RESEARCH ARTICLE

# Association Analysis of Single Nucleotide Polymorphisms in miR-146a and miR-196a2 on the Prevalence of Cancer in Elderly Japanese: A Case-Control Study

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## Abstract

**Background: Single nucleotide polymorphisms (SNPs) affecting microRNA (miR) sequences may influence carcinogenesis. Our current study primarily aimed to confirm previously conducted association studies between rs2910164 found on miR-146a, and rs11614913 located on miR-196a2 polymorphisms and cancer phenotypes in the Japanese elderly population. rs2910164 (G/C) and rs11614913 (T/C) polymorphisms were determined by genotyping on the samples collected from 1,351 consecutive autopsy cases registered in the Japanese SNPs for geriatric research (JG-SNP) data base. Cancer samples were systematically reviewed, pathologically verified and assessed with respect to miR-146a and miR-196a2 genotypic variation. The current study covered 726 males and 625 females with a mean age of 80.3±8.9 years. The study included 524 subjects without cancer and 827 subjects with at least one type of cancer, such as gastric (n=160), lung (n=148), colorectal (n=116) or others. Males with cancers (n=467) were more numerous than females (n=360). Both rs11614913 (CT: TT adjusted odds ratio (OR) 95% confidence interval (95%CI)=0.98 (0.75-1.28), p=0.873, CC: TT adjusted OR (95%CI)=1.06 (0.76-1.47), p=0.737, CT+CC: TT, adjusted OR (95%CI)=0.99 (0.77-1.29), p=0.990), and rs2910164 (CG: CC adjusted OR (95%CI)=1.12 (0.87-1.44), p=0.383, GG: CC adjusted OR (95%CI)=1.03 (0.71-1.48), p=0.887, CG+GG: CC adjusted OR (95%CI)=1.10 (0.87-1.39), p=0.446) polymorphisms did not show significant association with overall cancer in all subjects. However, "CC" genotype in rs11614913 polymorphism was significantly associated with increased gastric cancer (n=160) in all subjects (CC: CT+TT, adjusted OR (95%CI)=1.50 (1.02-2.22), p=0.040). We found that rs11614913 and rs2910164 do not pose general cancer risk, but rs11614913 may influence gastric cancer in Japanese elderly population. Confirmation of our study results requires further investigations with larger subject populations.**

**Keywords:** microRNAs -rs2910164 - rs11614913 SNPs - cancer risk - gastric cancer - elderly Japanese

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## Introduction

Human genome contains vast number and kind of non-coding RNAs (ncRNA) including micro RNAs (miRs) that directly or indirectly influence gene expression. MiRs are endogenous, single-stranded, and around 22-25 nucleotide long non-coding RNAs that exhibit various functions including inhibition of the expression of protein-coding genes by either translational repression or messenger RNA degradation (Pillai et al., 2005). Growing evidences have suggested that miRs regulate a wide range of biological processes, including development (Wienholds et al., 2005), proliferation and apoptosis (Brennecke et al., 2003; Ambros, 2004; Bartel, 2004). Some early studies confirmed that dysfunctional miR processes play critical roles in cancer (Hanahan et al., 2000; Sherr, 2004; Kumar et al., 2007). The gain or loss of function in specific miRs is involved as an oncogene or tumor formation factors (Esquela-Kercher et al., 2006; Kent et al., 2006).

Single nucleotide polymorphism (SNP) is the most common type of genetic variation in human genome. The polymorphisms located in miRs have been a major research topic for almost a decade. SNPs on miR (miR-SNP) are involved in transcription of pre-miR, miR processing, miR maturation, mRNA interactions (Duan et al., 2009), and may initiate an oncogene(s) also or tumor suppressor gene(s) depending on cell type and respective gene expression level (Sun et al., 2009). Functional miR-SNPs may act as analogous sequence to intervene mRNA formation either by creating new variants or destroying them as they are unable to maturate to become a functional mRNAs (Lu et al., 2005). The functional miR-SNPs are

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epigenetically involved in carcinogenesis by changing the methylation level at CpG sites of the respective target gene(s) (Lu et al., 2007; Lehmann et al., 2008). In evolutionary perspective the frequency of occurring a miR-SNP is 1% and there is a clear and stringent selection on deleterious alleles in seed sites where miRs binds to influence the mRNA biogenesis especially in humans (Ryan et al., 2010). Although the scarcity of the miR-SNP is evident, but the possibility of adverse influence is counterintuitively high. Despite of the numerous studies, the effect of miR-SNPs on cancer risk or susceptibility is still largely unknown.

