Influence of combined genotypes of *RETN* G62A with C-180G and G299A polymorphisms on colon cancer risk

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Abstract: Understanding the biology and genetic alterations of adipocytokines, cell-signaling proteins secreted by adipose tissue, provides new insights into the pathogenesis and treatment of several diseases including cancer. In the present study, we investigated the role of *RETN* gene G62A variant in the pathophysiology of colon cancer. A group of 120 Saudi volunteers (60-colon cancer patients and 60 disease-free controls) within the same age range was studied. PCR-RELP technique was used to determine the single nucleotide polymorphism (SNP). The results were compared with the control group. Analysis of SNP+62 genotypes results showed 100% normal (GG) in both the patients and the controls. Heterozygous (GA) and homozygous (AA) genotypes were not detected in all the patients and the controls. The interesting result in this study is the combined effect of G62A SNP with *RETN* C-180G and G299A SNPs. The results showed a decrease risk of colon cancer reflecting the protective role of SNP G62A in the development of colon cancer. These results suggest that these variants in *RETN* gene and their interactions are strongly associated with the development of colon cancer.
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1. Introduction

The global burden of cancer continues to increase largely. Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries (WHO., 2008). Colon cancer is one of the most frequently diagnosed cancers in females and males worldwide (Parkin *et al*., 2005; Jemal *et al*., 2008; Jemal *et a*l., 2011). Rates of colon cancer vary by race and ethnic status. In Saudi Arabia, the latest incidence report by the Saudi Cancer Registry (SCR) showed that colon cancer ranks the second most common malignancy among Saudis for all ages (10.3%) (Al-Eid *et al*., 2011). Researchers have indicated several risk factors that may increase a person's chance of developing colon cancer (Chan *et al*., 2010; Schlienger *et al*., 2009; Ma *et al*., 2013; Comstock *et al*., 2014). Obesity is a well-known risk factor for colon cancer, but until now, scientists were at a loss to explain why. A number of mechanisms have been proposed for the adverse effect of obesity on colon cancer risk. One of the major hypotheses is the adipocytokines, which are secreted by adipose tissue (Riondino *et al*., 2014; Sánchez *et al*., 2014). Researches have suggested that colon cancer risk rises with increasing weight and increasing the level of resistin (Kumor *et al*., 2009; Al-Harithy and Al-Ghafari., 2010; Gonullu *et al*., 2010).This finding points to a genetic reason for the link. Therefore; gaining a better understanding of the relationship between obesity and colon cancer using modern epidemiological studies that depends on genetic assays will provide new insight into the mechanisms of colon cancer pathogenesis.

Resistin, an adipocytokine, modulate metabolic and inflammatory responses (Steppan *et al*., 2001; Kaser *et al*., 2003; Bokarewa *et al*., 2005; Calabro *et al*., 2007; Filková *et al*., 2009). Human resistin is expressed mainly by macrophages and induced by TNF-α (Patel *et al*., 2003; Lehrke *et al*., 2004). Resistin encoded by the *RETN* gene that is located on chromosome 19p13.3, and spans approximately 1,750 base pairs (Wang *et al*., 2002; Ghosh *et al*., 2003). Single nucleotide polymorphisms (SNPs) have been detected in the gene encoding human resistin as well as the promoter region (Engert *et al*., 2002; Bouchard *et al*., 2004; Mattevi *et al*., 2004; Norata *et al*., 2007; Osawa *et al*., 2007). Resistin gene polymorphism at position G62A reported to acts as an independent contributing factor to T2DM and hypertension (Tan *et al*., 2003). Krízová and his group investigated in patients with obesity, anorexia nervosa, and in control healthy normal-weight women to determine G62A contribution to metabolic phenotype and found that this SNP could contribute to metabolic phenotype of patients with obesity and anorexia nervosa (Krízová *et al*., 2008). In 2012, Zhangbin and co-workers

