Association of resistin polymorphism, its serum levels and prevalence of stroke in Japanese type 2 diabetic patients

Eitaro Nakashima[1,2]*, Atsuko Watarai[1], Takayoshi Tsukahara[2], Yoji Hamada[2], Keiko Naruse[2], Hideki Kamiya[2], Jiro Kato[2], Norihiro Kato[4], Makoto Tomita[5], Yutaka Oiso[2], Jiro Nakamura[2]

ABSTRACT

Aims/Introduction: Resistin, an inflammatory cytokine, might be involved in the development of atherosclerosis. In a recent paper, we showed that resistin polymorphism might be a risk marker for stroke susceptibility in Japanese type 2 diabetic patients. We tested whether the serum resistin levels might be also a risk marker of stroke independently from *RETN* polymorphism.

Materials and Methods: Type 2 diabetic outpatients from our hospitals were enrolled. Patients (n = 89) with a history of coronary heart disease and stroke, and randomly selected controls (n = 178) matched for sex and age, but without a history of coronary heart disease and stroke, were examined for polymorphism -420 (C>G) and cytokines levels.

Results: Serum resistin levels were significantly higher in patients with cardiovascular diseases (CVD) than in those without CVD (P = 0.024), and were highest in patients with stroke among the CVD. In multiple logistic regression analysis, serum resistin levels was an independent risk marker of stroke even after adjusted by *RETN* polymorphism, age, sex, body mass index, HbA_{1c}, systolic and diastolic blood pressure, triglyceride, low density lipoprotein cholesterol, high density lipoprotein cholesterol, creatinine, history of coronary heart disease, treatment of insulin, sulfonylurea and aspirin (odds ratio 1.33, 95% confidence interval [CI] 1.02–1.73, P = 0.039). The enrolled patients were divided by their serum resistin levels (high or low group) and their genotypes (CC, CG, GG at -420) into six groups. Patients with the GG genotype and high resistin levels showed the highest odds ratio, 5.69 (95% CI 1.24–26.1), compared with the group with CC and low levels.

Conclusions: The results suggest that serum resistin levels might be a good marker of susceptibility to stroke as well as *RETN* polymorphism. (J Diabetes Invest, doi: 10.1111/j.2040-1124.00040.x, 2010)

KEY WORDS: Resistin, Type 2 diabetes, Stroke

INTRODUCTION

Resistin, an inflammatory cytokine expressed in human macrophages[1], has been reported to be elevated in subjects with obesity and inflammation[2,3]. It has direct action on the arterial wall[4,5] and might be involved in the development of atherosclerosis. Previously, the specific recognition of the -420G allele in the resistin gene (*RETN*) by Sp1/3 transcription factor was shown to increase its promoter activity[6]. The present authors and others have reported that serum resistin levels are increased in a genotype-dependent manner based on the *RETN* polymorphism at -420 (C>G)[7–9]. In a recent paper, the present authors have also shown that the genotyping of this polymorphism might provide a good risk marker for stroke susceptibility in Japanese type 2 diabetic patients[9]. However, there have been some conflicting reports that don't support a relationship between the blood resistin levels and susceptibility to cardiovascular diseases (CVD)[10–13]. In addition, not only genetic factors, but also systemic inflammation was suggested to affect the blood levels of resistin[14]. The aim of the present case–control study was thus to investigate, at first, the association between the serum resistin levels, inflammatory status and the prevalence of CVD, including coronary heart disease (CHD) and stroke, in Japanese type 2 diabetes patients. Therefore, we tested the serum resistin levels and *RETN* polymorphism at position -420 (C>G) as a risk marker of CVD.

[1]Diabetes Center, Chubu Rosai Hospital, Japan Labour Health and Welfare Organization, [2]Department of Endocrinology and Diabetes, Nagoya University Graduate School of Medicine, Nagoya, [3]School of Nutritional Sciences, Nagoya University of Arts and Sciences, Aichi, [4]Department of Gene Diagnostics and Therapeutics, International Medical Center of Japan, Tokyo, and [5]Department of Mathematical Sciences, Faculty of Mathematical Sciences and Information Engineering, Nanzan University, Seto, Japan
*Corresponding author. Eitaro Nakashima Tel.: +81-52-652-5511 Fax: +81-52-653-3533
E-mail address: eitaro@med.nagoya-u.ac.jp
Received 3 December 2009; revised 29 March 2010; accepted 23 April 2010

