*Physiol. Res. 59: 1029-1032, 2010*

**RAPID COMMUNICATION**

# The Connexin 37 (1019C>T) Gene Polymorphism Is Associated With Subclinical Atherosclerosis in Women With Type 1 and 2 Diabetes and in Women With Central Obesity

**J. PIŤHA[1], J. A. HUBÁČEK[1], P. PIŤHOVÁ[2]**

[1]Institute for Clinical and Experimental Medicine, Prague, Czech Republic, [2]Teaching Hospital Motol, Second Medical School, Department of Medicine, Prague, Czech Republic

Received May 31, 2010
Accepted June 25, 2010

**Summary**

The gene for connexin 37 (Cx37) is considered to be one of the candidate genes for cardiovascular disease. We evaluated the association between Cx37 (1019C>T) gene polymorphism (Pro319Ser) and ankle brachial blood pressure index (ABI) in women with type 1 (n=178) and type 2 (n=111) diabetes, and in women from general population (n=862). All women were genotyped for Cx37 polymorphism. In addition to traditional cardiovascular risk factors, ABI was analyzed. In women with type 1 diabetes, ABI significantly decreased from TT to CC carriers (p for trend= 0.008). A similar trend was seen in women with type 2 diabetes (p=0.050) and in women with waist circumference above 75$^{th}$ percentile (94 cm; n=208) of the general population (p=0.049). The gene for Cx37 was associated with subclinical atherosclerosis in women with type 1 and 2 diabetes and in women with advanced central obesity. The presence of C allele indicated increased risk.

**Key words**

Connexin 37 gene • Atherosclerosis • Ankle brachial index-women • Diabetes mellitus • Central obesity

**Corresponding author**

J. Pitha, Laboratory for Atherosclerosis Research, Institute for Clinical and Experimental Medicine, Vídeňská 1952/8, 140 21, Praha 4, Prague, Czech Republic. Fax + 0420 241 271 574. E-mail: japi@medicon.cz

The possible role of gap junctions in the process of atherosclerosis has been recently a matter of debate. An important factor in atherosclerosis development could be the altered communication between endothelial cells, vascular smooth muscle cells, and macrophages. The protein Connexin 37 (Cx37) has been suggested as one of the main participants in these inter-cell communications (Burnier *et al.* 2009). The protein is a member of the Connexin family forming gap-junction channels and hemichannels, distinctively affecting permeability for various signaling molecules. The 1019C>T polymorphism (Pro319Ser) of the Cx37 gene could change function of this protein and could be a possible accelerator of atherosclerosis and cardiovascular disease. The main mechanism proposed is the increased adhesion of macrophages to the vessel wall (Wong *et al.* 2006) and as recently described in the population of obese men, macrophages could play a complex role in the metabolic and inflammatory processes in humans including atherosclerosis (Broch *et al.* 2010). However, regarding association between this polymorphism and cardiovascular disease non-consistent data are presented (Yeh *et al*. 2001, Yamada *et al.* 2002, Hirashiki *et al.* 2003, Han *et al*. 2008)

Diabetes mellitus ranks among the strongest predictors of cardiovascular disease (Milicevic *et al.* 2008). In diabetic patients, smaller vessels are involved more extensively than in non-diabetic populations. Hypothetically, gap junctions could play a more important role in patients with diabetes and/or insulin resistance. Therefore, the gene for Cx37 could play important role in these populations.

We evaluated the association between the Cx37 gene polymorphism and ankle/brachial blood pressure

**Table 1.** Ankle-brachial blood pressure index in women without and with advanced central obesity, and in women with type 2 and 1 diabetes stratified by the Connexin 37 (1019C>T) gene polymorphism.

| Connexin 37 gene | Type 1 diabetes | Type 2 diabetes | General population Waist >94 cm | General population Waist < 94 cm |
|---|---|---|---|---|
| *n (TT/CT/CC)* | 18/79/81 | 13/45/53 | 21/97/90 | 80/300/274 |
| *%* | 10.1/44.4/45.5 | 11.7/40.6/47.7 | 10.1/46.6/43.3 | 12.2/45.9/41.9 |
| *TT* | 1.07 ± 0.12 | 1.00 ± 0.06 | 1.05 ± 0.11 | 1.06 ± 0.09 |
| *CT* | 1.01 ± 0.11 | 0.99 ± 0.13 | 1.06 ± 0.09 | 1.05 ± 0.09 |
| *CC* | 0.98 ± 0.12 | 0.96 ± 0.19 | 1.03 ± 0.08 | 1.06 ± 0.08 |
| *p value for trend* | 0.008 | 0.05 | 0.05 | 0.33 |

Data are mean ± S.D.

index (ABI) in women with type 1 and 2 diabetes and in women from the general population with markers of insulin resistance expressed as increased waist circumference.

