p53 codon 72 polymorphism and recurrent pregnancy loss: a meta-analysis

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Abstract

Background Arg72Pro polymorphism of the p53 tumour suppressor gene have been associated with recurrent pregnancy loss. However, results were inconsistent. We performed this metaanalysis to drive amore precise estimation of association between p53 codon 72 polymorphism and recurrent pregnancy loss.

Methods Electronic searche of PubMed was conducted to select studies. Case-control studies containing available genotype frequencies of Arg72Pro were chose, and Odds ratio (OR) with 95% confidence interval (CI) was used to assess the strength of this association.

Results 4 case-control studies including 523 cases and 378controls were identified. This meta-analysis showed that individuals with the homozygous Pro/Pro genotype had increased risk of recurrent pregnancy loss (OR=1.85, 95% CI: 1.078~3.173, $p=0.025$) in the pooled analyses. An symmetric funnel plot, the Egger's test ($P=0.497$), and the Begg- test ($P=0.85$) were all suggestive of the lack of publication bias.

Capsule This meta-analysis showed that the Pro/Pro geotype had increased risk of recurrent pregnancy loss (RPL), suggests the p53 Arg72Pro polymorphism may be associated with RPL.

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Conclusion This meta-analysis supports the idea that p53 condon72 Pro/Pro genotype is associated with increased risk of recurrent pregnancy loss. To draw comprehensive and true conclusions, further prospective studies with larger numbers of participants worldwide are needed to examine associations between p53 codon 72 polymorphism and recurrent pregnancy loss.

Keywords p53 · Meta-analysis · Recurrent pregnancy loss

Introduction

Recurrent pregnancy loss (RPL), defined as three or more first trimester spontaneous abortions prior to the 20th week of pregnancy is a common disorder. RPL is an important women's health problem. An underlying cause of recurrent miscarriage such as genetic, endocrine or autoimmune was found in more thar 50% of couples [1]. Several factors such as a variety of environmental as well as lifestyle factors have been considered for idiopathic recurrent miscarriage pathogenesis. Gene polymorphisms may predispose to an increased risk of RPL without consideration of the effects of environmental factors [2]. It was reportet that a significant association of the polymorphisms in genes such as CYP1A2,CYP17, CYP1A1, MTHFR and the risk of recurrent pregnancy loss [3–7]. However the polymorphism of MTHFR and eNOS gene are not associated with recurrent pregnancy loss in Japanese [8]. The RPL remains largely unexplained.

The low expression of angiogenesis- and apoptosis-related genes are associated with RPL [9]. Abnormal apoptosis may cause pregnancy loss during human pregnancy. It is well known that p53, the guardian of the genome, is a stress response protein that functions primarily as a tetrameric transcription factor which regulates a large

number of genes in response to various stresses, including oncogene activation and DNA damage [10]. p53 is involved in both the pro-survival response of cell cycle arrest and DNA repair, and the pro-death response of apoptosis [11]. If a mutation occurs, p53 may not only lose its normal functions, but also gain new abilities that promote tumorigenesis [12]. p53 is the most frequently mutated gene in human tumors; over 50% of tumors harbor mutations in the p53 gene [13]. Beside its role as a tumour suppressor gene, aberrant p53 expression may play a significant role in regulating angiogenesis [14, 15]. P53 was a potential mediator of pregnancy [16]. Some alleles of p53 and its negative regulator, MDM2, are under positive evolutionary selection [17–19]. Significant decreases in embryonic implantation, pregnancy rate and litter size were observed in matings with p53^{-/-} female mice but not with p53^{-/-} male mice. p53 can regulate both basal and inducible transcription of leukemia inhibitory factor (LIF) in mice [20]. These suggest that p53 could have a new function in reproduction [21].