METHODS

Subjects

A total of 267 type 2 diabetic outpatients (89 cases, 179 controls) who were consecutive visitors to Nagoya University Hospital and Chubu Rosai Hospital were enrolled. Cases were defined as all participants who had previously suffered from CVD (CHD and stroke). Controls were defined as participants with no record of CVD. Controls were randomly selected 2:1 from the enrolled

cohort in our hospitals after matching for sex and age. The assessment and definition of CVD were based on the following criteria. CHD was defined according to histories of physician-diagnosed ischemic heart disease. Strokes (ischemic cerebrovascular diseases) were diagnosed by means of neurological signs and symptoms, together with computed tomography or magnetic resonance imaging by neurologists. Only patients with a history of large vessel diseases and carotid stroke were enrolled, and patients with a history of cardioembolic and lacunar strokes were excluded. The study protocol and informed consent procedure were approved by the Ethics Committee of Nagoya University Graduate School of Medicine and Chubu Rosai Hospital and carried out in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Evaluated Parameters

Body mass index (BMI) and blood pressure (BP) were measured. Fasting blood samples were obtained, and sera were stored at −80°C. Blood glucose, HbA_{1c}, insulin, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), triglycerides (TG) and creatinine were measured in our hospital laboratory. Adipocytokines and C-reactive protein (CRP) were also analyzed by an enzyme-linked immunoassay (ELISA) kit (R&D, Minneapolis, MN, USA or American Research Products, Belmont, MA, USA).

Genotyping of Polymorphisms

DNA-fragments of the single nucleotide polymorphism (SNP)-420 were amplified from genomic DNA using polymerase chain reaction with a previously described procedure[9].

Statistical Analyses

Statistical analyses were carried out using the program SPSS (SPSS, Chicago, IL, USA). The normally distributed parameters were expressed as means ± standard deviations and evaluated by Student's *t*-test. The parameters that were not normally distributed were expressed as median and interquartile range, and evaluated by Mann–Whitney *U*-test. Correlations were sought

Table 1 | Baseline clinical characteristics in patients with or without cardiovascular diseases

	Control	Case	*P*
n	178	89	
No. females	65	31	
Age (years)	65.5 ± 9.4	67.0 ± 10.0	0.86
Duration of diabetes (years)	13.7 ± 9.4	17.3 ± 9.9	0.47
Body mass index (kg/m^2)	23.0 ± 3.5	23.4 ± 3.5	0.41
HbA_{1c} (%)	7.2 ± 1.2	7.5 ± 1.2	0.45
FBG (mmol/L)	8.2 ± 2.4	7.9 ± 2.2	0.69
Fasting insulin (μU/mL)	6.7 ± 5.5	7.8 ± 5.4	0.73
HOMA-R	2.5 ± 2.4	2.6 ± 1.8	0.27
SBP (mmHg)	131 ± 16	134 ± 16	0.86
DBP (mmHg)	74 ± 11	74 ± 11	0.65
Total cholesterol (mmol/L)	5.15 ± 0.92	5.24 ± 0.87	0.44
Triglycerides (mmol/L)	1.18 (0.88–1.64)	1.35 (1.10–2.28)	0.05[a]
HDL (mmol/L)	1.36 ± 0.40	1.28 ± 0.38	0.82
LDL (mmol/L)	3.10 ± 0.82	3.18 ± 0.75	0.35
Creatinine (μmol/L)	71 (62–80)	80 (71–102)	<0.001[a]
Adiponectin (μg/mL)	7.8 (4.9–12.8)	8.7 (5.5–14.6)	0.32[a]
Resistin (ng/mL)	10.8 (6.9–17.6)	14.4 (8.1–22.2)	0.02[a]
CRP (mg/dL)	0.052 (0.018–0.139)	0.076 (0.035–0.180)	0.046[a]
Smoking history (%)	48.6	57.1	0.33[b]
Medications			
Insulin (%)	22.3	43.4	0.02[b]
Sulfonylurea (%)	34.2	36.1	0.77[b]
Glitazone (%)	10.7	6.7	0.09[b]
Statin (%)	26.4	37.1	0.38[b]
Aspirin (%)	5.4	28.9	<0.001[b]

Data are presented as means ± SD or median (interquartile range).

CRP, C-reactive protein; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL, high density lipoprotein cholesterol; HOMA-R, homeostasis model assessment of insulin resistance; LDL, low density lipoprotein cholesterol; SBP, systolic blood pressure.

