RESEARCH COMMUNICATION

No Association of the Mitochondrial Genotype (Mt5178A/C) with Six Cancers in a Japanese Population

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Abstract

To examine an association between the mitochondrial DNA (mt5178) genotype and various cancers, we genotyped 1120 non-cancer controls and 930 cancer cases including esophageal, stomach, colorectal, lung, breast and malignant lymphoma in a sample of Japanese patients. The mt5178A/C was genotyped by the polymerase chain reaction with confronting two-pair primers (PCR-CTPP).

The frequency of mt5178A/C within the non-cancer and cancer groups, and age distribution of subjects with mt5178A and C were investigated. Odds ratios (ORs) of the mt5178A and C genotypes were also examined.
The frequency of mt5178A was 39.1 % in non-cancer subjects while frequencies in those having cancer included 39.0 % in breast, 37.4 % in colorectal, 45.1 % in esophageal, 38.0 % in lung, 41.5 % in malignant lymphoma, and 38.8 % in stomach cancer. There was no significant difference in the frequency of the mt5178 genotype among the six types of cancer studied. There was also no significant difference in the frequency of the mt5178 genotype between non-cancer and cancer subjects regardless of total age with the exception that ages 40-49 years (the frequency of the mt5178A was higher in cancer subjects). There was a significant interaction term between age and the mt5178 genotype in older (age>=60) lung cancer patients. The cumulative frequency of mt5178C increased more markedly than that of mt5178A after age 40 in non-cancer subjects, and after age 50 in cancer subjects. ORs of the genotype were not significant for all cancers combined or for any individual site of cancer.

In the present study, the mt5178 genotype seems to have no association with any of the cancers examined here. But an interaction term between the mt5178 genotype and aging on cancer was suggested within the Japanese population under study.

Key Words: mitochondrial genotype - Mt5178 - cancer - odds ratio

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Introduction

It is well known that Japan has the longest life expectancy in the world. Average life expectancy is 84.9 years for Japanese females and 78.1 years for Japanese males. It is believed that this longevity results from interactions among environmental conditions, lifestyle, and genetic factors. Oxidative damage to cells and tissues contributes to the natural aging process as well as several disease states. Mitochondrial DNA has been recently implicated as an

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important factor for the aging process; mitochondria are a source of reactive oxygen species (ROS) within the cell. Epidemiological studies have reported that the mitochondrial genotype mt5178A, derived from a C-to-A transversion at nucleotide 5178 of mtDNA causing a Leu-to-Met substitution within the NADH dehydrogenase subunit 2 (ND2) gene, is found frequently within the Japanese population (Cann et al., 1987; Shimokata et al., 2000; Tanaka et al., 1998; Yao et al., 2002). This genotype may be associated with longer life expectancy in the Japanese population. Previous studies have also reported a high percentage of subjects with mt5178A in the Japanese population (Shimokata et al., 2000; Tanaka et al., 1998). These studies concluded that mt5178A, as a potential genetic factor for longevity, might promote resistance to adult-onset diseases. Several studies found an association between the mt5178 genotype and type 2 diabetes (Matsunaga et al., 2001; Wang et al., 2001). (Wang et al., 2001) studied the occurrence and clinical features of type 2 diabetes and found that the mt5178C genotype might relate with a higher occurrence and a lower age of onset of type 2 diabetes. Matsunaga et al. (Matsunaga et al., 2001) also investigated the association between type 2 diabetes and mitochondrial genotype. Although they did not find significant differences in the frequency of mt5178A/C between type 2 diabetic patients and healthy control subjects, there was a significant difference in the mean intima-media thickness between mt5178A and mt5178C type 2 diabetics. Thus, mt5178A may contribute to longevity by conferring resistance to these diseases.

Cancer is the leading cause of death in Japan. It follows that the longevity of the Japanese population may be influenced by cancer. However, few studies have investigated the association between the mt5178 genotype and cancer. The purpose of this study is to examine associations between the mt5178 genotype and six cancers (esophageal, stomach, colorectal, lung, breast, and malignant lymphoma).

Subjects and Methods

Subjects

Patients with cancers of the esophageal, stomach, colon, rectum, lung, breast, and malignant lymphoma were diagnosed histologically. The patients were invited to participate in the present study by the investigating physicians. They were enrolled between March, 1999 and December, 2000.

