Comprehensive Association Analysis of 30 Single Nucleotide Polymorphisms Related with Metabolic Syndrome on Cancer Susceptibility in Japanese Population: A Case – Control Study

Cuneyd Parlayan[1*], Shinobu Ikeda[2], Noriko Sato[2], Motoji Sawabe[3], Masaaki Muramatsu[2] and Tomio Arai[4]

[1]Department of Biomedical Engineering, School of Engineering and Natural Science & Regenerative and Restorative Medicine Research Center (REMER), Istanbul Medipol University, Beykoz, İstanbul/Turkey

[2]Department of Molecular Epidemiology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan

[3]Department of Molecular Pathology, Graduate School of Health Care Sciences, Tokyo Medical and Dental University, Tokyo, Japan

[4]Department of Pathology, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan

[*]**Corresponding author**: Cuneyd Parlayan, Department of Biomedical Engineering, School of Engineering and Natural Science & Regenerative and Restorative Medicine Research Center (REMER), Istanbul Medipol University, Beykoz, 34810, İstanbul/Turkey, Tel: +905312613827, 5312613827; Fax: 5312613827; E-mail: cparlayan@medipol.edu.tr

Citation: Parlayan C, Ikeda S, Sato N, Sawabe M, Muramatsu M, et al. (2018) Comprehensive Association Analysis of 30 Single Nucleotide Polymorphisms Related with Metabolic Syndrome on Cancer Susceptibility in Japanese Population: A Case – Control Study. Health Sci J. Vol. 12 No. 1: 545.

Abstract

Single nucleotide polymorphisms (SNPs) related with metabolic syndrome may influence cancer. Our study aimed to replicate association studies of 30 selected polymorphisms pertaining to 21 genes and cancer phenotypes in Japanese elderly population. All polymorphisms were determined by genotyping on the samples collected from 1503 consecutive autopsy cases registered in the Japanese SNPs for geriatric research (JG-SNP) data base. Current study consisted of 807 (54%) males and 696 (46%) females with a mean age of 80.3 ± 8.9 years. Number of males with cancer was 520 (57%) and females 398 (43%) in total of 918 (61%) cancer present cases and 585 (39%) controls without cancer. Our pathological cancer phenotypes consisted of gastric cancer (n=178), lung cancer (n=163), colorectal (n=137) and others. Our results indicated that rs1800470 (P10L) located TGFβI gene has significant association with overall cancer (CT: CC adjusted odds ratio (OR) 95% confidence interval (95% CI)=1.386 (1.017 – 1.888), p=0.007, TT: CC adjusted OR (95% CI)=1.438 (1.106 – 1.870), p=0.039, CT + TT: CC, adjusted OR (95% CI)=1.421 (1.111 – 1.816), p=0.005), and also with lung cancer (CT + TT: CC adjusted OR (95% CI)=1.562 (1.024 – 2.383), p=0.039) in all subjects. We also found rs2243250 located in IL4 gene is associated with elevated risk of acute leukemia (TT + CT: CC adjusted OR (95% CI)=2.286 (1.243 – 4.206), p = 0.008). Furthermore, rs2077647 on ESRI gene was associated with decreased risk of acute leukemia (GG + AG: AA, adjusted OR (95% CI)=0.516 (0.319 – 0.835), p=0.007). We found that rs1800470, rs2243250 and

rs2077647 polymorphisms may influence cancer in Japanese elderly population. Our study warrants further confirmations with larger samples.

Keywords: Single nucleotide polymorphisms; Cancer risk; Acute leukemia; Lung cancer; Protective allele; TGFβ1; IL4; ESRI

Introduction

Cancer is the most feared and considerably complex malady made of multifaceted combinations of genetic, epigenetic and environmental oncogenic risk factors. According to World Health Organization's (WHO) estimation, there will be more than 13 million individuals affected by cancer in 2030 [1]. The recent studies clearly showed that Metabolic Syndrome (MetS) can be a major player creating carcinogenic risks [2-4]. Epidemiological studies indicated that diabetes obesity, dyslipidemia, hyperglycemia, inflammation, hyeperinsulinemia and other metabolic syndrome related diseases lead to cancer development [5,6]. Furthermore, there are number of evidences that specific cancer phenotypes including gastric cancer, prostate cancer, hepatocellular carcinoma, colorectal cancer, pancreatic cancer, and others demonstrated strong associations with metabolic syndrome [7-13].

Single nucleotide polymorphisms (SNPs) are the most common genetic modifications found in human genome. Recent genome wide association studies (GWAS) and candidate gene approach studies both indicated these genetic alterations, SNPs, influence cancer development [14,15]. The genetic modifications on metabolic syndrome related genes

such as rs2243250 on Interleukin type 4 (IL4), rs2077647 on estrogen receptor 1 (ESR1) and rs1800470 (P10L) found in transforming growth factor beta type I (TGFβI) genes have been reported to influence cancer [16-18]. The rs2243250 (C-524T) located in the IL4 gene has been reported to be associated with diabetes [19]. The other polymorphism rs2077647 is associated with obesity, and also rs1800470 (P10L) polymorphism located in the intronic region causing Proline (CCG) to Leucine (CTG) functional change at codon 10 reported to have an association with metabolic syndrome [20,21]. Both IL4 and TGFβI genes possess both pro/anti-inflammatory cytokines properties which are known to influence cancer development [22,23]. The rs1800470 polymorphism has been reported to effect the TGFβI gene expression level, and found to be associated with higher TGFβI serum level triggering the angiogenesis which often leads to cancer [24,25]. The rs2243250 (C-524T) polymorphism is located in the promoter region of IL4 which is a major cytokine involved in naive CD4 polarization, consequently binds to its receptor (IL4R) to cascade cytokine signalling that is a key part of the immune regulation, therefore aberrant expression of IL4 may affect the serum level of IL4 cytokine leading the cancer development [26-28]. On the other hand, rs2077647 (S10S) located on ESR1 gene causes synonymous codon alteration of serine (TCT) to serine (TCC) at the codon 10 position. Estrogen receptor 1 (ESR1) gene is not only involved in hormonal regulation, diabetes, insulin sensitivity and hypertension, but also endometrial carcinoma, cervical cancer, breast cancer, prostate cancer, and non-small cell lung cancer [29-37]. Moreover, rs1800470 (C29T) polymorphism interrupts the TGFβ signalling pathway disabling the tumor suppressing function, thus leads to oncogenesis including colorectal cancer, lung cancer, prostate pancreatic cancer, liver cancer and others [38-48]. Despite the numerous research efforts, the epidemiological association between metabolic syndrome and cancer development is still unclear and requires more scientific research.

Our objective was to investigate the metabolic syndrome associated SNPs illustrating clinical significance, whether they have influence on cancer in Japanese elderly population. In our study we examined 30 SNPs occurring on 21 different genes with varying functions including cytokines, enzymes, receptors and others that are related to metabolic syndrome but also have links or associations with cancer development. We focused on three SNPs, rs2243250 (C-524T), rs2077647 (S10S) and rs1800470 (P10L) which showed significant association(s) with overall cancer and specific cancer phenotypes.

Materials and Methods

1503 consecutive autopsy cases were analyzed, and mean age was 80.3 ± 8.9 years for all subjects. Our study included 807 males and 696 females. "C (+): cancer present" group is defined as any patient with at least one type of malignancy, and "C (-): cancer absent" group did not possess any type of malignancies. Cancer phenotypes were registered in the internet database of Japanese single-nucleotide

polymorphisms (SNPs) for geriatric research (JG-SNP) were matched with our genotyping results for all 30 SNPs [49].

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 previously reported, therefore our autopsy samples could be used [50,51]. All samples were pathologically reviewed and verified. 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 also "drinker" versus "non-drinker" by the alcohol consumption history of subjects [52-60].

Written informed consent was obtained from the bereaved family of each of the patients prior to the autopsy examination. The use of autopsy materials for medical education and research was permitted by the Act of Postmortem Examinations of Japan. This study was approved by the Tokyo Medical and Dental University Ethics Committee (approval No. 2009-19-4) and the Tokyo Metropolitan Geriatric Hospital Ethics Committee (approval No.230405).

Genotyping

Genomic DNA was extracted from renal cortex of subjects by conventional procedures. Genotyping was done by TaqMan assay (Applied BioSystems) according to the protocols described by the manufacturers. PCR was performed in 5 µl reaction mix including 10 ng genomic DNA. PCR was conducted with the Light Cycler 480 instrument (Roche Diagnostics). Allelic discrimination of each polymorphisms were determined with ≥ 98% accuracy criteria. The genotyping results were determined Light Cycler Genotyping software (Roche Diagnostics). Genotyping accuracy was verified by randomly selected samples [61-65].

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. In genetic models we used following genotypic groupings; additive model (AA, Aa, aa) "A"

is the dominant allele and "a" is the recessive allele. Dominant model was defined as (AA vs. Aa + aa), recessive model (aa vs. Aa + AA) and co-dominant model (AA vs. aa) [66-70].