[a]*P*-value by Mann–Whitney *U*-test.

[b]*P*-value by χ^2-test.

Non-labeled *P*-value by Student's *t*-test.

by use of Spearman's method. The association of serum resistin with stroke was assessed in multiple logistic regression models. A *P*-value <0.05 was considered statistically significant.

RESULTS

The baseline clinical characteristics of the study subjects are presented according to the presence or absence of CVD (Table 1). Cases had significantly higher TG, creatinine, resistin levels and CRP than controls. Other anthropometric data did not show any significant differences between the two groups. Serum resistin levels were significantly higher in patients with CVD than in those without CVD ($P = 0.024$). However, the levels of serum resistin were highest in patients with stroke among the CVD (stroke 16.5 [8.1–28.3] *vs* control, $P = 0.007$) (Figure 1a). The serum resistin levels had significantly univariate correlations with the levels of creatinine and CRP, but not with other anthropometric variables (Table 2).

As we reported previously, the serum resistin levels were significantly high ($P < 0.001$) according to the presence of the G allele (-420C/G). Next, we estimated serum resistin levels in multiple logistic regression analyses. The serum resistin levels were independently associated with stroke after adjustment for age, sex, BMI and genotype of *RETN* SNP -420 (Table 3; Model 1). After additional adjustment for HbA_{1c}, systolic BP, diastolic BP, LDL, HDL, triglyceride, and history of coronary disease (Table 3; Model 2), and even after further adjustment for creatinine, CRP, insulin treatment, sulfonylurea treatment and aspirin treatment (Table 3; Model 3), the significance still remained. Interestingly the genotype of *RETN* SNP -420 was not a significant factor in Model 1, but it was significant in Models 2 and 3 (CC *vs* GG).

Furthermore, we calculated the serum resistin levels in each genotype and found that serum resistin levels were significantly higher in cases of the CC and CG genotype groups than in controls (cases and controls; 14.2 ± 11.4 ng/dL *vs* 9.6 ± 6.1 [$P = 0.04$] in CC, 22.1 ± 13.1 *vs* 13.5 ± 6.9 [$P < 0.01$] in CG, 19.5 ± 12.2 *vs* 19.0 ± 11.0 [$P = 0.91$] in GG). In multiple regression analysis using serum resistin levels as the covariate in each genotype group, we found that high blood levels of resistin were a significant independent risk factor for stroke in the CG genotype group (odds ratio [OR] for stroke for 5-ng/mL increase in serum resistin levels, 1.66 [95% CI 1.03–2.68]) and showed a tendency for increased risk in the CC genotype group (OR 1.220, 95% CI 0.614–2.43). This means that, at least for diabetic patients with the CG genotype, measuring the serum resistin levels is more useful for detecting high-risk patients for susceptibility for stroke than just checking the genotype.

Based on these data, we divided the enrolled patients by their median serum resistin concentrations (High or Low) and their genotypes (CC, CG, GG) into six groups, just for an example. Odds ratios for stroke against the CC + Low group were calculated in each group by multivariate logistic-regression analysis (Figure 1b). The odds ratios increased according to the G allele mutation and high serum resistin concentration. Patients with the GG genotype and high serum resistin levels showed the highest odds ratio, 5.69 (95% CI 1.24–26.1), compared with the CC + Low group. However, we failed to show significance between High and Low within each genotype. Systolic blood pressure was also detected as a significant factor in this calculation.

