The association of arylendosulfatase 1 (*SULF1*) gene polymorphism with recurrent miscarriage

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Abstract

Purpose One of the most common problems in reproductive medicine is recurrent miscarriage (RM). There is increasing evidence showing genetic susceptibility of women is an important risk factor in the occurrence of RM. In recent years, there is a growing interest in sulfate and its role in fetal development. A novel mechanism of *SULF1* has been demonstrated for modifying the activities of some growth factors and signalling molecules that have major roles during embryogenesis. The aim of present study was to evaluate the association of *SULF1* gene polymorphism (rs6990375 G > A) in Iranian patients with RM.

Methods We established a case-control study of 200 Iranian women: 100 patients with the history of two or more RM as cases and 100 healthy women with at least two cases of successful pregnancy and no history of miscarriage as controls. The polymorphism was examined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. **Results** The genotypic analysis between case and controls showed significant differences (p -value=0.000). Allelic analysis showed no significant correlation ($\chi^2=3.36$, p -value=0.066). The heterozygous genetic variant was significantly higher among healthy women (OR=12.67, 95 % CI=6.47–24.79).

Capsule Our results indicated that the *SULF1* gene rs6990375 G > A polymorphism could be associated with increased risk for RM.

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Conclusions Our data showed that rs6990375 polymorphism of *SULF1* gene could be among one of the factors related to RM in Iranian women. Further evaluation of this polymorphism may be important and need further studies.

Keywords *SULF1* gene · Polymorphism · Recurrent miscarriage · Genetic susceptibility

Introduction

Habitual abortion, or recurrent pregnancy loss (RPL) or recurrent miscarriage (RM) is one of the most common problems in reproductive medicine. It is generally defined as two or more consecutive losses usually before 20 weeks of gestation. RM affects 3–5 % of couples attempting to bear children [1]. Several etiological factors potentially increase the risk of RM, including parental chromosomal anomalies, genetic disorders, uterine pathologies, endocrine dysfunctions, acquired autoimmune diseases, inherited thrombophilias and anatomic abnormalities [2].

However some couples never have a cause identified (at least 50 % of the RM cases), and are considered as idiopathic or unexplained cases. A wide variety of associated factors such as uterine abnormalities, luteal phase defect, hyper prolactinaemia, hyper androgenaemia, hyper homocysteinnemia, genital infection, maternal/paternal dysfunction and autoimmune dysfunction have been identified [3]. There is increasing evidence indicating genetic susceptibility of women is an important risk factor in occurrence of this multifactorial condition [4].

SULFs are arylendosulfatase which as post synthetic editors selectively liberate 6-O-sulfate groups from heparin sulfates and therefore alter the sulfation patterns of proteoglycans and the binding site of many growth factors [5]. With such unique regulatory activity, SULFs have an important role in many biological processes, such as angiogenesis, cell

signalling and embryogenesis [6–11]. SULF1 and SULF2 are produced in the large number of embryonic and adult tissues and have an important role in viability and embryonic development [12, 13]. Two naturally occurring SULF1 variants are SULF1A and SULF1B. There are specific changes in the proportions of SULF1A and SULF1B isoforms at both the mRNA and protein levels in many developing tissues. SULF1B promotes angiogenesis and is highly expressed in endothelial cells during early blood vessel development. SULF1A predominates in mature endothelial cells [14].

In order to understand essential role of SULFs in embryonic development, double knockout mice were produced. It was shown that genetically deficient mice for these sulfatases, show some abnormal phenotypes such as brain malformations, skeletal malformations, abnormal innervations of smooth muscle and embryonic lethality [15–17]. In one study mice were generated carrying loss of function alleles for the secreted Sulf1 and Sulf2. Analysis of these mice identified a highly redundant function of both genes. It was shown that with loss of increasing numbers of alleles, there is increased severity of the skeletal malformations. Additionally, double homozygous mutants were characterized by reduced body weight and size of the skeleton, in addition to different misshaped skeletal abnormalities [18].

In women with RM, there is a disability in successful pregnancy conservation, therefore considering the influence of SULFs deficiency on abnormal embryonic development and lethality, we hypothesis that deficiency of SULFs could have a role on RM in these patients. The present study aimed to evaluate the rate of one of the functional polymorphism of *SULF1* gene in an Iranian female population with or without RM.

We examined the common single nucleotide polymorphism (SNP) of rs6990375 G > A in a group of patients with at least two recurrent spontaneous miscarriages of unexplained etiology and with no previous live births as RM group. The results compared with a control group of women with no history of pregnancy related complications.

