

The MDM2 promoter T309G polymorphism was associated with preeclampsia susceptibility

Saeedeh Salimi^{1,2} · Abbas Mohammadpour-Gharehbagh^{1,2} · Mahnaz Rezaei^{1,2} ·
Mojtaba Sajadian^{1,2} · Batool Teimoori³ · Atefeh Yazdi³ · Mojgan Mokhtari³ ·
Minoos Yaghmaei⁴

Received: 15 February 2017 / Accepted: 30 April 2017 / Published online: 15 May 2017
© Springer Science+Business Media New York 2017

Abstract

Purpose Preeclampsia (PE) is a hypertensive disorder of pregnancy in which abnormal proliferation and apoptosis of placenta trophoblast has a pivotal role in its pathophysiology. The aim of the current study was to examine the association between Mouse Double Minute 2 (MDM2) T309G and 40 bp insertion/deletion (I/D) polymorphisms and PE risk.

Methods A case-control study was conducted on 208 PE women and 164 healthy pregnant women matching age, sex, and ethnicity. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and PCR methods were used for genotyping.

Results The MDM2 309GG genotype was associated with PE, and this genotype was found to be a risk factor for PE. There was no association between the MDM2 I/D polymorphism and PE. The haplotype-based association analysis revealed no association between MDM2 T309G and 40 bp I/D polymorphisms and PE. The frequency of TT-DD and GG-DD combined genotypes were significantly higher in PE women with marginal *P* values (*P* = 0.046).

Conclusions The MDM2 309GG genotype was associated with higher risk of PE. The TT-DD and GG-DD combined genotypes were higher in PE women.

Keywords Gene · MDM2 · Polymorphism · Preeclampsia

Introduction

Preeclampsia (PE) is a medical disorder of pregnancy characterized by the onset of hypertension and proteinuria during the second half of the gestation [1]. The occurrence of preeclampsia ranges from 3 to 7% for nulliparous and 1 to 3% for multiparous women, and it is dependent on ethnicity [2]. Preeclampsia is a leading cause of maternal mortality and morbidity, preterm birth, perinatal death, and intrauterine growth restriction [3]. There are several possible mechanisms of pathogenesis of preeclampsia such as uteroplacental ischemia, endothelial cell dysfunction, and exaggerated maternal inflammatory response to deported trophoblast, as well as apoptosis, but the underlying etiology of preeclampsia remains unknown [4]. Evidence shows the relation between genetic factors and preeclampsia because both the maternal and the paternal family histories of the disease can cause the preeclampsia [5]. Several studies have addressed the association between apoptosis disruption and PE [6]. The apoptosis or programmed cell death is a vital component of various processes including organ development, tissue remodeling, immune response, tumor suppression, and maintenance of tissue homeostasis [7, 8]. P53 is a necessary regulatory factor of apoptosis, triggering apoptosis in response to noxious stimulation by contributing factors including p21, APAF1, Puma, and Bax; these factors increase the activation of caspases [9, 10]. Under normal conditions, the human homolog of the mouse double minute 2 (MDM2) inhibits p53 by translocation

✉ Abbas Mohammadpour-Gharehbagh
am.mohammadpour@gmail.com

¹ Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

² Department of Clinical Biochemistry, School of Medicine and Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

³ Department of Obstetrics and Gynecology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

⁴ Department of Obstetrics and Gynecology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

of it to the cytoplasm and making it accessible for ubiquitination and targeting it for proteasomal degradation [11]. MDM2 is a member of E3 ubiquitin-protein ligase family, and its increased levels suppress the initiation of apoptosis and activate the cell cycle. Studies show that suppression of *MDM2* gene in mice increases the fetal mortality [12, 13]. *MDM2* gene is a target for tumor suppressor p53 so that p53 positively regulates *MDM2* expression while *MDM2* negatively regulates p53 levels and activity [11]. Although various studies have been performed on the effects of p53 levels on PE [14, 15], the studies about the correlation between *MDM2* and PE are rare [16]. In addition, there are few published reports on the association between MDM2 polymorphisms and PE [17].