The p53 tumor suppressor gene contains 11 exons, located on chromosome 17p13. The codon 72 polymorphism (rs1042522) is located in exon 4 with CGC to CCC transition, leading to an arginine to proline aminoacidic substitution in amino-acid position 72. To date, a number of studies have been conducted to investigate the potential association between this polymorphism and RPL risk in humans [22–24]. However, the results were inconsistent. Therefore, we conducted a meta-analysis to assess the effect of the p53 codon 72 polymorphism on the risk of RPL.

Materials and methods

Publication search

PubMed was searched using the search terms ‘p53’, ‘polymorphism’ and ‘recurrent pregnancy loss’ or ‘miscarriage’ (the last search update was on Jan 29, 2011). Case-control studies containing available genotype frequencies of Arg72Pro were chose. Additional studies were identified by a manual search of the references of original studies. Of the studies with overlapping data published by the same investigators, only the most recent or complete study was included in this meta-analysis.

Statistic analysis

For control group of each study, the observed genotype frequencies of the p53 codon 72 polymorphism were assessed for Hardy–Weinberg equilibrium using the χ^2 test. The strength of association between codon72 polymorphism of p53 gene and recurrent pregnancy loss was accessed by calculating crude odds ratios (ORs) with 95% confidence intervals (CIs). The pooled ORs were performed for codominant model (Pro/Pro vs. Arg/Arg; Pro/Arg vs. Arg/Arg), dominant model (Pro/Pro+Pro/Arg vs. Arg/Arg), and recessive model (Pro/Pro vs. Pro/Arg+Arg/Arg), additive genetic model (Pro vs. Arg) respectively. Heterogeneity assumption was checked by a chi-square based Q-test. A P-value of <0.05 for the Q-test indicated a lack of heterogeneity among the studies, the summary OR estimate of each study was calculated by the Mantel-Haenszel method [25]. The potential for publication bias was examined by a Begg’s test (funnel plot method) and Egger’s linear regression test ($P < 0.05$ considered representative of statistical significance) [26]. All statistical analyses were performed with Stata software (version 8.2; Stata Corporation, College Station, TX).

Result

Eligible studies

We identified 4 case–control studies on the association between p53 codon 72 polymorphism and recurrent pregnancy loss including 523 recurrent pregnancy loss cases and 387 controls in our meta-analysis (Table 1). The distribution of genotypes in the controls of all the studies was in agreement with Hardy–Weinberg equilibrium.

Meta-analysis

The results of the association between the p53 codon 72 polymorphism and recurrent pregnancy loss and the heterogeneity test were shown in Table 2. The recessive model (Pro/Pro vs. Pro/Arg+Arg/Arg) showed a significant association with recurrent pregnancy loss risk (OR=1.85,

Table 1 The distribution of the p53 codon 72 variant for cases and controls

Author	year	Case			Control			P ^a
		ArgArg	ArgPro	ProPro	ArgArg	ArgPro	ProPro	
Pietrowski D [27]	2005	83	70	22	83	50	10	0.52
Kay C [24]	2006	141	55	9	13	8	0	0.28
Firouzabadi RD [22]	2009	23	41	33	4	21	7	0.07
Kaare M [28]	2009	21	22	3	106	77	8	0.19

^a p value for Hardy–Weinberg equilibrium in control group.

Table 2 ORs and 95% CI for recurrent pregnancy loss and the p53 codon72 polymorphism under different genetic models

genetice model	Pooled OR[95%CI]p	Heterogeneity p-value	Begg's Test p-value	Egger's test p-value
Additive(Pro vs. Arg)	0.91[0.73~1.15]0.44	<0.001	0.042	0.152
Recessive (Pro/Pro vs. Arg-carriers)	1.85[1.08~3.17]0.025	0.996	0.497	0.85
Dominant(Pro-carriers vs.Arg/Arg)	1.240[0.9~1.71]0.19	0.155	0.042	0.06
codominant				
Pro/Arg vs. Arg/Arg	1.11[0.79~1.55]0.551	0.081	0.042	0.007
Pro/Pro vs. Arg/Arg	1.73[0.95~3.15]0.07	0.67	0.497	0.625

95% CI: 1.078~3.173, $p=0.025$, $P=0.996$ for heterogeneity, Fig. 1). No significant association was found in codominant model (Pro/Pro vs. Arg/Arg; Pro/ Arg vs. Arg/Arg), dominant model (Pro/Pro+Pro/Arg vs. Arg/Arg) and additive genetic model (Pro vs. Arg) respectively.