In our current study, we investigated both rs2910164 (G/C) located on miR-146a and rs11614913 (T/C) located on miR-196a2. The rs2910164 (G/C) polymorphism is a G to C nucleotide change leading to C:U mismatch on the stem region of miR-146a which is one of the variant of miR-146. The compiling evidence indicate that rs2910164 is associated with different types of cancer phenotypes including breast cancer (Shen et al., 2008) gastric cancer (Okubo et al., 2010; Zeng et al., 2010; Kogo et al., 2011), hepatocellular carcinoma (Xu et al., 2008), glioma (Permuth-Wey et al., 2011) prostate (Xu et al., 2010) and others (Wang et al., 2012). The G allele carriers were shown to have higher mature miR-146a synthesis (Xu et al., 2008). The rs2910164 (G/C) may involve in negative feedback regulation of Toll-like receptors (TLR) and cytokine receptor signalling (Taganov et al., 2006).

In the case of rs11614913 polymorphism in miR-196a2 has been reported to have associations with cancer prognosis and development (Feng et al., 2012; Zhang et al., 2012). The nucleotide change of T to C may affect the miR-196a2 expression (Saunders et al., 2007; Chu et al., 2011). Recent studies show that rs11614913 may influence lung cancer (Tian et al., 2009; Hong et al., 2011; Yuan et al., 2013), breast cancer (Hoffman et al., 2009; Linhares et al., 2012), head and neck cancer (Christensen et al., 2010), hepatocellular carcinoma (Xu et al., 2008; Li et al., 2010; Qi et al., 2010; Hao et al., 2013), gastric cancer (Peng et al., 2010), prostate cancer (George et al., 2011) and others (Zhang et al., 2012). On the contrary, there are also several studies for both rs2910164 and rs11614913 polymorphisms indicate conflicting results asserting either lack of association or decreased cancer risk (Xu et al., 2011; Hezova et al., 2012; He et al., 2012; Wang et al., 2012; Xu et al., 2013; Zhang et al., 2013). Despite the numerous studies, there is a plausible scientific need to clarify how miR-SNPs such as rs2910164 and rs11614913 effect cancer development or what kind of associations they may have with overall cancer and specific cancer types.

Our study primarily aimed to assess the association of rs2910164, and rs11614913 polymorphisms with overall cancer and different types of cancer risk in elderly Japanese population. To our knowledge, this study is the first attempt to characterize rs2910164 and rs11614913 miR-SNPs with respect to cancer in elderly Japanese population.

## Materials and Methods

*Study subjects*

1,351 consecutive autopsy cases were analyzed, average age was 80.3±8.9 years for all subjects. Our subjects consisted of 726 males and 625 females. "Cancer (+): cancer present" group is defined as any patient with at least one cancer type, and "Cancer (-): cancer absent" groups are defined as absence of any type of malignancies. Cancer phenotypes which were registered in the internet database of Japanese single-nucleotide polymorphisms (SNPs) for geriatric research (JG-SNP) (Sawabe et al., 2004) were matched with our genotyping results for both rs2910164 and rs11614913.

Pathological findings of presence or absence of cancers were determined from consecutive autopsy cases. The autopsies were performed on 40% of patients who died in the Tokyo Metropolitan Geriatric Hospital between 1995 and 2004. The study subjects had high resemblance with the figures of the death causes reported by National Cancer Center, Japan (Vital Statistics of Japan) (http://ganjoho.jp/professional/statistics/statistics.html) and previous studies (Oda et al., 2007; Takei et al., 2008) and, thus the geriatric autopsy samples could be used to analyze in our research. The pathological assessments and genotyping were done in different institutions in double-blind fashion. Clinical data such as, smoking and drinking status, were retrieved from the patient's medical records. Subjects were classified as "smokers" versus "non-smokers" by their smoking habit history, and likewise "drinker" versus "non-drinker" by the alcohol consumption history of subjects. Informed consents were obtained from the bereaved families at the time of autopsy. Our study and its protocol were approved by ethical Committees of the Tokyo Metropolitan Geriatric Hospital and the Tokyo Medical and Dental University.