performed meta-analysis for SNP G62A and observed no significant association with obesity (Zhangbin *et al*., 2012). Other SNP such as G+299A has been found to have an impact on the increased resistin concentrations and might influence susceptibility to type II Diabetes Mellitus (T2DM) in Thais (Osawa *et al*., 2002; Suriyaprom *et al*., 2009). Recently, homozygous (AA) genotype at position +299 in *RETN* gene was found to play a role in the pathogenesis and susceptibility to obesity, impaired glucose tolerance, and T2DM in the Egyptian population as well as higher risk to develop nonalcoholic fatty acids liver disease (NAFLD) in T2DM Chinese patients (El-Shal *et al*., 2013; Zhange *et al*., 2013). In contrast, studies involving Japanese patients showed that SNP G299A in the *RETN* gene did not have major effects on the susceptibility to insulin resistance syndrome associated with T2DM (Ochi *et al*., 2003). Regarding SNP C-180G in the *RETN* promoter region, several studies have focused on the role of resistin in the pathophysiology of insulin resistance, obesity and metabolic syndromes. The results from those studies were controversial (Smith *et al*., 2003; Ochi *et al*., 2007; Krízová *et al*., 2008; Ukkola *et al*., 2008; Dong *et al*., 2012; Liu *et al*., 2012; Wen *et al*., 2013).

Given the importance of obesity in colon cancer development and the fundamental role of resistin in obesity, it is reasonable to hypothesize that *RETN* gene SNPs may play a role in colon cancer susceptibility. Therefore, in this study we investigated the role of *RETN* gene G62A variant in the pathophysiology of colon cancer. The study also evaluated the combined effect of G62A SNP with two SNPs that have previous association with resistin circulating levels, *RETN* C-180G and G299A.

2. Materials and methods

Study subjects

Sixty randomly selected patients with colon cancer (31 males and 29 females) and 60 age matched control subjects (30 males and 30 females) were involved in this study. All the individuals were recruited from King Abdulaziz Hospital and Oncology Center in Jeddah, Kingdom of Saudi Arabia (KSA). Healthy subjects were judged to be in good health according to their medical history and routine laboratory tests. None were taking any medication. The ethical committee of King Abdulaziz University (KAU) has approved this study. All participants provided written informed consent.

Genotyping of *RETN* gene polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes using QIAamp DNA blood Mini kit (Qiagen, 51106). DNA samples were genotyped using PCR assays combined with restriction fragment length polymorphism (RELP). The genotype assay was used for analysis of the G299A and G62A variations. All of the PCR reactions were carried out by a Mastercycler gradient thermocycler (Eppendorf, Germany). Each 25 µl of the PCR reactions contained 2 µl genomic DNA (0.2 µg), 12.5 µl HotStar Taq Master Mix (Qiagen, 203445) in 10.1 µl RNase free water (Qiagen, 203445), and 0.2 µl of each primer (0.1 µM). The primer sequences and PCR- RFLP conditions used for SNPs G299A and G62A are mentioned in details in (Table 1 and Table 2) whereas, for SNP C-180G was published in (17, 23).

Statistical analysis

Genotype distributions, allele frequencies, odds ratio, and risk ratio between patients and controls were calculated by 2x2 contingency table. The Hardy-Weinberg equilibrium was tested for the genotypes. P-value < 0.05 was considered statistically significant.

3. Results

Genotype distribution and allele frequencies

The genotype and allele frequencies of G62A variant showed 100% normal (GG) in both patients and controls. Heterozygous (GA), and homozygous (AA) genotypes were not detected in all the patients and the controls. The genotype and allele frequencies of C-180G variant were previously examined and published (Al-Harithy *et al*., 2010). The genotype and allele frequencies of G299A variant were examined (Table 3). The genotypic frequencies of the patients were 5% (n=3) normal (GG), 85% (n=51) heterozygous (GA), and 10% (n=6) homozygous (AA). In controls, the results showed 25% (n=15) normal (GG), 65% (n=39) heterozygous (GA), and 10% (n=6) homozygous (AA). In patient subjects, the frequency of the G and A alleles were 47.5% and 52.5% respectively. In controls, the frequency of the G and A alleles were 57.5% and 42.5% respectively. Genotype distributions for colon cancer patients and controls were out of Hardy-Weinberg equilibrium (Goodness of fit X^2 = 29.83, df =1, P =0.0001) and (Goodness of fit X^2= 6.55, df =1, P =0.01), respectively.

