ARTICLE

MANBA polymorphism was related to increased risk of colorectal cancer in Swedish but not in Chinese populations

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Abstract

β -mannosidase, encoded by *MANBA*, has been suggested to be implicated in cancers, while genetic variations in the *MANBA* in relation to colorectal cancer (CRC) risk has not been examined. In this study, we investigated the relationship of a polymorphic CA repeat in *MANBA* gene with CRC risk in 152 Swedish CRC patients and 441 Swedish controls, and 196 Chinese CRC patients and 577 Chinese controls, as well as the clinicopathologic significance of this polymorphism on CRC patients, by using capillary electrophoresis. The *MANBA* genotypes were related to CRC risk in the Swedish population ($p=0.03$), but not in the Chinese population. In the Swedish population, individuals with <22 CAs/ <22 CAs had significantly increased risk for CRC compared with those with ≥ 22 CAs/ ≥ 22 CAs (gender-age-adjusted analysis: OR 1.93, 95% CI 1.06–3.51). There was no relationship between the polymorphism and clinicopathologic variables. These findings suggest the different susceptibilities of this polymorphism to CRC development in the two populations.

β -mannosidase is an exoglycosidase involved in the degradation of N-linked oligosaccharide moieties of glycoproteins. The gene, *MANBA*, encoding for β -mannosidase, comprises 17 exons and is located on chromosome 4q22-q25. It has been known that genetic deficiency of this enzyme results in beta-mannosidosis, a lysosomal storage disease that has a wide spectrum of neurological involvement [1]. Recently, several studies have demonstrated the implication of β -mannosidase in cancers. Bosmann et al. [2] reported elevated serum level of β -mannosidase in rats bearing transplantable Reuber H-35 hepatoma and mice bearing L1210 murine leukemia, suggesting that the tumor may release large quantities of β -mannosidase into the serum and affect the enzyme level in host organs. Sud et al. [3] observed the overexpression of β -mannosidase in human esophageal dysplasia and squamous cell carcinoma compared with normal esophageal epithelium, indicating that β -mannosidase might be

involved in human esophageal cancer development. However, the potential role of β -mannosidase and the genetic mechanism in tumor development and progression have not been reported.

Ota et al. [4] first discovered a polymorphic dinucleotide CA repeat, thought in the regulatory region of *NFKB1*. They also observed that this polymorphism had a high heterozygosity (0.813), suggesting that this polymorphism is a useful marker for studying genetic disorders affecting immune response and cell differentiation. Studies have investigated the implication of the polymorphic CA repeat in several immune-related diseases including type 1 diabetes, multiple sclerosis and celiac disease, as well as breast cancer [5–8]. Hegazy et al. found a significantly increased frequency of the allele 138 bp (24 CAs) and a decreased rate of the allele 146 bp (28 CAs) of the polymorphism in the patients with type 1 diabetes compared with the normal controls, suggesting the involvement of this polymorphism in

the susceptibility to type 1 diabetes [5]. However, this polymorphism did not associate with the susceptibility to multiple sclerosis, celiac disease and breast cancer [6–8]. Recently, this polymorphism was identified in the *MANBA* gene (GenBank accession no. AF224669), not in *NFKB1*, approximately 300 bp downstream to exon 17 of the *MANBA*. In the present study, we investigated whether this polymorphism was related to colorectal cancer (CRC) risk in two geographically and ethnically different populations: Swedish and Chinese populations, and the clinicopathologic significance of the polymorphism on CRC patients.

Materials and methods

Subjects

This study included 152 Swedish consecutive CRC patients and 441 Swedish healthy controls, and 196 Chinese consecutive CRC patients and 577 Chinese healthy controls. The baseline characteristics of the patients and controls are shown in Table I. Swedish patients consisted of 80 males and 72 females, aged 35–95 years (mean age, 71 years), diagnosed at Linköping Hospital, Linköping, or Vrinnevi Hospital, Norrköping, Sweden, between 1990 and 1999. The patients' gender, age, tumor location and stage (Dukes' and TNM stage) were taken from surgical and/or pathological records. Tumors from ascending and transverse colon were regarded as proximal tumors, while tumors from descending, sigmoid colon and rectum were considered as distal tumors. Tumor differentiation was graded as high (well + moderately) and low (poorly + mucinous) differentiated. No information was available about tumor location in two patients, Dukes' stage in eight, TNM

stage in 108, and differentiation in one. The patients were followed up until 2003, and 51 died of CRC by that time. Chinese patients consisted of 112 males and 84 females, aged 25–84 years (mean age, 57 years), diagnosed at the Department of Surgery, the Fourth Hospital, Hebei Medical University, Shijiazhuang, P. R. China, between 2001 and 2002, or Tangshan Worker's Hospital, Tangshan, P. R. China, between 2004 and 2005. The patients' gender, age, tumor location, Dukes' stage and differentiation were recorded by surgeons and pathologists, and confirmed by two of the present investigators. No information was available about age in one, tumor location in 40, Dukes' stage in 23, differentiation in 107, and TNM stage and survival status in all patients.