Based on the fact that *MDM2* plays an important role in the apoptosis, it was hypothesized that the functional polymorphisms in *MDM2* promoter (T309G and 40 bp insertion/deletion) may be associated with increased risk of PE formation.

Materials and method

Two hundred eight preeclamptic women and 164 normotensive pregnant women were recruited from the Department of Obstetrics and Gynecology of Ali-ebn-Abitaleb Educational Hospital of Zahedan University of Medical Sciences. Informed consent was obtained from all subjects, and the study protocol was approved by the Ethics Committee of Zahedan University of Medical Sciences and conducted in accordance with the Declaration of Helsinki.

Preeclampsia was defined as the presence of increased blood pressure (≥ 140 mmHg systolic blood pressure [SBP] or ≥ 90 mmHg diastolic blood pressure [DBP] on two or more measurements at least 6 h apart) and proteinuria ≥ 0.3 g/24 h or $\geq +1$ on a urine dipstick after 20 weeks of gestation. Women with chronic hypertension, underlying renal disease, and/or insulin-dependent diabetes twins or multiple pregnancies, hydatidiform mole, hydrops fetalis, diabetes, liver dysfunction, systemic lupus erythematosus, and all systemic diseases were excluded from the study. The PE was classified as severe hypertension and severe proteinuria (SBP ≥ 160 mmHg or DBP ≥ 110 mmHg and urinary protein excretion ≥ 5 g per 24 h).

Genomic DNA extraction and genotyping

Genomic DNA was extracted from 200 μ l EDTA-treated whole blood using the salting out method and stored at -20 °C until analysis. The analysis of MDM2 40-bp insertion/deletion (I/D) and T309G polymorphisms was performed by the polymerase chain reaction (PCR) or polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods, respectively. The fragment containing the MDM2 40 bp I/D polymorphism was amplified using the forward: 5'-TTTC

CTTTCTGGTAGGCTGG-3' and reverse: 5'-CACC TACTTCCCACAGAGA-3' primers. The primers used for the amplification of the fragment containing the MDM2 T309G polymorphism were forward: 5'-CGCG GGAGTTCAGGGTAAAG-3' and reverse: 5'-AGCT GGAGACAAGTCAGGACTTAAC-3' [18, 19]. The total volume of the PCR mixture was 25 μ l containing 200 ng genomic DNA, 25 pM of each primer, 0.1 mM dNTP, 1.5 mM MgCl₂, 2.5 μ l PCR buffer $\times 10$, and 1 U of Taq polymerase (Fermentas, Lithuania). Amplification was performed in a Bio-Rad thermal cycler using a thermal profile as previously described.

The 237-bp PCR product of the MDM2 T309G polymorphism was digested by MSPA1I restriction enzyme (Fermentas, Lithuania) and was incubated at 37 °C overnight. The digested products were separated by electrophoresis on 2.5% agarose gel. The MDM2 309T allele had no MSPA1 cleavage site and produced a 237-bp fragment only. However, the MDM2 309G had one MSPA1 cleavage site and was digested to 189 and 48 bp fragments. The PCR products of 40-bp I/D polymorphism were electrophoresed on 2.5% agarose gel and produced 262 and 222 bp fragments for I and D alleles, respectively.

Statistical analysis

Statistical analyses were performed using SPSS software (Version 20; SPSS Inc., Chicago, IL, USA). The clinical and demographic characteristics of the both groups were compared via independent Student's *t* test or Fisher's exact test. The independent effect of each polymorphism and haplotype on PE risk was assessed via logistic regression analysis. Haplotype frequency and LD were analyzed using Cube X software [20]. Values of $P < 0.05$ were considered statistically significant.

Results

Demographic and clinical characteristics of 208 PE women and 164 normotensive women are presented in Table 1. The maternal age, birth weight of neonates, and family history of PE did not differ between two groups. However, gestational age, systolic and diastolic blood pressures, and primiparity were significantly different between PE women and normotensive women.