Results

General characteristics of subjects

Demographics of our study are shown in **Table 1**. Our current study consisted of 1503 consecutive autopsy subjects including 918 (61%) cancer present and 585 (39%) cancer absent control subjects. Mean age was 80.3 ± 8.9 and also significantly different between cancer present and absent subjects (p<0.001). Males possessing cancer were 520 (57%) and females 398 (43%), gender parameter was found to be statistically significant between cancer present and absent subjects (p=0.004). Smokers, 460 (50%), were higher than non-smokers, 408 (44%) in cancer present subjects. Drinker subjects, 324 (35%) were less than non-drinker subjects, 533 (58%) in cancer bearing subjects. No statistical significance was found for both smoking (p=0.134) and drinking status (p=0.075). Cancer bearing subjects were further stratified; single cancer bearers were 689 (46%), and more than two cancer types' bearers were 229 (15%). Our pathologically verified cancer phenotypes included gastric cancer (n=178), lung cancer (n=163), colorectal (n=137) and others **(Table 1)**.

Table 1 Demographic variables of examined subjects.

Characteristics	Total, n (%) (n = 1503)	C (-)¹, n (%) (n = 585)	C (+)², n (%) (n = 918)	p*
Age at death, (years)	80.3 ± 8.9	81.3 ± 9.2	79.6 ± 8.6	<0.001
Gender, n (%)				
Male	807 (54)	287 (49)	520 (57)	
Female	696 (46)	298 (51)	398 (43)	0.004
Smoking status, n (%)				
Smoker	717 (48)	257 (44)	460 (50)	
Non-smoker	677 (45)	269 (46)	408 (44)	0.134
Missing	109 (7)	59 (10)	50 (6)	
Alcohol consumption, n (%)				
Drinker	499 (33)	175 (30)	324 (35)	
Non-drinker	887 (59)	354 (60)	533 (58)	
Missing	117 (8)	56 (10)	61 (7)	0.075
Cancer, n (%)				
0		585 (39)		
1			689 (46)	
≥ 2			229 (15)	
Cancer sites3, n				
Gastric			178	
Lung			163	
Colorectal			137	
Prostate			94	
Acute Leukemia			78	

±Standard Deviation, *p values for "age" was calculated by ANOVA and others were calculated by 2- sided Fisher's exact test, "C (-)" group includes only "cancer absent" subjects, "C (+)" group indicates "cancer present" patients with at least one type of cancer, Representative list of cancer sites, "( )" indicates "percentage" value in respective group.

Characteristics of examined polymorphisms

The general characteristics of polymorphisms are shown in **Table 2**. We analyzed 30 SNPs formerly found to have associations with metabolic syndrome or metabolic diseases including diabetes, myocardial infraction, arterial calcifications, cholestasis and others. Among these 30 polymorphisms, rs2077547, rs2243250 and rs1800470 SNPs illustrated significant results, thus we further focused on these three polymorphisms **(Table 2)**. The rs2077647 (A/G) polymorphism located in the 6th chromosome which causes synonymous change of Serine (S) to Serine (S) in the estrogen receptor 1 (ESR1) gene. The ESR1 (Gene ID: 2099) receptor / enzyme gene has clinical significance mainly in HDL metabolism and atherosclerosis, and is registered in Online Mendelian Inheritance in Man (OMIM, registry number: 133430). ESR1 has been reported to have association with breast cancer, endometrial cancer and involved in sexual development. The other SNP rs2243250 (C/T) polymorphism is located in the 5th chromosome and associated with Interleukin 4 (IL4) cytokine (Gene ID: 3565). IL4 is produced by activated T cells and key functions in immune system. IL4 possesses clinical significance (OMIM registry number: 147780). The rs1800470 is located on transforming factor beta type 1 (TGFβI) gene (Gene ID: 7040). In rs1800470 polymorphism C to T nucleotide modification causes Proline (P) codon change to Leucine (L) at the 10th codon (P10L). TGFβI gene has also clinical importance (OMIM registry number: 190180) and reported to have involvements in obesity and cancer [71-80].

Allelic and genotypic distributions of examined polymorphisms

The allelic and genotypic distributions of selected polymorphisms, rs2077647, rs2243250 and rs1800470, in all subjects are shown in **Table 3**, and the results of the entire polymorphisms. The allelic distribution of rs2077647's A: G was 1733 (58%): 1251 (42%), genotypic distribution of AA: AG: GG was 516 (35%): 701 (47%): 275 (18%) and observed minor allele frequency was 0.42. The allelic distribution of rs2243230's T: C was 2036 (68%): 948 (32%) genotypic

distribution of TT: CT: CC was 696 (47%): 644 (43%): 152 (10%) and observed minor allele frequency was 0.32. Finally, the allelic distribution of rs1800470's C: T was 1535 (52%): 1429 (48%), genotypic distribution of CC: CT: TT was 401 (27%): 733 (50%): 348 (23%) and observed minor allele frequency was 0.48. Due to stringent genotyping criteria some samples were either excluded or not successfully genotyped, these missing samples were as following; rs2077647 (n=11), rs2243250 (n=11) and rs1800470 (n=21). All of these three polymorphisms were consistent with Hardy-Weinberg Equilibrium (p=0.174, p=0.866 and p=0.713) respectively **(Table 3)**. All of our genotyped polymorphisms did not show any deviation from Hardy-Weinberg Equilibrium.
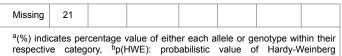
Table 2 General characteristics of rs2077647, rs2243250 and rs1800470 polymorphisms.

SNP	Allelic Change	Strand	Location (Ch:Loc)[1]	Target Gene	Gene ID[2]	Functional change	Functions of genes	Key features of genes[2]	OMIM[3]	MetS.[4]	Can.[5]
rs2077647	A/G	Reverse strand	6:152129077	Estrogen receptor (ESRI)	2099	(S10S)[*]	Receptor/ Enzyme	Estrogen receptor, breast cancer, endometrial cancer, sexual development	133430	70	71
rs2243250	C/T	Forward strand	5:132009154	Interleukin 4 (IL4)	3565		Cytokine/ hormone/ growth factor	Is a cytokine produced by activated T cells. Involved in immune regulatory signalling	147780	72	73
rs1800470	C/T	Reverse strand	19:41858921	Transforming growth factor, beta type I (TGFβI)	7040	(P10L)[*]	Cytokine/ hormone/ growth factor	Involved in proliferation, differentiation, adhesion, migration and other types of cellular functions.	190180	74	75

[1](Ch: Loc) indicates Chromosome: Location, [2]All information was extracted from dbSNP138 database, GRCh38 version (http://ncbi.nlm.gov/), [3]OMIM: Online Mendelian Inheritance in Man (http://omim.org/), [4]MetS. : Metabolic syndrome related publication, [5]Can.: Cancer related publication, [*]P: Proline, L: Luecine, S: Serine

Table 3 Allelic and genotypic distribution of rs2077647, rs2243250 and rs1800470 polymorphisms in all subjects.

SNP	Alleles		Genotypes			p(HWE)[b]	MAF
	A	G	AA	AG	GG		
rs2077647	1733	1251	516	701	275		
(%)[a]	-58	-42	-35	-47	-18	0.174	0.42
Missing	11						
	T	C	TT	CT	CC		
rs2243250	2036	948	696	644	152		
(%)	-68	-32	-47	-43	-10	0.866	0.32
Missing	11						
	C	T	CC	CT	TT		
rs1800470	1535	1429	401	733	348		
(%)	-52	-48	-27	-50	-23	0.713	0.48

Missing	21					

[a](%) indicates percentage value of either each allele or genotype within their respective category, [b]p(HWE): probabilistic value of Hardy-Weinberg equilibrium.

Genotypic distribution of examined polymorphisms in different inheritance models

We analyzed all SNPs with additive, dominant, recessive and co-dominant genetic models to find their relative effect in overall cancer in all subjects. The results of rs2077647, rs2243250 and rs1800470 is shown in **Table 4**, and the rest of the results. The rs2077647 polymorphism found in ESR1 gene showed significantly high frequency of AA genotype in cancer present subjects [81-86]. The additive model AA: AG: GG genotypic distribution in cancer present subjects was 335 (37%): 412 (45%): 167 (18%) and did not show significance (p=0.092). Dominant model AA: GG + AG genotypic distribution in cancer bearing subjects was 335 (37%): 579 (63%), and demonstrated statistical significance (p=0.039). Recessive model GG: AA + AG and co - dominant model AA: GG genotypic distributions in cancer present subjects was 167 (18%): 747 (82%) and 335 (67%): 167 (33%). Both recessive

and co-dominant models did not show statistical significance between cancer present and absent subjects (p=0.837 and p=0.246) respectively **(Table 4)**.