*Genotyping*

Genomic DNA was extracted from renal cortex of subjects by conventional procedure. Genotyping was done by TaqMan assay (Assay ID numbers for rs2910164: C__15946974_10 and for rs11614913: C__31185852_10, Applied BioSystems) according to the protocols described by the manufacturers. PCR was performed in 5 $\mu$l reaction mix including 10ng genomic DNA. PCR was conducted with the LightCycler 480 instrument (Roche Diagnostics) and settings were for pre-incubation at 95°C for 10 min., denaturation 92°C for 15 sec., annealing and extension at 60°C for 1 min. 40 cycles. Both rs2910164 and rs11614913 were genotyped with success rate of 100%. Allelic discrimination of each polymorphisms were determined with≥98% accuracy criteria. The genotyping results were determined LightCycler Genotyping software (Roche Diagnostics). Genotyping accuracy was verified by randomly selected samples.

*Statistical analysis*

Statistical analysis was done by using SPSS software (IBM) version 19. The p-value (p) lower than 0.05 ($p<0.05$) was considered as statistically significant.

Statistical significance in categorical values such as allelic distribution was calculated by 2-sided Fisher's exact test. Bonferroni correction for multiple testing was not applied in our statistical analysis for its conservative nature. Continuous values such as age was analyzed by analysis of variance (ANOVA). Hardy-Weinberg equilibrium (HWE) was assessed using permutation test. Chi-square test was used to determine significance in distribution of genotypes in cancer present or absent subjects. Odds ratio (OR), 95% Confidence interval (95%CI) values were calculated by binary logistic regression analysis. Confounding parameters such as "gender, smoking and drinking status, and age" were adjusted in regression analysis. Our study had 89.9% and 92.7% statistical power for rs2910164 polymorphism and rs11614913 polymorphism respectively.

## Results

*General characteristics of subjects*

Demographics of our study including number of cancer sites, number pathological of cancer types, and common risk factors such as smoking, and drinking status is shown in Table 1. The number of cancer present subjects was 827 and that of cancer absent subjects was 524 in the study population. Age, and gender parameters were found to be significant (p=0.001, and p=0.011, respectively) between cancer present versus cancer absent subjects. The number of "smokers" was 641 (48%) and "non-smokers" was 612 (45%). The "smoker" cancer present subjects were 411 (50%) and "non-smoker" cancer present subjects were 372 (45%). The number of "non-drinkers" was 806 (60%) and "drinkers" was 442 (33%). Neither "smoking status" (p=0.223), nor "alcohol consumption" (p=0.136) was found to be significant between "cancer present" and "cancer absent" subjects. Cancer present subjects with "single cancer" site were 621 (46%), "two cancer" sites were 160 (12%) and "three and more cancer" sites were 46 (3%) of the population. The highest number of cancer types are gastric (n=160), lung (n=148), and colorectal (n=116), (Table 1).

