Gynecological Endocrinology

ISSN: 0951-3590 (Print) 1473-0766 (Online) Journal homepage: https://www.tandfonline.com/loi/igye20

The molecular and genetic aspects of adolescent girls anomalous uterine bleeding: the role of endothelial dysfunction syndrome

O. A. Melkozerova, N. V. Bashmakova, E. V. Volkova, T. B. Tretyakova & P. B. Tsyvian

To cite this article: O. A. Melkozerova, N. V. Bashmakova, E. V. Volkova, T. B. Tretyakova & P. B. Tsyvian (2016) The molecular and genetic aspects of adolescent girls anomalous uterine bleeding: the role of endothelial dysfunction syndrome, Gynecological Endocrinology, 32:sup2, 23-26, DOI: 10.1080/09513590.2016.1232232

To link to this article: https://doi.org/10.1080/09513590.2016.1232232

Published online: 19 Oct 2016.

http://informahealthcare.com/gye
ISSN: 0951-3590 (print), 1473-0766 (electronic)

GYNECOLOGICAL ENDOCRINOLOGY

Gynecol Endocrinol, 2016; 32(S2): S23–S26

DOI: 10.1080/09513590.2016.1232232

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

The molecular and genetic aspects of adolescent girls anomalous uterine bleeding: the role of endothelial dysfunction syndrome

O. A. Melkozerova[1], N. V. Bashmakova[1], E. V. Volkova[1], T. B. Tretyakova[1], and P. B. Tsyvian[1,2]

[1]Federal State Budgetary Institution (FSBU) "Ural Research Institute for Maternal and Child Care" Ministry of Health of the Russian Federation, Yekaterinburg, Russia and [2]Ural State Medical University, Yekaterinburg, Russia

Abstract

The objective of the study is to assess NOS3 and ESR1 gene polymorphism in adolescent girls born with low birth weight (LBW) and suffered by anomalous uterine bleeding (AUB). A total 95 adolescent girls were studied including 32 born with LBW and AUB; 36 girls with normal birth weight and AUB; and 27 healthy girls. Single allele gene polymorphism NOS3 786T > C, 894G > T, ESR1 351A > G and 397T > C was studied. The existence of polymorphous allele C gene NOS3 786T > C (for homozygote OR = 2.03; 95% CI: 1.12–3.68; $p = 0.04$; for heterozygote OR = 1.68; 95% CI: 1.09–2.60; $p = 0.046$) and genotype Pvull-CC ESR1 (OR = 4.58; 95% CI: 0.97–21.68; $p = 0.04$) was detected in LBW girls with AUB. It was suggested that intrauterine programming of endothelial dysfunction syndrome could play a significant role in the development of AUB in adolescent girls born with LBW.

Keywords

Anomalous uterine bleeding in adolescent girls, intrauterine growth restriction, NOS3 and ESR1 gene polymorphism

Introduction

The problem of developmental origins of diseases, including intrauterine programming, is one of the most interesting and intriguing. The impact of maternal pregnancy and labor peculiarities on the development of reproductive health of the adolescent girls was demonstrated earlier [1–3]. It was shown that the menstrual abnormalities and anomalous uterine bleeding demonstrated more frequently in girls born from mothers who suffered by preeclampsia, pregnancy termination threat and diagnosed fetal intrauterine growth restriction during the pregnancy [2,4,5]. It was suggested that the trigger of above-mentioned obstetrical complications could be an endothelial dysfunction syndrome [6–8].

The role of NO synthase (NOS3) genetic polymorphism in the development of preeclampsia and placental dysfunction was demonstrated [9,10]. We suggested that gene polymorphism which could regulate the endothelial function may be inherited by LBW girls from mothers with pregnancies complicated by preeclampsia and placental dysfunction. Endothelial dysfunction in these girls could be phenotypically realized in AUB. These bleedings could be a result of endothelial growth factors dysbalance, changes in vascular tone, angiogenesis and endometrial local hemostasis [11]. The study of endothelial NO synthase gene polymorphism reveals functionally interrelated polymorphisms 894 G/T (rs1799983) and 786 T/C (rs2070744) [12]. The significant role of these polymorphisms demonstrated in cardiovascular diseases (ischemic heart disease, myocardial infarction, preeclampsia and stroke) and some other diseases (diabetes) [12,13].

