Original Article

Association of CYP11A1 Polymorphisms with Recurrent Pregnancy Loss in the Female Population of Punjab

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Abstract

Background: Recurrent pregnancy loss (RPL) is defined as the failure of two or more clinically recognised pregnancies before 20 weeks of gestation. The prevalence of clinically evident RPL is 1%–2% worldwide. The aetiologies of RPL include uterine anatomic anomalies, uncontrolled diabetes mellitus, untreated hypothyroidism, parental chromosomal abnormalities, antiphospholipid antibody syndrome, thrombophilia, genetic abnormalities and infections. **Aims:** This study was aimed at investigating the possible association between *CYP11A1* (rs11632698) and (rs4077582) polymorphisms with RPL in the female population of Punjab. **Settings and Design:** The case– control study was conducted on 170 subjects, of which 80 RPL cases and 90 controls were analysed. **Materials and Methods:** Genotypic analysis was performed using the polymerase chain reaction – restriction fragment length polymorphism. **Statistical Analysis Used:** Pearson's Chi-square test was used. **Results:** The genotypic frequency of *CYP11A1* (rs11632698) A > G polymorphism was statistically significantly different amongst cases and controls (P = 0.00001). It was observed that the presence of the G allele might increase the risk of RPL. A Chisquare analysis of *CYP11A1* (rs4077582) (*P* = 0.01) indicated a significant difference amongst the genotypes of cases and controls of RPL. **Conclusion:** *CYP11A1* variants (rs11632698 and rs4077582) may be useful markers in determining the genetic susceptibility to the pathogenesis of RPL. Keywords: *CYP11A1*, recurrent miscarriage, recurrent pregnancy loss, rs11632698, rs4077582, spontaneous abortion.

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Introduction

The loss of two or more consecutive pregnancies before 20 weeks of gestation is referred to as recurrent pregnancy. The incidence of recurrent pregnancy loss (RPL) was reported about 1%–2%, and the risk increased with increasing maternal age and number of previous pregnancy losses.[1] In women under 35 years of age, the risk of gestational loss is 9%–12%, whereas the risk increases up to 50% in those over 40 years of age.[2] RPL can be divided into three categories: primary RPL, women who have RPL without a previous viable pregnancy before 20 weeks of gestation; secondary RPL, women experiencing RPL after at least one viable pregnancy beyond 20 weeks of gestation; tertiary RPL, which refers to multiple pregnancy losses in between healthy pregnancies.[3,4] The aetiology of RPL may include chromosomal abnormalities, infections, thrombophilia, genetic factors, uterine anatomic abnormalities, antiphospholipid syndrome and exposure to environmental factors.[5]

CYP11A1 encodes a cholesterol side-chain cleavage (P450scc) enzyme. The cytochrome P450 proteins are monooxygenases which catalyse the synthesis

Received: 22-02-2023 **Revised:** 02-09-2023
Accepted: 03-09-2023 **Published:** 29-09-2023

Access this article online

Quick Response Code:

Website: www.jhrsonline.org

DOI: 10.4103/jhrs.jhrs_24_23

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How to cite this article: Rayat AK, Thapar P, Kaur M, Singh S, Kaur A. Association of CYP11A1 polymorphisms with recurrent pregnancy loss in the female population of Punjab. J Hum Reprod Sci 2023;16:242-5.

of cholesterol, steroids and other lipids.[6] This protein is localised to the inner membrane of mitochondria and catalyses the conversion of cholesterol to pregnenolone, the first and rate-limiting step in the synthesis of the steroid hormones.[7] Normal pregnancy is supported by the increased levels of progesterone, secreted by ovarian luteal cells through the enzymatic step catalysed by P450scc.[8] Progesterone produced through steroidogenesis is required for the maintenance of pregnancy, and foetal development as it suppresses uterine contractility and prevents spontaneous abortions.[9] It is responsible for preparation of the endometrium for implantation process and maintenance of gestational sac in the uterus, also by the modulation of the maternal immune system.[10] During pregnancy, there are two potential sources of progesterone – the corpus luteum of the mother's ovary and the placenta. The corpus luteum secretes progesterone only during the first trimester, whereas the placental progesterone becomes sufficient to maintain pregnancy after about 6 weeks of gestation, known as luteo-placental shift.[11] Disruption of P450 side-chain cleavage cytochrome enzyme due to deleterious mutation in the *CYP11A1* is thought to be incompatible with foetal survival due to impaired progesterone production by the foetoplacental unit.[12] The human foetus homozygous for a P450scc mutation will spontaneously abort at about 6–7 weeks when production of progesterone from the maternal corpus luteum wanes and the mutant placental system is unable to take over.[13]

Rationale of study

Women with RPL are increasing worldwide at an alarming rate. Therefore, it is important to characterise the genetic, anatomic and environmental factors that influence the disease susceptibility in females with RPL. Moreover, there are very less studies that describe the genetic mechanism of RPL amongst the North India population. Thus, the present study was aimed at investigating the possible association between *CYP11A1* (rs11632698 and rs4077582) and the risk of RPL in the female of Punjab.