Materials and methods

Participants The current study was conducted on 100 patients referred to a Yazd Research and Clinical Center for Infertility with a history of two or more RM. This group also has never had a live birth. The exclusion criteria were any known anatomical abnormalities, coagulopathies and/or chromosomal abnormalities causing RM. One hundred healthy, unrelated, age-matched individuals from the same geographical area with at least two cases of successful pregnancy and without any history of miscarriages and other gynecological complications were selected as the control group. This study was approved by the Ethics Committees of the Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. It

has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All persons gave their informed consent prior to their inclusion in the study.

Genetic analysis Whole blood samples (5 ml) were collected in EDTA coated collection tubes and genomic DNA was extracted using Bioneer DNA extraction kits based on the manufacturer protocol. *SULF1* gene SNP of rs6990375 was analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers used in PCR reactions consist of; forward: 5'-TGG CAA TTT TGC TCT TTT CC-3' and reverse: 5'-TGA CAT AGA GTG CCC AGG TG-3' to amplify a 227 bp fragment. The PCR conditions consist of an initial melting step of 95 °C for 3 min, followed by 35 cycles of denaturation (95 °C for 35 s), annealing (55 °C for 45 s), and extension (72 °C for 45 s), and a final extension step of 72 °C for 1 min. For detecting SNP, the PCR products were digested with restriction endonuclease HhaI. The digested products were detected on a 2 % agarose gel staining with ethidium bromide. A single 227 bp band was the indication of AA homozygous polymorphism of *SULF1* gene, while two bands of 128 and 99 bp on the gel revealed the GG homozygous polymorphism of *SULF1* gene and all three bands showed GA heterozygous polymorphism of *SULF1* gene.

Statistical analysis Both allelic and genotypic correlation between patients and controls were analyzed using chi-square test with Pearson and Yates correction and cross tabulation, where appropriate. Probability values of 0.05 or less were regarded as statistically significant. The odds ratio (OR) and the 95 % confidence intervals (CI) were calculated for all data. All analyses were performed using the SPSS Statistical Analysis software (version 16) and VassarStats: Website for Statistical Computation.

Results

In total 200 women of two groups, 100 healthy women with at least two cases of successful pregnancy and no history of miscarriages and 100 women with the history of RM were investigated. For reducing the effects of disturbing factors, the case and control groups were matched respecting the age and

Table 1 Characteristics of women with idiopathic recurrent miscarriage

	Age ± SD	BMI ± SD	Mean number of miscarriage	Mean number of children
Patients	30.93±5.17	29.30±5.24	3.07±1.26	0.00
Controls	29±4.38	22.74±2.65	0.00	2.05±0.96

Table 2 Genotypic and allelic analysis of *SULF1* polymorphism in Yazd population

SNP	Genotype frequency (%)			χ^2 (p -value)	Allele frequency (%)		χ^2 (p -value)	OR (95 % CI)
rs6990375	AA	AG	GG	−(0.000)	A	G	3.36 (0.066)	0.6875 (0.46–1.03)
Patients	52	24	24		128	72		
Controls	15	80	5		110	90		

ethnicity (Table 1). Our data showed that there is a seemingly large difference in the BMI of the patients (29) and the controls (22). To our knowledge this difference could not have had any impact on the results. The Genotypic and allelic analysis of *SULF1* rs6990375 G > A gene polymorphism is shown in Table 2. Allelic analysis showed no significant correlation ($\chi^2=3.36$, p -value=0.066). The genotypic analysis between case and controls showed significant differences (p -value=0.000). Further, CI and OR are presented in Table 3. Our data showed that the heterozygous genetic variant was significantly higher among healthy women (OR=12.67, 95 % CI=6.47–24.79).

Discussion

The goal of present research was to evaluate association of the *SULF1* gene polymorphism (rs6990375G > A) with increased risk of idiopathic recurrent miscarriage among patients referred to a Research and Clinical Center for Infertility. Previous studies have shown that RM is a multifactorial condition and many factors regarding this condition have been examined, such as genetics, age, antiphospholipid syndrome, uterine anomalies, thrombophilias, hormonal and metabolic disorder, infection, auto immunity, male factor and life style issues. There is no study investigating the relationship between SULFs and any of these various laboratory markers already associated with RPL specifically antiphospholipid antibodies, anti B2GP1, etc. Future studies should focus on the association of Sulfs with these other well described laboratory markers.

Genetic susceptibility is an important risk factor in occurrence of RM [3]. There is several studies that investigate the association between gene polymorphism and RM [19, 20]. Tempfer et al. showed that the endothelial nitric oxide synthase (*NOS3*) gene polymorphism may have an effect on the occurrence of RM [21]. Some other studies showed that

glutathione S-transferases (GST) M1 polymorphisms may have an increased risk of RM appearance [22].