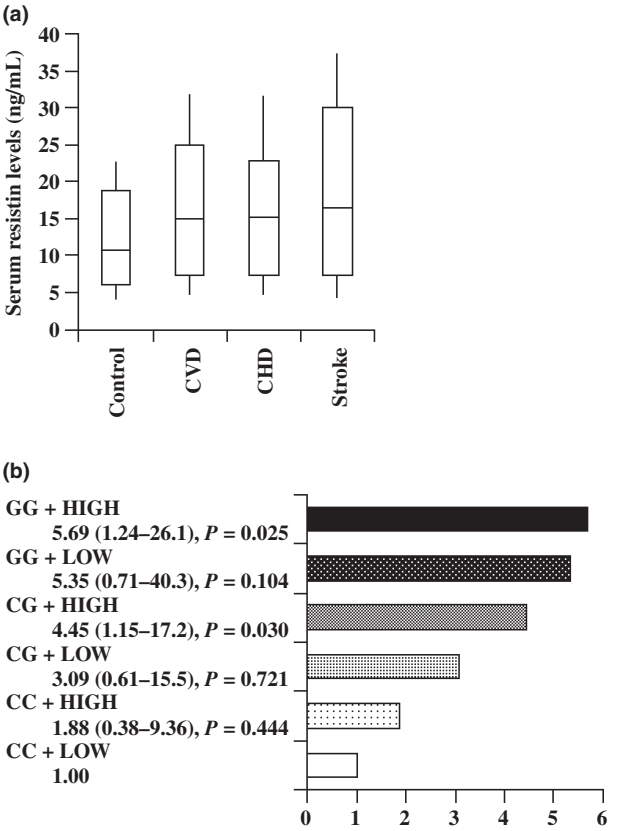

Figure 1 | (a) The resistin concentration (ng/mL) in control, total cardiovascular diseases (CVD) (coronary heart disease [CHD] + stroke) and each CVD. Box plots show median, interquartile range and non-outlier range. Extreme values are excluded from the box plots. (b) Odds ratio for stroke according to the combination of *RETN* genotype (-420C/G) and resistin levels (high or low) in multivariate logistic-regression analysis. The enrolled patients were divided by their serum resistin concentrations at median (high or low) and their genotypes (CC, CG, GG) into six groups. After adjustment for age, sex, body mass index, systolic blood pressure, serum levels of triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol, C-reactive protein and creatinine, the multivariate logistic-regression analysis were made and each odds ratio against the CC + Low group was calculated. Right column shows odds ratio (confidence intervals).

DISCUSSION

In the present study, both the serum resistin levels and its genotype at -420 (C>G) were associated with the prevalence of stroke in Japanese type 2 diabetic patients, even after adjustment for known atherosclerotic risk factors in the multiple logistic regression analysis. As serum resistin levels shows a significant odds ratio independently from its SNP-420, its measurement could

Table 2 | Spearman's correlation coefficients of serum resistin levels to anthropometric and biochemical variables in Japanese type 2 diabetic patients

	r	*P*
Age (years)	0.08	0.18
Duration of diabetes (years)	0.03	0.65
Body mass index (kg/m^2)	0.08	0.20
HbA_{1c} (%)	−0.03	0.60
Fasting blood glucose (mmol/L)	−0.15	0.08
Fasting insulin (μU/mL)	−0.06	0.57
HOMA-R	−0.13	0.21
Systolic blood pressure (mmHg)	0.05	0.41
Diastolic blood pressure (mmHg)	−0.07	0.25
Triglycerides (mmol/L)	−0.06	0.51
HDL-cholesterol (mmol/L)	−0.11	0.07
LDL-cholesterol (mmol/L)	−0.03	0.63
Creatinine (umol/L)	0.27	<0.001
Adiponectin	0.09	0.15
CRP	0.13	0.03

Spearman's *r* correlation across all cases and controls. *r* value with resistin. CRP, C-reactive protein; HDL, high density lipoprotein cholesterol; HOMA-R, homeostasis model assessment of insulin resistance; LDL, low density lipoprotein cholesterol.

also be helpful for the risk prediction of stroke in Japanese type 2 diabetic patients (Table 3).

Recent studies have shown that the resistin levels are significantly correlated with coronary artery calcification and are predictive of coronary atherosclerosis in humans[15,16]. Ukkola *et al.* and Norata *et al.*[17,18] described the association among this -420 (C>G) polymorphism, the resistin levels and cardiovascular risk factors. However, the association between the serum resistin levels and CHD seemed to be negative[10–13], and might be controversial for this polymorphism and CVD[17,19]. Differences in the cohorts might explain the different results, depending on which ethnic group was tested[8,20,21], or which diabetic cohort was explored. Indeed, methodological limitations in the commercially available ELISA assays might also result in variations among serum levels, which might cause difficulties when comparing results from different publications.

Although few studies have been carried out for stroke, our data showed that stroke was most strongly associated with the levels of resistin and its polymorphism (Figure 1).

After all, it is noteworthy that in a recent report by Efstathiou *et al.*[22], high resistin levels might have been strongly associated with an increased risk of 5-year mortality or disability after atherothrombotic ischemic stroke. However, because they did not measure the prestroke resistin levels, it is still unclear whether or not resistin is a key player in the pathogenesis of stroke or just a marker or indicator of the inflammatory status. To answer this, more studies will be required.