Methodology

The study was approved by the institutional ethical committee, with the principles embodied in the Declaration of Helsinki.[14] A signed informed consent was obtained from subjects for the use of anonymised data for research purposes.

Sample collection and selection criteria

After the approval given by the ethics review board of Guru Nanak Dev University, Amritsar (no: 820/HG), the present case–control study was conducted from 2021 to 2022. The archived blood samples from the our laboratory were used. The sample size was 170 females, including 80 cases and 90 controls for analysis of single-nucleotide polymorphisms (SNPs) in *CYP11A1* (rs11632698 and rs4077582). Couples with two or more consecutive pregnancy losses with or without a normal child were selected as cases, and for controls, normal healthy couples with at least one or more live births without any history of miscarriage were included in the study [Figure 1]. RPL couples with known cause of losses such as hormonal imbalances, blood grouping factors, chromosomal abnormalities, uterine anomalies, genital infections and endocrinological disorders were excluded from the study. After taking written informed consent from each participant, blood samples were obtained from women and were stored at − 20°C for further analysis. The DNA was extracted from the blood samples using organic method given by Adeli and Ogbonna with some modifications.[15] Quantification of DNA was done using NanoDrop and agarose gel

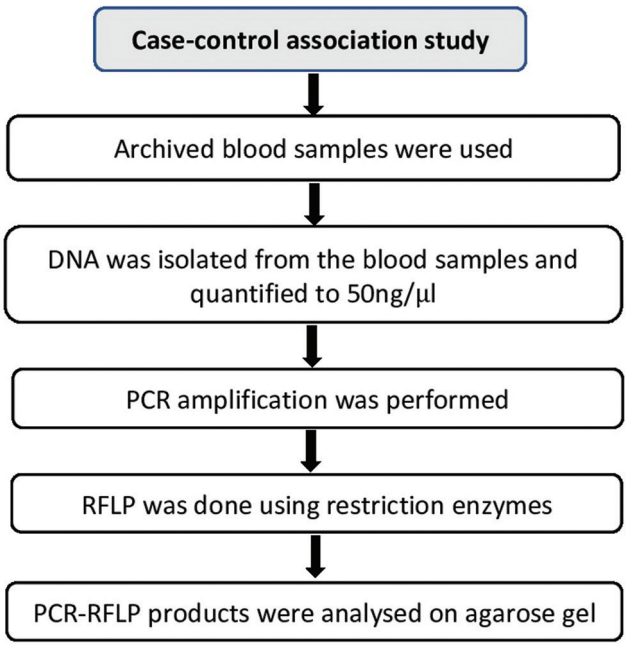

Figure 1: Flow chart depicting methodology

Table 1: Sequence of selected primers for polymerase chain reaction amplification

SNP	Primer sequence	Annealing temperature (°C)	Product size (bp)	Reference
rs11632698	5'-CATCCAGCACTCCCACCAG-3' 5'-TGGAGTGTCAAAGGTGAGCATC-3'	62	281	[16]
rs4077582	5'-GCCAGTCAGACAAGGGCACAGGAAG-3' 5'-GTGGCCGACTATGTAAACCAGAG-3'	62	212	[16]

SNP=Single-nucleotide polymorphism

electrophoresis. Genotype screening was performed for identification of *CYP11A1* polymorphisms [Table 1].[16]

Genotypic analysis

The present study was conducted on 170 females, 80 suffering from RPL and 90 healthy fertile females. The *CYP11A1* (rs11632698) polymorphism was analysed, and the products of 281 bp were obtained after polymerase chain reaction (PCR) amplification. The amplified products were analysed on 1.5% agarose gel. Restriction digestion was done at 37°C using *BanII* restriction enzyme. A single 281 bp band depicted the wild type (AA) homozygotes, whereas three bands of 281 bp, 179 bp and 102 bp indicated heterozygous (AG) genotype. The *CYP11A1* (rs4077582) polymorphism was analysed, and the products of 212 bp were obtained after PCR amplification. The products were analysed on 1.5% agarose gel. Restriction fragment length polymorphism was performed using a restriction enzyme, *Mly1*. The 212 bp band defined wild type (CC) genotype, and the three fragments of 212, 185 and 25 bp represented the (CT) heterozygous genotype.