Table 4 Genotypic distribution of rs2077647, rs2243250 and rs1800470 polymorphisms in four different models in overall cancer.

SNP (Gene)	Additive	C -a n (%)	C +a n (%)	Dominant	C - n (%)	C + n (%)	Recessive	C - n (%)	C + n (%)	Co-dominant	C - n (%)	C + n (%)
rs2077647 (ESRI) p b	AA	181 (31)	335 (37)	AA	181 (31)	335 (37)	GG	108 (19)	167 (18)	AA	181 (63)	335 (67)
	AG	289 (50)	412 (45)									
	GG	108 (19)	167 (18) 0.092	AG + GG	397 (68)	579 (63) 0.039	AG + AA	470 (81)	747 (82) 0.837	GG	108 (37)	167 (33) 0.246
rs2243250 (IL4) p b	TT	272 (47)	424 (47)	TT	272 (47)	424 (47)	CC	47 (8)	105 (12)	TT	272 (85)	424 (80)
	CT	261 (45)	383 (42)									
	CC	47 (8)	105 (11)	CT + CC	308 (53)	488 (53)	CT + TT	533 (92)	807 (88)	CC	47 (15)	105 (20)
			0.091			0.915			0.035			0.065
rs1800470 (TGFβI) p b	CC	177 (31)	224 (25)	CC	177 (31)	224 (25)	TT	126 (22)	222 (24)	CC	177 (58)	224 (50)
	CT	273 (48)	460 (51)									
	TT	126 (21)	222 (24)	CT + TT	399 (69)	682 (75)	CT + CC	450 (78)	684 (76)	TT	126 (42)	222 (50)
			0.038			0.012			0.258			0.03

aC -/+ indicates Cancer absent/present subjects, bp value for additive model was calculated by Chi-square and the other models were calculated by 2-sided Fisher exact test, ( ) indicates the percentage value of distribution in relevant polymorphisms

Genotypic distributions of rs2243250 located in IL4 gene in the cancer present subjects were as following; additive model TT: CT: CC was 424 (47%): 383 (42%): 105 (11%) and was not found to be significant (p=0.091), dominant model TT: CT + CC was 424 (47%): 488 (53%) and was not significant (p=0.915), recessive model CC: CT + TT was 105 (12%): 807 (88%) and CC genotype was significantly high in cancer present subjects (p=0.035), co-dominant model TT: CC was 424 (80%): 105 (20%) and was not significant (p=0.065), **(Table 4)**.

The rs1800470 located in TGFβI gene polymorphism's genotypic distributions of four different models in cancer bearing subjects were as following; additive CC: CT: TT was 224 (25%): 460 (51%): 222 (24%) and TT genotype was significantly higher in cancer present subjects (p=0.038), dominant model CC: CT + TT was 224 (25%): 682 (75%) and T allele was significantly high in cancer present subjects (p=0.012), recessive model TT: CT + CC was 222 (24%): 684 (76%) and no statistical significance was observed (p=0.258), co-dominant model CC: TT was 224 (50%): 222 (50%) and TT genotype was found to be statistically higher in cancer bearing subjects (p=0.030), **(Table 4)**.

Association analysis of rs2077647, rs2243250 and rs1800470 polymorphisms with overall cancer

To find out the possible association(s) of overall cancer risk with rs2077647, rs2243250 and rs1800470 polymorphisms, logistic regression analysis was applied and the results is shown in **Table 5**. The rs2077647 polymorphism case, AG, GG and GG + AG genotypes were tested with respect to major genotype AA, there were statistical significance in crude analysis AG: AA (p=0.031) and GG + AG: AA (p=0.032), however after adjustment with age, gender, smoking and drinking status, all examined genotypes including the GG + AG combination lost their statistical significance; AG: AA (p=0.051), GG: AA (p=0.261), GG + AG: AA (p=0.055). Therefore, there was no statistically reliable association between rs2077467 and overall cancer risk [87-93].

In rs2243250 polymorphism CC and CT was tested against to major genotype TT. There was no significant results in both in crude CT: TT (p=0.098) and CC: TT (p=0.452) and adjusted regression analysis CT: TT (p=0.107) and CC: TT (p=454). Furthermore, CC: CT + TT was not significant in crude (p=0.051) and adjusted (p=0.057) analysis. Therefore, no

statistical association was found between rs2243250 polymorphism and overall cancer risk [94-97].

The rs1800470 showed following results in the comparison of CT, TT and CT + TT with respect to major genotype CC. All three genotypic comparisons were statistically significant in crude results; CT: CC OR (95% CI)=1.402 (1.081 – 1.818), p=0.038. TT: CC OR (95% CI)=1.383 (1.018 – 1.879), p=0.011. CT + TT: CC OR (95% CI)=1.396 (1.094 – 1.780), p=0.007. In adjusted results the significant associations were also kept. CT: CC adjusted OR (95% CI)=1.386 (1.017 – 1.888), p=0.007. TT: CC adjusted OR (95% CI)=1.438 (1.106 – 1.870), p=0.039 [98-100].

CT + TT: CC adjusted OR (95% CI)=1.421 (1.111 – 1.816), p=0.005. Thus, rs1800470 polymorphism T allele is associated with 42% higher overall cancer risk in our samples.

Table 5 Association analysis of rs2077647, rs2243250 and rs1800470 polymorphisms with overall cancer in all subjects.

SNP (Gene)	Genotype	C -ᵃ n (%)	C +ᵃ n (%)	Crude OR (95% CI)	p*	Adjusted ORᵇ (95% CI)	p*
rs2077647 (ESRI)	AA	181 (31)	335 (37)	1.000 (reference)		1.000 (reference)	
	AG	289 (50)	412 (45)	0.761 (0.594 - 0.975)	0.031	0.780 (0.607 - 1.001)	0.051
	GG	108 (19)	167 (18)	0.812 (0.591 - 1.115)	0.198	0.832 (0.604 -1.147)	0.261
	AG + GG	397 (68)	579 (63)	0.775 (0.614 – 0.978)	0.032	0.794 (0.627 - 1.005)	0.055
rs2243250 (IL4)	TT	272 (47)	424 (47)	1.000 (reference)		1.000 (reference)	
	CT	261 (45)	383 (42)	1.393 (0.941 - 2. 062)	0.098	1.385 (0.932 - 2.058)	0.107
	CC	47 (8)	105 (11)	0.915 (0.727 - 1.152)	0.452	0.915 (0.725 - 1.155)	0.454
	CT + TT	533 (92)	807 (88)	1.000 (reference)		1.000 (reference)	
	CC	47 (8)	105 (12)	1.451 (0.998 - 2.118)	0.051	1.446 (0.989 - 2. 113)	0.057
rs1800470 (TGFβI)	CC	177 (31)	224 (25)	1.000 (reference)		1.000 (reference)	
	CT	273 (48)	460 (51)	1.402 (1.081 - 1.818)	0.038	1.386 (1.017- 1.888)	0.007
	TT	126 (21)	222 (24)	1.383 (1.018 - 1.879)	0.011	1.438 (1.106 - 1.870)	0.039
	CT + TT	399 (69)	682 (75)	1.396 (1.094 - 1.780)	0.007	1.421 (1.111-1.816)	0.005

ᵃC -/+ indicates Cancer absent/present subjects, ᵇThe odd ratio was adjusted by "age, gender, smoking and drinking status", *p value was calculated by binary logistic regression analysis, ( ) indicates the percentage value of distribution in relevant polymorphisms

Genotypic distribution of rs2077647, rs2243250 and rs1800470 polymorphisms in specific cancer phenotypes

We continued to investigate the genotypic frequencies in specific cancer phenotypes consisting of gastric cancer, lung cancer, colorectal cancer and acute leukemia in additive, dominant, recessive and co-dominant genetic models for all subjects and the results are shown in **Table 6**. Both rs2077647 and rs22432250 polymorphisms did not show any statistical significance in the specific genotypic distribution in each additive, dominant, recessive and co-dominant genetic models for gastric cancer, lung cancer and colorectal cancer **(Table 6)**. On the other hand, both rs2077647 and 22432250 polymorphisms had significant results in acute leukemia. The AA genotype frequencies in rs2077647 polymorphism were 37 (7%) in additive, recessive and co-dominant models and was found to be significantly higher in acute leukemia (p=0.023, p=0.014 and p=0.015 respectively) in all subjects. Additionally, CC genotype of rs2243250 polymorphism was 16 (10%) in additive, dominant and co-dominant models and had significantly high frequency (p=0.008, p=0.006 and p=0.010 respectively) in all subjects.

Although there were significant results in both rs2077467 and rs2243250 polymorphisms, one cautiously ought to consider the low sample number of acute leukemia (n=78) **(Table 7)**.

Table 6 Genotypic distribution of rs2077647, rs2243250 and rs1800470 polymorphisms in four different models in specific cancers.