Controls were sampled from patients at Aichi Cancer Center Hospital during the same period as cancer cases; 1) participants of a health checkup program provided by a local government and 2) first-visit outpatients enrolled in the HERPACC-II (Hospital-based Epidemiologic Research Program at Aichi Cancer Center-II). It was expected that about 20% of patients within the first-visit outpatient group would be diagnosed with some type of cancer. Therefore, the two control groups were regarded as 'non-cancer' groups. Since the hospital cancer registry collects data for cancer cases 1 year after the initial visit, the diagnosis was not available at the time of data analysis.

All subjects were asked to complete an informed consent form, to fill out a self-administered questionnaire, and to provide a 7-ml peripheral blood sample. In the informed consent form it was clearly outlined that the blood sample would be used for genetic polymorphism tests. The Ethics Committee of Aichi Cancer Center approved the present study for breast cancer patients (approval no. 12-20), esophageal cancer patients (approval no. 12-13), other cancer patients (approval no. 12-27), and the HERPACC-II participants (approval no. 41-2).

Laboratory Methods

DNA was extracted from the buffy coat fraction with a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA). DNA amplification was conducted by polymerase chain reaction with confronting two-pair primers (PCR-CTPP), using the primers F1: 5' TAC GCA AAA TCT TAG CAT ACT C and R1: 5' AAT TAA GGG TGT TAG TCA TGT TAG and F2P3: 5' TCT CGC ACC TGA AAC AAG A and R2: TTT GTG AAT TCT TCG ATA ATG. PCR-CTPP was conducted using a total reaction volume of 25μl. Each reaction mixture contained genomic DNA (30 to 100 ng), 0.18 mM dNTPs, 12.5 pmol of each primer, 0.5 units of AmpliTaq Gold, and 2.5 μl of GeneAmp 10 χPCR Buffer, containing 15 mM $MgCl_2$ (Perkin-Elmer, Foster City, CA, USA). Amplification conditions were as follows: 10 min of initial denaturation at 95˚C, followed by 35 cycles at 95˚C for 1 min, 1 min at 54˚C, and 1 min at 72˚C. A final 5-min extension was carried out at 72˚C. The amplified DNA was visualized on a 2% agarose gel via ethidium bromide staining. Genotyping was distinguished as follows: a 209-bp band for the C allele, a 123-bp band for the A allele, and a 292-bp band for the common band. Three samples of amplified DNA for each genotype were sequenced to assure correct genotyping by PCR-CTPP.

Statistical Analysis

All data were analyzed using the SAS statistical software, version 8.2 (SAS institute, Cary, N.C.). P values below 0.05 were regarded as statistically significant. Chi-square tests were performed to analyze the genotype frequency between cases and controls in all age groups combined and in each age group individually partitioned by decade. Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) of the mt5178C genotype for the six cancers studied. The interaction term between the mt5178 genotype and age for all six cancers was also computed using a logistic regression model. The interaction term between the mt5178 genotype and age was computed for two separate groups (young; age<60 yrs., and old; age>=60 yrs.)

The statistical power to detect a significant ($p<0.05$ by a two-sided test) OR for our samples with 930 cases and 1120 controls were 0.99 in case of OR=1.5 and 0.50 in case of

Table 1. Genotype Frequencies of Mt5178 for Cancer Casees and Controls

Subjects	Age	N	Sex ratio M/F	Genotypes of mt5178 A (%)	C (%)
Cases					
All Cancers	20-85	930	448/483	39.5	60.5
Esophageal	43-84	102	86/16	45.1	54.9
Stomach	33-85	147	104/43	38.8	61.2
Colorectal	27-82	147	84/63	37.4	62.6
Lung	31-84	192	115/77	38.0	62.0
Breast	28-72	236	0/236	39.0	61.0
Malignant Lymphoma	20-83	106	58/48	41.5	58.5
Non-cancer Control					
All Non-cancer	20-85	1120	552/872	39.1	60.9
Frist Visit Patients	20-79	672	312/360	36.8	63.2
Health Check-up	35-85	448	124/324	42.6	57.4

OR=1.2, when A allele was assumed to be 40% among the controls.

Results

Table 1 shows the mitochondrial genotype frequency for the two control groups and the 6 cancer groups. Mean ages of subjects in non-cancer and cancer groups were 56 and 60 years old, respectively.

In non-cancer controls, there was no significant difference in mt5178 genotype distribution between the health check-up group and the first-visit patient group. Also, no significant difference was found in the distribution of the mt5178 genotype among any of the 6 cancer groups, although the frequency of mt5178A in esophageal cancer patients tended to be greater than in the other cancers. In the comparison of mt5178A/C distribution among non-cancer controls and cancer cases, 39.1% of non-cancer subjects carried mt5178A and 39.5% of cancer subjects carried mt5178A. There was no significant difference between the non-cancer and cancer groups.