**Table 1. General Demographics of Subjects**

| Characteristics | Total n (%) (n=1351) | C (-)[1] n (%) (n=524) | C (+)[2] n (%) (n=827) | p* |
|---|---|---|---|---|
| Age at death, (years) | 80.3±8.9† | 81.4±9.2 | 79.7±8.7 | 0.001 |
| Gender | | | | |
| Male | 726 (54) | 259 (49) | 467 (57) | |
| Female | 625 (46) | 265 (51) | 360 (43) | 0.011 |
| Smoking status | | | | |
| Smoker | 641 (48) | 230 (46) | 411 (50) | |
| Non-smoker | 612 (45) | 240 (47) | 372 (45) | |
| Missing | 98 (7) | 54 (7) | 44 (5) | 0.223 |
| Alcohol consumption | | | | |
| Drinker | 442 (32) | 156 (30) | 286 (35) | |
| Non-drinker | 806 (60) | 319 (61) | 487 (59) | |
| Missing | 103 (8) | 49 (9) | 54 (6) | 0.136 |
| Cancer | | | | |
| 0 | | 524 (39) | | |
| 1 | | | 621 (46) | |
| ≥2‡ | | | 206 (15) | |
| Cancer sites[3], n | | | | |
| Gastric | | | 160 | |
| Lung | | | 148 | |
| Colorectal | | | 116 | |
| Prostate | | | 89 | |
| Acute leukemia | | | 72 | |

*p values for "age" was calculated by ANOVA test and others were calculated by 2- sided "Fisher's Exact Test"; †±indicates standard deviation (SD); [1]C-: Cancer absent subjects; [2]C+: Cancer present subjects; [3]Representative list of cancer phenotypes. All cancer phenotypes are pathological; ‡Multiple cancer bearing subjects who have at least 2 or more cancer types

*Genotypic results of rs2910164 and rs11614913 polymorphisms*

All genomic DNAs (n=1,351) were successfully genotyped. The allelic frequencies of rs2910164 located on miR-146a polymorphism were 1,712 (63%) for "C" allele, and 990 (37%) for "G" allele. Genotypic frequencies of each genotypes CC, CG and GG were 541 (41%), 630 (47%), and 180 (13%), respectively.

As for rs116149 polymorphism's allelic frequencies of "T" and "C" were 1,459 (54%) and 1,243 (46%). Genotypic frequencies of rs11614913 were 390 (29%) for "TT", 679 (50%) for "CT", and 282 (21%) for "CC" are shown in Table 2. Both rs2910164 (pHWE=0.873) and rs11614913 (pHWE=0.655) were consistent with Hardy-Weinberg equilibrium (Table 2).

*Genotypic distributions of different genetic models in cancer present and absent subjects for rs2910164 and rs11614913 polymorphisms*

Genotypic frequencies in additive, recessive, dominant and co-dominant inheritance models were calculated. and are shown. In rs2910164 polymorphism genotypic frequencies of additive model CC: CG: GG was 109 (13%): 393 (45%): 325 (42%), recessive model CC+CG: GG was 718 (86%): 109 (14%), dominant model GG+CG: CC was 502 (61%): 325 (39%) and co-dominant model GG: CC was 109 (61%): 71(39%) in cancer present subjects and there was no significant difference between cancer present and absent patients in all models ( p=0.708, p=0.870, p=0.494, p=0.930) respectively.

In rs11614913 polymorphism additive model frequencies of CC: CT: TT was 174 (21%): 409 (49%): 244 (30%), recessive model TT+CT: CC was 653 (79%): 174 (21%), dominant model CC+CT: TT was 583 (70%): 244 (30%) and co-dominant model TT: CC was 244 (58%): 174 (42%) in cancer present subjects. There was no statistical significance in additive, recessive, dominant and co-dominant models (p=0.740, p=0.891, p=0.538, p=0.872) respectively. Therefore, there was no statistical significance in four genetic models.

**Table 2. Allelic Count and Genotypic Results of rs2910164 and rs11614913 Polymorphisms for All Subjects**

| SNP | Alleles | | Genotypes | | | p (HWE)* |
|---|---|---|---|---|---|---|
| | C | G | CC | CG | GG | |
| rs2910164 | 1712 (63) | 990 (37) | 541 (41) | 630 (47) | 180 (13) | 0.873 |
| | T | C | TT | CT | CC | |
| rs11614913 | 1459 (54) | 1243 (46) | 390 (29) | 679 (50) | 282 (21) | 0.655 |

*p(HWE): Hardy-Weinberg Equilibrium. All subjects (n=1,351) were successfully genotyped (success rate: 100%); "( )" value indicates the "percentage" value of each allelic or genotypic group

