Genetican Alysis of the Role of Connexin 37 Polymorphism in Spontaneous Abortion

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Abstract

Introduction: Among unique cardiovascular risk factors in women are complications during pregnancy, including miscarriage. Important risk factor is also genetic background. One of powerful candidate genes for cardiovascular disease of atherosclerotic origin (aCVD) is gene for connexin 37 (Cx37) with strong gene-environment interaction including smoking status, that is also strong risk factor for complications in pregnancy including spontaneous abortion (SA). **Material and Methods:** We analysed association between SA and Cx37 gene polymorphism (1019C>T; Pro319Ser) in 547 foetuses and its potential interaction with smoking status of mothers. Using genetic analyses from women from general population as controls, ORs for T allele, found in our previous studies to be protective against a CVD, were calculated. This study was a secondary analysis of a randomized controlled trial. **Results and Conclusion:** T allele carriers (foetuses), had OR 0.91 (95 % CI 0.72-1.14) and no interaction with smoking was observed. In conclusion, no significant association between Cx37 polymorphism and SA was observed and no modifying effect of smoking status on this association was detected.

Keywords: *Connexin 37 gene, Gap junctions, Spontaneous abortion, Protective factor, Smoking, Cardiovascular disease, Candidate gene. aCVD: Cardiovascular disease of atherosclerotic origin, CI: Confidence interval, Cx37: Connexin 37, DNA: Deoxyribonucleic acid, OR: Odds ratio, SA: Spontaneous abortion, VEGF: Vascular endothelial growth factor*

Introduction

Regarding cardiovascular disease of atherosclerotic origin (aCVD) the annual incidence of cardiovascular diseases is age- dependently increasing both in men and women. The prevalence is higher in men until midlife[1]. However, women are affected similarly or more than men in older age. Despite the main cardiovascular factors are shared by both sexes, several unique cardiovascular factors were described in women as is miscarriage and other complications during pregnancy and metabolic disorders as gestational diabetes mellitus[2]. Therefore, detection of determinants of atherosclerotic disease in women including metabolic ones may help to identify and to prevent atherosclerotic process and its clinical complications. However, also better understanding of the complications in pregnancy might have similar preventative effect especially when accompanied by genetic analyses. Indeed, genetic studies could help to identify not only inherited causes of early pregnancy loss, extremely serious and emotional problem both for affected women, their partners and their physicians, but also for aCVD. One of powerful candidate genes for aCVD is gene for connexin 37 (C1019>T) (Cx37) with

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strong gene- environment interaction including smoking status. Connexins are the main proteins in gap junctions. Connexin 37 (Gap junction protein alpha; OMIM ID 121012; Cx37) is the main gap junction representative in the vessel wall

expressed in endothelial cells, smooth muscle cells but also in monocytes, therefore in the main cells involved in the process of atherosclerosis. Gene polymorphism-association studies have detected a link between the C1019>T single nucleotide polymorphism (SNP) in the human Cx37 gene and its protective role in cardiovascular disease[3]. This polymorphism results in a non-conservative amino acid change in the regulatory C-terminus of the Cx37 protein (P319>S). It was recently demonstrated that Cx37 hemichannels control the initiation of atherosclerotic plaque development by regulating ATP-dependent monocyte adhesion.

In addition to cells responsible for atherosclerosis and aCVD, Cx37 is also present in ovary, in which gap junctions mediate metabolic cooperation between granulosa cells and oocyte. These interactions are mediated not only by paracrine factors but also by gap junctions including Cx37. Preimplantation embryos express multiple connexins and assemble them into gap junctions.

The suppression of connexin expression seems to play an important role in embryo implantation in several species. The gap junction induction is one of the earliest known signs for a blastocyst-derived signal, which may be involved in preparing the endometrium for implantation[4]. It could help to regulate the invasion process by a coordinated cell death of the endometrial cells replaced by the invading trophoblast. However, despite the importance of the different connexin channels expressed in the endometrium this topic was not evaluated[5].