Both Swedish and Chinese controls were collected from the same residential area as their corresponding patients. They had neither any gastrointestinal disease nor a personal history of tumors. Swedish controls consisted of 218 males and 223 females, aged 22–77 years (mean age, 55 years) recruited from a regional population-based DNA bank created in the south-east region of Sweden. Chinese controls were 370 male and 206 female blood donors, aged 18–88 years (mean age, 37 years) collected from Blood Research Center of Hebei Province, Shijiazhuang, P. R. China. No information was available about gender in one Chinese control, and age in one Swedish control and one Chinese control, respectively. The study was approved by the ethical committee at the Faculty of Health Sciences, University of Linköping, Sweden, and the Health Bureau of Hebei Province, Shijiazhuang, P. R. China.

Table I. The baseline characteristics of patients and controls in the Swedish and Chinese populations.

	Swedish population		p	Chinese population		p
	Patients (n = 152)	Controls (n = 441)		Patients (n = 196)	Controls (n = 577)	
Gender			0.50			0.08
Male	80 (53)	218 (49)		112 (57)	370 (64)	
Female	72 (47)	223 (51)		84 (43)	206 (36)	
Age (years)						
Mean (range)	71 (35–95)	55 (22–77)	<0.0001	57 (25–84)	37 (18–88)	<0.0001
Tumor location						
Proximal	63 (42)			28 (18)		
Distal	87 (58)			128 (82)		
Dukes' stage						
A+B	73 (51)			107 (62)		
C+D	71 (49)			66 (38)		
Differentiation						
High	104 (69)			67 (75)		
Low	47 (31)			22 (25)		

DNA extraction

DNA from peripheral blood of the Swedish and Chinese controls, and the Chinese patients (who were from the Department of Surgery, the Fourth Hospital, Hebei Medical University, Shijiazhuang, China) was extracted by using the Wizard[®] Genome Purification Kit (Promega, Madison, WI). DNA from frozen normal colorectal mucosa of the Swedish patients, and Chinese patients (who were from Tangshan Worker's Hospital, Tangshan, China) was extracted by using Wizard[®] SV Genomic DNA Purification System according to the manufacturer's instructions (Promega).

Genotyping

Genotyping was examined by capillary electrophoresis with MegaBACE Fragment Profiler (Amersham Biosciences, Buckinghamshire, England) using the primers (forward primer FAM-labeled 5'-CTTCAGTATCTAAGAGTATCCT-3' and reverse primer 5'-CAAGTAAGACTCTACGGAGTC-3') described previously by Curran et al. [8].

Polymerase chain reaction (PCR) was performed in a 20 µl reaction mixture containing approximately 50 ng genomic DNA, 0.5 µM of each primer (Invitrogen, Carlsbad, CA), 1 × PCR buffer (Promega), 0.25 mM dNTPs (Invitrogen), 3.75 mM MgCl₂ (Promega) and 0.025 U/µl Taq polymerase (Promega) at 94°C for 5 min, 35 cycles at 94°C for 30 s, 58°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 5 min. Following PCR amplification, 1 µl of the PCR product was subjected to 25 µl distilled water with 0.25 µl of MegaBACE ET 400-R size standard (Amersham Biosciences) and the mix was injected to the capillary system of the MegaBACE instrument. Consequently, the polymorphic alleles of *MANBA* were analyzed by MegaBACE fragment profiler.

DNA sequencing

In order to confirm the number of CA repeats in *MANBA*, genomic DNA from two Swedish and two Chinese patients with homozygous alleles 18 CAs or 23 CAs was sequenced by a MegaBACE 1000 sequencing instrument. Briefly, following DNA amplification by PCR with the same primers (neither fluorescence-labeled) and PCR conditions above, 5 µl of the PCR product was cleaned up with 2 µl ExoSAP-IT (Amersham Biosciences) by incubation at 37°C for 15 min first and then followed by inactivation through heating to 80°C for 15 min. The PCR product was then subjected to a sequencing

reaction in a volume of 20 µl reaction mixture containing 7 µl PCR product, 1 µl reverse primer, and 8 µl sequencing reagent premix (Amersham Biosciences). A 25 cycles of the cycling program at 95°C for 20 s, 58°C for 15 s and 60°C for 1 min was performed. After purification, the sequencing reaction mixtures were detected by capillary electrophoresis by using the MegaBACE 1000 sequencing instrument and analyzed by MegaBACE 1000 sequencing profiler (Amersham Biosciences).