Women with type 1 (n=178) and type 2 diabetes (n=111) were examined in one center with regard to the presence of cardiovascular risk factors, genetic markers of atherosclerosis and the presence of preclinical atherosclerosis including ABI. The only exclusion criterion was participant´s age over 55 years and/or refusal to be included in the study. We also analyzed data from a random population sample of 874 women between 45-55 years old. The Institutional Ethical Committee approved the study and all participants signed informed consent forms.

All women were examined by identical protocol including a questionnaire focused on cardiovascular disease, cardiovascular risk factors, and treatment. In addition, anthropometric measurements (weight, height, waist and hip circumferences), and blood pressure measurements were obtained in the standard manner. Women with history of current and past regular smoking were defined as smokers. Body mass index was calculated as weight in kg over squared height in meters.

DNA was isolated from frozen EDTA blood (Miller *et al.* 1988). To genotype the C1019>T (Pro319>Ser) variant within Cx37 gene, oligonucleotides 5` CTGGACCCACCCCCTCAGAATGGCCAAAGA and 5` AGGAAGCCGTAGTGCCTGGTGG and restriction enzyme AasI (Fermentas, Lithuania) were used to distinguish the T (fragments of 240 bp and 35 bp) and C (275 bp) alleles. A set of 24 samples was analyzed three times within 3 weeks with 100 % confirmity.

ABI was calculated as the quotient of the mean of systolic blood pressures at four ankle arteries and the mean of systolic blood pressures of brachial arteries. Blood pressures were obtained in all arteries from all participating women. In women with type 1 and 2 diabetes, the measurements were done by continuous-wave Doppler ultrasound device with 10 MHz probe (SmartDop 50, Hadeco, Japan). In women from the general population, a continuous-wave Doppler ultrasound device with 10 MHz probe (Sonovit SV-1, Schiller AG, Switzerland) was used.

Descriptive data are presented as percentages for categorical variables and means with standard deviation for continuous ones. The differences between carriers of different genotypes were analyzed as p for the trend in the case of continuous variables; for discrete variables a Fisher exact $\chi^2$ test was applied (STATA software). To define high risk women from the general population, the $75^{th}$ percentile value of given continuous risk factors (body mass index, waist circumference, fasting glycemia, plasma lipids) was used as the cut off point.

Observed frequencies were similar to the frequencies published previously in other Caucasian populations (Boerma *et al.* 1999, Horan *et al*. 2006, Collings *et al*. 2007, Lanfear *et al*. 2007) and did not significantly differ between groups under study (Table 1).

The mean age of women from the general population, in women with type 2 and type 1 diabetes was 50.0±2.7, 48.9±7.3, and 36.9±10.2 years, respectively. The prevalence of smoking in women from general population, women with type 2 diabetes, and with type 1 diabetes was 49.9, 53.0, and 33.5 %, respectively. The mean duration of diabetes in women with type 2 and type 1 diabetes was 9.0±7.1 and 15.7±9.6 years, respectively. Prevalence of cardiovascular disease (ischemic heart disease, peripheral artery disease, and history of stroke) in women from the general population,

in women with type 2 diabetes, and in women with type 1 diabetes type was 5.5, 31.9, and 19.7 %, respectively.

As summarized in the Table 1, ABI significantly decreased from TT to CC carriers in type 1 diabetes (p= 0.008). A similar trend was observed in type 2 diabetes (p=0.050). Additionally, we analyzed data from general population with a focus on the women at high risk for insulin resistance ($75^{th}$ percentile cut point - fasting glycemia, body mass index, waist circumference and plasma lipids). In women with waist circumference above $75^{th}$ percentile, i.e. above 94 cm (n=208; TT/CT/CC: 10.1/46.6/43.3 %), a significant trend of decreasing ABI from TT to CC carriers was found (p=0.049).

In addition, in women who reported the presence of impaired fasting glycemia and at the same time had fasting glycemia above $75^{th}$ percentile, i.e. above 5.5 mmol/l, a similar trend of decreasing ABI from TT to CC carriers was observed (p=0.015). However, the number of these women was low (TT/ CT/ CC: 6/20/22).

No association between the Cx37 gene and other cardiovascular risk factors under study were found in women with type 1 diabetes and in women from the general population with increased waist circumference**.** In women with type 2 diabetes, we observed that age (TT, CT, and CC: 47.5±8.8, 47.5±7.4, and 50.2±6.9 years; p for trend = 0.049) and HDL cholesterol (TT, CT, and CC: 1.45±0.67, 1.32±0.42, and 1.56±0.57 mmol/l; p for trend = 0.050) increased from TT to CC carriers. In women with a waist circumference bellow $75^{th}$ percentile, waist circumference increased from TT to CC carriers (TT, CT, and CC: 79.4±7.5, 80.9±7.4, and 81.7±7.1 cm; p for trend = 0.046).