Based on these facts, we analyzed data from fetuses from women with spontaneous abortion (SA) for Cx37 gene polymorphism (1019C>T; Pro319Ser) including its potential interaction

Material and Methods

This study was a secondary analysis of a randomized controlled trial. Based on these facts, we analyzed data from fetuses from women with spontaneous abortion (SA) for Cx37 gene polymorphism (1019C>T; Pro319Ser) including its potential interaction with smoking, frequent risk factor for early pregnancy loss. In particular, we analysed if T allele of Cx37 gene, found to be protective against ischemic heart disease in our previous studies, could be protective also against SA and if this protection could be modified by smoking status[6].

Women with verified diagnosis of SA participated in the study after giving written informed consent. The study was approved by the Institutional ethics committees and was conducted according to the Good Clinical Practice guidelines. Participating women filled in questionnaire focused on history of SA and physical and ultrasound examination were performed[7].

Inclusion Criteria: The healthy pregnant women who were willing to participate in the study were included after obtaining informed consent.

Exclusion Criteria: Severe blood pressure, heart disease, pregnancy- induced hypertension patients were excluded

Study Design: randomized controlled trial

Statistical Analysis

In women with spontaneous abortion 5.6 % were smokers/past smokers (mean age 27.2±6.5 years) and 94.4 % were non-smokers (mean age 32.3±4.6 years) (difference for age, $p<0.001$). In the population sample 44.8 % women were smokers/past smokers (mean age 57±2.7 years)and 55.2 % non-smokers (mean age 57±2.7 years)[8].

Cx37 T allele was present in 55.2 % (mean age 32.1±4.7 years) of aborted foetuses, and in 57.5 % (mean age 57±2.7 years) of women from general population. In smoking women at least one T allele was present in general population[9]. In non- smoking women at least one T allele was present in 55.1 % of the foetuses, and in53.4

% of the foetuses, and in 56.1 % of women from 59.0 % of women from general population. We compared distribution of Cx37 gene polymorphism between foetuses from women older than 32 years with women younger than 32 years (median age). No difference in the prevalence of T allele was detected (56.9 % vs. 54.2 %, p=0.294). We also tested difference in prevalence of T allele between foetuses of women with one SA (n=448) and foetuses of women with more than 1 SA (n=115), but also in this case no significant difference was detected (54.3 % vs.

60.0 %, p=0.161)[10]. The only difference found was that women with more than one SA were older than women with one SA (34.1±4.7 vs. 31.6±4.8 years, p<0.001)[11].

Results

Using general population as a control sample, ORs for T allele carriers were calculated for the whole populations under study and for smoking and non-smoking women including similar stratification to smokers/past smokers and non-smokers as in the control group[12]. Foetuses from women suffering from SA with T allele have OR 0.91 (95 % CI 0.72-1.14) and there was no interaction with smoking status of mothers, OR 0.84 (0.64-1.11) for non-smokers vs. OR 0.89 (0.42-1.9) for smokers (Table 1)[13].

Table 1. Cx37 polymorphism in foetuses and population control. Smoking status in foetusescorrespond with this characteristics in mothers.

Entiregroups						
Cx37		**Controls**		**Abortions**	**OR**	**P**
	N	%	N	%		
CC	281	42.5	239	44.8	1.00	
CT	300	45.3	239	44.8	0.93(0.73-1.19)	0.28
TT	81	12.2	55	10.4	0.80(0.54-1.18)	0.25
+T	381	57.5	294	55.2	0.91(0.72-1.14)	0.41
Smokers/past smokers						
Cx37		**Controls**		**Abortions**	**OR**	**P**
N		%	N	%		
CC	126	43.9	14	46.7	1.00	
CT	121	42.2	14	46.7	1.04(0.48-2.28)	0.92
TT	40	13.9	2	6.7	0.45(0.10-2.07)	0.29
+T	161	56.1	16	53.4	0.89(0.42-1.90)	0.77
Neversmokers						
Cx37		**Controls**		**Abortions**	**OR**	**P**
N		%	N	%		
CC	144	40.7	232	44.9	1.00	
CT	172	48.6	231	44.7	0.83(0.63-1.11)	0.21
TT	38	10.4	54	10.4	0.88(0.55-1.40)	0.28
+T	210	59.0	285	55.1	0.84(0.64-1.11)	0.22