Genotype combinations of the *RETN* G299A and G62A polymorphisms

Genotype combinations were analyzed (GG/GG, GG/GA, GG/AA, GA/GG, GA/GA, GA/AA, AA/GG, AA/GA, and AA/AA) for G299A and G62A SNPs (Table 4). Combination between the genotypes of the two SNPs did not show a correlation with colon cancer risk except for combination between the homozygous genotype for G299A and the normal genotype for G62A (AA/GG) SNPs. The result showed non-significant decrease to the risk of colon cancer (P=0.687).

Genotype combinations of the *RETN* C-180G and G62A polymorphisms

Genotype combinations were analyzed (CC/GG, CC/GA, CC/AA, CG/GG, CG/GA, CG/AA, GG/GG, GG/GA, and GG/AA) for C-180G and G62A SNPs (Table 5). Combination between several genotypes of the two SNPs did not show a correlation with colon cancer risk except for combination between the homozygous genotype for C-180G and the normal genotype for G62A (GG/GG) SNPs. The result showed non-significant decrease to the risk of colon cancer (P=0.109).

Table 1: The primer sequences and PCR- RFLP conditions used for G299A SNP

Forward primer	(5'-GAGAGGATCCAGGAGGTCG-3')	
Reverse primer	(5'-GTGAGACCAAACGGTCCCT-3')	
PCR conditions	96˚C for 5 min	1x
	96˚C for 35 S	40x
	56˚C for 35 S	40x
	72˚C for 35 S	40x
	72˚C for 4 min	1x
Amplified product length	373 bp	
Restriction enzyme	*AluI* (Thermo Scientific, FERFD0014)	
RFLP condition	37˚C for 2 hours	
RFLP products length	Normal (GG)	243 and 55 bp
	Heterozygous (GA)	243, 158, 85, and 55 bp
	Homozygous (AA)	158, 85, and 55 bp
Reference	(9)	

Table 2: The primer sequences and PCR- RFLP conditions used for G62A SNP

Forward primer	(5'-AGAGTCCACGCTCCTGTGTT-3')	
Reverse primer	(5'- CATCTCCAGGTTTATTTCCAGC-3')	
PCR conditions	96˚C for 5 min	1x
	96˚C for 35 S	40x
	55˚C for 35 S	40x
	72˚C for 35 S	40x
	72˚C for 4 min	1x
Amplified product length	249 bp	
Restriction enzyme	*BseR*I (New England BioLabs, R0581L)	
RFLP condition	37˚C for 2 hours	
RFLP products length	In the presence of G allele	249 bp
	In the presence of A allele	238 and 11 bp
Reference	(25)	

Table 3: Genotypes and allele frequencies of *RETN* G299A SNP for patients and controls

***RETN* polymorphism Genotypes**	**Frequencies %**		***P* value [1]**	**Odds Ratio (95% CI)**	**Risk Ratio (95% CI)**
	Patients (n=60)	**Controls (n=60)**			
GG Normal	5.0 (n=3)	25.0 (n=15)		1.00 (Reference)	1.00 (Reference)
GA Heterozygous	85.0 (n=51)	65.0 (n=39)	0.002 [a]	6.5 (1.77 – 24.18)	1.31 (1.09 – 1.56)
AA Homozygous	10.0 (n=6)	10.0 (n=6)	0.11 [b]	5.0 (0.93 – 26.79)	2.33 (1.03 – 5.29)
GA + AA	95.0 (n=57)	75.0 (n=45)	0.002 [a]	6.33 (1.73 – 23.23)	1.27 (1.08 – 1.48)
***RETN* polymorphism Alleles**					
G	47.5	57.5		1.00 (Reference)	1.00 (Reference)
A	52.5	42.5	0.12 [a]	1.49 (0.89 – 2.49)	1.24 (0.94 – 1.62)