Genotypes

The genotype frequencies of MDM2 T309G polymorphism were in Hardy-Weinberg equilibrium in the PE and normotensive women. Although the MDM2 40 bp I/D polymorphism was in Hardy-Weinberg equilibrium in normotensive women, it was deviated from Hardy-Weinberg equilibrium in PE women ($P = 0.002$).

Table 1 Demographic characteristics of PE women and controls

Variable	Controls <i>n</i> = 164	PE <i>n</i> = 208	<i>P</i> value	OR (95% CI)
Maternal age (mean ± SD, years)	26.7 ± 6.4	27.6 ± 7.5	NS	
Gestation age (mean ± SD, days)	268 ± 19	260 ± 24	<0.001	
Birth weight (mean ± SD, g)	2932 ± 470	2807 ± 459	0.01	
SBP (mean ± SD, mmHg)	115 ± 9.3	144 ± 22.2	<0.0001	
DBP (mean ± SD, mmHg)	71.6 ± 11.6	90.7 ± 13.8	<0.0001	
Primiparity, <i>n</i> (%)	46 (28)	91 (44)	0.002	2 (1.3–3.1)
Family history of PE, <i>n</i> (%)	54 (33)	84 (40)	NS	

NS not significant, SD standard deviation

Table 2 shows the allele and genotype frequencies of MDM2 T309G and MDM2 40 bp I/D polymorphisms in the PE women and healthy controls.

The frequency of MDM2 309 TG genotypes was not different between PE and control groups; however, the frequency of MDM2 309 GG genotype was statistically higher in PE women, and MDM2 309 GG genotype was associated with 2.3-fold higher risk of PE [OR 2.3 (95% CI 1.1–4.6); *P* = 0.03]. MDM2 309-G allele was not more frequent in PE women compared to normotensive women.

The frequency of I/I, I/D, and D/D genotypes and D allele of MDM2 40 bp I/D polymorphism were not different between two groups.

Haplotype analysis of MDM2 T309G and 40 bp I/D polymorphisms

Table 3 presents the frequency of four haplotypes of MDM2 T309G and 40 bp I/D polymorphisms. Although the

Table 2 Allelic and genotypic frequency of MDM2 polymorphisms in PE women and control group

	PE (<i>N</i> = 208)	Control (<i>N</i> = 164)	<i>P</i> value	OR (95% CI)
<i>MDM2 T309G</i>				
TT, <i>n</i> (%)	85 (41)	73 (44.5)	1	
TG, <i>n</i> (%)	89 (43)	78 (47.5)	0.93	1 (0.6–1.5)
GG, <i>n</i> (%)	34 (16)	13 (8)	0.03*	2.3 (1.1–4.6)
Allele				
T, <i>n</i> (%)	259 (62.3)	224 (68)	1	
G, <i>n</i> (%)	157 (37.7)	104 (32)	0.09	1.3 (1–1.8)
<i>MDM2 40 bp</i>				
I/I, <i>n</i> (%)	112 (53.8)	93 (57)	1	
I/D, <i>n</i> (%)	68 (32.7)	59 (36)	0.9	1 (0.6–1.5)
D/D, <i>n</i> (%)	28 (13.5)	12 (7)	0.08	1.9 (0.9–4)
Allele				
I, <i>n</i> (%)	292 (70)	245 (75)	1	
D, <i>n</i> (%)	124 (30)	83 (25)	0.2	1.3 (0.9–1.7)

**p* < 0.05

frequency of T-D and G-I haplotypes were higher in the PE women compared to the controls, the haplotype-based association analysis revealed that these differences were not statistically significant. Linkage disequilibrium was calculated for MDM2 T309G and 40 bp I/D polymorphisms *D'* = 0.26, *r*² = 0.05 in PE and *D'* = 0.36, *r*² = 0.1 in control women.