In summary, in our study we found no significant differences in Cx37 gene variability between fetuses analysed after SA and general population of middle aged women[14]. These results were not modified by smoking status. Therefore, in contrast to our previous findings in women with acute coronary syndrome Tallele does not seem to be neither protective against SA, nor its effect is strongly modified by smoking status. Therefore, mechanisms leading to cardiovascular disease and complications during pregnancy including SA seem to be different regarding mechanism mediated by gap junctions involved in differentiation processes by mediating exchanges between mother and foetus cells, affecting the maternofetal blood flow interrelationships, trophoblast invasiveness and the formation of a syncytiotrophoblast[15]. Expression of some connexins, among them also Cx37, has been shown to reflect maturity of luteinized follicles in animal models and Cx43 expression was associated with better prognosis in in vitro fertilization[16]. Transcription factors of several connexins were detected in human embryos, e.g. Cx43 was indicated in all embryonic developing stages [17]. The expression of Cx43, together with VEGF, is significantly reduced in chorionic villi and decidua in women with spontaneous abortion. This may be caused by the influence on angiogenesis of placenta and developing embryo[18]. It was reported, that mouse embryos cultured with some gap junctions' inhibitors presented frequent collapses and developmental delay. Functional and structural abnormalities of Cx43 might also play an important role in heart diseases. Because of this finding and possible similarity with Cx43, we focused on Cx37 polymorphism on spontaneous abortion. Inaddition, Cx37 polymorphism is also supposed to play important role in cardiovascular events. Nevertheless, we did not found an association in the case of Cx37 gene as for other studies describing different connexins polymorphisms and spontaneous abortion [19].

Regarding the (absence of) effect of smoking in our study, the idea was that adhesion properties of vessels wall caused by Cx37 gene polymorphism could favour not only macrophage accumulation in the atherosclerotic lesions but also endothelial dysfunction induced by smoking initiated by reduced nitric oxide bioavailability and further by the increased expression of adhesion molecules Therefore protective effect of the Cx37 T allele might be strongly modified by smoking in atherosclerosis. But according to our data the deleterious effect of smoking on pregnancy, particularly on SA seems not to be mediated through Cx37 gene[20].

The limitations of our study are incomplete data in women with history of SA and focus only on one polymorphism of one gene from rather large connexin family. On the other hand, the strengths of the study include high number of studied women and numerous representative control group from the population sample in Prague, which could reflect real population background for our findings in contrast to cases and controls usually used for such comparisons. In addition, selecting only Cx37 gene was based on knowledge, that connexin 37 is on one hand the main protein of gap junctions in cells involved in the process of atherosclerosis, but on the other hand it is also present in maternofetal organs[21]. Moreover, data indicated that the strongest protective effect of the Cx37 T allele was detected in the non- smoking patients without diabetes mellitus and hypertension and that effect could be mediated through stem cells[22]. Therefore, we have chosen Cx37 polymorphism as genepotentially covering both cardiovascular and pregnancy complications[23]. Another limitation is the lack of data from mothers/ fathers and absence of information regarding other potential modifying factors including diabetes mellitus; however, women with these characteristics were excluded to detect only the effect of this particular gene variability and smoking population, women (after exclusion of diabetics) with the history of one or more SA (n=51) were compared to women without history of SA with history of pregnancy (n=233) of similar age, no difference in the presence of T allele was observed (66.7 % vs. 60.1 %; p=0.67[24].

Conclusion

In summary, to the best of our knowledge, only a

few studies have demonstrated effect of connexins on spontaneous abortion and no studies analysed parallel effect of particular connexin gene polymorphism on cardiovascular disease and SA[25]. In our study, no effect of Cx37 polymorphism measured in foetuses was observed on abortion and no interaction with smoking status on this association was proved.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: It was given by the institution.

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