

The role of connexin 37 gene polymorphism (1019C > T; Pro319Ser) in cardiovascular disease

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In spite of the strong prognostic value of all traditional cardiovascular risk factors, still striking differences exist in the prevalence of clinical events between patients at apparently similar risk. One of the main reasons is different genetic background. One of recently discussed candidate genes for cardiovascular disease is the gene for the protein Connexin 37 (Cx37). This protein is a part of gap junctions responsible for communications between cells including cells in the vessel wall. Studies focused on the association between Cx37 gene polymorphism (1019C > T; Pro319Ser) and cardiovascular disease demonstrate inconsistent results. Our findings in 1.316 men and women indicated that the Cx37 gene polymorphism (genotype CC) is significantly associated with acute coronary syndrome in non-smoking women. In addition, in urban and rural women from general population (n = 1.056) with impaired fasting glycaemia the same genotype is associated with increased intima media thickness of carotid arteries measured by ultrasound. Finally, in 289 women with diabetes type 1 or 2, and in 208 women from general population with central obesity, the CC genotype was associated with lower ankle brachial blood pressure index. These data indicate that Cx37 gene polymorphism could have gender- and smoking-dependent effects on acute coronary events and glucose dependent effect on atherosclerosis in women.

Keywords: connexin 37 gene polymorphism, atherosclerosis, acute coronary syndromes, women smoking, glycaemia.

Atherosclerosis and its main consequence – ischemic heart disease – is the leading cause of mortality in European countries [1]. The high incidence and prevalence of cardiovascular disease is associated with several main risk factors like age, male sex, genetic factors, smoking, dyslipidemia, hypertension and diabetes mellitus.

Assessment of cardiovascular risk in particular person is obtainable from the medical history, basic physical examination, and widely available blood tests. However, in spite of the strong prognostic value of all these factors, still striking differences exist in the prevalence of clinical events between patients at apparently similar risk. One of the main reasons is different

genetic background of particular persons at risk or patients with cardiovascular disease. Because atherosclerosis is a complex disease caused by the interplay between many genetic and environmental factors, identification of single genetic trait is not possible and the research is focused on many candidate genes.

Advances in molecular approach focused on genetic markers of cardiovascular disease allow better understanding of differences between individuals and could potentially offer more effective detection of individuals at risk and more effective therapeutic strategies to prevent life threatening clinical events. In addition, it could reveal mechanisms responsible for development of atherosclerosis and its clinical consequences. Many new candidate genes have been identified using geno-

me wide association studies and single nucleotide polymorphism studies [2–4].

Two extreme models of common disease development are suggested. Both apply for cardiovascular disease as well. One model is based on a large number of alleles, having minor effects, composing different genes interacting with each other and with the environment to cause cardiovascular disease (polygenic model). The second model is based on rather rare alleles, with major effect, composing a large number of genes which cause cardiovascular disease (monogenic model). In the case of atherosclerosis, it has been known, that this complex model involves a combination of both possibilities [5].

Because of the high number of candidate genes and putative high-risk gene polymorphisms, the identification of genetic variants that play a role in pathogenesis of CAD is difficult. One possibility for candidate gene identification is to determine their disadvantageous variations/polymorphisms. The effect of these variants is being assessed worldwide in on-going studies with different designs.

Many epidemiological studies have already been published. On one hand, these studies have proved the strong genetic determination of coronary events and their complications. On the other hand, results of these studies did not find unequivocal results.

There are several reasons for these discrepancies. One of the most important one is the fact that many genetic studies have had insufficient numbers of patients and controls – averaging between 100–300 individuals; the power of such a study is, therefore, low and the risk of false positive or false negative results is high. Another reason for these discrepancies is not sufficient clinical information about persons/patients under study including risk profile of given populations and factors that could modify effect of given gene variant – gene-environment interaction [6, 7].

One of recently discussed candidate genes for atherosclerosis and cardiovascular disease is the gene for the protein Connexin 37 (Cx37). This protein is a part of gap junctions responsible for communications between cells including cells in the vessel wall.

The possible role of gap junctions in atherosclerotic process is recently discussed [8, 9].