Combination effects of MDM2 T309G and 40 bp I/D polymorphisms on PE risk

The association between nine combined genotypes of MDM2 T309G and 40 bp I/D polymorphisms and PE risk is shown in Table 4. The frequency of TT-DD and GG-DD combined genotypes were significantly higher in PE women; however, the *P* values for these differences were marginal (*P* = 0.046).

Discussion

The human placenta is surfaced by a continuous layer of epithelial cells called the trophoblast and plays an essential role in embryo implantation and interaction with the maternal uterus. After fertilization, these cells proliferate and differentiate into two cell layers, cytotrophoblast (inner layer) and syncytiotrophoblast (outer layer). The syncytiotrophoblasts directly contact with the maternal blood; therefore, they are responsible for the fetal-maternal transfer of gases, nutrients, and wastes [21, 22].

Oxidative and nitrate stresses created by exogenous stimuli affect placental development and lead to placental injury. Evidences show that oxidative stresses and placental maldevelopment or damage are associated with pregnancy complications such as preeclampsia [23, 24].

On the other hand, impairment in trophoblast invasion and remodeling of maternal uterine spiral arteries that occur in the first 20 weeks of pregnancy have been introduced as a major accepted concept in PE etiology. Therefore, the factors that disrupt the balance between trophoblast proliferation and apoptosis could play an essential role in this complication [25].

Apoptosis is an active process by which excessive and dysfunctional cells are removed for the preservation of normal

Table 3 Haplotype frequency of *MDM2* T309G and 40 bp I/D polymorphisms in PE women and control group

<i>MDM2</i> T309G	<i>MDM2</i> 40 bp I/D	PE (<i>N</i> = 208)	Control (<i>N</i> = 164)	<i>P</i> value	OR (95% CI)
T	I	203 (48.7)	188 (57.3)		1
T	D	59 (14)	36 (11)	0.08	1.5 (1–2.4)
G	I	87 (21)	57 (17.3)	0.09	1.4 (1–2.1)
G	D	68 (16.3)	47 (14.4)	0.2	1.3 (0.9–2)

**p* < 0.05

tissue function. During the embryonic development, apoptosis is introduced as a critical process. In normal conditions, apoptosis is often stimulated in response to stresses. The apoptosis acts at least through the extrinsic pathway using cell-surface receptors and the intrinsic pathway that contains mitochondrial depolarization [26]. Different studies have proved an increased level of villous trophoblast apoptosis in pregnancies complicated by preeclampsia [6, 14].

MDM2 as an apoptosis-related factor is the major regulator of p53 protein. The levels of both MDM2 and p53 proteins are strongly regulated. In response to genotoxic stresses, p53 induces MDM2 transcription; however, MDM2 inhibits and directs p53 for proteasomal degradation. Therefore, MDM2 overexpression has been proposed as an effective mechanism in p53 inactivation [27]. Taking the effects of p53 and MDM2 proteins on apoptosis, several studies have investigated the expression levels of p53, MDM2, and other apoptotic proteins in the placenta of PE women. Sharp et al. illustrated higher expression of p53, p21, and Bax proteins in the placental villous tissue of PE women. However, Mdm2 protein levels significantly decreased in placental villous tissue of PE women [10]. Heazell et al. showed that the expression of p53, p21, and MDM2 elevated in trophoblast following exposure to hypoxia [28].

MDM2 gene has two promoters: the basal promoter (P1) situated at upstream of exon 1 and the alternative promoter (P2) presented in intron 1. P1 regulates MDM2 expression in non-stress states; however, in presence of p53-responsive element in P2 region, it regulates MDM2 expression in cells under cellular stress [29].

The T-to-G transversion at nucleotide 309 (T309G, rs2279744) in the P2 promoter increases the binding affinity of the transcriptional activator Sp1 and leads to increased production of MDM2 protein. In addition, the 40-bp insertion/deletion (I/D) polymorphism (–1208 to –1169, rs3730485) has been found in the promoter region of the MDM2 gene, which may alter the expression of MDM2 gene [18, 30].