There are some limitations in the interpretation of the present study. First, we examined type 2 diabetic patients in Japan who were relatively lean compared with those in other developed countries. It would be hard to extrapolate the results of the present study to non-diabetic patients, obese diabetic patients or other

Table 3 | Multiple logistic regression analyses of serum resistin levels with history of stroke

Adjusted for	Model 1		Model 2		Model 3	
	Odds ratio (CI)	*P*	Odds ratio (CI)	*P*	Odds ratio (CI)	*P*
Resistin	1.32 (1.09–1.59)	0.004	1.34 (1.06–1.69)	0.013	1.33 (1.02–1.73)	0.039
Genotype of *RETN* SNP-420						
CC *vs* CG	1.55 (0.63–3.84)	0.34	1.34 (0.44–4.09)	0.61	1.43 (0.46–4.50)	0.54
CC *vs* GG	1.75 (0.56–5.42)	0.34	3.67 (1.02–13.2)	0.046	3.81 (1.03–14.1)	0.046
Age	1.03 (0.99–1.08)	0.13	1.02 (0.97–1.08)	0.38	1.03 (0.97–1.09)	0.35
Sex	1.18 (0.51–2.72)	0.69	1.03 (0.35–3.02)	0.96	1.02 (0.35–3.04)	0.97
BMI	1.06 (0.95–1.18)	0.32	1.01 (0.88–1.14)	0.93	1.02 (0.89–1.16)	0.81
HbA_{1c}			0.79 (0.51–1.22)	0.28	0.74 (0.46–1.20)	0.22
SBP			1.04 (1.01–1.07)	0.02	1.04 (1.00–1.07)	0.045
DBP			0.98 (0.93–1.04)	0.51	0.99 (0.94–1.04)	0.63
LDL			1.01 (0.99–1.02)	0.53	1.01 (0.99–1.02)	0.51
HDL			0.97 (0.94–1.01)	0.14	0.97 (0.94–1.01)	0.15
Triglycerides			1.00 (1.00–1.01)	0.33	1.00 (1.00–1.01)	0.31
History of CHD			1.95 (0.71–5.33)	0.20	1.68 (0.56–5.06)	0.36
Creatinine					0.95 (0.63–1.41)	0.78
CRP					1.01 (0.85–1.21)	0.89
Insulin therapy					1.95 (0.47–8.14)	0.36
Sulfonylurea therapy					1.80 (0.54–6.01)	0.34
Aspirin therapy					1.15 (0.34–1.41)	0.82

Odds ratio and 95% confidence interval (CI) for the existence of stroke for 5-ng/mL increase in serum resistin levels.
BMI, body mass index; CHD, coronary heart diseases; CRP, C-reactive protein; DBP, diastolic blood pressure; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; SBP, systolic blood pressure; SNP, single nucleotide polymorphism.

ethnic groups. Second, this was a kind of cross-sectional study at the point of estimating serum resistin levels; we cannot determine any cause–effect relationship based on this study design. Third, the results were influenced by survivor effects, and the true prevalence of atherosclerotic diseases might be underestimated.

Some previous reports showed that the blood levels of resistin were positively related to the systemic inflammatory status. Although we cannot change our genotype to lower our susceptibility for stroke, there is the possibility of lowering the risk of stroke in diabetic patients, even those with the CG genotype, by multifactorial intervention aimed at reducing the systemic inflammation status, that is, by treatment with glitazone. Our results suggest that using the genotype and serum levels of resistin to discriminate between diabetic responders and non-responders will contribute to the development of effective strategies and improve the prognosis in this population. In conclusion, the present results suggest that serum resistin levels might be also a good marker of susceptibility to stroke as well as *RETN* polymorphism, and the measurement of both *RETN* gene polymorphism and serum resistin levels might be useful to detect the susceptibility to stroke and might provide an incremental value in the risk prediction for stroke, beyond the current approaches, among Japanese type 2 diabetic patients. Our findings need to be confirmed by further studies.

ACKNOWLEDGEMENTS

We thank Miss Kimiko Sato and Miss Yuko Maehata for technical support. This work was supported by a grant from the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBI). We have no conflict of interest in this work.