[a] Two sided X^2 test
[b] Two sided Fisher exact test

Table 4. Genotype combinations of G299A and G62A SNPs in the *RETN* gene

G299A/G62A Genotypes	**Frequencies %**		**Odds Ratio (95% CI)**	**Risk Ratio (95% CI)**	***P* Value**
	Patients (n=60)	**Controls (n=60)**			
Normal/ Normal GG/GG	0.05 (n=3)	0.25 (n=15)	1.00 (Reference)	1.00 (Reference)	
Normal/ Hetero GG/GA	0	0	0	0	1 [b]
Normal/ Homo GG/AA	0	0	0	0	1 [b]
Hetero/ Normal GA/GG	0.85 (n=51)	0	0	0	1 [b]
Hetero/ Hetero GA/GA	0	0	0	0	1 [b]
Hetero/ Homo GA/AA	0	0	0	0	1 [b]
Homo/ Normal AA/GG	0.1 (n=6)	0.75 (n=45)	0.6667 (0.1481-3)	0.9444 (0.7507-1.1882)	0.687 [b]
Homo/ Hetero AA/GA	0	0	0	0	1 [b]
Homo/ Homo AA/AA	0	0	0	0	1 [b]

(**Homo**- Homozygous, **Hetero**- Heterozygous)
[a] Two sided X^2 test
[b] Two sided Fisher exact test

Table 5: Genotype combinations of SNPs C-180G and G62A in the *RETN* gene

C-180G/G62A Genotypes	Frequencies %		Odds Ratio (95% CI)	Risk Ratio (95% CI)	*P* Value
	Patients (n=60)	Controls (n=60)			
Normal/ Normal CC/GG	27 (n=16)	0.7 (n=24)	1.00 (Reference)	1.00 (Reference)	
Normal/ Hetero CC/GA	0	0	0	0	1[b]
Normal/ Homo CC/AA	0	0	0	0	1[b]
Hetero/ Normal CG/GG	55 (n=33)	0	0	0	1[b]
Hetero/ Hetero CG/GA	0	0	0	0	1[b]
Hetero/ Homo CG/AA	0	0	0	0	1[b]
Homo/ Normal GG/GG	18.33 (n=11)	60 (n=36)	0.4583 (1.5-3.2727)	0.7833 (0.6-0.766)	0.109[a]
Homo/ Hetero GG/GA	0	0	0	0	1[b]
Homo/ Homo GG/AA	0	0	0	0	1[b]

(**Homo**- Homozygous, **Hetero**- Heterozygous)
[a] Two sided X^2 test
[b] Two sided Fisher exact test

4. Discussion

Our study showed an interesting finding in the combined effect of the *RETN* G62A with G299A and C-180G SNPs on colon cancer risk. Data analysis revealed that the risk of colon cancer for patients with normal (GG) G62A genotype decreased when combined with either homozygous (AA) G299A or homozygous (GG) C-180G genotypes. Indicating that, G at single nucleotide polymorphism 62 is required for A at 299 or G at -180 in *RETN* gene to reduce the risk of colon cancer in Saudi patients. SNPs combination is an excellent method that gives clues for analyzing complex diseases and understanding the effect of SNPs. There have been no reports on the combination effect of the *RETN* G62A with G299A and C-180G SNPs on colon cancer risk to date.

There has been controversy regarding the role of resistin in humans. Polymorphisms in the gene encoding resistin are important to clarify resistin role. *RETN* G299A polymorphism was found to be associated with coronary artery disease (Hussain *et al*., 2011). Other group suggested that resistin gene polymorphisms might play an important role in pathogenesis and susceptibility to obesity, impaired glucose tolerance, and T2DM in the Egyptian population (El-Shal *et al*., 2013). Also in T2DM patients, Zhang and his team found an association with increases in the risk of the nonalcoholic fatty liver disease development among 299AA genotype carriers (Zhang *et al*., 2013). On the other hand, Tan and his group reported that G62A acts as an independent contributing factor to T2DM and hypertension (Tan *et al.,* 2003). Krízová *et al.,* 2008 found that SNP of the resistin gene G62A was associated with lower HbA1c in normal weight and higher cholesterol concentrations in obese group. Moreover, carriers of the minor A allele in the locus 62 of the resistin gene had significantly higher cholesterol levels than obese G/G homozygotes, while the presence of the minor A allele in healthy normal-weight women was accompanied by lower HbA1c levels. Zhangbin *et al.,* performed meta-analysis and observed no significant association with obesity (Zhangbin *et al.,* 2012). In addition, Boumaiza *et al.,* failed to contribute genetic variant G62A in *RETN* gene with obesity and metabolic syndrome in Tunisian population (Boumaiza *et al*., 2012).