One of the key roles could be played by the altered communication between endothelial cells, vessel smooth muscle cells and macrophages. The protein Cx37 has been proposed to be one of the main participants in these inter cell communications. This protein is a member of Connexin protein family forming gap-junctions channels and hemichannels affecting distinctively permeability for various signaling molecules [10, 11].

The polymorphism (serin or proline at position 39) of this protein was evaluated as a possible risk factor for atherosclerosis and cardiovascular disease. Therefore, the Cx37 gene polymorphism (1019C > T; Pro319Ser) has attracted attention as a possible prognostic marker for atherosclerosis and cardiovascular events.

However, studies focused on association between this polymorphism and atherosclerosis/cardiovascular disease serve as a good example of inconsistent results found in most of studies focused on gene-disease associations.

In experimental settings TT genotype of the gene for Cx37 was indicated as proatherogenic, because it was associated with increased adhesion of macrophages to the vessel wall [12]. Nevertheless, controversial data were obtained from human studies. Even in the similar ethnic groups, different Cx37 genotypes were associated with cardiovascular disease. In Taiwan population CC genotype was associated with coronary artery disease [13], in contrast, in Japanese population TT genotype was associated with coronary artery disease [14]. In Caucasian population from Switzerland, CC genotype was associated with myocardial infarction [15], in contrast, in population from Sicily the TT genotype was suggested as a risk factor [16]. Furthermore, no association was found between Cx37 genotype and coronary artery disease in Ireland [17]. Just recently, study from Taiwan found T allele of Cx37 gene polymorphism to be associated with subclinical atherosclerosis (expressed as carotid intima-media thickening measured by ultrasound) and subsequent ischemic stroke [18].

In our population based study with almost two thousand participants, we did not find an association between Cx37 (and other gene) polymorphism(s) and coronary events [19].

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 [23]

Connexin 37 gene	Diabetes type 1	Diabetes Type 2	General population, waist > 94 cm	General population, waist < 94 cm
TT/CT/CC, n %	18/79/81 10.1/44.4/45.5	13/45/53 11.7/40.6/47.7	21/97/90 10.1/46.6/43.3	80/300/274 12.2/45.9/41.9
TT, mean (SD)	1.07 (0.12)	1.00 (0.06)	1.05 (0.11)	1.06 (0.09)
CT, mean (SD)	1.01 (0.11)	0.99 (0.13)	1.06 (0.09)	1.05 (0.09)
CC, mean (SD)	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

All these discrepancies could be caused by different patient and control populations with different background of the main cardiovascular risk factors. In addition, the weakness of studying the association between genes and clinical events could be in already present subclinical disease in control population.

Therefore, from this point of view more reliable approach could be achieved via detection of subclinical atherosclerosis. However, even in this field of research no consistent findings are available. Using ultrasound measurements of carotid plaques in hypertensive men from Sweden, an association of TT alleles of Cx37 gene polymorphism with subclinical atherosclerosis was described already in 1999 [20]. In contrast, no association between Cx37 gene polymorphism and subclinical atherosclerosis expressed as carotid intima-media thickness and flow-mediated dilatation of brachial arteries was found in a recent large and population based study from Finland [21].

The complexity of the association between particular gene and (sub)clinical atherosclerosis could be explained by the role of particular gene as a modifier of the disease process, even when not being in direct causal relationship to it. From this point of view, interesting association was found in another study from Finland which found the Cx37 gene polymorphism to be modifier of the effect of smoking and homocyst(e)in on endothelial dysfunction [22].

In our recent studies we evaluated the association between Cx37 gene polymorphism and preclinical atherosclerosis expressed as ankle brachial blood pressure index (ABI) or intima media thickness of carotid arteries measured by ultrasound.

The study with ABI and the Cx37 gene was performed in women with type 1 ($n = 178$) and 2 ($n = 111$)

diabetes, and in women from general population ($n = 862$) [23]. Observed frequencies of carriers of TT/CT/CC Cx37 genotypes were similar to the frequencies published previously in other Caucasian populations [20, 22] and did not significantly differ between groups under study.

As shown in Table 1, in women with type 1 diabetes, ABI significantly decreased from TT to CC carriers. A similar trend was seen in women with type 2 diabetes ($p = 0.050$) and in women with waist circumference above 75th percentile (94 cm; $n = 208$) of the general population ($p = 0.049$). Based on these results, the Cx37 gene polymorphism 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.

