The G972R Polymorphism in the Insulin Receptor Substrate (IRS)-1 Gene does not Correlate with Angiographic Presence of Significant Coronary Artery Disease

Strohmer B, Hölzl B, Paulweber B, Reiter R

Homepage:

www.kup.at/jcbc

**Online Data Base Search
for Authors and Keywords**

The G972R Polymorphism in the Insulin Receptor Substrate (IRS)-1 Gene does not Correlate with Angiographic Presence of Significant Coronary Artery Disease

B. Strohmer[1], B. Hölzl[2], R. Reiter[2], B. Paulweber[2]

A base change at codon 972 of the insulin receptor substrate-1 (IRS-1) gene resulting in an arginine for glycine substitution has been found to be associated with insulin resistance and predisposition to non-insulin dependent diabetes mellitus. Some data exist about the possible contribution of the G972R mutation to development of coronary artery disease (CAD). We conducted a case-control study in order to test the frequency and possible association of this common mutation with the presence of CAD in 436 consecutive Austrian subjects undergoing coronary angiography. Genotypes of IRS-1 polymorphism were determined in 215 patients with significant (stenosis > 50 %) CAD and in 221 controls free of coronary atherosclerosis.

*The frequency of G972R mutation carriers was identical in the patient and control group (12.1 % vs. 12.2 %, p = n.s.) except for a higher prevalence in a small subgroup of male subjects with diabetes. Consequently, regression analysis revealed no association between this common mutation and CAD independent of typical risk factors in both gender. The group of CAD contained 44 (20.5 %) patients with diabetes, whereas only 19 (8.6 %) diabetics were found in the control arm (p <0.001). Among 63 diabetic individuals of the total study population the frequency of the IRS-1 mutation was almost 2-times higher than among non-diabetics (20.6 % vs. 10.7 %, p < 0.003). Male mutation carriers showed significantly higher values of fasting insulin, female mutation carriers of triglycerides and LDL cholesterol. Data of the present study do not suggest that the G972R mutation is a genetic marker for the presence of significant CAD in patients stratified by coronary angiography. **J Clin Basic Cardiol 2005; 8: 61–4.***

***Key words:** IRS-1 gene polymorphism, coronary artery disease, coronary angiography, insulin resistance, diabetes*

Coronary artery disease (CAD) is a multifactorial disorder resulting from an interaction between genetic background, conventional risk factors and environmental factors [1]. The insulin resistance syndrome is considered a potent risk factor for the development of atherosclerotic vascular disease [2–4]. A base change at codon 972 of the insulin receptor substrate-1 (IRS-1) gene resulting in an arginine for glycine substitution has been found to be associated with the insulin resistance syndrome and predisposition to non-insulin dependent diabetes mellitus (NIDDM) [5, 6]. Some investigators reported that the G972R mutation in the IRS-1 gene was significantly associated with increased risk of CAD, particularly in subgroups of individuals with obesity and insulin resistance syndrome [7]. These results suggested that the IRS-1 gene variant might be a new genetic risk factor for CAD independent of diabetes or hyperlipidaemia. However, there is evidence that the frequency of the G972R mutation in the IRS-1 gene is variable in different ethnic and regional populations [8, 9] complicating the interpretation of the influence of this polymorphism on CAD risk [10, 11].

We performed a case-control study in order to test the frequency and possible association of this common mutation with the presence of CAD in the Austrian population.

Methods

Study Population
The study population comprised a total of 436 consecutive unrelated subjects who underwent coronary angiography at our hospital. The patient group consisted of 215 subjects with CAD (163 male, 52 female patients, mean age 58 ± 8 years). Diagnostic criteria for recruitment to the patient cohort were one or more stenoses > 50 % in at least one major coronary artery. The control group consisted of 221 subjects (110 males, 111 females, mean age 60 ± 8 years), which showed a smooth outline of the epicardial arteries without visible signs of atherosclerotic lesions in the standard coronary angiogram. All individuals studied were of Austrian descent. Subjects were considered as hypertensive if their blood pressure readings exceeded 140/90 mm Hg at two separate checkups. The diagnosis of diabetes was established according to WHO criteria.