In the present study, we found that G299A, a SNP in the non-coding region of intron 2, increases the risk of colon cancer in colon cancer patients. Carriers of the heterozygous (GA) genotype of 299 (OR=6.5, 95%CI 1.77-24.18, *P*=0.002) and the homozygous (AA) genotype (OR=5.0, 95% CI 0.93-

26.79, *P*=0.11) had a significantly higher colon cancer risk than carriers of normal (GG) genotype. Hence, *RETN* 299 GA and AA variants can be used as indicators for colon cancer due to their association. Concerning C-180G, a SNP in the promoter region, we found in a previous work that this variant increases the risk of colon cancer (Al-Harithy *et al*., 2010). Therefore, at this point, it appears that G299A and C-180G can be considered as promising candidates for causal variants, with the possibility that certain combination with G299A and C-180G SNPs may increase or reduce colon cancer risk. To test this hypothesis, we evaluated the combined effect of *RETN* G62A with G299A and C-180G SNPs on the risk of colon cancer. The results showed an impact of combined effect of *RETN* G62A with C-180G and G299A SNPs on reducing the risk of colon cancer. These results suggest that these variants in *RETN* gene and their interactions are strongly associated with the development of colon cancer.

The findings in this study should be considered in light of the small number of subjects that limit the statistical power. Despite that, the design of this study is relatively strong because the controls were recruited from the same cohort as the colon cancer patients. In addition, the cases and controls have been matched by age and sex. Moreover, we evaluated the influence of polymorphisms in the *RETN* gene, not only based on SNP, but also on combined genotypes. The combination approach yielded a more informative relationship between the *RETN* and colon cancer.

In conclusion, our study provides an estimate of the *RETN* alleles frequencies in Saudi Arabia. It also indicates the influence of combined genotypes on colon cancer risk. The function of *RETN* SNPs, including interactions with other SNPs, remains to be elucidated. Further experiments will be required to clarify these points.

Competing interests:

The author declares that there are no competing interests.

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References

1. Al-Eid HS, Bazarbashi S, Al-Zahrani A. Cancer Incidence Report Saudi Arabia 2005, Saudi Cancer Registry website, (2011) Available at: http://www.scr.org.sa/reports/. Assessed on October 29, 2011.
2. Al-Harithy RN, Al-Ghafari AB. Resistin in human colon cancer. Increased expression independently of resistinpromoter C-180G genotype. Saudi Med J 2010;31(5):495-500.
3. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. Resistin, an adipokine with potent proinflammatory properties. J Immunol 2005;174:5789–5795.
4. Bouchard L, Weisnagel SJ, Engert JC, Hudson TJ, Bouchard C, Vohl MC, Perusse L. Human resistin gene polymorphism is associated with visceral obesity and fasting and oral glucose stimulated C-peptide in the Quebec Family Study. J Endocrinol Invest 2004;27:1003–1009.
5. Boumaiza I, Omezzine A, Rejeb J, Rebhi L, Ben Rejeb N, Nabli N, Ben Abdelaziz A, Bouslama A. Association between four resistin polymorphisms, obesity, and metabolic syndrome parameters in Tunisian volunteers. Genet Test Mol Biomarkers 2012;16(12):1356-1362.
6. Calabro P, Yeh ET. Obesity, inflammation, and vascular disease: the role of the adipose tissue as an endocrine organ. Subcell Biochem 2007;42:63-91.
7. Chan AT, Giovannucci EL. Primary prevention of colorectal cancer. Gastroenterology 2010;138(6):2029-2043.
8. Comstock SS, Xu D, Hortos K, Kovan B, McCaskey S, Pathak DR, Fenton JI. Association of insulin-related serum factors with colorectal polyp number andtype in adult males. Cancer Epidemiol Biomarkers Prev 2014 Jun 24. pii: cebp.0249.2014. [Epub ahead of print]
9. Dong XQ, Du Q, Yu WH, Zhang ZY, Zhu Q, Che ZH, Wang H, Chen J, Yang SB, Wen JF. Plasma resistin, associated with single nucleotide polymorphism -420, is correlated with C-reactive protein in Chinese Han patients with spontaneous basal ganglia hemorrhage. Genet Mol Res 2012;11(3):1841-1850.
10. El-Shal AS, Pasha HF, Rashad NM. Association of resistin gene polymorphisms with insulin resistance in Egyptian obese patients, Gene 2013;515(1):233-238.
11. Engert JC, Vohl M-C, Williams SM, Lepage P, Faith J, Dore C, Renaud Y, Burtt NP, Villeneuve A, Hirschhorn JN, Altshuler D, Groop LC, Despres J-P, Gaudet D, Hudson TJ. 5′ flanking