Based on our data, the gene for Cx37 was associated with preclinical atherosclerosis in women with type 1 and 2 diabetes and in women from the general population with advanced central obesity reflecting increased risk for insulin resistance. In all risk groups under study, the carriers of CC genotype had the lowest values of ABI. While this association was weak in women with advanced obesity and women with type 2 diabetes, a very strong association was found in women with type 1diabetes. This indicates a possible important role of hyperglycemia as a strong modifier of Cx37 gene effect on macrovascular disease which could play important role especially in type 1 diabetes. Therefore, further confirmation of the hypothesis regarding the association between Cx37 genotype and the extent of macrovascular disease could be important for future studies**.**

## Conflict of Interest

There is no conflict of interest.

## Acknowledgements

Supported by grant NR/9026-4/2006 (Internal Grant Agency of the Ministry of Health of the Czech Republic).

## References

BOERMA M, FORSBERG L, VAN ZEIJL L, MORGENSTERN R, DE FAIRE U, LEMNE C, ERLINGE D, THULIN T, HONG Y, COTGREAVE IA: A genetic polymorphism in Connexin 37 as a prognostic marker for atherosclerotic plaque development. *J Intern Med* **246:** 211-218, 1999.

BROCH M, RAMÍREZ R, AUGUET MT, ALCAIDE MJ, AGUILAR C, GARCIA-ESPANA A, RICHART C: Macrophages are novel sites of expression and regulation of retinol binding protein-4 (RBP4). *Physiol Res* **59**: 299-303, 2010.

BURNIER L, FONTANA P, ANGELILLO-SCHERRER A, KWAK BR: Intercellular communication in atherosclerosis. *Physiology (Bethesda)* **24**: 36-44, 2009.

COLLINGS A, ISLAM MS, JUONALA M, RONTU R, KÄHÖNEN M, HUTRI-KÄHÖNEN N, LAITINEN T, MARNIEMI J, VIIKARI JS, RAITAKARI OT, LEHTIMÄKI TJ: Associations between connexin37 gene polymorphism and markers of subclinical atherosclerosis: the Cardiovascular Risk in Young Finns study. *Atherosclerosis* **195:** 379-384, 2007.

HAN Y, XI S, ZHANG X, YAN C, YANG Y, KANG J: Association of connexin 37 gene polymorphisms with risk of coronary artery disease in northern Han Chinese. *Cardiology* **110:** 260-265, 2008.

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* **42:** 1429-1437, 2003.

HORAN PG, ALLEN AR, PATTERSON CC, SPENCE MS, McGLINCHEY PG, McKEOWN P: The connexin 37 gene polymorphism and coronary artery disease in Ireland. *Heart* **92**: 395-396, 2006.

LANFEAR DE, JONES PG, MARSH S, CRESCI S, SPERTUS JA, McLEOD HL: Connexin37 (GJA4) genotype predicts survival after an acute coronary syndrome. *Am Heart J* **154**: 561-566, 2007.

MILICEVIC Z, RAZ I, BEATTIE SD, CAMPAIGNE BN, SARWAT S, GROMNIAK E, KOWALSKA I, GALIC E, TAN M, HANEFELD M: Natural history of cardiovascular disease in patients with diabetes: role of hyperglycemia. *Diabetes Care* **31** (Suppl 2): S155-S160, 2008.

MILLER SA, DYKES DD, POLESKY HF: A simple salting out procedure for DNA extraction from human nucleated cells. *Nucleic Acid Res* **16:** 1215, 1988.

YAMADA Y, IZAWA H, ICHIHARA S, TAKATSU F, ISHIHARA H, HIRAYAMA H, SONE T, TANAKA M, YOKOTA M: Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. *N Engl J Med* **347:** 1916-1923, 2002.

YEH HI, CHOU Y, LIU HF, CHANG SC, TSAI CH: Connexin 37 gene polymorphism and coronary artery disease in Taiwan. *Int J Cardiol* **81**: 251-255, 2001.

WONG CW, CHRISTEN T, ROTH I, CHADJICHRISTOS CE, DEROUETTE JP, FOGLIA BF, CHANSON M, GOODENOUGH DA, KWAK BR: Connexin 37 protects against atherosclerosis by regulating monocyte adhesion. *Nat Med* **12:** 950-954, 2006.