**Table 3. Association Analysis of rs11614913 with Gastric Cancer in All Subjects**

| Cancer type | C+, n (%) | Genotype | Crude OR† (95% CI) | p* | Adjusted OR‡ (95% CI) | p* |
|---|---|---|---|---|---|---|
| Gastric | 116 (11) | TT+CT | 1.00 (reference) | | 1.00 (reference) | |
| | 44 (16) | CC | 1.52 (1.04-2.32) | 0.031 | 1.50 (1.02-2.22) | 0.040 |

†OR: Odds ratio, 95%CI: 95 percent confidence interval; *p value is calculated by binary logistic regression; ‡ORs are adjusted by age, gender, smoking and drinking status; "( )" value indicates the "percentage" value of both combined genotype and each genotypes within gastric cancer present group

*Association analysis results of both rs2910164 and rs11614913 in overall cancer*

Association analysis results of rs2910164 and rs11614913 polymorphisms in overall cancer are shown. In rs2910164 polymorphism the comparison was done with respect to major genotype CC, overall cancer risk was examined with both crude and adjusted odds ratios by regression analysis. The adjusted results are CG: CC OR (95% CI)=1.118 (0.870-1.436), GG: CC OR (95% CI)=1.027 (0.714 -1.476) and CG+GG OR (95% CI)=1.096 (0.866-1.388), statistical significance was not found in all genotypic comparisons (p=0.383, p=0.887 and 0.446) respectively.

For rs11614913 polymorphism the major genotype, TT, was set as reference and other comparisons were made. The adjusted OR values of overall cancer are CT: TT, OR (95% CI)=0.978 (0.746-1.283), CC: TT, OR (95% CI)=1.058 (0.759-1.475) and CC+CT: TT OR (95% CI)=0.998 (0.773-1.289). In all examined models, we could not find any significant associations with overall cancer (p=0.873, p=0.737 and p=0.990) respectively.

*Allelic and genotypic frequencies in selected cancer phenotypes for both rs2910164 and rs11614913 polymorphisms*

We further investigated specific cancer phenotypes consisting of gastric, lung, colorectal, prostate and acute leukemia which have highest number of samples among other cancer phenotypes. The frequency of each allele and genotype in gastric, lung, colorectal, prostate and acute leukemia is shown. In rs2910164 polymorphism C allele was dominant in all selected cancer phenotypes; gastric (63%), lung (60%), colorectal (69%), prostate (65%) and acute leukemia (62%). The genotypic distributions of CC: CG: GG were following; gastric cancer 61 (38%): 79 (49%): 20 (13%), lung cancer 56 (38%): 67 (45%): 25 (17%), colorectal 58 (45%): 50 (43%): 12 (12%), prostate cancer 37 (42%): 41 (46%): 11 (12%). The allelic distribution of C and T in rs11614913 was similar. T allele frequencies were following; gastric (50%), lung (53%), 127 (55%), 96 (54%) and acute leukemia (49%). Genotypic distribution was rather well distributed among all genotypes. Genotypic distributions of CC: CT: TT were following; gastric 44 (28%): 72 (44%): 44 (28%), lung 29 (19%): 81 (55%): 38 (26%), colorectal 23 (20%): 59 (51%): 34 (29%), prostate 17 (19%): 48 (54%): 24 (27%) and acute leukemia 21 (29%): 31 (43%): 20 (28%).

*Genotypic distributions of selected cancer phenotypes for both rs2910164 and rs11614913 polymorphisms*

In rs2910164 additive models of each specific cancer phenotypes indicated no statistical significance in gastric (p=0.753), lung (p=0.401), colorectal (p=0.549), prostate (p=0.910) and acute leukemia (p=0.901). Similarly, no statistical significance was observed in any other models.

In rs11614913 polymorphism additive, dominant and co-dominant models did not show any statistical significance. In recessive model CC genotype frequency (16%) was significantly higher in gastric cancer than TT+CT genotype carriers (11%), (p=0.038). Although, C allele frequency was found to be significant, the conclusive interpretations are hard to construct, due to low number of gastric cancer (n=160).