Publication bias

Funnel plot and Egger's test were done to estimate the publication bias of literatures. The results of Egger's test provided statistical evidence for funnel plot symmetry (for Pro/Pro vs.Pro/Arg+Arg/Arg: $P=0.85$), suggesting the absence of publication bias.

Discussion

Human recurrent pregnancy loss is important clinical problem. The mechanism of RPL is still relatively unknown. Single nucleotide polymorphisms (SNPs) can be used as a tool in investigating genetic variations and disease susceptibility. The frequency of homozygous Pro/Pro genotypes was found

significantly higher among the women with RPL than the other two groups indicated that p53 codon 72 polymorphism would be expected to play a major role to bring about RPL [22]. In Pietrowski study, an over-representation of the Pro allele of the p53 gene in women with idiopathic recurrent miscarriages supports to the theory that p53 has a potential role during pregnancy [27]. On the contrary, the other studies reported an insignificant association between the Pro allele and RPL [24, 28]. The summary OR from our meta-analyses revealed that individuals with the homozygous Pro/Pro genotype had increased risk of recurrent pregnancy loss (OR=1.85, 95% CI: 1.078~3.173, $p=0.025$).

Arg72Pro of p53 gene is a common coding polymorphism that results in either proline or arginine at amino-acid position 72. Significant ethnic differences were found in the codon 72 polymorphism. The Pro72 allele frequency increasing in populations as they near the equator is closely linked to latitude [29]. There is a tight association between cold winter temperature and p53 Arg72 in Eastern Asia that suggests causative selection [19]. The codon 72 polymorphism in p53 gene is evolutionarily balanced and maintained by natural

Fig. 1 Forest plot of ORs of recurrent pregnancy loss for Pro/Pro genotype when compared to the Arg allele carriers (Pro/Pro+Pro/Arg). The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the study-specific weight. The diamond represents the pooled OR and 95% CI

selection indicated the association between codon 72 polymorphism and fertility in humans.

A balance in apoptosis and proliferation plays a crucial role in embryonic development during pregnancy. Once implanted, the embryo continues to survive and grow by stimulating its own blood supply through angiogenesis. A good exchange between fetus and mother is necessary to ensure the normal fetal growth, therefore cell arrest in cytotrophoblasts and blood vessels can result in imbalances of cell differentiation leading to miscarriage. Significant differences in the codon 72 polymorphic form of p53 gene might affect the biological activity of p53. The Arg72 variant of p53 protein is markedly better than the Pro72 form in inducing apoptosis and suppressing cellular transformation [30–32], while the Pro72 form appears to induce a higher level of G1 arrest than the Arg72 form [33]. The lower level of apoptosis in p53 Pro allele carriers might cause misguided growth of cells or tissues leading to miscarriage. The increasing cells arrested at the G1 checkpoint might cause inadequate trophoblastic growth leading to abortion. G1 arrest or the potential to resist apoptosis might be the explanation that Pro/Pro carriers are more associated with RPL than with Arg allele carriers. However, these possible explanations are largely speculative and more studies on how the p53 Pro variant contributes to the complex pathogenesis of RPL is required [27].