SULF1 are arylendosulfatase which alter the sulfation patterns of proteoglycans and the binding site of many growth factors [23]. They have an important role in embryogenesis.

An important nutrient for human growth and development is Sulfate (SO₄^{2−}) which is obtained from the diet. In addition, the intra-cellular metabolism of sulfur-containing amino acids such as methionine and cysteine can produce sulfate. But during pregnancy, fetal tissues cannot produce enough sulfate, therefore the fetus must obtain the sulfate from the maternal circulation. In recent years, there is a growing interest in sulfate and its role in fetal development [24]. It is the possibility that the addition of heparin sulfate could theoretically influence the alteration of sulfation patterns and therefore this can be used as a theoretical approach to manage RM.

As mentioned, previous studies have investigated the effects of reduction of *SULF1* and *SULF2* in abnormal embryonic development [15–17]. Therefore, we hypothesises that SULFs polymorphism may have an effect on recurrent pregnancy losses in RM patients. In order to assess the above hypothesis, rs6990375 SNP frequencies were calculated using PCR-RFLP among two groups included RM patients and healthy women. This is the first report of *SULF1* gene polymorphisms association with RM. The survey about polymorphisms of *SULF1* gene, for the first time was performed by Han et al. who genotyped five common single nucleotide polymorphisms (SNPs) in *SULF1* gene to evaluate associations between these functional *SULF1* SNPs and the risk of ovarian cancer [23]. They found that some of the *SULF1* gene SNPs can have one of following predicted functionalities: (1) affecting transcription factor binding sites (TFBS) activity in the putative promoter region, (2) affecting splicing activity, or (3) affecting the microRNA binding sites activity. According to their study the rs6990375 is a functional SNP affecting the micro RNA binding site activity [23]. Recently numerous studies have shown that miRNAs have important role in the

Table 3 Genotypic analysis and confidence intervals

Genotype	AA	95 %CI	OR (95 % CI)	AG	CI	OR (95 % CI)	GG	95 %CI	OR (95 % CI)
Patients	52	0.42–0.62	0.16 (0.08–0.32)	24	0.16–0.34	12.67 (6.47–24.79)	24	0.16–0.34	0.17 (0.06–0.46)
Controls	15	0.09–0.24		80	0.70–0.87		5	0.02–0.12	

occurrences of gynecological disorders such as endometriosis, preeclampsia, infertility and recurrent miscarriages. The targets of many miRNAs are molecules that are closely associated with processes such as cell proliferation and differentiation.

Because *SULF1* as a regulator is associated with many signalling molecules such as BMPs, fibroblast growth factors (FGFs), hedgehogs, and Wnts, therefore it is a possibility that *SULF1* can be one of these target molecules [25]. Heparan sulfate proteoglycans (HSPGs), when sulfated, serve as co-receptors for many growth factors and cytokines. *SULF1* desulfate cellular HSPGs. Therefore *SULF1* seems to modulate growth factor and cytokine signaling. In facts, *SULF1* inhibits the co-receptor function of HSPGs in different growth factors such as fibroblast growth factor 2, vascular endothelial growth factor, hepatocyte growth factor, PDGF, and heparin-binding epidermal growth factor (HB-EGF). Therefore, a novel mechanism of *SULF1* has been demonstrated for modifying the activities of some growth factors and signalling molecules that have major roles during embryogenesis. The examples of these growth factors and signaling molecules are Wnt signalling, BMP signaling, and fibroblast growth factor regulation. It was shown that forced expression of *SULF1* decreases cell proliferation, migration, and invasion [26].

Our results represent a new genetic association between *SULF1* and recurrent miscarriage as shown; the frequency of GG and AA homozygous genotypes is higher among patients, and then women with these genotypes were significantly more susceptible to RM. On the other hand, the increasing frequency of AG genotype in control group suggesting that this genotype is associated with more chance for successful pregnancy. Why heterozygotes do better? Further studies must concentrate on the difference between the function of this heterozygosity. One possible explanation is that the expression level of *SULF1* for embryogenesis is best when there is heterozygosity, not higher or lower expression.

In summary, our findings reveal a new genetic association between occurrences of recurrent miscarriage and *SULF1* gene mutation. But our study had some limitation therefore we suggest doing further genetic studies with a larger sample size to confirm and clarify this association. Investigating *SULF1* gene expression patterns in RM patients, and also study of overlapping *SULF1* and *SULF2* roles in development could help this confirmation. Further evaluation of this polymorphism may be important and need further studies.

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

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