Significance of genetic polymorphism investigations in pregnancy complications

AGNIESZKA SEREMAK-MROZIKIEWICZ

Abstract

Rapid and extensive development of molecular biology methods have big impact on obstetrics and gynecology. There have been revealed numerous gene polymorphisms which have more significant impact on some pregnancy complications and gynecological diseases. It is also suggested that some genetic variants may be the markers of increased risk of several diseases in pregnant women as well as in gynecologic disturbances. Special attention in this review has been devoted to polymorphism that could have impact to develop personalized medicine application in obstetrics. It is thought that in many obstetrical complications very important is over activity of intravascular coagulation process in utero-placental unit enhanced in women with genetically conditioned thrombophilia. These are recurrent miscarriages, unexplained intrauterine fetal death, preeclampsia, eclampsia, fetal hypotrophy and preterm placental ablation. The importance of genetic polymorphisms has been also revealed in the etiology of preterm delivery, intraamniotic infection and preterm premature rupture of membranes. There is expected that in the future the development of medicine based on individual genetic prevention and diagnostics will be possible. Among others detection and understanding of function of several DNA polymorphic sites has impact on better understanding of etiology and give promises of individualization therapy in several obstetrical diseases.

Key words: gene polymorphisms, genetic analysis, pregnancy complications

Introduction

The rapid accumulation of advanced knowledge in genetics and molecular biology as well as fast progress in diagnostic methods enabled to distinguish a new research field called molecular medicine. Nowadays molecular biology methods are the core applications used in investigations concerning etiology of numerous complex human diseases and contributes to gene therapy development. A key method used in that kind of diagnostics are polymerase chain reaction (PCR) – based applications which are characterized by much higher sensitivity and specificity than classical diagnostics [1].

Many issues of molecular biology consider problems from obstetrical and gynecological field. There have been revealed numerous gene polymorphisms which are favorable to clear some pregnancy complications and gynecological diseases [1, 2].

It is also suggested that some genetic variants may be the markers of increased risk of several diseases in pregnant women as well as in gynecologic disturbances. Typical example of the practice of molecular biology methods are investigations on the osteoporosis field. Nowadays studies show that osteoporosis is a multifactorial disease with significant genetic contribution. Indeed, researches concerning candidate genes of osteoporosis and osteopenia in women in peri- and postmenopausal age have been performed. Most frequently considered gene polymorphisms are vitamin D receptor gene (*Bsm*I, *Apa*I, *Taq*I, *Fok*I), estrogen receptor – alpha (*Pvu* II, *Xba* I, (TA)n), calcitonine receptor (*Alu*I) and collagen I alpha 1 (Sp 1) [3].

Similarly, in case of studies on breast cancer many papers covered mutations in suppressor genes *BRCA1, BRCA2, TP53*, proto-oncogenes *Ki-ras* and *Ha-ras*, gene polymorphisms of estrogen receptor (*Pvu* II, *Xba* I) and progesterone receptor (*Taq*I) and are helpful to clarify the etiology and possible therapeutic targets [4].

There are also been held intensive researches on gene polymorphisms of enzymes involved in metabolism of drugs and some hormones including estrogens. These observations applies first of all to genes of P-450 cytochrome family (CYP1A1, CYP1B1, CYP2E1, CYP19, CYP17) and are important step in therapy individualization [5].

Above examples shows how comprehensive is the usage of molecular biology in gynecology. The following part of this paper summarizes an evidence for similar importance of molecular biology investigations in obstetrics.

Inherited thrombophilia

Current studies found that increased activity of intravascular coagulation process in utero-placental unit is

Division of Perinatology and Women's Diseases, Poznań University of Medical Sciences, Poznań, Poland

enhanced in women with inherited thrombophilia. The main causes of this condition are inherited polymorphisms of genes coding for factor V (FV) and factor II (prothrombin, PTM) in the coagulation cascade, as well as 5,10-methylenetetrahydrofoliate reductase (MTHFR) gene mutation (*677C>T*). The rare, but also serious for etiology are inherited protein C and S deficiency, and anythrombin III deficiency [2].

The most frequently investigated polymorphism of factor V (FV) is Leiden variant – presence of single point mutation with a replacement of guanine to adenine (*1691G>A*, FVL) that causes the amino acid exchange in protein chain (*Arg506Glu*) of FV molecule connected with heritable predisposition to activated protein C (APC) resistance [6]. The next one of prothrombotic polymorphism is situated in prothrombin gene (PTM) and is the transition of guanine to adenine at the 20210 position (*20210G>A*) in 3'-untranslated region. The presence of mutated *20210A PTM* allele is related with longer mRNA stabilization and increased prothrombin concentration in serum [7].