Leukaemia inhibitory factor (LIF) is the cytokine produced and secreted by the endometrial glands of uterus, regulating implantation through its influence on the luminal epithelium and stromal decidualization, but also its influence on reproductive tract cells such as leukocytes and glandular epithelium, during the pre-implantation phase of pregnancy [34]. Sufficient uterine LIF protein is an essential condition for implantation, and the LIF production in fertile women was greater than in the infertile women has been reported [35]. P53 plays a significant role in maternal reproduction, through the regulation of LIF. Loss of p53 decreased both the level and function of LIF in uteri. In the absence of p53, low LIF is produced, the uterus does not become adequately receptive and implantation of embryos is impaired [20, 21]. Arg72 was clearly more active than p53 Pro72 in transactivating LIF [19]. Pro72 may alter the activity of p53, and in turn result in the reduced uterine LIF levels and decreased implantation rates in women.

In conclusion, this meta-analysis showed that the homozygous Pro/Pro genotype had increased risk of recurrent pregnancy loss (OR=1.85, 95% CI: 1.078–3.173, $p=0.025$), suggests the p53 codon 72 polymorphism may be associated with the risk of recurrent pregnancy loss. Future well designed large studies might be necessary to validate this association in different populations incorporated with environmental factors in the susceptibility of recurrent pregnancy loss.

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References

1. Clifford K, Rai R, Watson H, Regan L. An informative protocol for the investigation of recurrent miscarriage: preliminary experience of 500 consecutive cases. *Hum Reprod*. 1994;9:1328–32.
2. Yamada H, Sata F, Saijo Y, Kishi R, Minakami H. Genetic factors in fetal growth restriction and miscarriage. *Semin Thromb Hemost*. 2005;31:334–45.
3. Sata F, Yamada H, Suzuki K, et al. Caffeine intake, CYP1A2 polymorphism and the risk of recurrent pregnancy loss. *Mol Hum Reprod*. 2005;11:357–60.
4. Suryanarayana V, Deenadayal M, Singh L. Association of CYP1A1 gene polymorphism with recurrent pregnancy loss in the South Indian population. *Hum Reprod*. 2004;19:2648–52.
5. Sata F, Yamada H, Yamada A, et al. A polymorphism in the CYP17 gene relates to the risk of recurrent pregnancy loss. *Mol Hum Reprod*. 2003;9:725–8.
6. Govindaiah V, Naushad SM, Prabhakara K, Krishna PC, Radha Rama Devi A. Association of parental hyperhomocysteinemia and C677T Methylene tetrahydrofolate reductase (MTHFR) polymorphism with recurrent pregnancy loss. *Clin Biochem*. 2009;42:380–6.
7. Kim SY, Park SY, Choi JW, et al. Association Between MTHFR 1298A > C Polymorphism and Spontaneous Abortion with Fetal Chromosomal Aneuploidy. *Am J Reprod Immunol* 2011.
8. Makino A, Nakanishi T, Sugiura-Ogasawara M, Ozaki Y, Suzumori N, Suzumori K. No association of C677T methylenetetrahydrofolate reductase and an endothelial nitric oxide synthase polymorphism with recurrent pregnancy loss. *Am J Reprod Immunol*. 2004;52:60–6.
9. Choi HK, Choi BC, Lee SH, Kim JW, Cha KY, Baek KH. Expression of angiogenesis- and apoptosis-related genes in chorionic villi derived from recurrent pregnancy loss patients. *Mol Reprod Dev*. 2003;66:24–31.
10. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature*. 2000;408:307–10.
11. Li Z, Ni M, Li J, Zhang Y, Ouyang Q, Tang C. Decision making of the p53 network: Death by integration. *J Theor Biol* 2010.
12. Brosh R, Rotter V. When mutants gain new powers: news from the mutant p53 field. *Nat Rev Cancer*. 2009;9:701–13.
13. Bennett WP, Hussain SP, Vahakangas KH, Khan MA, Shields PG, Harris CC. Molecular epidemiology of human cancer risk: gene-environment interactions and p53 mutation spectrum in human lung cancer. *J Pathol*. 1999;187:8–18.
14. Ravi R, Mookerjee B, Bhujwalla ZM, et al. Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. *Genes Dev*. 2000;14:34–44.
15. Yuan A, Yu CJ, Luh KT, Kuo SH, Lee YC, Yang PC. Aberrant p53 expression correlates with expression of vascular endothelial growth factor mRNA and interleukin-8 mRNA and neoangiogenesis in non-small-cell lung cancer. *J Clin Oncol*. 2002;20:900–10.
16. Sivaraman L, Conneely OM, Medina D, O'Malley BW. p53 is a potential mediator of pregnancy and hormone-induced resistance to mammary carcinogenesis. *Proc Natl Acad Sci U S A*. 2001;98:12379–84.
17. Atwal GS, Bond GL, Metsuyanin S, et al. Haplotype structure and selection of the MDM2 oncogene in humans. *Proc Natl Acad Sci U S A*. 2007;104:4524–9.
18. Murphy ME. Polymorphic variants in the p53 pathway. *Cell Death Differ*. 2006;13:916–20.
19. Shi H, Tan SJ, Zhong H, et al. Winter temperature and UV are tightly linked to genetic changes in the p53 tumor suppressor pathway in Eastern Asia. *Am J Hum Genet*. 2009;84:534–41.