Considering the effects of MDM2 on apoptosis, several studies have investigated the association between MDM2 polymorphisms and different diseases including cancers [31, 32] and uterine leiomyoma [33].

In the current study, it was found that MDM2 309 GG but not MDM2 309 TG genotype was associated with 2.3-fold higher risk of PE. MDM2 309 G allele was also associated with PE. MDM2 40 bp I/D polymorphism was not associated with PE. However, the synergic effects of TT/DD and GG/DD genotypes of MDM2 T309G and I/D polymorphisms were associated with PE risk with marginal *P* values.

The findings regarding the association between MDM2 309 GG genotype and PE risk are in accordance with the effect of MDM2 309 G allele on increased production of MDM2 and inhibition of apoptosis in PE patients. To the best of our knowledge, there is only one published report on the association between MDM2 T309G polymorphism and PE [17], and there is no published report on the association between MDM2 40 bp I/D polymorphism and PE. Conversely, Busatto et al. showed no association between MDM2 T309G polymorphism and PE in Brazil [17]. However, Fang et al. found an association between MDM2309 GG genotype and risk of missed abortion. They showed higher levels of

Table 4 The combination effects of MDM2 T309G and 40 bp I/D polymorphisms on PE risk

<i>MDM2</i> T309G	<i>MDM2</i> 40 bp	PE (<i>N</i> = 208)	Control (<i>N</i> = 164)	<i>P</i> value	OR (95% CI)
TT	II	56 (26.9)	55 (33.5)		1
TT	ID	19 (9.1)	16 (9.8)	0.69	1.2 (0.5–2.5)
TT	DD	10 (4.8)	2 (1.2)	0.046*	4.9 (1–23.4)
TG	II	43 (20.7)	27 (16.5)	0.15	1.6 (0.9–2.9)
TG	ID	41 (19.7)	43 (26.2)	0.82	0.9 (0.5–1.7)
TG	DD	8 (3.8)	8 (4.9)	0.97	1 (0.3–2.8)
GG	II	13 (6.3)	11 (6.7)	0.74	1.2 (0.5–2.8)
GG	ID	8 (3.8)	0 (0)	1	–
GG	DD	10 (4.8)	2 (1.2)	0.046*	4.9 (1–23.4)

**p* < 0.05

MDM2 mRNA and protein in women carrying MDM2 309GG genotype [34]. Moreover, several studies have reported an association between other apoptotic genes such as FAS, FAS ligand [35], and caspase 8 [36].

The present study demonstrated an association between MDM2 309 GG and PE susceptibility. The synergic effects of TT/DD and GG/DD genotypes of MDM2 T309G and I/D polymorphisms were associated with PE risk. Since these associations are not strong, further studies in other countries and ethnic groups are recommended to confirm or refute the current results.

Acknowledgements The study was supported by the Research Center of Research Deputy in Zahedan University of Medical Sciences (Registered No. 1395.18).

Compliance with ethical standards Informed consent was obtained from all subjects, and the study protocol was approved by the Ethics Committee of Zahedan University of Medical Sciences and conducted in accordance with the Declaration of Helsinki.

Conflict of interest The authors declare that they have no conflict of interest.