The above mentioned genetic variants lead to exacerbation of the coagulation cascade activity and have clinical consequences. Moreover, impact of thrombotic risk factors might be intensified by pathophysiological processes and circumferential factors. The carriage of several thrombophilic mutations is connected with augmented risk of gestational complications and extensively influence perinatal result. The adverse outcome of pregnancy (recurrent miscarriages, fetal hypotrophy, placenta abruption, unexplained intrauterine fetal death, and susceptibility to develop preeclampsia/eclampsia) could be notably linked with presence of thrombotic polymorphic inherited variants which act as single risk factor or, moreover could collaborate together [8-10]. Separate problem of pregnant women with thrombophilia is venous thromboembolism [11].

On the other hand MTHFR is an enzyme involved in methionine and homocysteine metabolism. The *677C>T* polymorphism is well-known and results the amino acid exchange (*Ala222Val*) in catalytic domain of enzyme. This variant decreases activity of enzyme that *677TT* genotype carriers have only about 30% of normal enzyme activity. It results in significantly increased homocysteine concentration, decreased folate level in plasma and in erythrocytes. Negative effects of folate insufficiency and homocysteine accumulation on female reproductive function are extensively discussed [8, 12] as a reason of endothelial cells damage, depressed nitric oxide and thrombomodulin synthesis, induction of oxidative stress and inflammatory response. The possible suggested mechanism of pathologic changes also is induction of coagulation cascade in placental vessels, failure in fibrinolysis and clots forming. There are the signs of thrombosis in spiral arteries and fibrin deposited in intravillous space, infarctions of placental villous followed by utero-placental insufficiency. It is noteworthy that in placental changes fetal and maternal coagulation system may be involved [9, 10, 12].

Recurrent miscarriages

Studies of last years revealed that in women with recurrent miscarriages inherited thrombophilia is often present [11]. Most researches concern thrombophilia and miscarriages in second and third trimester of pregnancy, which are probably caused by activation of coagulation system in placental vessels. Numerous studies focused on importance of factor V Leiden showing high frequency in women with recurrent miscarriages from 8 to 30% of cases and even 2-5 fold higher risk of recurrent miscarriages [8, 9, 13].

In presence of PTM mutation it was suggested 2-3 fold increase risk of miscarriages in women carriers of PTM mutation. These observations concern early and late recurrent miscarriages and late non-recurrent miscarriages [14]. The frequency of PTM in general population is 1-3% and in patients with venous thromboembolism frequency is much higher – 6-18%. PTM is related to 2-5 fold increase of thrombosis occurrence [9]. It is thought that PTM leads to forming of clots in arterial and venous blood vessels, moreover increased prothrombin concentration is correlated with failed placental function [9-11].

Currently it is suggested that hyperhomocysteinemia conditioned by *677C>T MTHFR* polymorphism is an independent risk factor of first trimester recurrent miscarriages. The *1298A>C* and *1793G>A* are other investigated polymorphisms of *MTHFR* gene that may be also related to the risk of recurrent miscarriages [15-17].

One of the biggest summary concerning miscarriages is meta-analysis performed by Rey et al. (3000 women with recurrent miscarriages). The authors indicated that early miscarriages in the first trimester of pregnancy are strictly correlated with the occurrence of factor V Leiden, resistance to active C protein and *G20210A* prothrombin gene polymorphism. Recurrent miscarriages in the second trimester seemed to be more correlated with factor V Leiden than miscarriages in the early stages of pregnancy. As to the late non-recurrent miscarriages there were correlation with factor V Leiden,

prothrombin gene mutations and protein S deficiency. However, this analysis did not reveal any significant meaning of *677TT* genotype of *MTHFR* gene in the etiology of miscarriages [13].

Studies of last years have been also focused on the significance of the factor VII activity changes in the etiology of recurrent miscarriages. The majority genetic variants is connected with a factor VII deficiency being a cause of exaggerated bleedings at patients. However, a few polymorphisms influencing the activity and the concentration of the factor VII in the serum which increase the risk of thrombotic changes occurrence. In consequence the occurrence of recurrent miscarriages have been described. The greatest significance is being assigned to the functional polymorphism causing the exchange of the amino acid of arginine for the glutamine in position 353 of the factor VII protein chain (*Arg 353Gln, R353Q*) [18].