Statistical analysis

Power estimates (at a significant level of 5%) for the case-control analysis were calculated by one of the co-authors (Rosell, J, statistician) using the formulae described by Kirkwood BR [9]. The student t-test was used to assess the age difference between patients and controls. The χ^2 test was used to determine difference in the frequency of genotypes or alleles between patients and controls, between the two populations, as well as the relationship of the polymorphism with clinicopathologic variables. The logistic regression was used to calculate odds ratio (OR) and 95% confidence intervals (CI) in both univariate and multivariate analyses including gender and age for assessing the association of genotypes with CRC risk. Cox's Proportional Hazard Model was used to test the relationship between the polymorphism and the survival of patients. Kaplan-Meier method was used to calculate survival curves. Two-sided p-values of less than 5% were considered statistically significant.

Results

Power analysis

Under the case-to-control ratio of 1 to 2.9 (i.e., 152 Swedish patients versus 441 controls and 196 Chinese patients versus 577 controls), and the assumption of a prevalence of "bad" genotype of 17% in the Swedish control and 21% in the Chinese control, and a true odds ratio for cancer of 2, the sample size was associated with a power of about 90% for the Swedish and 95% for the Chinese study.

The distribution of *MANBA* alleles in the Swedish and Chinese populations

The age distribution between the patients and controls in both the Swedish and Chinese populations was significant difference ($p < 0.0001$, Table I). There was no gender difference between the patients and controls in both the populations ($p > 0.05$, Table I).

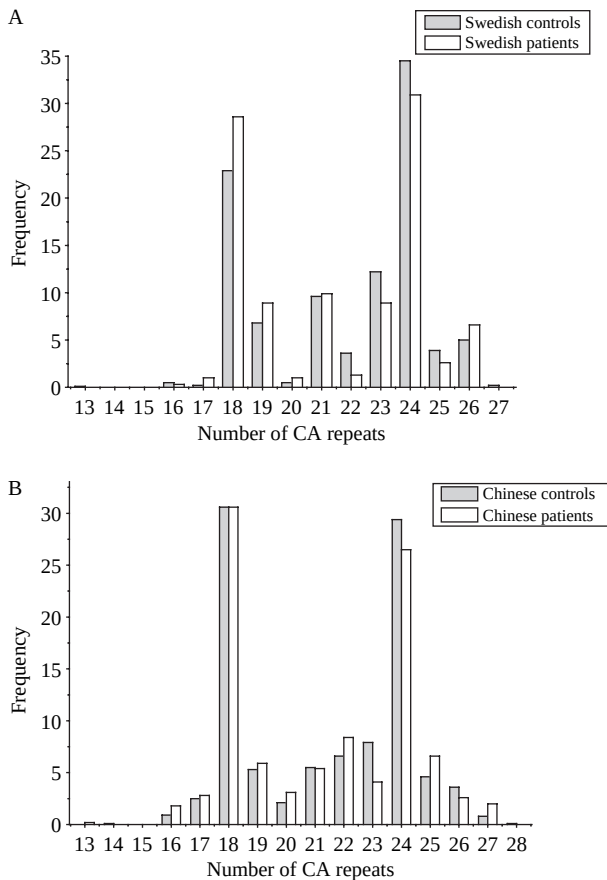


Figure 1. Frequency distribution (%) of number of CA repeats in patients and controls in the Swedish (A) and Chinese (B) populations.

Thirteen polymorphic alleles, ranging from 116 to 144 bp corresponding to 13–27 CA repeats, were detected in the Swedish population (patients, 16–26 CAs, and controls, 13–27 CAs, Figure 1A, Table II), and 15 alleles, ranging from 116 to 146 bp corresponding to 13 to 28 CA repeats, in the Chinese population (patients, 13–27 CAs, and controls, 14–28 CAs, Figure 1B, Table II). The distribution of the alleles in both the populations was bimodal, with two peaks located at 18 CAs and at 24 CAs. However, the most common allele between the two populations was different: in the Swedish population, 24 CAs were the most common allele followed by 18 CAs, whereas in the Chinese population 18 CAs were the most common allele followed by 24 CAs. The distribution of the alleles between the two populations, either patients versus patients ($\chi^2 = 59.37$, $\gamma = 12$, $p < 0.0001$), or controls versus controls ($\chi^2 = 83.56$, $\gamma = 14$, $p < 0.0001$), was significantly different (Table II). There was no difference in the frequency of alleles between the patients and controls either in the Swedish ($\chi^2 = 18.77$, $\gamma = 12$, $p = 0.09$) or in the Chinese populations ($\chi^2 = 22.51$,

$\gamma = 14$, $p = 0.07$, data not shown). In addition, there was no gender-polymorphism association in the two populations ($p > 0.05$, data not shown).