DNA Analysis
After informed consent genotypes of IRS-1 polymorphism were determined in all subjects by nested polymerase chain reaction and BstN1 restriction enzyme digestion, as described previously [7].

Measurement of Lipoproteins and Biochemical Analysis
All blood samples were drawn after a fasting period of at least 12 hours and not less than 6 weeks after myocardial infarction. Fasting glucose, total cholesterol, serum triglycerides, and HDL cholesterol levels were determined enzymatically with commercial kits. Serum levels of apolipoprotein B, apolipoprotein A-1 and lipoprotein (a) were measured by commercially available immunoassays (Hoffmann-LaRoche GmbH, Vienna, Austria). Serum fibrinogen levels were determined by an automated coagulation test based on the method of Clauss. Plasma homocysteine levels were measured by HPLC (Chromsystems Inc. M. Chem. GmbH, Munich, Germany).

Statistical Analysis
The STATISTICA statistical software package (version 5.5, StatSoft, Tulsa, OK, U.S.A.) was used for all calculations.

Mean levels of all tested clinical and biochemical parameters in the group of non-carriers were compared to mean levels in the group of carriers separated by gender using one-way ANOVA. Continuous variables were compared by t-test and categorical variables by χ^2-test. Data for continuous variables were expressed as mean ± SD. The criterion for statistical significance was p < 0.05. The Bonferroni adjustment was applied for each post-hoc comparison of a single parameter to end up with a reasonable overall type I error rate of 5 %. Logistic regression analysis was used for calculation of significant predictors of CAD.

Results

Clinical characteristics and biochemical parameters of the study population are summarised in Table 1. The patient group (n = 215) comprised 163 male and 52 female subjects with angiographically verified CAD. The control group (n = 221) consisted of 110 male and 111 female subjects free of CAD documented by normal angiograms. Classical risk factors, such as cigarette smoking, hypertension, and diabetes mellitus, were more prevalent in the patient than in the control group, though with considerable gender differences. Patients of both gender had significantly higher levels of lipoprotein (a), female patients of triglycerides and male patients of fibrinogen compared to control subjects. Total cholesterol did not differ between the two groups, whereas HDL and LDL cholesterol levels followed the expected distributions among CAD patients. No differences existed between the two groups as far as plasma levels of fasting insulin, fasting glucose and homocysteine were concerned.

The genotype distribution of the G972R polymorphism in the IRS-1 gene was determined for all subjects. Genotype and allele frequencies of G972R mutation in CAD patients and controls are reported in Table 2. The genotype distribution was in Hardy-Weinberg equilibrium. In the total study cohort of 436 individuals 49 (11.2 %) subjects were found to be heterozygous for the G972R mutation in the IRS-1 gene. Overall, four homozygous carriers were found, two in the male patient group and two in the female control group. The frequency of the G972R mutation carriers in the patient group was 12.1 %, and in the control group 12.2 % (p = n.s.).

Table 3 compares various biochemical parameters between non-carriers and carriers of the IRS-1 mutation for male and female subgroups. Female mutation carriers presented significant higher levels of triglycerides and LDL. Fasting-glucose concentrations did not differ between mutation carriers and non-carriers. However, male carriers showed significantly higher values of fasting-insulin, a parameter serving as surrogate for insulin sensitivity. Other biochemical data did not reach statistical significance when compared to the presence of the G972R mutation.

The whole study group comprised 63 (14.5 %) patients with type II diabetes mellitus. The patient group contained 44 (20.5 %) diabetics, whereas the control group contained only 19 (8.6 %) diabetics (p < 0.001). Diabetes was significantly more prevalent among male mutation carriers compared to non-carriers (p = 0.005). Among all diabetic individuals the frequency of IRS-1 mutation was significantly higher than in patients without diabetes (20.6 % [13/63] vs. 10.7 % [40/373], p < 0.03). As far as hypertension was concerned no difference existed between mutation carriers and non-carriers.