The researches on recurrent miscarriages and genetically conditioned thrombotic disorders could improve the knowledge about etiology of these disorders. Nevertheless most important problem is inclusion of thromboprophylaxis with the usage of low-molecular heparin in women carriers of thrombotic polymorphism. The clear situation is connected with prophylaxis and treatment in pregnant women with venous thrombosis or with previous personal history in this direction. The elucidation and development of guidelines are required in women with recurrent miscarriages. Despite the current recommendation concerning the prophylaxis in women with recurrent miscarriages are very careful the final decision to include thromboprophylaxis belongs to the obstetrician and depends of clinical conditions in pregnant women [1, 2].

Preeclampsia

Nowadays the analysis of candidate genes is one of the study way of genetic background of etiology in preeclampsia (PE). The candidate gene is a gene of documented biological activity involved in the PE pathways, with polymorphic activity. This process is one of the condition of inter-individual variability. At present there are more than sixty candidate genes to PE development. It was distinguished some groups of candidate genes in PE pathophysiology [19, 20]. The most frequently investigated genes encoding the factors from rennin-angiotensin system (angiotensinogen, angiotensin converting enzyme, angiotensin receptor type II), from thrombophilia (factor V Leiden, prothrombin, MTHFR), genes including in endothelin system (endothelin 1, endothelial nitric oxide synthase), oxidative stress (xantine oxidase and superoxide dismutase) and several genes coding for cytokines (interleukin 6, tumor necrosis factor-alpha) [19, 21].

Disturbances of endothelin system are thought to be very important factor of preeclampsia development. Endothelin gene polymorphisms influence protein concentration and indirectly – the preeclampsia risk. It was suggested that endothelin 1 concentration in plasma and amniotic fluid is significantly higher in women with preeclampsia. Recently attention has also been paid to genetic variants of endothelin-1 (ET-1) and endothelin-1 converting enzyme (ECE-1). These are for example *Lys-198Asn* (ET-1 gene) and *Thr341Ile* (ECE-1 gene). There was investigated genetic variability of endothelin-1 system in gestational hypertension and preeclampsia. The authors revealed lack of direct correlation of mentioned polymorphisms and the risk of preeclampsia in the population of Polish women. But the results of this study suggest protective role of coexistence of genotypes *ECE-1 CT / ET-1 GT* of *Thr341Ile ECE-1* and *Lys198Asn ET-1* polymorphisms lowering the risk of preeclampsia development [22].

Many studies focused on correlation of preeclampsia and inherited thrombophilia. Most of the performed analysis suggest 2-9 fold higher risk of preeclampsia at carriers of factor V Leiden. It is shown in many large meta-analysis that factor V Leiden is significantly linked with preeclampsia appearance [23-25]. Researches on polymorphisms of MTHFR and prothrombin genes and protein C or S deficiency revealed positive contribution, as well as negative correlation with preeclampsia etiology [26].

It is also suggested the interaction of genetic, epigenetic and environmental factors in PE etiology. Many attention was paid on epigenetic marks that at present seems to help to decipher the pathophysiological processes in PE [27, 28]. Otherwise, in complex human diseases such as PE genome wide scanning is method often used to achieve the desired results on disease susceptibility. Genome-wide scanning, large study of genetic variations detects the genetic loci contributing of susceptibility to complex human diseases and identifies susceptibility loci for development of disease [29].

Preterm delivery, intra-amniotic infection, preterm premature rupture of membranes

Most commonly genetic analysis in relation to preterm delivery concerns polymorphisms of genes involved in inflammatory response. The most frequent studied

are genetic variants of cytokines involved in preterm premature rupture of membranes and intraamniotic infection – a well-known risk factors of preterm delivery. Most often analyzed cytokines are interleukin (IL) 1, 4, 6, 10 and tumor necrosis factor alpha (TNF-alpha). These substances were shown to play an essential role in the numerous immune mechanisms, augment the level of acute phase proteins and act synergistically to induce inflammatory reactions [30, 31]. These factors are increased in amniotic fluid and plasma of women with preterm delivery and intraamniotic infection. The genetic polymorphisms could induce the differences in concentration and activity of cytokine proteins. There was concluded that maternal cytokine gene polymorphisms could be linked with severity of IAI because of certain genetic variants are connected with enlarged production of proinflammatory cytokines or with decreased production of anti-inflammatory factors, thereby possibly promoting IAI [30-32].