References

- Bulletins—Obstetrics ACoP. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. *Obstet Gynecol* 2002;99(1):159–167.
- Zhang J, Zeisler J, Hatch MC, Berkowitz G. Epidemiology of pregnancy-induced hypertension. *Epidemiol Rev*. 1997;19(2): 218–32.
- Carty DM, Delles C, Dominiczak AF. Preeclampsia and future maternal health. *J Hypertens*. 2010;28(7):1349–55. doi:10.1097/HJH.0b013e32833a39d0.
- Dekker G, Sukcharoen N. Etiology of preeclampsia: an update. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet*. 2004;87(Suppl 3):S96–103.
- Esplin MS, Fausett MB, Fraser A, Kerber R, Mineau G, Carrillo J, et al. Paternal and maternal components of the predisposition to preeclampsia. *N Engl J Med*. 2001;344(12):867–72. doi:10.1056/NEJM200103223441201.
- Mendilcioglu I, Karaveli S, Erdogan G, Simsek M, Taskin O, Ozekinci M. Apoptosis and expression of Bcl-2, Bax, p53, caspase-3, and Fas, Fas ligand in placentas complicated by preeclampsia. *Clinical and experimental obstetrics & gynecology*. 2011;38(1): 38–42.
- Whitley GS, Dash PR, Ayling LJ, Prefumo F, Thilaganathan B, Cartwright JE. Increased apoptosis in first trimester extravillous trophoblasts from pregnancies at higher risk of developing preeclampsia. *Am J Pathol*. 2007;170(6):1903–9. doi:10.2353/ajpath.2007.070006.
- Sharp AN, Heazell AE, Crocker IP, Mor G. Placental apoptosis in health and disease. *Am J Reprod Immunol*. 2010;64(3):159–69. doi:10.1111/j.1600-0897.2010.00837.x.
- Prives C, Hall PA. The p53 pathway. *J Pathol*. 1999;187(1):112–26. doi:10.1002/(SICI)1096-9896(199901)187:1<112::AID-PATH250>3.0.CO;2-3.
- Sharp AN, Heazell AE, Baczyk D, Dunk CE, Lacey HA, Jones CJ, et al. Preeclampsia is associated with alterations in the p53-pathway in villous trophoblast. *PLoS One*. 2014;9(1):e87621. doi:10.1371/journal.pone.0087621.
- Wu X, Bayle JH, Olson D, Levine AJ. The p53-mdm-2 autoregulatory feedback loop. *Genes Dev*. 1993;7(7A): 1126–32.
- Jones SN, Roe AE, Donehower LA, Bradley A. Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. *Nature*. 1995;378(6553):206–8. doi:10.1038/378206a0.
- Meek DW. The p53 response to DNA damage. *DNA Repair (Amst)*. 2004;3(8–9):1049–56. doi:10.1016/j.dnarep.2004.03.027.
- Gao Q, Zhu X, Chen J, Mao C, Zhang L, Xu Z. Upregulation of P53 promoted G1 arrest and apoptosis in human umbilical cord vein endothelial cells from preeclampsia. *J Hypertens*. 2016;34(7): 1380–8. doi:10.1097/HJH.0000000000000944.
- Berks D, Duvetkot JJ, Steegers EA, Visser W. P53. Association between thrombophilia and preeclampsia. *Pregnancy hypertension*. 2011;1(3–4):298. doi:10.1016/j.preghy.2011.08.114.
- Lucas Rosa Fraga MB. Polymorphisms of the apoptotic genes TP53 and MDM2 and preeclampsia development. *Journal of Fertilization: In Vitro - IVF-Worldwide, Reproductive Medicine, Genetics & Stem Cell Biology*. 2014;03(01). doi:10.4172/2375-4508.1000135.
- Busatto M FL, Boquett JA. Polymorphisms of the apoptotic genes TP53 and MDM2 and preeclampsia development. *JFIV Reprod Med Genet*. 2014;3(1).
- Lalonde ME, Ouimet M, Lariviere M, Kritikou EA, Sinnett D. Identification of functional DNA variants in the constitutive promoter region of MDM2. *Human genomics*. 2012;6:15. doi:10.1186/1479-7364-6-15.
- Ohmiya N, Taguchi A, Mabuchi N, Itoh A, Hirooka Y, Niwa Y, et al. MDM2 promoter polymorphism is associated with both an increased susceptibility to gastric carcinoma and poor prognosis. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2006;24(27):4434–40. doi:10.1200/JCO.2005.04.1459.
- Gaunt TR, Rodriguez S, Day IN. Cubic exact solutions for the estimation of pairwise haplotype frequencies: implications for linkage disequilibrium analyses and a web tool ‘CubeX’. *BMC bioinformatics*. 2007;8:428. doi:10.1186/1471-2105-8-428.
- Burton GJ, Woods AW, Jauniaux E, Kingdom JC. Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. *Placenta*. 2009;30(6):473–82. doi:10.1016/j.placenta.2009.02.009.
- Mayhew TM. Villous trophoblast of human placenta: a coherent view of its turnover, repair and contributions to villous development and maturation. *Histol Histopathol*. 2001;16(4):1213–24.
- Longtine MS, Chen B, Odibo AO, Zhong Y, Nelson DM. Villous trophoblast apoptosis is elevated and restricted to cytotrophoblasts in pregnancies complicated by preeclampsia, IUGR, or preeclampsia with IUGR. *Placenta*. 2012;33(5):352–9. doi:10.1016/j.placenta.2012.01.017.
- Myatt L. Review: reactive oxygen and nitrogen species and functional adaptation of the placenta. *Placenta*. 2010;31(Suppl):S66–9. doi:10.1016/j.placenta.2009.12.021.
- Roberts JM, Escudero C. The placenta in preeclampsia. *Pregnancy hypertension*. 2012;2(2):72–83. doi:10.1016/j.preghy.2012.01.001.
- Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol*. 2007;35(4):495–516. doi:10.1080/01926230701320337.
- Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature*. 1997;387(6630):296–9. doi:10.1038/387296a0.
- Heazell AE, Lacey HA, Jones CJ, Huppertz B, Baker PN, Crocker IP. Effects of oxygen on cell turnover and expression of regulators of apoptosis in human placental trophoblast. *Placenta*. 2008;29(2): 175–86. doi:10.1016/j.placenta.2007.11.002.