It is well known that estrogens are important regulators of the endothelial function. These hormones can activate and release NO, prostacycline, hyperpolarizing endothelial factor and decrease the release of the vasoconstricting factors (endothelin I and angiotensin II) [10,14–16]. Estrogens impact could be realized via specific nuclear and membrane nongenomic receptors which are located in vascular smooth muscle cells and endothelium. There are two estrogen receptors ERα and ERβ which are the parts of nuclear steroid hormone receptors family. ERα plays the most significant role in the endothelium signal transmission [17].

There is limited information on molecular mechanisms of these polymorphisms that influence on receptors activation. ESR1 PvuII polymorphism could impact ESR1 gene expression through changes in transcriptional factors binding, and could influence the ESR1 splicing [18]. Some vascular protective effects of estrogens could be mediated through nongenomic mechanisms, including activation ways: MAPK (mitogen-activated protein kinase) and phosphatidyl-inositol-3-kinase. That is the way of NO (NOS3) endothelial synthase activation in combination with genomic effect of estradiol on NO synthesis [19,20].

Several aspects of intragenomic interaction of polymorphic NOS genes and role of estradiol receptors genes in number of diseases, including pathology of reproduction, are still unknown. This problem needs further clarification.

The objective of this study is to assess NOS3 and ESR1 genes polymorphic associations in adolescent girls with different birth weight suffering from anomalous uterine bleeding.

Materials and methods

A total 95 adolescent girls were studied. There were three groups. Group 1 consisted of 32 girls with AUB who were born with low

Address for correspondence: O. A. Melkozerova, MD, PhD, Head of Gynecological Department, Mother and Child Care Research Institute, Repin str1, Yekaterinburg, 620028, Russia. Tel: +7(343)371 8768. Fax: +7(343) 371 8773, E-mail: abolmed1@mail.ru

birth weight (2367.3 ± 34.6 g). Group 2 consisted of 36 girls suffered from AUB and normal birth weight (3436.4 ± 54.3 g, $p < 0.001$). Control group consisted of 27 healthy girls with normal birth weight (3416.0 ± 177.2 g, $p < 0.001$). The main causes of low birth weight of the Group 1 girls were maternal preeclampsia and placental dysfunction.

Mean gestational age at the moment of birth for all girls was 37 weeks and more. All groups were comparable according to maternal parity, gestational age and mode of delivery. All girls and their parents gave informed consent for participation in the study. The information on girls' birth weight and length was obtained from medical documentation related to birth certificate kept by the girls' parents.

Gene polymorphism study

Genomic DNA was extracted from peripheral blood samples using standard techniques. DNA amplification was performed using ''Probe-GS-Genetics'' (DNA Technologies, Russia) diagnostic kit from 0.5 ml of blood with EDTA (to prevent coagulation). DNA amount was measured by ''KBM'' (DNA Technologies, Russia) kit for PCR reactions. The sample of 1.0 ng of genomic DNA was used for each reaction. Allele-specific PCR method was used for the samples genotyping according to allele variants of genes in real time with assessment of the melting curves of amplification products. The PCR analysis was performed automatically using detecting amplifier ''DT-96'' (DNA Technologies, Russia).

Statistical analysis was performed by ''Statistica-7.0'' package. Data were compared using Student's *t*-criterion. Nonparametric variables were assessed using χ^2 Pearson's criterion. The odds ratio (OR) was determined with 95% confidence interval (95% CI). Null hypothesis was rejected at $p < 0.05$. The Hardy–Weinberg law test on genotype distribution was performed by χ^2 criterion using Hardy–Weinberger equilibrium program.

Results

The mean age of Group 1 girls was 14.1 ± 0.3 years, girls from Group 2 were 13.5 ± 0.2 years old and girls from control group were 14.6 ± 0.4 years old $p > 0.05$. All groups were comparable by the age. The body mass index (BMI) of Group 1 girls was 24.3 ± 1.8 kg/cm^2, which was more than BMI in Group 2 (22.1 ± 1.3 kg/cm^2, $p = 0.04$) and in control group (20.1 ± 1.7 kg/cm^2, $p = 0.008$). The height of Group 1 girls (161.0 ± 1.8 cm) was less than in Group 2 (163.3 ± 1.6 cm, $p = 0.03$) and of control group girls (164.0 ± 2.1 cm, $p = 0.02$).