variants of resistin are associated with obesity. Diabetes 2002;51:1629–1634.
12. Filková M , Haluzík M, Gay S, Senolt L.The role of resistin as a regulator of inflammation: Implications for various human pathologies. Clin Immunol 2009;133(2):157-170.
13. Ghosh S, Singh A, Aruna A, Mukhopadhyay S, Ehtesham N. The genomic organization of mouse resistin reveals major differences from the human resistin: functional implications. Gene 2003; 305(1):27-34.
14. Gonullu G, Kahraman H, Bedir A, Bektas A, Yücel I. Association between adiponectin, resistin, insulin resistance, and colorectal tumors. Int J Colorectal Dis 2010; 25(2):205-212.
15. Hussain S, Bibi S, Javed Q. Heritability of genetic variants of resistin gene in patients with coronary artery disease: a family-based study. Clin Biochem 2011;44(8-9):618-622.
16. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun M. Cancer statistics, 2008. CA Cancer J Clin 2008;58:71-96.
17. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61(2):69-90.
18. Kaser S, Kaser A, Sandhofer A, Ebenbichler CF, Tilg H, Patsch JR. Resistin messenger-RNA expression is increased by proinflammatory cytokines in vitro. Biochem Biophys Res Commun 2003;309:286–290.
19. Krízová J, Dolinková M, Lacinová Z, Sulek S, Dolezalová R, Housová J, Krajícková J, Haluzíková D, Bosanská L, Papezová H, Haluzí K M. Adiponectin and Resistin gene polymorphisms in patients with anorexia nervosa and obesity and its influence on metabolic phenotype. Physiol Res 2008;57:539-546.
20. Kumor A, Daniel P, Pietruczuk M, Małecka-Panas E. Serum leptin, adiponectin, and resistin concentration in colorectal adenoma and carcinoma (CC) patients. Int J Colorectal Dis 2009; 24(3):275-281.
21. Lehrke M, Reilly MP, Millington SC, Iqbal N, Rader DJ, Lazar MA. An inflammatory cascade leading to hyperresistinemia in humans. PLoS Med 2004; 1(2):e45.
22. Liu R, He B, Gao F, Liu Q, Yi Q. Association of the resistin gene promoter region polymorphism with Kawasaki disease in Chinese children. Mediators inflamm 2012; 2012:356362.
23. Ma Y, Yang Y, Wang F, Zhang P, Shi C, Zou Y, Qin H. Obesity and risk of colorectal cancer: a systematic review of prospective studies. PLoS One 2013;8(1):e53916.
24. Mattevi VS, Zembrzuski VM, Hutz MH: A resistin gene polymorphism is associated with body mass index in women. Hum Genet 2004;115:208–212.
25. Norata GD, Ongari M, Garlaschelli K, Tibolla G, Grigore L, Raselli S, Vettoretti S, Baragetti I, Noto D, Cefalu AB, Buccianti G, Averna M, Catapano AL. Effect of the −420C/G variant of the resistin gene promoter on metabolic syndrome, obesity, myocardial infarction and kidney dysfunction. J Intern Med 2007;262:104–112.
26. Ochi M, Osawa H, Onuma H, Murakami A, Nishimiya T, Shimada F, Kato K, Shimizu I, Shishino K, Murase M, Fujii Y, Ohashi J, Makino H. The absence of evidence for major effects of the frequent SNP +299 G>A in the resistin gene on susceptibility to insulin resistance syndrome associated with Japanese type 2 diabetes. Diabetes Res Clin Pract 2003;61(3):191-198.
27. Ochi M, Osawa H, Hirota Y, Hara K, Tabara Y, Tokuyama Y, Shimizu I, Kanatsuka A, Fujii Y, Ohashi J, Miki T, Nakamura N, Kadowaki T, Itakura M, Kasuga M, Makino H. Frequency of G/G genotype of resistin single nucleotide polymorphism at -420 appears to be increased in younger-onset type 2 diabetes. Diabetes 2007;56 (11):2834-2838.
28. Osawa H, Onuma H, Murakami A, Ochi M, Nishimiya T, Kato K, Shimizu I, Fujii Y, Ohashi J, Makino H. Systematic search for single nucleotide polymorphisms in the resistin gene: the absence of evidence for the association of three identified single nucleotide polymorphisms with Japanese type 2 diabetes. Diabetes 2002;51(3):863-866.
29. Osawa H, Tabara Y, Kawamoto R, Ohashi J, Ochi M, Onuma H, Nishida W, Yamada K, Nakura J, Kohara K, Miki T, Makino H. Plasma resistin, associated with single nucleotide polymorphism-420, is correlated with insulin resistance, lower HDL cholesterol, and high-sensitivity C-reactive protein in the Japanese general population. Diabetes Care 2007;30:1501–1506.
30. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics 2002. CA Cancer J Clin 2005; 55(2):74-108.
31. Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, Macphee CH, Smith SA. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochem Biophys Res Commun 2003;300(2):472-476.
32. Riondino S, Roselli M, Palmirotta R, Della-Morte D, Ferroni P, Guadagni F. Obesity and