SNP (Gene)	Cancer type	Additive				Dominant			Recessive			Co-dominant		
		AA	AG	GG	pa	AA	AG + GG	pb	GG	AG + AA	pb	AA	GG	pb

Gene	Cancer type				p			p			p			p
rs2077647 (ESRI)	Gastric	67 (13)	81 (12)	29 (11)	0.567	67 (13)	110 (11)	0.355	29 (11)	148 (12)	0.536	67 (13)	29 (11)	0.361
	Lung	60 (12)	67 (10)	36 (13)	0.241	60 (12)	103 (11)	0.542	36 (13)	127 (11)	0.201	60 (12)	36 (13)	0.568
	Colorectal	47 (9)	63 (9)	26 (10)	0.976	47 (9)	89 (9)	1	26 (10)	110 (9)	0.817	47 (9)	26 (10)	0.898
	Acute Leukemia	37 (7)	32 (5)	8 (3)	0.023	37 (7)	40 (4)	0.014	8 (3)	69 (6)	0.069	37 (7)	8 (3)	0.015
		TT	CT	CC		TT	CT + TT		CC	CT + TT		TT	CC	
rs2243250 (IL4)	Gastric	81 (12)	85 (13)	12 (8)	0.184	81 (12)	97 (12)	0.81	12 (8)	166 (12)	0.114	81 (12)	12 (8)	0.2
	Lung	72 (10)	79 (12)	12 (8)	0.244	72 (10)	91 (11)	0.561	12 (8)	151 (11)	0.271	72 (10)	12 (8)	0.454
	Colorectal	62 (9)	53 (8)	19 (12)	0.253	62 (9)	72 (9)	1	19 (12)	115 (9)	0.132	62 (9)	19 (12)	0.173
	Acute Leukemia	32 (5)	30 (5)	16 (10)	0.008	32 (5)	46 (6)	0.352	16 (10)	62 (5)	0.006	32 (5)	16 (10)	0.01
		CC	CT	TT		CC	CT + TT		TT	CT + CC		CC	TT	
rs1800470 (TGFβI)	Gastric	43 (11)	82 (11)	51 (15)	0.183	43 (11)	133 (12)	0.418	51 (15)	125 (11)	0.072	43 (11)	51 (15)	0.122
	Lung	32 (8)	97 (13)	32 (9)	0.013	32 (8)	129 (12)	0.031	32 (9)	129 (11)	0.279	32 (8)	32 (9)	0.601
	Colorectal	36 (9)	68 (9)	30 (9)	0.936	36 (9)	98 (9)	1	30 (9)	104 (9)	0.831	36 (9)	30 (9)	0.898
	Acute Leukemia	16 (4)	35 (5)	24 (7)	0.169	16 (4)	59 (6)	0.287	24 (7)	51 (5)	0.092	16 (7)	24 (7)	0.102

[a]p value is calculated by Chi-square, [b]p value is calculated by 2-sided Fisher exact test, ( ) indicates the percentage value of distribution in relevant polymorphisms

Association analysis of rs2077647, rs2243250 and rs1800470 polymorphisms with specific cancer phenotypes

We further investigated the possible association(s) of the selected polymorphisms, rs2077647, rs2243250 and rs1800470, with specific cancer phenotypes, and significant results are shown in **Table 7**. All 30 SNPs were screened through for association(s) of specific cancer phenotypes (data not shown) however, only three SNPs illustrated significant association with acute leukemia and lung cancer **(Table 7)**. The rs2077647 polymorphism located on ESRI indicated that AG + GG: AA comparison both in crude analysis OR (95% CI)=0.493 (0.307 – 0.792) and adjusted analysis Adjusted OR (95% CI)=0.516 (0. 319–0. 835) found to be statically associated (p=0.003 and p=0.007 respectively) with reduced acute leukemia. Thus, G allele had a protective feature and may reduce the acute leukemia risk up to 48% in all subjects **(Table 7)**.

Table 7 Association analysis of rs2077647, rs2243250 and rs1800470 polymorphisms with acute leukemia and lung cancer in all subjects.

SNP (Gene)	Cancer type	Genotype	C +[a] n (%)	Crude OR (95% CI)	p*	Adjusted OR[b] (95% CI)	p*
		AA	37 (7)	1.000 (reference)		1.000 (reference)	
rs2077647 (ESRI)	Acute Leukemia	AG + GG	40 (4)	0.493 (0.307 -0.792)	0.003	0.516 (0.319 -0.835)	0.007
		CT + TT	162 (5)	1.000 (reference)		1.000 (reference)	
rs2243250 (IL4)	Acute Leukemia	CC	6 (10)	2.322 (1.280 - 4.213)	0.006	2.286 (1.243 - 4.206)	0.008
		CC	32 (8)	1.000 (reference)		1.000 (reference)	

| rs1800470 (TGFβI) | Lung | CT + TT | 129 (12) | 1.560 (1.031 - 2.359) | 0.035 | 1.562 (1.024 - 2.383) | 0.039 |

aC + indicates Cancer present subjects, bThe odd ratio was adjusted by "age, gender, smoking and drinking status", *p value was calculated by binary logistic regression analysis, ( ) indicates the percentage value of distribution in relevant polymorphisms

The rs2243250 polymorphism located in IL4 showed that CC: CT + TT ratio both in crude analysis OR (95% CI)=2.322 91.280 – 4.213) and adjusted analysis adjusted OR (95% CI)=2.286 (1.243 – 4.206) was found to be significantly associated (p=0.006 and p=0.008 respectively) with the increased risk of acute leukemia **(Table 7)**. Although, CC genotype significantly increased the risk of acute leukemia, the low number of acute leukemia and its effect ought to be considered to make a concrete conclusion.

Finally, rs1800470 polymorphism located on TGFβI demonstrated that CT + TT: CC genotypic comparison both in crude analysis OR (95% CI)=1.560 (1.031 – 2.359) and adjusted analysis adjusted OR (95% CI)=1.562 (1.024–2.382) were significantly (p=0.035 and p=0.039 respectively) associated with increased lung cancer. As a result, T carriers increased 56% lung cancer occurrence in all subjects **(Table 7)**.

Discussion

In our current research, we hypothesized if the metabolic syndrome related SNPs may influence primarily overall cancer risk and secondarily the risk of specific cancer phenotype(s) in elderly Japanese population. Our study population consisted of 1503 individuals in which 61% had cancer, with mean age of over 80 years old **(Table 1)**. 30 SNPs found in 21 encoding genes having clinical significance and associated with metabolic syndrome were analyzed **(Table 2)**. Among all 30 genotyped SNPs, we focused on three polymorphisms, rs2077547, rs2243250, rs1800470 polymorphisms **(Table 3)** and examined in additive, dominant, recessive and co-dominant genetic models **(Table 4)**. Then, these three polymorphisms were investigated for other specific cancer phenotypes including gastric cancer, lung cancer, colorectal cancer, and acute leukemia **(Table 6)**, other cancer phenotypes such as liver, pancreas etc. were also analyzed but no significance was detected (data not shown). Finally, the association(s) between rs2077547, rs2243250, and rs1800470 polymorphisms and overall cancer and selected specific cancer phenotypes including acute leukemia and lung cancers were analyzed **(Tables 5 and 7)**.

We found that G allele in rs2077647 (S10S) polymorphism had significantly higher frequency in control group in dominant model **(Table 4)**. Furthermore, there was no association between rs2077647 and overall cancer risk **(Table 5)**. Our findings indicated that G allele was significantly higher in control subjects in both additive and dominant model for acute leukemia **(Table 6)**. As we further investigated the relationship between rs2077647 G allele carriers with acute leukemia, G allele appeared to be associated with reduced level of acute leukemia, thus had a "protective" effect against acute leukemia in all subjects **(Table 7)**. Our findings are consistent with previous reports; Nicolaiew et al., Wang et al.

and Chae et al. found no significant association between ESR1 related polymorphisms such as rs2077647 with cancer more specifically prostate cancer [100-102]. Fernandez et al. found similar result that rs2077647 (S10S) polymorphism had a protective function against breast cancer in Spanish population [103] and a similar report from a Taiwanese group, Hsiao et al. S10S variant showed protective property [104]. The underlying reason(s) of this observed protective property of G allele against acute leukemia is still unclear, however, rs2077647 may have unknown tumour suppressor effect either by itself or with other SNPs having high linkage disequilibrium or may interact with other genetics factors to supress the oncogenic formation. Also, there are some reports asserted that consumption of phytoestrogens or isoflavone for instance soy beans, a dominant dietary intake in Japan, has protective effect against androgenic pathway related cancer types such as prostate cancer [105]. Thereby, G carriers may initiate analogous pathway(s) to reduce the risk of tumour development.