Table 2 shows the distribution of the mt5178 genotype for various age groups divided over 7 decades (age 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, over 80). No cancer subjects within the age 20-29 group had the mt5178A genotype. There was no consistent tendency in the frequency of mt5178A with age for either the non-cancer or cancer subjects. There was no significant difference in mt5178A/C ratio between non-cancer and cancer subjects in any of the age groups studied with the exception of ages 40-49 years. The percentage of subjects carrying mt5178A was significantly higher in cancer cases than that in non-cancer subjects. A greater distribution of mt5178A within the non-cancer group than in the cancer group was found in those over 80 years old(62.5% in non-cancer, 36.8% in cancer). However, this difference between non-cancer and cancer groups was not statistically significant due to the small number of subjects within this age group.

Age and sex adjusted odds ratios (ORs) of the genotype for each site of cancer are shown in Table 3. The two non-cancer groups were combined for the OR estimation, since the difference in the genotype distribution was not substantial. There was no significant OR of mt5178C for any of the cancer types surveyed.

Table 2. Genotype Frequencies of Mt5178 (A/C ratio) in Each Dacade.

Age group	Non-cancer		Cancer		All Subjects	
	N	% of mt5178A	N	% of mt5178A	N	% of mt5178A
20-29	34	29.4	9	-	43	23.3
30-39	141	46.8	28	46.4	169	46.7
40-49	157	37.6	123	48.0*	280	42.1
50-59	309	36.6	280	35.7	589	36.2
60-69	325	39.1	314	42.4	639	40.7
70-79	146	39.7	157	35.0	303	37.3
Over 80	8	62.5	19	36.8	27	44.4
All	1120	39.1	930	39.5	2050	39.3

*$p<0.05$ significant difference between non-cancer and cancer

Table 3. Age and Sex Adjusted Odds Ratios (ORs) 95% Confidence Intervals (95% CIs) of Mt5178 Genotype

Subjects	OR (95% CI)	
	Mt5178A	Mt5178C
All Cancers	1	0.980 (0.817-1.173)
Esophageal	1	0.767 (0.500-1.175)
Stomach	1	1.001 (0.694-1.443)
Colorectal	1	1.063 (0.741-1.525)
Lung	1	1.027 (0.741-1.423)
Breast	1	1.060 (0.782-1.433)
Malignant Lymphoma	1	0.885 (0.589-1.329)

The two control groups (First visit patients and Health Check-up) were combined for the OR estimation, because the difference in the genotype distribution was not substantial.

There was no consistent tendency with age in the ORs of the genotype for each site of cancer. In lung cancer subjects, ORs of the mt5178A genotype increased with age, but this increase was not significant. The interaction term between age, the mt5178 genotype, and cancer, was only significant in older (age>=60 yrs.) lung cancer subjects ($p<0.023$, OR 1.093, CI 1.012-1.181).

Figure 1 demonstrates the relationship between age and genotype distribution in non-cancer and cancer subjects. Age is plotted on the X axis while the Y axis shows the cumulative percentage of subjects carrying mt5178A and mt5178C. Although the percentages of mt5178A and C were similar among non-cancer and cancer subjects, the patterns of increase in each genotype were different between the two groups. In non-cancer subjects, the percentages of subjects with mt5178A and mt5178C were comparable until age 30. After age 30, the percentage of subjects with mt5178C increased more sharply than those with mt5178A. Similar percentages of cancer subjects had mt5178A and mt5178C before age 40. After age 50, the percentage of subjects with mt5178C increased more markedly than those with mt5178A.

Discussion

Recently it has been reported that the mt5178A genotype may contribute to longevity (Gong et al., 1998; Tanaka et al., 1998; Tanaka et al., 2000). Tanaka et al. suggested that mt5178A may promote resistance to adult-onset diseases by suppressing obesity and atherosclerosis (Tanaka et al., 2000). The Japanese population has the longest life expectancy in the world, and a higher frequency of the mt5178A genotype is found within the Japanese population compared to other populations (Cann et al., 1987). Shimokata et al. reported that the proportions of mt5178A and mt5178C within the Japanese population were 42.1% and 57.9% respectively, with no gender differences between the two genotypes (Shimokata et al., 2000). Kokaze et al. showed that the percentage of subjects with mt5178A was 40.9% in men and 44.4% in women (Kokaze et al., 2001). Tanaka et al. found that 45% of their healthy Japanese subjects had the mt5178A