The age of onset of menarche was different in the girls of all groups. This age was 10.8 ± 0.3 years for girls of Group 1, 11.7 ± 0.2 years for Group 2 and 12.4 ± 0.5 years, $p < 0.01$ for the girls of control group. In 85.5%, the anomalous uterine bleeding was the actual start of menarche in girls of Group 1 and in 57.4% AUB repeated several times in this group.

The genotyping results demonstrated that alleles and genotype distribution in both (1 and 2) groups correspond to Hardy–Weinberg equilibrium. The possible influence of the genotype peculiarities on the endothelial functional state was assessed according the general, multiple and dominant models. The genotype distribution analysis according to polymorphous gene variant of NOS3 and ESR1 (Table 1) demonstrated significant increase in genotype occurrence frequency containing polymorphous alleles 786C of gene NOS3T > C in homozygote and heterozygote state in Group 1 girls (for homozygote, OR = 2.03; 95% CI: 1.12–3.68; $p = 0.046$; for heterozygote, OR = 1.68; 95% CI: 1.09–2.60; $p = 0.046$; Table 1). The frequency of alleles NOS3 and ESR1 genes distribution demonstrated more frequent occurrence of allele 786C of NOS3 gene in girls of Group 1 (NOS3:786T > C: OR = 3.46; 95% CI: 1.16–10.34; $p = 0.02$; Table 2). The allele polymorphism NOS3 and ESR1 of Group 1 and 2 girls demonstrated no significant difference in polymorphous allele 786C in NOS3 (Table 2).

Table 1. NOS3 and ESR1 genotypes frequencies distribution in LBW girls with AUB (Group 1) and control group girls (general model).

	Frequencies						
Genotype	Group 1	Control	χ^2	*p*	OR	95% CI	
ESR1 351 AA	0.556	0.500	0.75	0.69	1.25	0.74	2.10
ESR1 351 AG	0.407	0.500			0.69	1.65	0.29
ESR1 351 GG	0.037	0.000			–	–	–
ESR1 397 TT	0.462	0.286	1.97	0.37	2.14	0.71	6.44
ESR1 397 TG	0.500	0.714			0.40	1.50	0.11
ESR1 397 GG	0.038	0.000			–	–	–
NOS3 786 TT	0.069	0.357	5.78	0.046	0.13	0.72	0.02
NOS3 786 TC	0.483	0.357			1.68	1.09	2.60
NOS3 786 CC	0.448	0.286			2.03	1.12	3.68
NOS3 894 GG	0.517	0.571	0.52	0.77	0.80	1.49	0.43
NOS3 894 GT	0.448	0.357			1.46	0.50	4.26
NOS3 894 TT	0.034	0.071			0.46	4.01	0.05

Recessive model of inheritance reveals difference in polymorphous gene loci NOS3 and ESR1 occurrence in adolescent girls groups. The allele 786C in NOS3 gene in homozygote and heterozygote states was found more frequently in girls of Group 1 (OR = 2.56; 95% CI: 1.01–6.5; $p = 0.04$; Table 2) and Group 2 (OR = 3.46; 95% CI: 1.16–10.34; $p = 0.02$; Table 2) than in control group. NOS3:786TT genotype was detected more frequently in the girls of control group (OR = 0.12, 95% CI: 1.04–0.01; $p = 0.04$; Table 1).

We suggest that polymorphous allele C in locus 786 of endothelial NO synthase is associated with increased risk of AUB in adolescent girls. Also, the dominant model of NOS3 and ESR1 genotypes reveals more frequent occurrence of ESR1:397CC genotype with polymorphous locus 397C in estrogen receptors alpha gene in Group 1 girls (OR = 4.58; 95% CI: 0.97–21.68; $p = 0.04$; Table 3).

Therefore, polymorphous allele 397C in ESR1 gene additionally increases the risk of anomalous uterine bleedings in low birth weight girls.