*Association analysis of rs11614913 polymorphism with gastric cancer*

Among all selected cancer phenotypes, gastric cancer showed significant association with CC genotype. The regression analysis showed CC: CT+TT adjusted OR (95% CI)=1.504 (1.020-2.218), p=0.040. Thus, the gastric cancer risk was increased 50% by CC genotype in rs11614913 polymorphism (Table 3). No other significant association with specific cancer types was found (data not shown).

## Discussion

In our current study, our primary hypothesis was whether rs2910164 and rs11614913 have effect and association on total cancer and secondary question was if these miR-SNPs have association with individual cancer types in elderly Japanese population.

Our results indicated that rs2910164 and rs11614913 miR-SNPs did not show any differential frequency significance in any genotypic models for overall cancer. Furthermore, no significant association was found in both polymorphisms in overall cancer. Our results are consistent with previous studies (Christensen et al., 2010; Xu et al., 2011; Hoffman et al., 2009; Ma et al., 2013).

We analysed the genotypic frequencies in additive, recessive, dominant and co-dominant models for both rs2910164 and rs11614913 polymorphisms in specific cancer phenotypes such as gastric, lung, colorectal, prostate, acute leukemia which were the top five highest malignancy phenotypes in our subjects (Table 1), and found that rs2910164 did not show any significant results in all specific cancers as well. The rs2910164 (G/C) is located on stem region causing C:U mismatch in miR-146a precursor. Consequently, rs2910164 (G/C) alters the mRNA processing, reduction in miR-146a and ultimately induces risk for cancer (Srivastava et al., 2012). One rare feature of rs2910164 is GC heterozygote genotype can cause papillary thyroid tumors (Ryan et.al., 2010). This G to C genetic modification reduces the secondary structure stability, since C has lower free energy (ΔG=-40 kcal/mol) than G allele (ΔG=-42 kcal/mol) showed reduced mature miR-146a adversely influencing the inhibition of the target tumor suppressor genes such as IL-1 receptor-associated kinase 1 (IRAK1), TNF receptor-associated factor 6 (TRAF6), and papillary thyroid carcinoma 1 (PTC1), G allele down regulates the mature miR-146a and increased cell proliferation homozygous GG carriers

had higher matured miR-146a expression level leading to higher cancer susceptibility (Xu et al., 2008) Furthermore, rs2910164 CC genotype was associated with breast cancer by increasing the binding affinity to BRCA1 and/or BRCA2 oncogenes and alternatively may disturb the well-known NF-kB apoptotic pathway (Taganov et al., 2006). On the contrary, our results indicated that rs2910164 has no statistical significance with over all cancer which is consistent with previous studies indicating that either rs2910164 (G/C) polymorphism has no significant association or decreasing/protective effect to cancer development (Xu et al., 2013; Xu et al., 2011) even more specifically with liver cancer (Zhou et al., 2011; Wang et al., 2012), colorectal cancer (Hezova et al., 2012, Du et al., 2014), digestive system including gastric cancer (Kupcinskas et al., 2013; Li et al., 2014).

The rs11614913 is located the passenger strand of the 3' end of pre-miR-196a2 sequence, which negatively effects the miR-196a2 maturation and its interaction(s) with other miRs or gene(s) and also mature miR-196a2 level was reduced in CC homozygous variant than TT homozygous genotype was found in previous studies (Duan et al., 2008). Previously, CC homozygous genotype was found to be associated with increased cancer risk (Feng et al., 2012). Hong et al., also found that TC+CC carriers were associated with Non-small cell lung cancer (NSCLC) in Korean population (Hong et al., 2011). On the other hand, few studies indicated that TT homozygote genotype was also associated with cancer development including oesophageal cancer in Caucasian males (Ye et al., 2008), gastric cancer (Okubo et al., 2010), and liver cancer (Li et al., 2010). The rs11614913 was also associated with poor lung cancer survival, hence it was the first miR-SNP used for prognosis on lung cancer (Hu et al., 2008). However, our results indicated both rs2910164 and rs11614913 did not demonstrate significant associations with overall cancer. Our results are supported by several previous results. Wang et al. found two common miR-SNPs, rs2910164 and 11614913, are not linked with hepatocellular carcinoma in Asian population (Wang et al., 2012). Additionally, Wu et al. found no significant association between rs2910164 with digestive cancer (Wu et al., 2013). Hezova et al. also found no allelic or genotypic association with rs2910164 and rs11614913 polymorphisms with colorectal cancer in Caucasians (Hezova et al., 2012), Wu et al. found no statistical significance