The other important finding was regarding to rs2243250 (C-534T) polymorphism found on IL4 gene. C allele was minor allele **(Table 3)** and found significantly frequent in case subjects in recessive model **(Table 4)**. However, no significant association was found with overall cancer risk **(Table 5)**. As we further checked the possibilities of rs2243250 (C-534T) polymorphism for other specific cancer phenotypes, C allele appeared to be the risk allele in additive and dominant models of acute leukemia **(Table 6)**, consequently the C carriers found to be significantly associated with acute leukemia **(Table 7)**. Our results are aligned with the previous reports such, De Guia et al. found rs2243250 (C-534T) polymorphism, C carriers had higher serum level of IgE leading to higher chance of getting allergy, thus leading to cancer [106]. There are other reports indicated that T to C allelic alteration in rs2243250 may lead to aberrant T-helper 2 cells, then C or CC carriers have higher tendency to have cancers such oral, head and neck and leukemia [107,108]. Moreover, there are some conflicting results reported by other research groups indicating that T allele had significant oncogenic effect [27]. Additionally, Zhenzhen et al. found CC homozygote associated with increased renal cancer, but decreased oral cancer risk in Asian population [26]. The underlying reasons or mechanisms of the oncogenic effect of rs2243250 (C-534T) polymorphism remains largely unknown, however some possible explanations can be asserted; first, the aberrant T-helper type 2 cells may disturb the immune surveillance causing less defensive immune system against either cancer via allergies or directly cancer, more specifically leukemia or haematological malignancies [109]. Second, IL-4 is also a soluble pro-inflammatory cytokine, thus the dysfunctional cytokine pathway (s) due to polymorphic allele alteration may lead to immunosuppressive state coupling with tumour initiating factors, and thereby oncogenesis occurs [110]. Third, there may be ethnical

differences as in the Zhenzhen et al. report in which Caucasians and Asian possess differential risk factors for different types of cancers caused by aberrant IL-4 gene [26].

Our final and most critical results are pertaining to rs1800470 (P10L) polymorphism. There is a functional change at the 10th codon on transforming growth factor beta type I (TGFβI) gene from proline (P) to leucine (L) amino acids located on chromosome 19. T allele was minor allele **(Table 3)**. T allele and TT homozygote genotype showed significantly high presence in additive, dominant and co-dominant models in overall cancer risk **(Table 4)**. Furthermore, T allele carriers had significant association with overall cancer risk in Japanese elderly population **(Table 5)**. T allele was also found in high frequency in lung cancer case both in additive and dominant models **(Table 6)**. Finally, the T carriers found to be associated with 56% higher lung cancer risk **(Table 7)**. Our results indicated that 10L (Leucine) substitution had significant association with overall and lung cancer risk.

There are numerous reports about TGFβI, which is most abundant form of TGFβ gene family, associated polymorphisms and TGFβ signalling pathway and their oncogenic effects in the literature [23-25,40-48,111-122]. The rs1800470 polymorphism causing cysteine to thymine (C→T) at 29th nucleotide resulting the change of proline (CCG) amino acid to leucine (CTG) at 10th codon (P10L). T allele carriers were previously reported to have higher plasma concentration of TGFβI [42,111]. The Our results were consistent with previous reports such as Pooja et al. found T allele carriers had higher risk and significant association with breast cancer in Indian population [112]. Falleti et al. found TT genotype carriers had significantly higher in hepatocellular carcinoma [45]. Three Japanese groups also confirmed that T or TT carriers had high risk of developing prostate cancer, gastric cancer and hepatocellular carcinoma [113-115]. There are conflicting results, despite of the several meta-analysis using considerably higher number of samples [18]. Zhang et al. found in their meta-analysis that rs1800469 (-509°C/T), which is in considerably similar linkage disequilibrium (LD) block with rs1800470, is associated with upper digestive tract cancer but not rs1800470 polymorphism [116]. One study conducted in Spain asserted that pro-and anti-inflammatory cytokine gene polymorphisms is not linked with gastric cancer [117]. Other groups also indicated rs1800470 T carriers have either no association or association with decreased level of prostate, colorectal and gastric cancer [39,42,118]. It is still largely unknown why there are several conflicting reports, however, there are possible reasons that may be asserted; firstly, the ethnical back ground is a key factor of these different results [18]. Secondly, type of the study i.e., hospital based, multicenter based, population based etc. may increase the selection bias, heterogeneity of the sampling, testing errors, or false positives [119]. Thirdly, genetically related diseases some genotypes may have "low-penetrance" effect, thus either C carriers (Proline) or T carriers (Leucine) may have low penetrance which may create differing results in cancer phenotypes [120]. Fourthly, the unique dual character of TGFβI gene might have been the reason. TGFβI can either suppress or cause oncogenesis, thereby T carriers could have oncogenic

property of TGFβI gene resulting overall cancer risk by creating aberrant TGFβ pathway signalling specifically effecting either SMAD protein or receptor (TGFβRI/II) binding [23,25,46,48].

Among other results pertaining to rs1804700 polymorphism on TGFβI gene, T carrier was also associated with elevated lung cancer **(Table 7)**. Our finding was supported by two studies done in Asian population; both studies indicated T or TT carriers have elevated risk of lung cancer as compared with other genotypes or alleles [40,41]. Although the reasons behind lung cancer risk caused by rs1800470 is still world-wide undergoing research topic, there are possible reasons; firstly the genetic alteration may initiate the epithelial-mesenchymal transition (EMT) like growth which often leads to cancer, besides the lung tissues is known for the vulnerable nature for the invasive epithelial growth [121-123]. Secondly, canonical SMAD 2/3 then SMAD4 proteins binding(s) may have been hindered then it leads to either perturbed or loss of TGFβ signalling causing higher tendency of metastatic invasion or inhibition of tumor supressing function of TGFβ protein families enabling cancer development [23-25]. Finally, rs1800470 may interact with other unknown SNP(s) or gene(s) to create genetic or epigenetic oncogenic risk factor(s) coupled with the aberrant TGFβ signalling.

Another interesting findings were rs1042522 polymorphism located on Tumour protein p53 (TP53) gene involved in cell cycle arrest and DNA repair, and rs1799964 polymorphism occurring on Tumor Necrosis Factor (TNF) gene involved in proliferation and apoptosis, did not show any significant result, despite the fact, both genes and SNPs were well-known for their oncogenic affects [124,125]. Counter intuitively, genetic modifications on both TNF and TP53 genes did not show any significant association(s) or cancer risk in our current study.

Our study had some limitations; our samples were hospital based consecutive autopsy cases, this fact may cause selection bias due to chance of admission, age bias having elderly individuals, survival bias associated with other disease(s) causing cancer development in particular genotype. Our data lacked the cancer progression which may have provided some critical depth in our results. Furthermore, the life style information for instance exercising and dietary habits were not included in our data. There was also no data on hematological properties i.e., blood proteins, lipid concentration etc. which could provide important insights for better understanding of cancer development [51].

Conclusion

Our results indicated that rs2077647 (S10S) and rs2243250 (C-534T) polymorphisms did not show overall cancer risk, but rs2077647 may reduce and rs2243250 may increase acute leukemia risk. On the other hand, rs1800470 (P10L) polymorphism may pose overall cancer and lung cancer risk in Japanese elderly population. Although, these three SNPs showed significant results, the concrete conclusions cannot be made due to the low sample numbers. Our findings shed some light on the understanding of common genetic modifications

such as rs2077647, rs2243250 and rs1800470 polymorphisms associated with metabolic syndrome may influence cancer development. Further studies accompanied with functional analysis with larger samples are warranted to verify our current results.

Authors' Contributions

CP conducted genotyping analysis, statistical analysis, and drafted the manuscript. MS and TA provided pathological & clinical data, and also provided advice on the manuscript. SI assisted on data retrieval and gave genotyping analysis. NS provided critical advice. MM proofread, edited, and provided critical comments and guidance.

Acknowledgment

References

1. http://www.who.int/mediacentre/factsheets/fs297/en/index

2. Cowey S, Hardy RW (2006) The metabolic syndrome: A high-risk state for cancer? Am J Pathol 169: 1505-1522.

3. Russo A, Autelitano M, Bisanti L (2008) Metabolic syndrome and cancer risk. Eur J Cancer 44: 293-297.

4. Seyfried TN, Shelton LM (2010) Cancer as a metabolic disease. Nut & Metab 7: 7.

5. Zelenko Z, Galagher EJ (2014) Diabetes and cancer. Endorinol Metab Clin N Am 43: 167-185.

6. Braun S, Bitton-Worms K, LeRoith D (2011) The link between the metabolic syndrome and cancer. Int J Biol Sci 7: 1003-1015.

7. Tseng CH, Tseng FH (2014) Diabetes and gastric cancer: The potential links. World J Gastroenterol 20: 1701-1711.

8. Pelucchi C, Serraino D, Negri E, Montella M, Dellanoce C, et al. (2011) Metabolic syndrome and risk of prostate cancer in Italy. AEP 21: 835-841.