The possibility of modifying effect of impaired glucose metabolism was confirmed in another population study focused on intima-media thickness of common carotids arteries measured by ultrasound (unpublished data – accepted for publication to International Angiology in 2010).

A 5 % population sample of urban women aged 45–54 years ($n = 892$) and a 1 % representative sample of rural women aged 33–72 years ($n = 160$) were examined. These women were examined using an identical protocol and genotyped for Cx37 gene polymorphism. The association between the Cx37 gene polymorphism and intima-media thickness in common carotid arteries measured by ultrasound was studied. Between groups comparison of continuous variables was calculated using Mann Whitney t -test. For discrete variables Pearson χ^2 test was applied, or Yates χ^2 test in the case of low numbers in analyzed subgroups. All results were considered to be statistically significant at $p < 0.05$.

Table 2

Distribution of connexin 37 gene polymorphism (1019C > T; Pro319Ser) among representative population sample and among men and women with acute coronary syndrome (ACS) in men and women with regard to smoking behavior

Carriers	General population, men + women MONICA, n = 2,559, %	General population, women from Prague, n = 898, %	ACS, men+ women, n = 1,316, %	ACS, women smokers, n = 248, %	ACS, women non-smokers, n = 116, %
TT	9.5	11.3	9.4	14.2	6.9*
CT	44.0	46.0	41.1	43.2	31.9*
CC	46.5	42.8	49.5	42.6	61.2*

χ^2 test p = 0.003: women with ACS non- smokers vs. women with ACS smokers.

The threshold for fasting glycaemia was defined as 75. percentile of values obtained in urban middle aged women (5.5 mmol/l).

In both groups of women with fasting glycaemia higher than 5.5 mmol/l, we observed a significant increase in carotid intima-media thickness from the TT to CC genotypes, while no such trend was found in those with fasting glycaemia less than 5.5 mmol/l. Again, in those women with fasting glycaemia higher than 5.5 mmol/l, T allele seemed to be protective against subclinical atherosclerosis in common carotid arteries. Therefore, the Cx37 gene polymorphism may exert completely different effects in the artery wall, depending on glycaemia. These differences were detected in both groups of urban and rural women and also when both groups were merged. These results were not altered when age, urban/rural origin and when traditional risk factors were included into the analysis. No such association was found in men from the same population (data not published).

This relation of gene effect to the particular risk factor could explain different results obtained in populations at different cardiovascular risk. The limitation of all these studies was their cross-sectional design, not allowing us to detect reliably cause-and-effect relationships and low number of participants in selected subgroups under study (smokers, higher fasting glycaemia). On the other hand, possible role of glycaemia in gene expression was already confirmed in the literature [24].

In our already mentioned population based study focused on patients with acute coronary syndromes [19] in subsequent subanalysis we observed trend suggesting possible association between Cx37 gene polymorphism and acute coronary events in non-

smoking women (Table 2). However, because of rather low number of participants in subgroups under study these data need to be confirmed in studies with higher number of participants.

In all studies the Cx37 gene data were obtained by the same methodology. DNA was isolated from frozen EDTA blood [25]. To genotype the 1019C > T (Pro319 > Ser) variant within Cx37 gene, oppositely-oriented oligonucleotides 5' CTGGACCCACCCCCTCAGAA-TGGCCAAAGA and 5' AGGAAGCCGTAGTGCCT-GGTGG and restriction enzyme *AasI* («Fermentas, Lithuania») were used to distinguish the T (fragments of 240 and 35 bp) and C (275 bp) alleles. A set of 24 samples was analysed three times within 3 weeks with 100 % conformity.

Conclusion. Using genetic markers in cardiovascular research is producing new insights concerning the etiology of cardiovascular disease. Recent genome-wide association studies demonstrate clear associations between single nucleotide polymorphisms and important cardiovascular phenotypes. However, risk alleles for the single nucleotide polymorphisms in question do not explain a sufficient portion of individual risk to be useful for screening purposes [26].

Genome-wide association studies, and to certain extent also single gene polymorphism studies could be valuable tool to detect processes and gene-environment interactions. The Cx37 gene polymorphism could be, therefore, one of genetic markers fulfilling criteria for gene-environment interaction including gender oriented approach. Studying this gene including its expression and phenotype could be rewarding in evaluating the biology of the vessel wall and atherosclerosis.