These conflicting results deserve more detailed investigation, however there are some possible reasons; first, miR-SNPs in general may affect the incidence of cancer in substantially varied manner in different races and ethnicities, thus both rs2910164 and rs11614913 may have differential results in different ethnical backgrounds; second, most of the previous studies that are indicating the oncogenic implications are meta-analysis which inherently consist of publication, heterogeneity, stratification biases, public vs. hospital based cohort bias and some other calculation/statistical biases may have an accumulated effect on the data analysis (He et al., 2012). Finally, rs2910164 and rs11614913 polymorphism may have unknown target mRNA(s) or gene(s) other than known miR-146a and miR-196a2 eventually, causing differential expression(s) and outcomes in different ethnicities, thus may initiate unidentified pathway(s) leading either tumour suppression or initiation in varying degrees in different types of tissues.

We also analyzed other types of cancer such as gastric, lung, colorectal and others (Table 1), however, only rs11614913 polymorphism showed that homozygous CC genotype was significantly high in gastric cancer, however, and did not show any other significant results in other cancer types. Subsequently, CC genotype compared with both CT+TT genotypes, was associated with 50% increased gastric cancer in all subjects (Table 3). Our finding is consistent with previous studies. Guo et al. found C allele and CC genotype increased the risk of digestive system cancers including gastric in Asian population (Guo et al., 2012). Recent studies by Tian et al., and Yuan et al. both found C allele or CC genotype increased the risk of lung cancer in Asian population (Yuan et al., 2013). In detailed clinicopathological analysis of rs11614913, Okubo et al. found CC genotype may be involved in gastric cancer characterization including histological morphology (Okubo et al., 2010). There are some other conflicting reports such as Zhang et al. found C was not a risk allele for gastric, liver or esophageal cancer type in Asian population. The reasons for the conflicting reports are still in question, however, sample size, ethnical background and age can be crucial parameters in determining the risk factors.

The underlying mechanism of how or why rs11614913 CC genotype increases the gastric cancer risk still remains unclear. However, there are some possible explanations; first, C allele carriers have been reported to have oncogenic role and unregulated in breast cancer (Hoffman et al., 2009). Second, miR-196a2's target genes include Homeobox HOX gene family (Types: B8, C8, D8 and A7) and rs11614913 CC genotype may upregulate or cause aberrant HOX gene expression leading to tumorgenesis (Calvo et al., 2000; Miller et al., 2003; Yekta et al., 2004). Third, other known or unknown SNPs or miR-SNPs in similar linkage disequilibrium (LD) value (D') may also be involved in the gastric cancer development process. Fourth, miR-196a2-gene-environment interactions that increases the gastric cancer susceptibility. Finally, possible unknown epigenetic interaction (s) may effect differential methylation on the miR-196a2's target gene(s) consequently leading to gastric cancer development.

There are certain limitations in our study. Our subjects were hospital based consecutive autopsy cases, thus may have selection bias due to chance of admission, cause of death, and autopsy practice, and aged population. Furthermore, the survival bias which may be associated with other disease influencing cancer development in particular genotype and can manipulate subjects' life span. Our collected data did not include cancer progression for different stages of malignancies. It also lacks the life style information, exercising habits and blood samples which can be used for better understanding of cancerous disease states (Oda et al., 2007; Takei et al., 2008).

In conclusion, rs11614913 and rs2910164 miR-SNPs did not illustrate statistically significant overall cancer

risk or association, but rs11614913 may have influence in increasing the gastric cancer risk in Japanese elderly population. Although the statistical significance is evident, it is hard to come to conclusive statement of gastric cancer association with rs11614913 polymorphism, due to low sample number. Our current findings shed some light on how miR-SNPs may influence cancer development and require further investigations with higher sample numbers and functional analysis.

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