29. Zauberman A, Flusberg D, Haupt Y, Barak Y, Oren M. A functional p53-responsive intronic promoter is contained within the human mdm2 gene. *Nucleic Acids Res.* 1995;23(14):2584–92.
30. Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, et al. A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell.* 2004;119(5):591–602. doi:10.1016/j.cell.2004.11.022.
31. Xue Z, Zhu X, Teng Y. Relationship between murine double minute 2 (MDM2) T309G polymorphism and endometrial cancer risk: a meta-analysis. *Medical science monitor : international medical journal of experimental and clinical research.* 2016;22:3186–90.
32. Lv J, Zhu B, Zhang L, Xie Q, Zhuo W. MDM2 SNP309 variation confers the susceptibility to hepatocellular cancer: a meta-analysis based on 4271 subjects. *Int J Clin Exp Med.* 2015;8(4):5822–30.
33. Salimi S, Hajizadeh A, Khodamian M, Pejman A, Fazeli K, Yaghmaei M. Age-dependent association of MDM2 promoter polymorphisms and uterine leiomyoma in South-East Iran: a preliminary report. *J Obstet Gynaecol Res.* 2015;41(5):729–34. doi:10.1111/jog.12625.
34. Fang Y, Kong B, Yang Q, Ma D, Qu X. The p53-HDM2 gene-gene polymorphism interaction is associated with the development of missed abortion. *Hum Reprod.* 2011;26(5):1252–8. doi:10.1093/humrep/der017.
35. Salimi S, Moudi B, Farajian Mashhadi F, Tavilani H, Hashemi M, Zand H, et al. Association of functional polymorphisms in FAS and FAS ligand genes promoter with pre-eclampsia. *J Obstet Gynaecol Res.* 2014;40(5):1167–73. doi:10.1111/jog.12327.
36. Orlando Junior IC, Tanaka SC, Balarin MA, da Silva SR, Pissetti CW. CASPASE-8 gene polymorphisms (rs13416436 and rs2037815) are not associated with preeclampsia development in Brazilian women. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet.* 2017:1–15. doi:10.1080/14767058.2017.1285882.