Discussion

We suggest that the birth of the girls with small-for-gestational age weight from mothers with endothelial dysfunction could be determined by intrauterine programming changes of endothelial function of these girls. These changes could be realized as anomalous uterine bleeding at the moment of menarche beginning in adolescence. We have studied 786 T > C, 894G > T and ESR1 351A > G (Xbal) и 397T > C (Pvull) gene polymorphism and some endothelial function markers in LBW girls. The analysis of distribution frequencies of NOS3 and ESR1 gene alleles demonstrates that the frequency of NOS3 786T>C gene occurrence significantly higher in LBW girls with AUB. It was demonstrated that allele C existence in 786T > C NOS3 gene promoter would lead to eNOS suppression and activity decrease [13,16,20,21]. These changes in eNOS could decrease NO synthesis and release, which determines an endothelial dysfunction.

Low nitrites blood level and diminished compensatory brachial artery blood flow activation by acetylcholine injection and estrogens were reported in persons with allele C of 786C NOS3 gene [22]. The relationship between existence of polymorphous

Table 2. NOS3 and ESR1 genes alleles frequencies distribution in girls with AUB (Groups 1 and 2) and in girls of control group (recessive model).

Groups	Genes	Frequencies: AUB girls	Frequencies: Control group	χ^2	*p*	OR	95% CI	
Group 1 in comparison with control group	ESR1 351 A	0.759	0.750	0.01	0.93	1.05	0.36	3.08
	ESR1 351 G	0.241	0.250			0.95	2.79	0.32
	ESR1 397 T	0.712	0.643	0.40	0.53	1.37	0.51	3.70
	ESR1 397 G	0.288	0.357			0.73	1.97	0.27
	NOS3 786 T	0.310	0.536	4.06	0.04	0.39	0.99	0.15
	NOS3 786 C	0.690	0.464			2.56	1.01	6.50
	NOS3 894 G	0.741	0.750	0.01	0.93	0.96	2.74	0.33
	NOS3 894 T	0.259	0.250			1.05	0.37	3.00
Group 2 in comparison with control group	ESR1 351 A	0.844	0.750	0.82	0.37	1.80	0.49	6.60
	ESR1 351 G	0.156	0.250			0.56	2.04	0.15
	ESR1 397 T	0.781	0.600	2.39	0.12	2.38	0.78	7.31
	ESR1 397 G	0.219	0.400			0.42	1.29	0.14
	NOS3 786 T	0.250	0.536	5.16	0.02	0.29	0.86	0.10
	NOS3 786 C	0.750	0.464			3.46	1.16	10.34
	NOS3 894 G	0.813	0.750	0.34	0.56	1.44	0.41	5.07
	NOS3 894 T	0.188	0.250			0.69	2.43	0.20

Table 3. NOS3 and ESR1 genotypes frequencies distribution in girls with AUB (Groups 1 and 2) and in girls of control group (dominant model).

Genotype	Frequencies: AUB girls	Frequencies: Control group	χ^2	*p*	OR	95% CI	
ESR1 351 AA	0.688	0.500	1.09	0.30	2.20	0.47	10.31
ESR1 351 AG + GG	0.313	0.500			0.45	2.13	0.10
ESR1 397 CC	0.625	0.267	4.01	0.04	4.58	0.97	21.68
ESR1 397 TC + TT	0.375	0.733			0.22	1.03	0.05
NOS3 786 TT	0.063	0.357	4.05	0.04	0.12	1.04	0.01
NOS3 786 TC + CC	0.938	0.643			8.33	0.96	72.10
NOS3 894 GG	0.625	0.571	0.09	0.77	1.25	0.27	5.77
NOS3 894 GT + GG	0.375	0.429			0.80	3.69	0.17