Logistic regression analysis revealed no association between the G972R mutation and CAD status independent of other risk factors, neither for male (p = 0.46) nor for female (p = 0.40) subjects. No significant interaction was observed between IRS-1 type and risk of CAD in obese patients (body mass index/BMI > median). The probability of a stenosis > 50 % given that the mutation is present and that the BMI is

Table 2. Genotype and allele frequency of the G972R mutation in the IRS-1 gene among subjects with and without coronary artery disease (CAD)

Variables	Subjects w/o CAD n/N	%	Subjects with CAD n/N	%	p-value
Genotypes					
GG	194/221	87.8	189/215	87.9	
GR	25/221	11.3	24/215	11.2	n.s.
RR	2/221	0.9	2/215	0.9	
Alleles					
G	413/442	93.4	402/430	93.5	
R	29/442	6.6	28/430	6.5	n.s.

Table 1. Clinical and biochemical characteristics of subjects with significant (stenosis > 50 %) coronary artery disease (CAD) and without angiographic evidence of CAD

	Male					Female				
	w/o CAD		with CAD			w/o CAD		with CAD		
Clinical Characteristics	Mean	SD	Mean	SD	p-value	Mean	SD	Mean	SD	p-value
Age (years)	57.7	7.6	58.1	7.9	0.6	62.7	8.1	57.8	8.4	0.0004*
Smokers (n/total)	25/110	–	54/163	–	0.07	6/111	–	13/52	–	0. 0004*
BMI (kg/m^2)	26.5	3.6	26.8	3.4	0.6	27.2	4.6	27.5	4.7	0.72
Hypertension (n/total)	39/110	–	80/163	–	0.028	57/111	–	33/52	–	0.15
Diabetes (n/total)	10/110	–	29/163	–	0.04	9/111	–	15/52	–	0.0006*
F-insulin (µU/mL)	12.9	9.2	14.3	17.4	0.47	13.9	20.1	12.2	9.5	0.62
F-glucose (mg/dL)	91.5	15.5	92.2	16.5	0.76	87.0	11.1	87.0	15.9	0.99
Total cholesterol (mg/dL)	229.8	51.3	233.4	47.6	0.618	242.0	41.6	246.4	68.6	0.712
Triglycerides (mg/dL)	186.5	289.6	164.4	73.3	0.486	139.0	50.7	179.0	95.5	0.010
HDL chol. (mg/dL)	51.2	14.5	44.9	13.4	0.003*	62.7	17.7	50.9	22.1	0.013
LDL chol (mg/dL)	142.7	38.5	154.8	40.0	0.040	153.2	39.3	159.4	57.3	0.566
Apo B (mg/dL)	122.6	33.2	130.7	27.9	0.096	125.3	27.2	130.5	38.0	0.518
Apo A-1 (mg/dL)	155.7	34.7	137.0	29.7	< 0.001*	178.8	61.6	143.5	29.9	0.024
Lp (a) (mg/dL)	24.4	39.4	41.6	49.7	0.019	16.2	28.8	60.6	64.8	< 0.001*
Fibrinogen (mg/dL)	332.2	122.2	396.4	172.2	0.007	322.6	96.9	366.6	136.0	0.104
Homocysteine (µmol/L)	12.1	5.0	11.1	3.5	0.203	10.6	3.6	10.9	4.4	0.736

*p-values indicate a significant difference (p-value adjustment for each sub-table with the Bonferroni-method)

Table 3. Clinical and biochemical characteristics among carriers and non-carriers of the G972R mutation in the IRS-1 gene.