Our data indicated Cx37 gene polymorphism could have gender- and smoking-dependent effects on acute

coronary events and glucose dependent effect on earlier stages of atherosclerosis in women. To confirm, if these results could be generalized, larger studies from different populations with respect to their risk status are needed.

Acknowledgements. The work was supported by institutional finance support from the Institute for Clinical and Experimental Medicine (MZO 00023001), by research grant 1M0510 (Ministry of Education, Youth and Sports of the Czech Republic), and by Regional Cooperation for Health, Science and Technology (RECOOP HST) Consortium.

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Роль поліморфізму гена конексину 37 (1019C > T; Pro319Ser) у серцево-судинних захворюваннях

Резюме

Незважаючи на однозначність прогностичного значення усіх традиційних факторів ризику серцево-судинних захворювань, існують значні розбіжності у їхніх клінічних проявах у різних пацієнтів. Однією з основних причин цього є неоднаковий генетичний фон. З-поміж недавно обговорюваних генів-кандидатів, які впливають на серцево-судинні захворювання, обрано ген білка конексину 37 (Cx37). Цей білок відповідає за міжклітинні взаємодії, включаючи клітини стінок кровоносних судин. Дослідження, зосереджені на пошуку зв'язку між поліморфізмом гена конексину 37 (1019C > T; Pro319Ser) та серцево-судинними захворюваннями, показують суперечливі результати. Наші дослідження 1316 чоловіків і жінок демонструють, що поліморфізм гена Cx37 (генотип CC) значною мірою пов'язаний з гострим коронарним синдромом у жінок, які не курять. Крім того, у міських і сільських жінок із загальної популяції (n = 1056) з порушеною глікемією цей же генотип пов'язаний із збільшенням товщини інтими сонних артерій, виміряної ультразвуком. У 289 жінок з діабетом 1-го або 2-го типу і у 208 жінок із загальної популяції з ожирінням генотип CC виявився зв'язаний з нижчим щиколотко-плечовим індексом тиску крові. Такі дані показують, що стать і куріння діють на гострі коронарні прояви, обумовлені поліморфізмом гена Cx37, для жінок виявлено також глюкозозалежний вплив на атеросклероз.

Ключові слова: поліморфізм гена конексину 37, атеросклероз, гострий коронарний синдром, куріння жінок, глікемія.

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Роль поліморфізма гена конексина 37 (1019C > T; Pro319Ser) в серцево-судинних захворюваннях

Резюме

Несмотря на однозначность прогностического значения всех традиционных факторов риска сердечно-сосудистых заболеваний, по-прежнему существуют значительные отличия в их клинических проявлениях у разных пациентов. Одной из основных причин этого является неодинаковый генетический фон. Среди недавно обсуждаемых генов-кандидатов, влияющих на

сердечно-сосудистые заболевания, выбран ген белка коннексина 37 (Cx37). Этот белок ответствен за межклеточные взаимодействия, включая клетки стенок кровеносных сосудов. Исследования, сосредоточенные на поисках связи между Cx37 полиморфизмом гена (1019C > T; Pro319Ser) и сердечно-сосудистыми заболеваниями, показывают противоречивые результаты. Наши исследования 1316 мужчин и женщин демонстрируют, что полиморфизм гена Cx37 (генотип CC) в значительной степени связан с острым коронарным синдромом у не-курящих женщин. Кроме того, у городских и сельских женщин из общей популяции (n = 1056) с нарушенной гликемией тот же генотип связан с увеличением толщины интими сонных артерий, измеренной ультразвуком. У 289 женщин с диабетом 1-го или 2-го типа и у 208 женщин из общей популяции с ожирением генотип CC оказался связанным с более низким лодыжечно-плечевым индексом давления крови. Эти данные показывают, что пол и курение действуют на острые коронарные проявления, обусловленные полиморфизмом гена Cx37, для женщин выявлен также глюкозозависимое влияние на атеросклероз.

Ключевые слова: полиморфизма гена коннексина 37, атеросклероз, острый коронарный синдром, курение женщин, гликемия.

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UDC 616.1 + 577.21
Received 10.01.11