The correlation between the MANBA genotypes and CRC risk

Comparing the frequency of the same allele in the Swedish patients with the corresponding controls (e.g., at 13 CAs, 0 versus 0.1%, Figure 1A), we found that almost all the alleles with < 22 CAs (except 13 CAs and 16 CAs) appeared more frequently in the patients than the controls (e.g., the frequency of allele with 21 CAs was higher in the patients (9.9%) than that in the controls (9.6%)), and an opposite trend was seen in alleles with ≥ 22 CAs (except 26 CAs) (e.g., the frequency of allele with 22 CAs was lower in the patients (1.3%) than that in the controls (3.6%)). Considering this trend of the allelic frequency difference between the Swedish patients and controls, together with the bimodal distribution of the CA repeats, a model adapted from previous analyses of dinucleotide microsatellite polymorphisms [10–12], we divided the alleles into two subclasses using 22 CAs as a cut-off point to consequently obtain three groups of genotype, < 22 CAs/ < 22 CAs, < 22 CAs/ ≥ 22 CAs and ≥ 22 CAs/ ≥ 22 CAs, for further analysis. In the Swedish population, the genotype of the *MANBA* was significantly related to CRC risk with a dosage effect of allele with < 22 CAs (trend test in gender-age-adjusted analysis: $p = 0.03$, Table III). Further analysis showed that individuals with < 22 CAs/ < 22 CAs had a significantly increased risk for CRC compared with those with ≥ 22 CAs/ ≥ 22 CAs (OR 2.07, 95% CI 1.22–3.50, $p = 0.01$ for univariate analysis). Since the mean age of the Swedish patients differed from that of the controls ($p < 0.0001$, Table I), we further did a multivariate analysis adjusting for gender and age, and observed the similar results (OR 1.93, 95% CI 1.06–3.51, $p = 0.03$, Table III).

There was no association of the *MANBA* genotypes with gender, age, tumor location, Dukes' stage and TNM stage, grade of differentiation, and survival of the Swedish patients ($p > 0.05$, data not shown).

In the Chinese population, using the same cut-off point and subsequent genotype classification as above, we did not see any difference in the genotype frequency between the patient and controls ($p > 0.05$ for both univariate and multivariate analyses, Table III), or association of the genotypes with gender, age, tumor location, Dukes' stage, and grade of differentiation of the Chinese patients ($p > 0.05$, data not shown).

Table II. Frequency of *MANBA* alleles in patients and controls in the Swedish and Chinese populations.

Groups	MANBA Allele (No. of CA repeat)																Total
	13	14	16	17	18	19	20	21	22	23	24	25	26	27	28		
Patients																	
Swedish (No.)	0	0	1	3	87	27	3	30	4	27	94	8	20	0		304	
(%)			(0.3)	(1.0)	(28.6)	(8.9)	(1.0)	(9.9)	(1.3)	(8.9)	(30.9)	(2.6)	(6.6)	(0)			
Chinese (No.)	1	0	7	11	120	23	12	21	33	16	104	26	10	8		392	
(%)	(0.2)	(0)	(1.8)	(2.8)	(30.6)	(5.9)	(3.1)	(5.4)	(8.4)	(4.1)	(26.5)	(6.6)	(2.6)	(2.0)			
Controls																	
Swedish (No.)	1	0	4	2	202	60	4	85	32	108	304	34	44	2	0	882	
(%)	(0.1)		(0.5)	(0.2)	(22.9)	(6.8)	(0.5)	(9.6)	(3.6)	(12.2)	(34.5)	(9)	(5.0)	(0.2)			
Chinese (No.)	0	1	10	29	353	61	24	64	76	91	340	53	41	10	1	1154	
(%)		(0.1)	(0.9)	(2.5)	(30.6)	(5.3)	(2.1)	(5.5)	(6.6)	(7.9)	(29.4)	(4.6)	(3.6)	(0.8)	(0.1)		

Discussion

In the present study, the allele size and its corresponding CA repeat number (e.g., 126 bp was corresponding to 18 CAs, 138 bp was corresponding to 24 CAs) detected were consistent with the previous studies in *MANBA* (rather than in *NFKB1*) in a Japanese and a Spanish population [4,7]. The distribution of the alleles in both the Swedish and Chinese populations was bimodal, with two peaks located at 18 CAs and at 24 CAs. However, the most common alleles between the two populations were different. In the Swedish population, the most common alleles were 24 CAs followed by 18 CAs, which were consistent with previous studies in Spanish population [7]. However, in the Chinese population, the most frequent alleles were 18 CAs followed by 24 CAs. These results indicate that the distribution of the polymorphism among different ethnic populations may be different.