Statistical analysis

The primary purpose of this study is not hypothesis testing, and therefore, the sample size is not calculated. The relative association between patients and controls for genotypic and allelic frequencies was assessed by the Pearson's Chi-square test. The corresponding odds ratios and confidence intervals (95% confidence interval) were calculated**.**

Results

The study consists of 170 subjects, including 80 cases and 90 controls. The genotypic frequency of *CYP11A1* (rs11632698) A >G polymorphism was significantly different amongst cases and controls (P = 0.000016) [Table 2]. The odds ratio of (AG + GG) dominant model is 8.0 (2.96–21.5) [Table 2] indicating that these genotypes increase the risk of having RPL by 8 folds, whereas the odds ratio of G allele is 3.3 (1.63–6.79) [Table 2], and thus, G allele confers a 3-fold increase in risk of having RPL. A Chi-square analysis of *CYP11A1* (rs4077582) C >T (P = 0.01296) [Table 2] indicated a significant difference amongst the genotypes of cases and controls (P = 0.01296) [Table 2]. The odds ratio of (CT) heterozygous genotype is 2.9 (1.08–8.16) and that of (TT) genotype is 4.1 (1.13–15.32) [Table 2] indicating that these genotypes increase the risk of RPL by 2.9 and 4 folds, respectively. The odds ratio of the T allele is 1.9 (1.05–3.64) [Table 2], and thus, the T-allele increases the risk of having RPL by 1.9 folds.

Discussion

RPL is one of the most distressing conditions faced by the couples trying to conceive as they go through typical stages of grief and loss. The spontaneous miscarriage rate varies from 10% to 20% of all clinically recognised pregnancies. The pathophysiology underlying RPL is incredibly diverse which involves haematology, endocrinology, immunology and genetics; hence, it is necessary to determine the cause behind RPL.[17] In the first and rate-limiting phase of steroidogenesis, *CYP11A1* encodes the P450scc enzyme that catalyses the conversion of cholesterol to pregnenolone. All steroid hormones, including progesterone, which is necessary to maintain a term pregnancy, become deficient as a result of defective enzyme activity.

A significant association of *CYP11A1* (rs4077582) polymorphism in RPL was found in our study, which is in concordance with the study done by Xu *et al*.[18] to explore the genetic relationships between *LHR1* (rs2816948), *CYP19* (rs727479 and rs700518) and P450scc (rs4077582) as a potential mechanism behind unexplained recurrent spontaneous abortions in a Chinese Han population. A case–control study was conducted on patients with unexplained recurrent miscarriage (n = 82, abortion group) and those who voluntary surrendered to a normal early pregnancy (n = 97, control group). There were significant genotypic differences (P < 0.05) for P450scc (rs4077582), but no significant differences for its allelic distribution (P > 0.05).[18]

Table 2: Genotypic and allelic frequency distribution of *CYP11A1* (rs11632698) and (rs4077582) polymorphisms in cases and controls

Polymorphisms	Genotype/allele	Cases (%)	Controls (%)	χ^2 (P)	OR (95% CI)	P
rs11632698	AA	8 (20.0)	30 (66.6)	0.00001*	Reference	-
	AG + GG	32 (80.0)	15 (33.3)		8.0 (2.96–21.57)	0.0001*
	A	48 (60.0)	75 (83.3)	0.0006*	Reference	-
	G	32 (40.0)	15 (16.6)		3.3 (1.63–6.79)	0.0009*
rs4077582	CC	6 (15.0)	20 (44.4)		Reference	-
	CT	24 (60.0)	17 (37.7)	0.012*	2.9 (1.08–8.16)	0.03*
	TT	10 (25.0)	8 (17.7)		4.1 (1.13–15.32)	0.03*
	C	36 (45.0)	57 (63.3)	0.016*	Reference	-
	T	44 (55.0)	33 (36.6)		1.9 (1.05–3.64)	0.03*

*P<0.05 was considered to be statistically significant. OR=Odds ratio, CI=Confidence interval

Our study found a significant role of *CYP11A1* (rs11632698) polymorphism in RPL, and a finding that is in concordance with a study conducted on *CYP11A1* transgenic female mice by Chien *et al*.[9] observed that progesterone insufficiency led to a large proportion of impaired implantation in *CYP11A1* transgenic mothers, although these females had normal folliculogenesis and ovulation ability. In *CYP11A1* transgenic mothers, the defective differentiation of corpora lutea resulted in P4 insufficiency, decreased implantation rates and aberrant placentation.

Limitations

The current study was conducted on a limited sample size; therefore, it was not possible to generalise the findings. A large-scale investigation is required to substantiate the role of *CYP11A1* polymorphisms in RPL.

Conclusion

To the best of my knowledge, the present study is first of its kind to examine the association of *CYP11A1* (rs11632698) polymorphism with RPL worldwide. The *CYP11A1* (rs4077582) polymorphism is the first to be investigated for its association with recurrent miscarriages in India. These SNPs (rs11632698 and rs4077582) may be useful markers in determining the genetic susceptibility to the pathogenesis of RPL. Identification of genetic markers through case–control associations and further functional studies are needed to analyse the potential factors for RPL.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Data availability statement

The data used in the present study are available by the corresponding author on request.

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