9. Xiang YZ, Xiong H, Cui ZL, Jiang SB, Xia QH, et al. (2013) The association between metabolic syndrome and the risk of prostate cancer, high-grade prostate, advanced prostate cancer, prostate cancer-specific mortality and biochemical recurrence. J Exp Clin Canc Res 32: 9.

10. Smith Robert J (2013) Nutrition and metabolism in hepatocellular carcinoma. Hepatobiliary Surg Nutr 2: 89-96.

11. Mills KT, Bellows CF, Hoffman AE, Kelly TN, Gagliardi G, et al. (2013) Diabetes mellitus and colorectal cancer prognosis: a meta-analysis. Dis colon rectum 56: 1304-1319.

12. Luo J, Lin HC, He K, Hendryx M (2014) Diabetes and prognosis in older persons with colorectal cancer. Br J Cancer, pp: 1-8.

13. Esposito K, Chiodini P, Colao A, Lenzi A, Giugliano D (2012) Metabolic syndrome and risk of cancer. Diabetes Care 35: 2402-2411.

14. Phaorah PDP, Dunning AM, Ponder BAJ, Easton DF (2004) Association studies for finding cancer-susceptibility genetic variants. Nat Rev Cancer 4: 850-860.

15. Chung CC, Chanock SJ (2011) Current status of genome-wide association studies in cancer. Hum Genet 130: 59-78.

16. Tindall EA, Severi G, Hoang HN, Ma CS, Fernandez P, et al. (2010) Comprehensive analysis of the cytokine-rich chromosomes 5q31.1 region suggests a role for IL-4 gene variants in prostate cancer risk. Carcinogenesis 31: 1748-1754.

17. Anghel A, Narita D, Seclaman E, Popovici E, Anghel M, et al. (2010) Estrogen receptor alpha polymorphisms and the risk of malignancies. Pathol Oncol Res 16: 485-496.

18. Wei BB, Xi B, Wang R, Bai JM, Chang JK, et al. (2010) TGFβ1 T29C polymorphism and cancer risk: A meta-analysis based on 40 case-control studies. Cancer Genet Cytogen 196: 68-75.

19. Alsaid A, El-Missiry M, Hatata ES, Tarabay M, Settin A (2013) Association of IL-4-590 C>T and IL-13-1112 C>T gene polymorphism with susceptibility to Type 2 Diabetes Mellitus. Dis Markers 4: 243-247.

20. Sertic J, Juricic L, Ljubic H, Bozina T, Lovric J, et al. (2009) Variants of ESRI, APOE, LPL, and IL-6 loci in young healthy subjects: association with lipid status and obesity. BMC Research Notes 2: 203.

21. McCarthy JJ, Meyer J, Moliterno DJ, Newby K, Rogers WJ, et al. (2003) Evidence for substantial effect modification by gender in a large genetic association study of metabolic syndrome among coronary heart disease patients. Hum Genet 114: 87-98.

22. Seruga B, Zhang H, Bernstein LJ, Tannock IF (2008) Cytokines and their relationship to the symptoms and outcome of cancer. Nat Rev Cancer 8: 887-899.

23. Bierie B, Moses HL (2006) TGFβ: the molecular Jekyll and Hyde of cancer. Nat Rev Cancer 6: 506-520.

24. Padua D, Massugue J (2009) Roles of TGFβ in metastasis. Cell Res 19: 89-102.

25. Akhurst RJ, Derynck R (2001) TGF-β signalling in cancer-a double-edged sword. Trends Cell Biol 11: S44-S51.

26. Zhenzhen L, Xianghua L, Qingwei W, Zhan G, Ning S (2013) Three common polymorphisms in the IL-4 gene and cancer risk: A meta-analysis involving 5,392 cases and 6,930 controls. Tumuor Biol 34: 2215-2224.

27. Schoof N, von Bonin F, Zeynalova S, Ziepert M, Jung W, et al. (2009) Favorable impact of the interleukin-4 receptor allelic variant I75 on the survival of diffuse large B-cell lymphoma patients demonstrated in a large prospective clinical trial. Ann Oncol 20: 1548-1554.

28. Vairaktaris E, Yannopoulos A, Vassiliou S, Serefoglou Z, Vylliotis A, et al. (2007) Strong association of interleukin-4 (-590 C/T) polymorphism with increased risk for oral squamous cell carcinoma in Europeans. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 104: 796-802.

29. Gallagher CJ, Langefeld CD, Gordon CJ, Campbell JK, Mychalecky JC, et al. (2007) Association of the Estrogen Receptor-α gene with the metabolic syndrome and its component traits in African-American families. Diabetes 56: 2135-2141.

30. Lo JC, Zhao X, Scuteri A, Brockwell S, Sowers MR (2006) The association of genetic polymorphisms in sex hormone biosynthesis and action with insulin sensitivity and diabetes mellitus in women at midlife. Am J Med 119: S69-S78.

31. Kelly TN, Rebholz CM, Gu D, Hixson JE, Rice TK et al. (2013) Analysis of sex hormone genes reveals gender differences in the genetic etiology of blood pressure salt sensitivity: The GenSalt study. Am J Hypertens 26: 191-200.

32. Rahman MT, Nakayama K, Rahman M, Ishikawa M, Katagiri H, et al. (2013) ESR1 gene amplification in endometrial carcinomas: A clinicopathological analysis. Anticancer Res 33: 3775-3782.

33. Kirn V, Zaharieva I, Heublein S, Thangarajah F, Friese K, et al. (2014) ESR 1 promoter methylation in squamous cell cervical cancer. Anticancer Res 34: 723-728.

34. Mizoo T, Taira N, Nishiyama K, Nogami T, Iwamoto T, et al. (2013) Effects of lifestyle and single nucleotide polymorphisms on breast cancer risk: A case-control study in Japanese women. BMC Cancer 15: 565.

35. Segal CV, Dowsett M (2014) Estrogen receptor mutations breast cancer-new focus on to Old target. Clin Cancer Res 20: 1-3.

36. Atmaca A, Al-Batran S, Wirtz RM, Werner D, Sabine Z, et al. (2013) The validation of estrogen receptor 1 mRNA expression as a predictor of outcome in patients with metastatic non-small lung cancer. Int J Cancer 134: 2314-2321.

37. Holt SK, Kwon EM, Fu R, Kolb S, Feng Z, et al. (2013) Association of variants in Estrogen-related pathway genes with prostate cancer. Prostate 73: 1-10.

38. Crivello A, Giacalone A, Vaglica M, Scola L, Forte GI, et al. (2006) Regulatory cytokine gene polymorphisms and risk of colorectal carcinoma. Ann NY Acad Sci 1098: 98-103.

39. Zhang Y, Liu B, Jin M, Ni Q, Liang X, et al. (2008) Genetic polymorphisms of transforming growth factor- β1 and its receptors and colorectal cancer susceptibility: A population-based case-control study in China. Cancer Lett 275: 102-108.

40. Park KH, Han SGL, Whang YM, Lee HJ, Yoo YD, et al. (2006) Single nucleotide polymorphisms of the TGFβ1 gene and lung cancer risk in Korean population. Cancer Genet Cytogen 169: 39-44.

41. Kang H-G, Chae MH, Park JM, Kim EJ, Park JH, et al. (2008) Polymorphisms in TGF-β1 gene and the risk of lung cancer. Lung Cancer 52: 1-7.

42. Ewart-Toland A, Chan JM, Yuan J, Balmain A, Ma J (2004) A gain of function TGFB1 polymorphism may be associated with late stage prostate cancer. Cancer Epidemiol Biomerkaers Prev 13: 759-764.

43. Tindall EA, Hayes VM, Petersen DC (2010) Inflammatory genetic markers of prostate cancer risk. Cancer 2: 1198-1220.

44. Javle M, Li Y, Tan D, Dong X, Chang P, et al. (2014) Biomarkers of TGF-β signaling pathway and prognosis of pancreatic cancer. PLoS One 9: e85942.

45. Falleti E, Fabris C, Toniutto P, Fontanini E, Cussigh A, et al. (2008) TGF-β1 genotypes in cirrhosis: Relationship with the occurrence of liver cancer. Cytokine 44: 256-261.

46. Levy L, Hill CS (2005) Alterations in components of the TGF-β superfamily signalling pathways in human cancer. Cytokine Growth F R 17: 41-58.

47. Ikushima H, Miyazono K (2010) TGF-β signalling: A complex web in cancer progression. Nat Rev Cancer 10: 415-424.

48. Pardali K, Moustakas A (2006) Actions of TGF-β as tumour suppressor and pro-metastatic factos in human cancer. Biochim Biophys Acta 1775: 21-62.

49. Sawabe, M, Arai T, Kasahara I, Esaki Y, Nakahara K, et al. (2004) Developments of geriatric autopsy database and Internet-based database of Japanese single nucleotide polymorphisms for geriatric research (JG-SNP). Mech Ageing Dev 125: 547-552.