In the promoter region of TNF-alpha gene there have been described several polymorphisms, of which *–238G>A, –308G>A, –376G>A* and *–863G>A* may be involved in the etiology of preterm delivery. These variants probably interfere the TNF-alpha gene transcription. For instance, there have been noticed a positive correlation of *–308G>A* polymorphism with a preterm delivery preceded by bacterial vaginosis and preterm premature rupture of membranes [33]. Most commonly investigated polymorphisms of IL-6 gene in relation to preterm delivery etiology are *–174G>C, –572G>C* and *–597G>A* [34]. But so far, the results have not clearly confirmed correlation of these genetic variants with direct risk of preterm delivery.

Toll-like receptors (TLR) -2 and -4 are a part of basic defensive mechanism against bacterial infection recognizing microbial products and increasing immune response of host organism. TLR influence the activation of nuclear factor kappa-B, which indirectly regulates expression of genes encoding cyclooxygenase-2 (COX-2), IL-1-beta, IL-8, metaloproteinase-8 (MMP-8) and oxytocin receptor. The relationship between expression of TLR and the occurrence of intraamniotic infection and preterm delivery has been demonstrated. According to this fact the correlation of functional *Arg753Gln TLR-2* and *Asp299Gly TLR-4* polymorphisms with premature rupture of membranes, intraamniotic infection and preterm delivery is suggested [35].

Conclusions

The purpose of numerous genetic researches is identification of polymorphic variants responsible for the diseases development. This procedure will probably allow to understand the etiology of the particular disturbances and find exact prophylaxis and probably also treatment methods. There are already known some monogenic diseases, however many other ailments are multifactorial, influenced by genetic polymorphisms and environmental factors. Individual response to treatment strategy conditioned by genetic polymorphisms has been also concerned. Participation of genetic factors in mentioned processes remains often unclear, but numerous studies concerning this issue probably bring us closer to its explanation. There is expected that in the future the development of medicine based on individual genetic prophylaxis and diagnostics will be possible.

References

[1] Ward K. (2008) *Genetic factors in common obstetric disorders*. Clin. Obstet. Gynecol. 51: 74-83.
[2] Varga E. (2007) *Inherited thrombophilia: key points for genetic counseling*. J. Genet. Couns. 16: 261-277.
[3] Massart F., Marcucci G., Brandi M.L. (2008) *Pharmacogenetics of bone treatments: the VDR and ERalpha gene story.* Pharmacogenomics 9: 733-746.
[4] Narod S.A. (2010) *BRCA mutations in the management of breast cancer: the state of the art*. Nat. Rev. Clin. Oncol. 7: 702-707.
[5] Roots I., Laschinski G., Arjomand-Nahad F. et al. (2007) *Genotype and phenotype relationship in drug metabolism*. Ernst. Schering. Res. Found. Workshop. 59: 81-100.
[6] Bertina R.M., Koeleman B.P., Koster T. et al. (1994) *Mutation in blood coagulation factor V associated with resistance to activated protein C.* Nature 369: 64-67.
[7] Poort S.R., Rosendaal F.R., Reitsma P.H. et al. (1996) *A common genetic variation in the 3-prime-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis*. Blood 88: 3698-3703.
[8] Jivraj S., Makris M., Saravelos S. Et al. (2009) *Pregnancy outcome in women with factor V Leiden and recurrent miscarriage.* BJOG 116: 995-998.
[9] Mohamed M.A., El Moaty M.A., El Kholy A.F et al. (2010) *Thrombophilic gene mutations in women with repeated spontaneous miscarriage.* Genet. Test. Mol. Biomarkers. 14: 593-597.
[10] Lund M., Nielsen H.S., Hviid T.V., Steffensen R. et al. (2010) *Hereditary thrombophilia and recurrent pregnancy loss: a retrospective cohort study of pregnancy outcome and obstetric complications*. Hum. Reprod. 25: 2978-2984.
[11] Kovac M., Mitic G., Mikovic Z. et al. (2010) *Thrombophilia in women with pregnancy-associated complications: fetal loss and pregnancy-related venous thromboembolism*. Gynecol. Obstet. Invest. 69: 233-238.
[12] Kupferminc M.J. (2005). *Management of thrombophilia in women with PVC*. Thromb. Res. 115: 46-50.
[13] Rey E., Kahn S.R., David M. et al. (2003) *Thrombophilic disorders and fetal loss: a meta-analysis*. Lancet 361: 901-908.