To our knowledge, the present study is the first investigation on the association of the polymorphic CA repeats in *MANBA* with CRC risk. A previous study has investigated this polymorphism for involvement breast cancer in the Australian population [8]. In accordance with their finding, where the polymorphisms of the alleles (they did not analyze genotypes) were not related to risk for breast cancers [8], we did not either find an association of the *MANBA* alleles with risk for CRC in both the Swedish and Chinese populations. Interestingly, when we used 22 CAs as a cut-off point to set three genotypes, <22 CAs/ <22 CAs, <22 CAs/ ≥22 CAs and ≥22 CAs/ ≥22 CAs, genotype <22 CAs/ <22 CAs was related to CRC risk compared with ≥22 CAs/ ≥22 CAs in the Swedish population. These results indicated that not only the polymorphisms but also the CA repeat number played a role in the susceptibility to CRC development.

It is notable that this polymorphism was related to CRC risk in the Swedish but not in the Chinese population. Swedish and Chinese populations are two geographically and ethnically different populations with marked differences in CRC incidence rate, culture and life style. For example, the age-adjusted incidence rate of CRC among Swedish population (33.4/100 000 in males and 26.2/100 000 in females) is considerably higher than incidence among Chinese population (13.6/100 000 in males and 9.3/100 000 in females) [13]. Swedish people have a higher red meat consumption and lower intake of plant foods while Chinese have opposite. Studies of migrants moving from a low- to a high-risk area have suggested that the variation in CRC incidence among populations is mainly due to difference of environmental factors and life style,

Table III. Association of *MANBA* genotypes with colorectal cancer risk in the Swedish and Chinese populations.

Genotypes	Patients n (%)	Controls n (%)	Univariate analysis		Gender-age-adjusted analysis	
			OR (95% CI)	p	OR (95% CI)	p
Swedish						
≥ 22 CAs/ ≥ 22 CAs	39 (26)	156 (35)	Referent		Referent	
< 22 CAs/ ≥ 22 CAs	75 (49)	212 (48)	1.41 (0.91–2.18)	0.13	1.43 (0.87–2.33)	0.16
< 22 CAs/ < 22 CAs	38 (25)	73 (17)	2.07 (1.22–3.50)	0.01	1.93 (1.06–3.51)	0.03
Trend test			p = 0.01		p = 0.03	
Chinese						
≥ 22 CAs/ ≥ 22 CAs	57 (29)	155 (27)	1.00		1.00	
< 22 CAs/ ≥ 22 CAs	83 (42)	302 (52)	0.75 (0.51–1.10)	0.14	0.77 (0.49–1.21)	0.25
< 22 CAs/ < 22 CAs	56 (29)	120 (21)	1.26 (0.81–1.95)	0.31	1.23 (0.73–2.07)	0.43
Trend test			p = 0.37		p = 0.48	

most likely diet [14,15]. Furthermore, accumulating evidence has shown that the interaction of environmental exposures with genetic polymorphisms may influence the susceptibility of polymorphisms to cancer [16]. In the present study, we observed the different pattern of the allelic distribution between the Swedish and Chinese population, and statistical analyses revealed a significant difference of the frequency of *MANBA* alleles between the two populations. Taken together, the genetic difference attributable to different populations as well as environmental exposures may partly explain the discrepancy of the *MANBA* polymorphism in susceptibility to CRC as well as CRC incidence diversity between the two populations. On the other hand, there was an obvious age-difference between the patients and controls in the two populations, which may influence the evaluation of the effect of *MANBA* polymorphism on the susceptibility to CRC, although age-adjusted analysis has been done. In addition, polymorphisms in other genes or loci within the *MANBA* gene that were in linkage disequilibrium with the CA repeat polymorphism may also have impact on the susceptibility of the polymorphism to CRC in the two populations.

In conclusion, the association of the CA repeat polymorphism of *MANBA* gene with CRC risk in the Swedish but not in Chinese population suggests the different susceptibilities of this polymorphism to CRC development in the two populations. Further analysis containing strictly selective subject with matched age, genetic linkage, gene expression and environmental exposure in different populations would contribute to our understanding the function of the *MANBA* polymorphism.

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