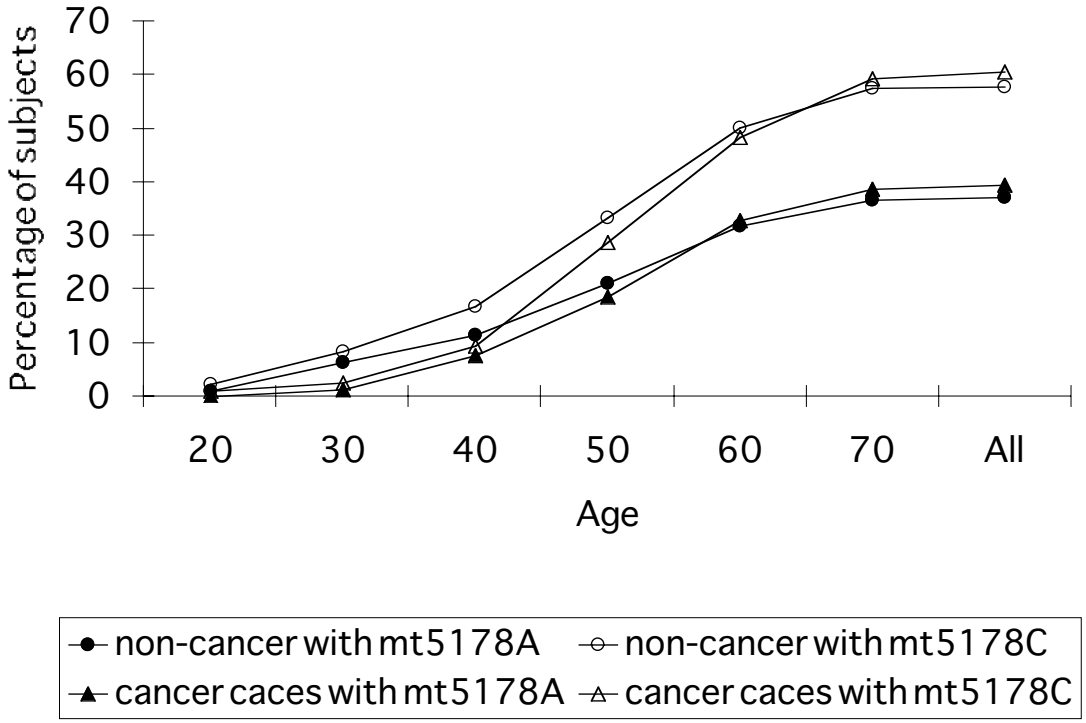

Figure 1. Cumulative Percentage of Subjects Carrying mt5178A or mt5178C in Non-cancer Control and Cancer Cases.

genotype (Tanaka et al., 1998). They also demonstrated a remarkably higher frequency of mt5178A in centenarians (62%) compared to that in the general Japanese population (45%). In addition, they discovered a higher frequency of C-to-T transitions at np 8414 within the ATP synthase subunit 8 gene and a G-to-A transitions at np 3010 within the 16S rRNA gene in centenarians compared to healthy controls (mt8414T in 7/11 centenarians vs 11/43 controls, mt3010A in 7/11 centenarians vs 11/42 controls). In that study, all of the 7 centenarians with both mt8414T and mt3010A also carried mt5178A.

In the present study, we found that 39.1% of non-cancer subjects and 39.5% of cancer subjects carried mt5178A. This result confirmed that carrying the mt5178A genotype is relatively frequent in the Japanese population as reported previously (Gong et al., 1998; Shimokata et al., 2000; Tanaka et al., 1998; Tanaka et al., 2000).

The mt5178A genotype is caused by a C-to-A transition on the NADH dehydrogenase subunit 2 genes (ND2). This results in a Leu-to-Met substitution at amino acid 237 of the ND2. It remains unclear how mt5178 contributes to longevity, although Tanaka suggested that the Leu237Met replacement in the ND2 subunit might have a protective effect against oxidative damage to mitochondria (Tanaka, 2002). Several previous studies (Kokaze et al., 2002; Kokaze et al., 2001; Matsunaga et al., 2001; Wang et al., 2001) have demonstrated relationships between mt5178 and diseases. Kokaze et al. reported that serum triglyceride concentration in Japanese females carrying mt5178A was significantly lower than that in Japanese females carrying mt5178C. This study also reported that serum HDL-cholesterol concentrations were higher in Japanese men carrying mt5178A than in men carrying mt5178C (Kokaze et al., 2001). Matsunaga et al. investigated the relationship between mt5178 and serum lipids in Japanese patients with type 2 diabetes (Matsunaga et al., 2001). They demonstrated that serum cholesterol level in diabetic patients carrying mt5178A was significantly lower than that in patients carrying mt5178C, and that the mean intima-media thickness (IMT) at six sites in the bilateral carotid arteries was significantly smaller in diabetic patients with mt5178A than that in patients with mt5178C. Also, Wang et al. reported that the age at onset of type 2 diabetes was significantly lower in patients with mt5178C than in patients with mt5178A (Wang et al., 2001). Thus, it is possible that the mt5178C genotype may predispose individuals to disorders in glucose and/or lipid metabolism.

