CD46 polymorphism: A probable risk factor for recurrent spontaneous abortion in a northern Indian population

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ABSTRACT

Background. CD46 is a complement regulatory glycoprotein. Certain polymorphic forms of the CD46 gene have been associated with recurrent pregnancy loss in the Caucasian population. We assessed the role of CD46 polymorphism in recurrent spontaneous abortion in our setting, as this has not been done on Indian subjects till date.

Methods. Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) was carried out on 44 samples each from women with recurrent spontaneous abortion and normal pregnancy. Genotyping of the CD46 gene was done using 2.5% agarose gel. Statistical analysis was done using the TFPGA software.

Results. The absence of CD46H*1 homozygosity was more pronounced in women with recurrent spontaneous abortion in the Indian population. Of recurrent aborters, 9% had the H*1/1 genotype as compared to 30% of normal pregnant women.

Conclusion. Although our data did not fit the Hardy–Weinberg equilibrium, this pilot study indicates that further increasing the sample size might clarify whether polymorphism in the first intron of the CD46 gene can be regarded as a risk factor for recurrent spontaneous abortion.

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INTRODUCTION

Recurrent spontaneous abortion (RSA), defined as 3 consecutive pregnancy losses prior to the 20th week of gestation, occurs in 3%–4% of women.1 In a number of studies, 2 consecutive pregnancy losses prior to the 20th week of gestation have also been included as RSA.2,3 Women with RSA can be classified into primary and secondary aborters.4 Primary aborters are those who have lost all previous pregnancies and have no live birth while secondary aborters are those who have at least 1 successful pregnancy irrespective of the number of pregnancies lost. Globally, 5 of 100 women are reported to have RSA.5 The aetiology is idiopathic.6

The complement system is often referred to as a double-edged sword. On the one hand, it plays an important role in host defense against infectious agents and in promoting the removal of immune complexes and apoptotic cells. On the other hand, unrestrained activation of the complement system has a major role in inflammatory diseases such as systemic lupus erythematosus (SLE) and even RSA.7,8 Because of the risks of complement-dependent damage to foetal tissue, expression of all 3 complement-regulatory proteins DAF, CD46 and CD59 in the cytotrophoblast and syncytiotrophoblast is thought to be one of the strategies of the maternal immune system to counter the ill-effects of uncontrolled complement activation.9

CD46 is a membrane-bound complement-regulatory glycoprotein, also known as membrane cofactor protein (MCP) or trophoblast leukocyte common (TLX).10 It is a cofactor for factor I-dependent proteolytic cleavage of C3b and C4b.11 Except for red blood cells, nearly every cell in the body expresses CD46 on its surface.12 The human CD46 gene has been localized to chromosome 1 at band q 32. It exists as a single copy gene of ~43 kb and contains 14 exons.13,14

Studies on polymorphism of the CD46 gene in the Caucasian population indicate that certain forms of the gene make an individual more susceptible to RSA.1,4 Wang et al.1 also reported that the frequency and homozygosity of one allele (CD46H*2) were significantly increased in patients with RSA (27.4% v. 9.7% in fertile controls). We, therefore, studied CD46 polymorphism and its role in the pathophysiology of RSA in Indian women.

CD46 shows 2 protein isoforms in the peripheral blood lymphocytes; Mr 66,000 (α) and Mr 56,000 (β), determined by differential splicing of mRNA. All individuals express both the forms; the relative quantity of the higher and lower Mr forms expressed, however, differs and is inherited in an autosomal, codominant manner as

1. U  upper Mr form predominant,
2. E  lower and upper Mr forms expressed in equal amounts,
3. L  lower Mr form predominant.

In American Caucasian populations, the percentage distribution of the U, E and L forms was 65%, 30% and 5%, respectively.16 Studies indicate a strong correlation between restriction fragment length polymorphism (RFLP) patterns and expression of CD46 protein isoforms.13,15 It has been proposed that point mutations in the CD46 gene (within exons or introns) may affect the splicing process and bias the relative proportions of different RNA transcripts.15,17 The gene for CD46 is polymorphic with a biallelic polymorphism at the restriction enzyme HindIII site in its first intron between exon 1 (codes for 5´UT/signal peptide) and exon 2 (codes for the first short consensus repeat).13,15

Using polymerase chain reaction (PCR), the sequence around intron 1 of the gene was amplified by Bora et al.13 and Wang et al.1 to study HindIII polymorphism. In the study done by Wang et al.1 on a much larger sample size of both patient and normal population, a single 243 bp band was obtained after PCR. In the case of the U protein phenotype, HindIII digestion of the amplicon resulted in 2 bands 188 and 55 bp in size. In the L protein phenotype, there was no change in the size of the 243 bp segment. For the E phenotype, the PCR product was partially cleaved and bands were observed at the 243, 188 and 55 bp positions. DNA from the U phenotype demonstrated an intact HindIII site (AAGCTT) whereas in case of DNA from the L phenotype, guanine was substituted for a cytosine in the recognition sequence. So, the sequence for the L phenotype was AAGGT'T. This change in sequence is designated as IVS1-1724 C>G.