REFERENCES

1. Patel L, Buckels AC, Kinghorn IJ, *et al.* Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem Biophys Res Commun* 2003; 300: 472–476.
2. Degawa-Yamauchi M, Bovenkerk JE, Juliar BE, *et al.* Serum resistin (FIZZ3) protein is increased in obese humans. *J Clin Endocrinol Metab* 2003; 88: 5452–5455.
3. Vozarova de Courten B, Degawa-Yamauchi M, Considine RV, *et al.* High serum resistin is associated with an increase in adiposity but not a worsening of insulin resistance in Pima Indians. *Diabetes* 2004; 53: 1279–1284.
4. Kawanami D, Maemura K, Takeda N, *et al.* Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine-endothelial cell interactions. *Biochem Biophys Res Commun* 2004; 314: 415–419.
5. Burnett MS, Lee CW, Kinnaird TD, *et al.* The potential role of resistin in atherogenesis. *Atherosclerosis* 2005; 182: 241–248.
6. Osawa H, Yamada K, Onuma H, *et al.* The G/G genotype of a resistin single-nucleotide polymorphism at -420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/3. *Am J Hum Genet* 2004; 75: 678–686.
7. Cho YM, Youn BS, Chung SS, *et al.* Common genetic polymorphisms in the promoter of resistin gene are major determinants of plasma resistin concentrations in humans. *Diabetologia* 2004; 47: 559–565.
8. Osawa H, Onuma H, Ochi M, *et al.* Resistin SNP-420 determines its monocyte mRNA and serum levels inducing type 2 diabetes. *Biochem Biophys Res Commun* 2005; 335: 596–602.
9. Tsukahara T, Nakashima E, Watarai A, *et al.* Polymorphism in resistin promoter region at -420 determines the serum resistin levels and may be a risk marker of stroke in Japanese type 2 diabetic patients. *Diabetes Res Clin Pract* 2009; 84: 179–186.
10. Burnett MS, Devaney JM, Adenika RJ, *et al.* Cross-sectional associations of resistin, coronary heart disease, and insulin resistance. *J Clin Endocrinol Metab* 2006; 91: 64–68.
11. Lim S, Koo BK, Cho SW, *et al.* Association of adiponectin and resistin with cardiovascular events in Korean patients with type 2 diabetes: The Korean atherosclerosis study (KAS) A 42-month prospective study. *Atherosclerosis* 2008; 196: 389–404.
12. Pilz S, Weihrauch G, Seelhorst U, *et al.* Implications of resistin plasma levels in subjects undergoing coronary angiography. *Clin Endocrinol (Oxf)* 2007; 66: 380–386.
13. Weikert C, Westphal S, Berger K, *et al.* Plasma resistin levels and risk of myocardial infarction and ischemic stroke. *J Clin Endocrinol Metab* 2008; 93: 2647–2653.
14. Lehrke M, Reilly MP, Millington SC, *et al.* An inflammatory cascade leading to hyperresistinemia in humans. *PLoS Med* 2004; 1: e45.
15. Reilly MP, Lehrke M, Wolfe ML, *et al.* Resistin is an inflammatory marker of atherosclerosis in humans. *Circulation* 2005; 111: 932–939.
16. Mohty D, Pibarot P, Despres JP, *et al.* Age-related differences in the pathogenesis of calcific aortic stenosis: The potential role of resistin. *Int J Cardiol* 2009; in press.
17. Norata GD, Ongari M, Garlaschelli K, *et 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.
18. Ukkola O, Kunnari A, Kesaniemi YA. Genetic variants at the resistin locus are associated with the plasma resistin concentration and cardiovascular risk factors. *Regul Pept* 2008; 3: 56–59.
19. Kunnari A, Ukkola O, Kesaniemi YA. Resistin polymorphisms are associated with cerebrovascular disease in Finnish Type 2 diabetic patients. *Diabet Med* 2005; 22: 583–589.
20. Menzaghi C, Coco A, Salvemini L, *et al.* Heritability of serum resistin and its genetic correlation with insulin resistance-related features in nondiabetic Caucasians. *J Clin Endocrinol Metab* 2006; 91: 2792–2795.
21. Hivert MF, Manning AK, McAteer JB, *et al.* Association of variants in RETN with plasma resistin levels and diabetes-related traits in the Framingham Offspring Study. *Diabetes* 2009; 58: 750–756.
22. Efstathiou SP, Tsiakou AG, Tsioulos DI, *et al.* Prognostic significance of plasma resistin levels in patients with atherothrombotic ischemic stroke. *Clin Chim Acta* 2007; 2: 78–85.