allele C in 786T > C NOS3 gene and increased coronary arteries tone, altered reaction of coronary arteries on acetylcholine injection, was demonstrated in meta-analysis of 20 studies including 11 236 patients [12]. Therefore, polymorphous allele 786C of NOS3 gene in heterozygote and homozygote states (which was detected in LBW girls with AUB) is associated with endothelial dysfunction risk. In addition, we demonstrated the high frequency of ESR1 Pvull-CC occurrence in alpha estrogen receptors in LBW girls with AUB. It was shown that ESR1 Pvull-CC genotype is associated with high risk of endothelial dysfunction, arterial hypertension, ischemic heart disease and increase in arterial intima thickness in women [10,23,24]. Also this genotype is associated with significant decrease in NO-mediated blood flow increase and NO bioavailability [13]. It was suggested that endothelial dysfunction can cause a significant decrease in endometrial stroma blood flow and tissue hypoxia [25,26]. Tissue hypoxia can trigger the anomalous angiogenesis by vascular cells which are increasing the vascular endothelial growth factor (VEGF) synthesis. It was also shown that the hypoxia decreases the endometrial blood flow and increases the production of oxygen active forms at endothelial dysfunction syndrome [27,28]. This process can also increase the VEGF and angiopoetin-2 (Ang-2) synthesis in endometrial endothelial cells [11,29]. All these events can make the endometrial vascular wall more fragile and stimulate uncontrolled endometrial angiogenesis, which in concert cause AUB.

We can suggest that such proangiogenic reaction could be a part of the compensatory mechanisms to protect optimal endometrial perfusion at this particular condition.

Conclusion

We suggest that AUB in LBW girls born from mothers suffered from preeclampsia and placental dysfunction could be a result of intrauterine programming of endothelial dysfunction associated with NOS3 and ESR1 gene polymorphism which lead to angiogenesis and vascular tone alterations. Further research on molecular and genetic origins of AUB will be essential for prediction and prevention of uterine bleeding in adolescent girls.

Declaration of interest

The authors report no declaration of interest.

References

1. Baranov AA, Kuchma VR, Namazova-Baranova LS. Zdorov'e i razvitie podrostkov Rossii [Health and development of Russian teenagers]. Moscow: NCZD RAMN; 2010:102 (in Russian).
2. Kokolina VF. Ginekologija detskogo i podrostkovogo vozrasta [Gynecology of childhood and adolescence]. Moscow: Medpraktika; 2006:670 (in Russian).
3. Uvarova EV. Abnormal uterine bleeding puberty [Reproduktivnoe Zdorov'e Detej I Podrostkov]. 2013;3:73–87.
4. Dan'kova IV, Bashmakova NV, Chistjakova GN. The course of puberty in adolescent girls who were born with the manifestations of intrauterine growth retardation [Reproduktivnoe Zdorov'e Detej I Podrostkov]. 2013;1:44–52 (in Russian).
5. Lobodina IM. Uterine bleeding puberty (literature review) [Reproduktivnoe Zdorov'e Detej I Podrostkov]. 2007;4:55–71 (in Russian).
6. (a) Bisadze VO, Makacarija AD, Hizroeva HD, Jashenina EV. Thrombophilia as the most important link in the pathogenesis of complications of pregnancy. [Prakticheskaja medicina]. 2012;5: 22–9. (b) Kupferminc MJ. Thrombophilia and pregnancy. Reprod Biol Endocrinol 2003;1:111. doi: 10.1186/1477-7827-1-111.
7. Rey E, Garneau P, David M, et al. Dalteparin for the prevention of recurrence of placental-mediated complications of pregnancy in women without thrombophilia: a pilot randomized controlled trial. J Thromb Haemost 2009;7:58–64.
8. Kireeva OV, Sutulina IM. Biochemical markers of endothelial dysfunction and myocardial damage in newborns from mothers with preeclampsia. *Mat'i ditja: mat. XIV Vseros. nauch. foruma* [Mother