[14] Larciprete G., Rossi F., Deaibess T. et al. (2010) *Double inherited thrombophilias and adverse pregnancy outcomes: fashion or science?* J. Obstet. Gynaecol. Res. 36: 996-1002.
[15] Wiwanitkit V. (2005). *Roles of methylenetetrahydrofolate reductase C677T polymorphism in repeated pregnancy loss*. Clin. Appl. Thromb. Hemost. 11: 343-345.
[16] Ren A., Wang H. (2006) *Methylenetetrahydrofolate reductase C677T polymorphism and the risk of unexplained recurrent pregnancy loss: a meta-analysis*. Fertil. Steril. 86: 1716-1722.
[17] Mtiraoui N., Zammiti W., Ghazouani L. et al. (2006) *Methylenetetrahydrofolate reductase C677T and A1298C polymorphism and changes in homocysteine concentrations in women with idiopathic recurrent pregnancy losses.* Reproduction 131: 395-401.
[18] Seremak-Mrozikiewicz A., Drews K., Kurzawińska G. et al. (2009) *The connection between Arg353Gln polymorphism of coagulation factor VII and recurrent miscarriages*. Ginekol. Pol. 80: 8-13.
[19] Williams P.J., Broughton Pipkin F. (2011) *The genetics of pre-eclampsia and other hypertensive disorders of pregnancy*. Best. Pract. Res. Clin. Obstet. Gynaecol. 25: 405-417.
[20] Jebbink J., Wolters A., Fernando F. et al. (2012) *Molecular genetics of preeclampsia and HELLP syndrome – a review.* Biochim. Biophys. Acta 1822: 1960-1969.
[21] Williams P.J., Morgan L. (2012) *The role of genetics in pre-eclampsia and potential pharmacogenomic interventions. Pharmgenomics.* Pers. Med. 5: 37-51.
[22] Seremak-Mrozikiewicz A., Barlik M., Perlik M. et al. (2011) *Genetic variability of endothelin-1 system in gestational hypertension and preeclampsia.* Ginekol. Pol. 82: 363-370.
[23] Kosmas I.P., Tatsioni A., Ioannidis J.P. (2003) *Association of Leiden mutation in factor V gene with hypertension in pregnancy and pre-eclampsia: a meta-analysis*. J. Hypertens. 21: 1221-1228.
[24] Nurk E., Tell G.S., Refsum H. et al. (2006) *Factor V Leiden, pregnancy complications and adverse outcomes: the Hordaland Homocysteine Study*. QJM 99: 289-298.
[25] Dudding T., Heron J., Thakkinstian A. et al. (2008) *Factor V Leiden is associated with pre-eclampsia but not with fetal growth restriction: a genetic association study and meta-analysis*. J. Thromb. Haemost. 6: 1869-1875.
[25] Rath W. (2011) *Pre-eclampsia and inherited thrombophilia: a reappraisal*. Semin. Thromb. Hemost. 37: 118-124.
[27] Millis R.M. (2011) *Epigenetics and hypertension*. Curr. Hypertens. Rep. 13: 21-28.
[28] Alfirevic A., Alfirevic Z., Pirmohamed M. (2010) *Pharmacogenetics in reproductive and perinatal medicine.* Pharmacogenomics 11: 65-79.
[29] Fitzpatrick E., Göring H.H., Liu H. et al. (2004) *Fine mapping and SNP analysis of positional candidates at the preeclampsia susceptibility locus (PREG1) on chromosome 2*. Hum. Biol. 76: 849-862.
[30] DeFranco E., Teramo K., Muglia L. (2007) *Genetic influences on preterm birth*. Semin. Reprod. Med. 25: 40-51.
[31] Nesin M. (2007) *Genetic basis of preterm birth*. Front. Biosci. 12: 115-124.
[32] Esplin M.S. (2006) *Preterm birth: a review of genetic factors and future directions for genetic study*. Obstet. Gynecol. Surv. 61: 800-806.
[33] Macones G.A., Parry S., Elkousy M. et al. (2004) *A polymorphism in the promoter region of TNF and bacterial vaginosis: preliminary evidence of gene-environment interaction in the etiology of spontaneous preterm birth*. Am. J. Obstet. Gynecol. 190: 1504-1508.
[34] Simhan H.N., Krohn M.A., Roberts J.M. et al. (2003) *Interleukin-6 promoter –174 polymorphism and spontaneous preterm birth*. Am. J. Obstet. Gynecol. 189: 915-918.
[35] Łukaszewski T., Barlik M., Seremak-Mrozikiewicz A. et al. (2009) *Polymorphism in the genes of Toll-like receptors type 2 and type 4 (TLR-2 and TLR-4) and the risk of premature rupture of the membranes – preliminary study*. Ginekol. Pol. 80: 914-919.

✉ Agnieszka Seremak-Mrozikiewicz
Department of Perinatology and Women's Diseases
University of Medical Sciences in Poznań
60-535 Poznań, ul. Polna 33, Poland
e-mail: asm@data.pl