50. Takei K, Ikeda S, Arai T, Tanaka N, Muramatsu M, et al. (2008) Lympotoxin-alpha polymorphisms and presence of cancer in 1,536 consecutive autopsy cases. BMC Cancer 8: 235.

51. Parlayan C, Ikeda S, Sato N, Sawabe M, Muramatsu M, et al. (2014) Association analysis of single nucleotide polymorphisms in miR-146a and miR196a2 on the prevalence of cancer in elderly Japanese: a case-control study. Asian Pac J Cancer Prev 15: 2101-2107.

52. Kurokawa N, Young EH, Oka Y, Satoh H, Wareham NJ, et al. (2008) The ADRB3 Trp64Arg variant and BMI: a meta-analysis of 44833 individuals. Int J Obesity 32: 1240 -1249.

53. Perrone MG, Notarnicola M, Caruso MG Tutino V, Scilimati A (2008) Upregulation of beta3-adrenergic receptor mRNA in human colon cancer: a preliminary study. Oncology 75: 224-229.

54. Liang X, Qiua J, Liua X, Li X, Zhao S, et al. (2013) Polymorphism of angiotensinogen gene M235T in myocardial infarction and brain infarction: a meta-analysis. Gene 529: 73-79.

55. Shimomoto T, Ohmori H, Luo Y, Chihara Y, Denda A, et al. (2012) Diabetes-associated angiotensin activation enhances liver metastasis of colon cancer. Clin Exp Metastasis 29: 915-925.

56. Nakamura A, Niimura H, Kuwabara K, Takezaki T, Morita E, et al. (2013) Gene-Gene combination effect and interactions among ABCA1, APOA1, SR-B1 and CETP polymorphisms for serum high-density lipoprotein-cholesterol in the Japanese population. PLoS One 8: e82046.

57. Báez S, Tsuchiya Y, Calvo A, Pruyas M, Nakamura K, et al. (2010) Genetic variants involved in gallstone formation and capsaicin metabolism, and the risk of gallbladder cancer in Chilean women. World J Gastroenterol 16: 372-378.

58. Malfait F, Symoens S, De backer J, Hermanns-Le T, Sakalihasan N, et al. (2007) Three arginine to cyctine substitutions in the Pro-alpha (I)- Collagen chain cause Ehlers-Danlos syndrome with the propensity to arterial rupture in early adulthood. Hum Mutat 28: 387-395.

59. Hu K, Chen F (2012) Identification of significant pathways in gastric cancer based on protein - protein interaction networks and cluster analysis. Genet Mol Biol 35: 701-708.

60. Nikitin AG, Chistiakov DA, Minushkina LO, Zateyshichikov DA, Nosiko VV (2010) Association of the CYBA, PPARGC1A, PPARG3, and PPARD gene variants with coronary artery disease and metabolic risk factors of coronary atherosclerosis in a Russian population. Heart Vessels 25: 229-236.

61. Li Q, Fu G-B, Zheng JT, He J, Niu XB, et al. (2013) NADPH oxidase subunit p22phox -mediated reactive oxygen species contribute to angiogenesis and tumor growth through AKT and ERK1/2 signaling pathways in prostate cancer. Biochim Biophys Acta 1833: 3375-3385.

62. Achour A, Zaag I, Gueddah L, Trimeche B, Slama FBH, et al. (2011) Role of CYP1A1 (T6235C) polymorphism and cigarette smoking in the development of coronary heart disease in Tunisian population. J Genet 90: 303-307.

63. Ji YN, Wang Q, Lin XQ, Suo LJ (2012) CYP1A1 MspI polymorphisms and lung cancer risk: An updated meta-analysis involving 20,209 subjects. Cytokine 59: 324-334.

64. Park S, Seo EJ, Yoo HW, Kim Y (2006) Novel mutations in the human elastin gene (ELN) causing isolated supravalvular aortic stenosis. Int J Mol Med 18: 329-332.

65. Starcher B, Sauter E, Ho C (2013) Elastin overturn in malignant solid tumors. Connect Tissue Res 54: 314-319.

66. Zhou L, Cai J, Liu G, Wei Y, Tang H (2012) Associations between Interleukin-1 gene polymorphisms and coronary heart disease risk: a meta-analysis. PLoS One 7: e45641.

67. Xu J, Yin Z, Cao S, Gao W, Liu L, et al. (2013) Systematic review and meta-analysis on the association between IL-1B polymorphisms and cancer risk. PLoS One 8: e63654.

68. Yin YW, Hu AM, Sun QQ, Zhang BB, Liu HL, et al. (2013) Association between interleukin 10 gene -1082 A/G polymorphism and the risk of type 2 diabetes mellitus: A meta-analysis of 4250 subjects. Cytokine 62: 226-231.

69. Sun JM, Li Q, Gu HY, Chen YJ, Wei JS, et al. (2013) Interleukin 10 rs1800872 T>G polymorphism was associated with an increased risk of esophageal cancer in a Chinese population. Asian Pacific J Cancer Prev 14: 3443-3447.

70. Lucas G, Lluis-Ganella C, Subirana I, Senti M, Willenborg C, et al. (2011) Post-genomic update on a classical candidate gene for coronary artery disease: ESR1. Circ Cardiovasc Genet 4: 647-654.

71. Toy W, Shen Y, Won H, Green B, Sakr RA, et al. (2013) ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. Nat Genet 45: 1439-1444.

72. Ho KT, Shiau MY, Chang YH, Chen CM, Yang SC, et al. (2010) Association of interleukin-4 promoter polymorphisms in Taiwanese patients with type 2 diabetes mellitus. Metabolism 59: 1717-1722.

73. Zhu J, Ju X, Yan F, Qin C, Wang M, et al. (2010) Association of IL-4 -590 T>C polymorphism and risk of renal cell carcinoma in a Chinese population. Int J Immunogenet 37: 459-465.

74. Herder C, Carstensen M, Ouwens DM (2013) Anti-inflammatory cytokine and risk of type 2 diabetes. Diabetes Obes Metab 1: 39-50.

75. Hu S, Zhou G, Zhang L, Jiang H, Xiao M (2012) The effects of functional polymorphisms in the TGFβ1 gene on nasopharyngeal carcinoma susceptibility. Otolaryng Head Neck 146: 579.

76. Haglund S, Taipalensuu J, Peterson C, Almer S (2007) IMPDH activity in thiopurine-treated patients with inflammatory bowel disease – relation to TPMT activity and metabolite concentrations. Br J Clin Pharmacol 65: 69-77.

77. Penuelas S, Noe V, Ciudad CJ (2005) Modulation of IMPDH2, survivin, topoisomerase I and vimentin increases sensitivity to methotrexate in HT29 human colon cancer cells. FEBS J 272: 696-710.

78. Olza J, Aguilera CM, Gil-Campos M, Lei R, Bueno G, et al. (2012) Myeloperoxidase is an early biomarker of inflammation and cardiovascular risk in prepubertal obese children. Diabetes Care 35: 2373-2376.

79. Yang JP, Wang WB, Yang XX, Yang L, Ren L, et al. (2013) The MPO2463G.A polymorphism and lung cancer risk: A meta-analysis based on 22 case–control studies. PLoS One 8: e65778.

80. Uehara SK, Rosa G (2008) Association of homo cysteinemia with high concentrations of serum insulin and uric acid in Brazilian subjects with metabolic syndrome genotyped for C677T polymorphism in the methylene tetrahydrofolate reductase gene. Nutr Res 28: 760-766.

81. Senses KM, Gonen M, Barutcu AR, Kalaylioglu Z, Isbilen M, et al. (2013) Cancer-testis gene expression is associated with the methylenetetrahyrofolate reductase 677 C>T polymorphism in non-small cell lung carcinoma. BMC Med Genet 14: 97.

82. Liu CS, Huang RJ, Sung FC, Lin CC, Yeh CC (2013) Association between endothelial nitric oxide synthase polymorphisms and risk of metabolic syndrome. Dis Markers 34: 187-197.

83. Lee SA, Lee KM, Yoo KY, Noh DY, Ahn SH, et al. (2012) Combined effects of antioxidant vitamin and NOS3 genetic polymorphisms on breast cancer risk in women. Clin Nutr 31: 93-98.

84. Robinette SL, Holmes E, Nicholson JK, Dumas ME (2012) Genetic determinants of metabolism in health and disease: from biochemical genetics to genome–wide associations. Genome Medicine 4: 30.

85. Wang L, Tang S, Wang Y, Xu S, Xu J, et al. (2013) Ecto-50-nucleotidase (CD73) promotes tumor angiogenesis. Clin Exp Metastasis 30: 671-680.

86. Gonul N, Kadioglu E, Kocabas NA, Ozkaya M, Karakaya AE, et al. (2012) The role of GSTM1, GSTT1, GSTP1, and OGG1 polymorphisms in type 2 diabetes mellitus risk: A case–control study in a Turkish population. Gene 505: 121-127.