colorectal cancer: role of adipokines in tumor initiation and progression. World J Gastroenterol 2014;20(18):5177-5190.
33. Sánchez R C, Ibáñez C, Klaassen J. The link between obesity and cancer. Rev Med Chil 2014;142(2):211-221.
34. Schlienger JL, Luca F, Vinzio S, Pradignac A. Obesity and cancer. Rev Med Interne 2009; 30(9):776-782.
35. Smith SR, Bai F, Charbonneau C, Janderová L, Argyopoulos G. A promoter genotype and oxidative stress potentially link resistin to human insulin resistance. Diabetes 2003;52(7):1611-1618.
36. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA. The hormone resistin links obesity to diabetes. Nature 2001;409:307–312.
37. Suriyaprom K, Phonrat B, Namjuntra P, Chanchay S, Tungtrongchitr R. The +299(g>a) resistin gene polymorphism and susceptibility to type 2 diabetes in Thais. J Clin Biochem Nutr 2009; 44(1):104-110.
38. Tan M, Chang S, Chang D, Tsai J, Lee Y. Association of resistin gene 3'-untranslated region +62G-->A polymorphism with type 2 diabetes and hypertension in a Chinese population. J Clin Endocrinol Metab 2003;88:1258-1263.
39. Ukkola O, Kunnari A, Kesäniemi YA. Genetic variants at the resistin locus are associated with the plasma resistin concentration and cardiovascular risk factors. Regulatory Peptide 2008;149(1-3):56-9.
40. Wang H, Chu W, Hemphill C, Elbein S. Human resistin gene: molecular scanning and evaluation of association with insulin sensitivity and type 2 diabetes in Caucasians. J Clin Endocr Metab 2002; 87:2520-2524.
41. Wen Y, Lu P, Dai L. Association between resistin gene -420 C/G polymorphism and the risk of type 2 diabetes mellitus: a meta-analysis. Acta Diabetol 2013;50(2):267-272.
42. World Health Organization. The Global Burden of Disease: 2004 Update. Geneva: World Health Organization; 2008.
43. Zhang LY, Jin YJ, Jin QS, Lin LY, Zhang DD, Kong LL. Association between resistin +299A/A genotype and nonalcoholic fatty liver disease in Chinese patients with type 2 diabetes mellitus. Gene 2013;529(2):340-344.
44. Zhangbin Y, Shuping H, Xingguo C, Chun Z, Xuejie W, Xirong G. Genetic polymorphisms in adipokine genes and the risk of obesity: A systematic review and meta-analysis. Obesity 2012;20 (2):396-406.

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