and Child: Materials XIV All-Russia Science Forum]. Moscow, 2013;441–2 (in Russian).
9. L'vova TJ, Stepanova OI, Postolicyna EA. The formation of vascular endothelial cells line EA.NY926 tubes in the presence of secretory placental tissue products. *Mat'i ditja: mat. XIV Vseros. nauch. Foruma* [Mother and Child: Materials XIV All-Russia Science Forum]. Moscow, 2013;111–12 (in Russian).
10. Clapauch R, Mourão AF, Mecenas AS, et al. Endothelial function and insulin resistance in early postmenopausal women with cardiovascular risk factors: importance of ESR1 and NOS3 polymorphisms. PLoS One 2014;9:e103444. doi:10.1371/journal.pone.0103444.
11. Arnal JF, Fontaine C, Billon-Galés A, et al. Estrogens receptors and endothelium. Arterioscler Thromb Vasc Biol 2010;30:1506–12.
12. Doshi AA, Ziolo MT, Wang H, et al. Promoter polymorphism of the endothelial nitric oxide synthase gene is associated with reduced mRNA and protein expression in failing human myocardium. J Card Fail 2010;16:314–19.
13. Gilchrist M, Shore AC, Benjamin N. Inorganic nitrate and nitrite and control of blood pressure. Cardiovasc Res 2011;15:492–8.
14. Masood D, Roach EC, Beauregard KG, Khalil RA. Impact of sex hormone metabolism on the vascular effects of menopausal hormone therapy in cardiovascular disease. Curr Drug Metab 2010;11:693–714.
15. Knowlton AA, Lee AR. Estrogen and the cardiovascular system. Pharmacol Ther 2012;135:54–70.
16. Alevizaki M, Saltiki K, Cimponeriu A, et al. Severity of cardiovascular disease in postmenopausal women: associations with common estrogen receptor alpha polymorphic variants. Eur J Endocrinol 2007;156:489–96.
17. Heldring N, Pike A, Andersson S, et al. Estrogen receptors: how do they signal and what are their targets. Physiol Rev 2007;87:905–31.
18. Bollig A, Miksicek RJ. An estrogen receptor-α splicing variant mediates both positive and negative effects on gene transcription. Mol Endocrinol 2000;14:634–49.
19. Chow RW, Handelsman DJ, Ng MK. Minireview: rapid actions of sex steroids in the endothelium. Endocrinology 2010;151:2411–22.
20. Entringer S, Buss C, Swanson JM, et al. Fetal programming of body composition, obesity, and metabolic function: the role of intrauterine stress and stress biology. J Nutr Metab 2012;2012:632548. doi:10.1155/2012/632548.
21. Asif AR, Oellerich M, Armstrong VW, et al. T-786C Polymorphism of the nos-3 gene and the endothelial cell response to fluid shear stress – a proteome analysis. J Prot Res 2009;8:3161–8.
22. Erbs S, Möbius-Winkler S, Linke A, et al. Both T-786C and G894T polymorphism of endothelial nitric oxide synthase affect in-vitro endothelium-dependent relaxation of internal mammary artery rings from patients with coronary artery disease. Eur J Cardiovasc Prev Rehabil 2006;13:826–31.
23. Shen C, Chen J, Fan S, et al. Association between the polymorphism of estrogen receptor α and coronary artery disease in a Chinese population. Eur J Intern Med 2012;23:175–8.
24. Casey M, MacDonald P. Modulation of endometrial blood flow: regulation of endothelin-1 biosynthesis and degradation in human endometrium. In: Alexander N, d'Arcangues C, eds. Modulation of endometrial blood flow: regulation of endothelin-1 biosynthesis and degradation in human endometrium. Washington (DC): AAAS Press; 1992. p. 209.
25. Cao Y, Linden P, Shima D, et al. In vivo angiogenic activity and hypoxia induction of heterodimers of placenta growth factor/vascular endothelial growth factor. J Clin Invest 1996;98:2507–11.
26. Zil'ber MJ, Grebneva OS, Cyganenko SV. Status of the receptor apparatus of the endometrium in acute placental insufficiency in history. *Mat'i ditja: mat. XIV Vseros. nauch. foruma* [Mother and Child: Materials XIV All-Russia Science Forum]. Moscow, 2013:69–70 (in Russian).
27. Lockwood CJ, Nemerson Y, Guller S, et al. Decidualized human endometrial stromal cells mediate hemostasis, angiogenesis, and abnormal uterine bleeding. Reprod Sci Feb 2009;16:162–70.
28. Nofer JR. Estrogens and atherosclerosis: insights from animal models and cell systems. J Mol Endocrinol 2012;48:R13–29.
29. Lockwood CJ. Mechanisms of normal and abnormal endometrial bleeding. Menopause 2011;18:408–11.