87. Wei B, Zhou Y, Xu Z, Xi B, Cheng H, et al. (2011) The effect of hOGG1 Ser326Cys polymorphism on cancer risk: evidence from a meta-analysis. PLoS One 6: e27545.

88. Huen K, Harley K, Beckman K, Eskenazi B, Holland N (2013) Associations of PON1 and genetic ancestry with obesity in early childhood. PLoS One 8: e62565.

89. Eroglu M, Yilmaz N, Yalcinkaya S, Ay N, Aydin O, et al. (2013) Enhanced HDL-cholesterol-associated antioxidant PON-1 activity in prostate cancer patients. Kaohsiung J Med Sci 29: 368-373.

90. Chan K-hK, Brennan K, You N-cY, Lu X, Song Y, et al. (2011) Common variations in the genes coding for C - reactive protein (CRP), Tumor Necrosis Factor Alpha (TNF-α), and Interleukin-6 (IL-6) and risk of clinical diabetes in the Women's Health Initiative Observational Study (WHI-OS). Clin Chem 57: 317-325.

91. Danforth KN, Rodriguez C, Hayes RB, Sakoda LC, Huang WY, et al. (2008) TNF polymorphisms and prostate cancer risk. The Prostate 68: 400-407.

92. Reiling E, Lyssenko V, Boer JMA, Imbolz S, Verschuren MM, et al. (2012) Codon 72 polymorphism (rs1042522) of TP53 is associated with changes in diastolic blood pressure over time. Eur J Hum Genet 20: 696-700.

93. Cheng C, Lingyan W, Yi H, Cheng Z, Huadan Y, et al. (2014) Association between TLR2, MTR, MTRR, XPC, TP73, TP53 genetic polymorphisms and gastric cancer: A meta-analysis. Clin Res Hepatol Gastroenterol 38: 346-359.

94. Muiya N, Al-Najai M, Tahir A, Elhawari S, Gueco D, et al. (2013) The 3-UTR of the adiponectin Q gene harbours susceptibility loci for atherosclerosis and its metabolic risk traits. BMC Med Genet 14: 127.

95. Ye C, Wang J, Tan S, Zhang J, Li M, et al. (2013) Meta-analysis of adiponectin polymorphisms and colorectal cancer risk. Int J Med Sci 10: 1113-1120.

96. Hardwick JP, Eckman K, Lee YK, Abdelmegeed MA, Esterle A, et al. (2013) Eicosanoids in metabolic syndrome. Adv Pharmacol 66: 157-266.

97. Bagnoli M, Beretta GL, Gatti L, Pilotti S, Alberti P, et al. (2013) Clinicopathological impact of ABCC1/MRP1 and ABCC4/MRP4 in epithelial ovarian carcinoma. Biomed Res Int 2013: 143202.

98. Chai J, Luo D, Wu X, Wang H, He Y, et al. (2011) Changes of organic anion transporter MRP4 and related nuclear receptors in human obstructive cholestasis. J Gastrointest Surg 15: 996-1004.

99. Copsel S, Garcia C, Diez F, Vermeulen M, Baldi A, et al. (2011) Multidrug Resistance Protein 4 (MRP4/ABCC4) regulates cAMP cellular levels and controls human leukemia cell proliferation and differentiation. J Biol Chem 286: 6979-6988.

100. Nicolaiew N, Cancel-Tassin G, Azzouzi AR, Legrand B, Mangin P, et al. (2008) Association between estrogen and androgen receptor genes and prostate cancer risk. Eur J Endocrinol 160: 101-106.

101. Wang YM, Liu ZW, Guo JB, Wang XF, Zhao XX, et al. (2013) ESR1 gene polymorphisms and prostate cancer risk: A HuGE review and meta- analysis. PLoS One 8: e66999.

102. Chae YK, Huang HY, Strickland P, Hoffman SC, Helzlsouer K (2009) Genetic polymorphisms of estrogen receptors α and β and the risk of developing prostate cancer. PLoS One 4: e6523.

103. Fernandez LP, Milne RL, Barroso E, Cuadros M, Arias JI, et al. (2006) Estrogen and progesterone receptor gene polymorphisms and sporadic breast cancer risk: a Spanish case-control study. Int J Cancer 119: 467-471.

104. Hsiao WC, Young KC, Lin SL, Lin PW (2004) Estrogen receptor-a polymorphism in a Taiwanese clinical breast cancer population: a case control study. Breast Cancer Res 6: 180-186.

105. Lee MM, Gomez SL, Chang JS, Wey M, Wang RT, et al. (2003) Soy and isoflavone consumption in relation to prostate cancer risk in China. Cancer Epidemiol Biomarkers Prev 12: 665-668.

106. De Guia RM, Ramos JD (2010) The -590C/T IL4 single-nucleotide polymorphism as a genetic factor of atopic allergy. Int J Mol Epidemiol Genet 1: 67-73

107. Sokol CL, Barton GM, Farr AG, Medzhitov R (2008) A mechanism for the initiation of allergen-induced T helper type 2 responses. Nat Immunol 9: 310-318.

108. Gaur P, Mittal M, Mohanti B, Das S (2011) Functional variants of IL4 and IL6 genes and risk of tobacco-related oral carcinoma in high-risk Asian Indians. Oral Dis 17: 720-726.

109. Soderberg KC, Hagmar L, Schwartzbaum J, Feychting M (2004) Allergic conditions and risk of hematological malignancies in adults: a cohort study. BMC Public Health 4: 51.

110. Turner MC, Chen Y, Krewski D, Ghadirian P (2006) An overview of the association between allergy and cancer. Int J Cancer 118: 3124-3132.

111. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, et. al. (1986) Transforming growth factor type β: Rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci USA 83: 4167-4171.

112. Pooja S, Francis A, Rajender S, Tamang R, Rajkumar R, et al. (2013) Strong impact of TGF- β1 gene polymorphisms on Breast cancer risk in Indian women: a case-control and Population-Based Study. PLoS One 8: e75979.

113. Li Z, Habuchi T, Tsuchiya N, Mitsumori K, Wang L, et. al. (2004) Increased risk of prostate cancer and benign prostatic hyperplasia associated with transforming growth factor gene polymorphism at codon 10. Carcinogenesis 25: 237-240.

114. Lin Y, Kikuchi SY, Yagyu OK (2006) Serum levels of transforming growth factor beta1 are significantly correlated with venous invasion in patients with gastric cancer. J Gastroenterol Hepatol 21: 432-437.

115. Migita K, Miyazoe S, Maeda Y, Daikoku M, Abiru S, et al. (2005) Cytokine gene polymorphisms in Japanese patients with hepatitis B virus infection-association between TGF-β1 polymorphisms and hepatocellular carcinoma. J Hepatol 42: 505-510.

116. Zhang CF, Wang ZW, Hou MX, Li K, Zhou X, et al. (2012) Transforming growth factor β1 -509C/T and +869T/C polymorphisms on the risk of upper digestive tract cancer: a meta-analysis based on 10,917 participants. Ann Hum Genet 76: 363-376.

117. Garcia MA, Lanas GA, Quintero E, Nicolas D, Parra-Blanco A, et al. (2007) Gastric cancer susceptibility is not linked to pro-and anti-inflammatory cytokine gene poly- morphisms in whites: a nationwide multicenter study in Spain. Am J Gastroenterol 102: 1878-1892.

118. Balding DJ (2006) A tutorial on statistical methods for populations association studies. Nat Rev Gen 7: 781-791.

119. Guan X, Zhao H, Niu J, Tang D, Ajani JA, et al. (2009) The VEGF -634 G>C promoter polymorphism is associated with risk of gastric cancer. BMC Gastroenterol 9: 77.

120. Cooper DN, Krawczak M, Polychronakos C, Tyler-Smith C, Kehrer-Sawatzki H (2013) Where genotype is not predictive of phenotype: towards an understanding of the molecular basis of reduced penetrance in human inherited disease. Hum Genet 132: 1077-1130.

121. Oft M, Hider KH, Beug (1998) TGFβ signalling is necessary for carcinoma cell invasiveness and metastasis. Curr Biol 8: 1243-1252.

122. Toonkel RL, Borczuk AC, Powell CA (2010) TGF-β signalling pathway in lung adenocarcinoma invasion. J Thorac Oncol 5: 153-157.

123. Laballe M, Begum S, Hynes RO (2011) Direct signalling between platelets and cancer cells induces and Epithelial-Mesenchymal-Like Transition and promotes metastasis. Cancer Cell 20: 576-590.

124. Imyanitov EN (2009) Gene polymorphisms, apoptotic capacity and cancer risk. Hum Genet 125: 239-246.

125. Grivennikov SI, Karin M (2011) Inflammatory cytokines in cancer: Tumour necrosis factor and interleukin 6 take the stage. Ann Rheum Dis 70: i104-i108.