On the other hand, there are negative reports regarding the contribution of mt5178A to diseases or longevity. Matsunaga et al. suggested that mt5178A may not be associated with the onset and/or progression of type 2 diabetes, although this genotype was found to be associated with carotid IMT and serum cholesterol level in type 2 diabetic patients (Matsunaga et al., 2001). Yao et al. failed to find a higher frequency of mt5178A in older (age>70 yrs.) than in younger (age 24-37 yrs.) subjects within a Chinese population (Yao et al., 2002). Although the older subjects were not centenarians, the authors emphasized that the older subjects were representative of long-lived individuals within the general Chinese population. In European populations, only one mtDNA polymorphism, a nucleotide substitution at nt 9055, has been linked to longevity (Ivanova et al., 1998). Thus, it is not clear whether mt5178 relates to the onset and/or progression of diseases that affect life span.

In the present study, we found no remarkable association of mt5178A with several types of cancers. There was no significant difference in the frequency of mt5178A/C among patients representing six different sites of cancer and non-cancer controls. Also, ORs of cancer patients carrying mt5178C were not significant. There was no evidence that mt5178C subjects were more susceptible to any cancer examined in this study. Because environmental conditions, including lifestyle, are considered to largely contribute to the development of cancer, (Lichtenstein et al., 2000), the contribution of mt5178 genotype may be minimal to none. However, due to the associations among mt5178 genotype and glucose and/or lipid metabolism, it is possible that mt5178 genotype affects cancer recovery rates and/or age of cancer-related mortality . In this respect, a longitudinal analysis may be able to elucidate the relationships among longevity, cancer and mt5178 genotype.

In the present study, we examined the relationship between age and genotype frequency in non-cancer and cancer groups (Figure 1). In non-cancer subjects, the percentage of subjects carrying mt5178C increased more markedly than that of mt5178A after age 40. In the cancer group, the percentage of subjects carrying mt5178C was similar to that of mt5178A up to age 40. After age 40, the frequency of cancer patients with mt5178C increased more markedly than that of mt5178A. Thus, the frequency of mt5178A/C changed after age 40 in the non-cancer group, and after age 50 in the cancer group. Several previous studies have reported a relationship between age and the frequency of mt5178A/C (Gong et al., 1998; Tanaka et al., 1998). Tanaka et al. (1998) studied the age distribution of subjects with mt5178A or mt5178C in inpatients and outpatients in a hospital setting. They found that the cumulative number of subjects with mt5178C increased more markedly than that of mt5178A after age 45, whereas the frequency of mt5178C was similar to that of mt5178A before age 45. In the present study, only non-cancer and cancer patients aged 40-49 years showed a significant difference in the percentage of subjects carrying the mt5178A genotype. Even though it remains unclear why remarkable differences between non-cancer and cancer subjects occur in this age-group, this result is consistent with previous findings (Tanaka et al., 1998). In addition, we found a significant interaction term between age and genotype on lung cancer in the older age-group (age<=60 years). Thus, the effects of mt5178 genotype on cancer may be realized with age, since mitochondrial function contributes to the normal aging process .

In the present study, a remarkably higher frequency of mt5178A was found in non-cancer subjects aged>=80

compared to the other age groups. This may be in agreement with the findings of Tanaka et al. (1998) that centenarians carry mt5178A more frequently than the general healthy population. Individuals who live over 80 years without cancer may be regarded as a kind of healthy elite. Therefore, it is possible that the frequency of mt5178A in subjects aged over 80 is greater, even if they are not yet centenarians. Since illness normally occurs more frequently in individuals over 80, the higher frequency of mt5178A in this group could be a protective factor against ill health.

In conclusion, our results confirm the previously reported high frequency of mt5178A within the Japanese population. There was no evidence that the mt5178 genotype contributes to the development of any of the six types of cancer examined within this study. However, an interaction term between aging and mt5178 genotype on cancer, particularly on lung cancer, was suggested. A future longitudinal prospective analysis is needed to examine the relationship between mt5178 genotype and the development and/or progression of cancer.

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