| G972R mutation | Male | | | | | Female | | | | |
| | Non-Carriers | | Carriers | | | Non-Carriers | | Carriers | | |
	Mean	SD	Mean	SD	p-value	Mean	SD	Mean	SD	p-value
Age (years)	57.7	7.7	59.7	8.1	0.67	61.2	8.7	60.7	7.3	0.77
Smokers (n/total)	72/244	–	7/29	–	0.57	14/139	–	5/24	–	0.61
BMI (kg/m^2)	26.6	3.5	26.9	3.2	0.73	27.2	4.7	27.9	4.4	0.53
Hypertension (n/total)	106/244	–	13/29	–	0.91	78/139	–	12/24	–	0.58
Diabetes (n/total)	30/244	–	9/29	–	0.005*	20/139	–	4/24	–	0.79
F-insulin (µU/mL)	13.0	8.1	21.1	42.9	0.024	13.9	19.2	10.3	3.7	0.065
F-glucose (mg/dL)	91.8	16.0	93.2	16.8	0.714	86.5	13.1	89.8	8.3	0.136
Total cholesterol (mg/dL)	230.2	47.4	240.8	62.6	0.34	239.1	41.9	263.2	66.6	0.051
Triglycerides (mg/dL)	171.2	200.4	211.1	305.6	0.42	137.9	51.5	190.1	92.3	0.001*
HDL-chol. (mg/dL)	48	13.8	50.1	18	0.52	60.6	17.3	61	26.9	0.935
LDL-chol (mg/dL)	148.8	39.1	145.4	43.9	0.72	150.3	36.4	176.8	64.5	0.017
Apo B (mg/dL)	126.6	30	128.5	37.3	0.8	124.9	27.7	136.1	39.6	0.243
Apo A-1 (mg/dL)	144.9	33	155.8	36.1	0.18	173.5	59.3	158.4	50.9	0.444
Lp (a) (mg/dL)	30	43.8	49.3	52.5	0.08	21.9	36.2	45.6	68.6	0.069
Fibrinogen (mg/dL)	370.2	155.3	346.2	123.8	0.4	330.6	100.9	327.1	105.3	0.9
Homocysteine (µmol/L)	12.1	5.0	11.1	3.5	0.203	10.6	3.6	10.9	4.4	0.736

*p-values indicate a significant difference (p-value adjustment for each sub-table with the Bonferroni-method)

above the median was 63 % for male and 35.2 % for female subjects. The probability of a stenosis > 50 % given that the mutation is absent and the BMI is above the median was 56.3 % for male and 35.7 % for female subjects. Both probabilities, for male (63 % vs. 56.3 %) and for female (35.2 % vs. 35.7 %) subjects did not differ significantly (p = 0.61 and p = 0.97, respectively).

Discussion

The present study was conducted to determine the frequency of the G972R polymorphism and the relationship to the presence of significant CAD in a cross-sectional Austrian population. 215 patients with angiographically proven CAD were compared with 221 consecutive controls free of CAD as far as the G972R mutation, clinical and biochemical characteristics were concerned. Of note, we found no significant difference in genotype frequency between carriers and non-carriers with respect to clinical characteristics (such as BMI or hypertension) except for a higher prevalence in the small subgroup of male subjects with diabetes. Diabetes is a strong risk factor for CAD, and previous studies have reported that the G972R mutation is almost twice as frequent in NIDDM patients as in control subjects [8, 12]. We confirmed this finding by the observation that the IRS-1 gene variant was present in diabetics in about 21 % vs. 11 % in non-diabetics.

Insulin resistance syndrome and type II diabetes are nowadays thought to result from polygenic interactions of several inherited gene mutations. The IRS-1 gene may act as susceptibility gene predisposing to manifestation of NIDDM [12–15]. It has been suggested previously to include angiographically documented CAD in the disease cluster comprising the insulin resistance syndrome [16].

A group of investigators reported that the frequency of the G972R mutation in 318 CAD patients was 2.5-times higher than in 208 control individuals [7]. After adjustment for other risk factors, the relative risk for angiographic coronary disease attributable to the IRS-1 polymorphism was 3-fold, and even increased to 7-fold and 27-fold, respectively, in obese patients and in patients with the insulin resistance syndrome.