20. Hu W, Feng Z, Teresky AK, Levine AJ. p53 regulates maternal reproduction through LIF. *Nature*. 2007;450:721–4.
21. Hu W, Feng Z, Atwal GS, Levine AJ. p53: a new player in reproduction. *Cell Cycle*. 2008;7:848–52.
22. Firouzabadi RD, Ghasemi N, Rozbahani MA, Tabibnejad N. Association of p53 polymorphism with ICSI/IVF failure and recurrent pregnancy loss. *Aust N Z J Obstet Gynaecol*. 2009;49:216–9.
23. Coulam CB, Kay C, Jeyendran RS. Role of p53 codon 72 polymorphism in recurrent pregnancy loss. *Reprod Biomed Online*. 2006;12:378–82.
24. Kay C, Jeyendran RS, Coulam CB. p53 tumour suppressor gene polymorphism is associated with recurrent implantation failure. *Reprod Biomed Online*. 2006;13:492–6.
25. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst*. 1959;22:719–48.
26. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 1997;315:629–34.
27. Pietrowski D, Bettendorf H, Riener EK, et al. Recurrent pregnancy failure is associated with a polymorphism in the p53 tumour suppressor gene. *Hum Reprod*. 2005;20:848–51.
28. Kaare M, Butzow R, Ulander VM, Kaaja R, Aittomaki K, Painter JN. Study of p53 gene mutations and placental expression in recurrent miscarriage cases. *Reprod Biomed Online*. 2009;18:430–5.
29. Sjalander A, Birgander R, Kivela A, Beckman G. p53 polymorphisms and haplotypes in different ethnic groups. *Hum Hered*. 1995;45:144–9.
30. Dumont P, Leu JI, Della Pietra 3rd AC, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat Genet*. 2003;33:357–65.
31. Sullivan A, Syed N, Gasco M, et al. Polymorphism in wild-type p53 modulates response to chemotherapy in vitro and in vivo. *Oncogene*. 2004;23:3328–37.
32. Bergamaschi D, Samuels Y, Sullivan A, et al. iASPP preferentially binds p53 proline-rich region and modulates apoptotic function of codon 72-polymorphic p53. *Nat Genet*. 2006;38:1133–41.
33. Pim D, Banks L. p53 polymorphic variants at codon 72 exert different effects on cell cycle progression. *Int J Cancer*. 2004;108:196–9.
34. Kimber SJ. Leukaemia inhibitory factor in implantation and uterine biology. *Reproduction*. 2005;130:131–45.
35. Hambartsoomian E. Endometrial leukemia inhibitory factor (LIF) as a possible cause of unexplained infertility and multiple failures of implantation. *Am J Reprod Immunol*. 1998;39:137–43.