In contrast to the cited study, it is noteworthy that our control group consisted of patients who underwent coronary angiography with normal results. However, it is conceivable that

some of those patients may present cardiac syndrome X, which is known to occur more frequently in patients with classical risk factors, such as high fasting insulin and lipoprotein levels [17–19]. Our results suggest that the prevalence of the IRS-1 variant in patients with normal epicardial coronaries seems to be as high as in patients with angiographic evidence of CAD. Most likely, discrepancies in genetic epidemiological association studies may be explained by selection criteria of the control group, ethnic divergence as well as differences in sample size and power of the study.

In conclusion, present data do not suggest that the G972R mutation is associated with an increased risk of CAD independent of diabetes in an ethnically homogenous population. This finding is in line with previously published data indicating that the IRS-1 gene may not be implicated directly in the pathogenesis of atherosclerotic vascular disease [11, 20–23]. As far as the contribution of a single genetic polymorphism to CAD risk is concerned, we believe that the interplay between several environmental and genetic factors needs to be strongly considered.

References:
1. Hirashiki A, Yamada Y, Murase Y, Suzuki Y, Kataoka H, Morimoto Y, Tajika T, Murohara T, Yokota M. Association of gene polymorphisms with coronary artery disease in low- or high-risk subjects defined by conventional risk factors. J Am Coll Cardiol 2003; 42: 1429–37.
2. Bressler P, Bailey SR, Matsuda M, DeFronzo RA. Insulin resistance and coronary artery disease. Diabetologia 1996; 39: 1345–50.
3. Laasko M. Insulin resistance and coronary heart disease. Curr Opin Lipidol 1996; 7: 217–26.
4. Baroni MG, Arca M, Sentinelli F, Buzzetti R, Capici F, Lovari S, Vitale M, Romeo S, Di Mario U. The G972R variant of the insulin receptor substrate-1 (IRS-1) gene, body fat distribution and insulin-resistance. Diabetologia 2001; 44: 367–72.
5. Almind K, Bjorbaek C, Vestergaard H, Hansen T, Echwald S, Pedersen O. Aminoacid polymorphisms of insulin receptor substrate-1 in non-insulin-dependent diabetes mellitus. Lancet 1993; 342: 828–32.
6. Clausen JO, Hansen T, Bjorbaek C, Echwald SM, Urhammer SA, Rasmussen S, Andersen CB, Hansen L, Almind K, Winther K. Insulin resistance: interactions between obesity and a common variant of insulin receptor substrate-1. Lancet 1995; 346: 397–402.
7. Baroni MG, D'Andrea MP, Montali A, Pannitteri G, Barilla F, Campagna F, Mazzei E, Lovari S, Seccareccia F, Campa PP, Ricci G, Pozzilli P, Urbinati G, Arca M. A common mutation of the insulin receptor substrate-1 gene is a risk factor for coronary artery disease. Arterioscler Thromb Vasc Biol 1999; 19: 2975–80.
8. Hitman GA, Hawrami K, McCarthy MI, Viswanathan M, Snehalatha C, Ramachandran A, Tuomilehto J, Tuomilehto-Wolf E, Nissinen A, Pedersen O. Insulin receptor substrate-1 gene mutations in NIDDM; implications for the study of polygenic disease. Diabetologia 1995; 38: 481–6.

9. Chuang LM, Lai CS, Yeh JI, Wu HP, Tai TY, Lin BJ. No association between the Gly971Arg variant of the insulin receptor substrate 1 gene and NIDDM in the Taiwanese population. Diabetes Care 1996; 19: 446–9.

10. Delafontaine P. IRS-1 variant: A new risk factor for coronary artery disease? Arterioscler Thromb Vasc Biol 2000; 20: 283–4.

11. Tsai CT, Hwang JJ, Lai LP, Chiang FT, Tseng YZ. IRS-1 Gly971Arg variant is not a new risk factor for coronary artery disease in the Taiwanese population. Arterioscler Thromb Vasc Biol 2002; 22: 194.

12. Zhang Y, Wat N, Stratton IM, Warren-Perry MG, Orho M, Groop L, Turner RC. UKPDS 19: heterogeneity in NIDDM: separate contributions of IRS-1 and beta 3-adrenergic-receptor mutations to insulin resistance and obesity respectively with no evidence for glycogen synthase gene mutations. UK Prospective Diabetes Study. Diabetologia 1996; 39: 1505–11.

13. Marchetti P, Lupi R, Federici M, Marselli L, Masini M, Boggi U, Del Guerra S, Patane G, Piro S, Anello M, Bergamini E, Purrello F, Lauro R, Mosca F, Sesti G, Del Prato S. Insulin secretory function is impaired in isolated human islets carrying the Gly(972) – Arg IRS-1 polymorphism. Diabetes 2002; 51: 1419–24.

14. Marini MA, Frontoni S, Mineo D, Bracaglia D, Cardellini M, De Nicolais P, Baroni A, D'Alfonso R, Perna M, Lauro D, Federici M, Gambardella S, Lauro R, Sesti G. The Arg972 variant in insulin receptor substrate-1 is associated with an atherogenic profile in offspring of type 2 diabetic patients. J Clin Endocrinol Metab 2003; 88: 3368–71.

15. Laukkanen O, Pihlajamaki J, Lindstrom J, Eriksson J, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Tuomilehto J, Uusitupa M, Laakso M; Finnish Diabetes Prevention Study Group. Common polymorphisms in the genes regulating the early insulin signalling pathway: effects on weight change and the conversion from impaired glucose tolerance to

Type 2 diabetes. The Finnish Diabetes Prevention Study. Diabetologia 2004; 47: 871–7.

16. Fuster V, Gotto AM, Libby P, Loscalzo J, McGill HC. 27th Bethesda Conference: matching the intensity of risk factor management with the hazard for coronary disease events. Task Force 1. Pathogenesis of coronary disease: the biologic role of risk factors. J Am Coll Cardiol 1996; 27: 964–76.

17. Ley CJ, Swan J, Godsland IF, Walton C, Crook D, Stevenson JC. Insulin resistance, lipoproteins, body fat and hemostasis in nonobese men with angina and a normal or abnormal coronary angiogram. J Am Coll Cardiol 1994; 23: 377–83.

18. Botker HE, Frobert O, Moller N, Christiansen E, Schmitz O, Bagger JP. Insulin resistance in cardiac syndrome X and variant angina: influence of physical capacity and circulating lipids. Am Heart J 1997; 134: 229–37.

19. Al Suwaidi J, Higano ST, Holmes DR Jr, Lerman A. Pathophysiology, diagnosis, and current management strategies for chest pain in patients with normal findings on angiography. Mayo Clin Proc 2001; 76: 813–22.

20. Grant PJ, Stickland MH, Mansfield MW. Insulin receptor substrate-1 gene and cardiovascular risk factors in NIDDM. Lancet 1995; 346: 841–2.

21. Ossei-Gerning N, Mansfield MW, Stickland MH, Grant PJ. Insulin receptor substrate-1 gene polymorphism and cardiovascular risk in non-insulin dependent diabetes mellitus and patients undergoing coronary angiography. Clin Lab Haematol 1997; 19: 123–8.

22. Holzl B, Iglseder B, Stadlmayr A, Hedegger M, More E, Reiter R, Sandhofer F, Paulweber B. Intima media thickness of carotid arteries is reduced in heterozygous carriers of the Gly972 Arg variant in the insulin receptor substrate-1 gene. Eur J Clin Invest 2003; 33: 110–6.

23. Strohmer B, Reiter R, Holzl B, Paulweber B. Lack of association of the Gly972 Arg mutation of the insulin receptor substrate-1 gene with coronary artery disease in the Austrian population. J Intern Med 2004; 255: 146–7.