METHODS

Collection of samples from patients and normal pregnant women

Patients were selected from Sai Ashirwad Medical Centre (Noida)
A reaction volume of 50 µl was used which included 2 mM MgCl₂. Genomic DNA obtained from the samples was used as a template. PCR analysis of DNA (optimum concentration) was taken for each reaction. Recording the absorbance at 260 nm and 280 nm. About 10–15 ng to extract genomic DNA according to a previously standardized and 44 women with RSA. The lymphocytes were then processed from patients 6 weeks after they had aborted and from the control group when the pregnancy was a mean (SD) of 12 (2) weeks old. Parity and gravidity of the patient population was 0 and 2+, respectively. All patients filled a detailed questionnaire of their current condition, the treatment they were on, a review of their lifestyle, nutrition and a report of any previous illness. All samples were taken after informed consent.

Preparation of genomic DNA as a template for PCR–RFLP Plasma was separated from the lymphocytes of 44 normal pregnant and 44 women with RSA. The lymphocytes were then processed to extract genomic DNA according to a previously standardized protocol of our laboratory. The DNA was obtained by recording the absorbance at 260 nm and 280 nm. About 10–15 ng of DNA (optimum concentration) was taken for each reaction.

PCR analysis
Genomic DNA obtained from the samples was used as a template. A reaction volume of 50 µl was used which included 2 mM MgCl₂, 200 µM of each dNTP, 0.5 µM primer concentration and 1.25 U of Taq DNA polymerase. We used the primer sequence mentioned by Wang et al. PCR generated amplicons were ethanol precipitated, resuspended and digested with HindIII. The digested products were visualized on 2.5% agarose gel. The marker used was ΦX174 DNA digested with BsuRI (HaeIII).

Statistical analysis
The allelic frequencies of the CD46 polymorphic forms (U, L and E phenotypes) were calculated using descriptive statistics. Hardy–Weinberg equilibrium analysis was done to ascertain that the two alleles CD46H*1 and CD46H*2 behaved in a co-dominant fashion. Observed and expected values of different genotypes were constructed and the level of significance was analysed using the chi-square test. All the analyses were done using the TFPGA software package.

RESULTS
PCR of CD46 gene
This was done on 44 samples from women with RSA and 44 samples from women with a normal pregnancy. A 243 bp product was obtained, which was visualized on 2.5% agarose gel (Fig. 1a).

Restriction digestion of the amplified fragments
Restriction digestion of the PCR product was done using the HindIII restriction enzyme. The recognition site for HindIII is 5’AGGCCT 3’. The digested products were visualized on 2.5% agarose gel on which all 3 genotypes could be easily distinguished. The U protein phenotype (CD46H*1/H*1) showed a band at 243 bp and 55 bp. The L protein phenotype (CD46H*2/H*2) showed a band that remained undigested at 243 bp. Individuals bearing E protein phenotype (CD46H*1/H*2) showed partial digestion pattern and three bands of 243, 188 and 55 bp were obtained (Fig. 1b).

DISCUSSION
Our results confirmed the presence of biallelic polymorphism for CD46 at the HindIII site. The three different genotypes observed on 2.5% agarose gel were designated as H*1/*1 (U), H*2/*2 (L) and H*1/*2 (E) (Fig. 1b).

In 1991, Risk et al. showed that a striking absence of some of the smaller HindIII fragments (U protein phenotype) occurred more frequently in patients with RSA than in normal women (the frequency of the H*1 allele was reported to be 0.59 in women with RSA and 0.83 in normal women). Wang et al. reported that the frequency of the CD46H*2 allele and its homozygosity were significantly increased in women with recurrent pregnancy loss/RSA, when compared with fertile controls. The allelic frequency of CD46H*1 was found to be 0.64 in fertile controls while in women with RSA it was 0.52. The allelic frequency of CD46H*2, on the other hand, was reported to be 0.36 in fertile controls while in those with RSA it was 0.48. CD46H*2 homozygosity was also shown to be highly associated with RSA (27.5% v. 9.7% in fertile controls, p<0.0045).

The data that we obtained revealed that the allelic frequency of CD46H*1 was 0.63 in fertile controls while in RSA cases, it was 0.49, and the allelic frequency of CD46H*2 was 0.38 in fertile controls while in those with RSA it was 0.51. The percentage distribution of the three genotypes showed that although CD46H*2 homozygosity was associated with RSA (11% v. 5% in normal pregnant controls), it was the absence of CD46H*1 homozygosity that was more pronounced in Indian women with RSA, i.e. only 9% showed the H*1/*1 genotype compared with 30% of normal pregnant women (Fig. 2). Therefore, we found the results of Risk et al. to hold good for the Indian population. Our data, however,
could not fit the Hardy–Weinberg equilibrium. The two studies which showed that the allele follows the equilibrium have used 100 or more samples.\textsuperscript{1,16} The other reasons for our data not fitting the Hardy–Weinberg equilibrium could be mutation, gene flow, genetic drift, non-random mating and natural selection. One or more of these ‘disturbing influences’ could be affecting the population and could be responsible for the observed deviation.

We consider our work to be a pilot study. Increasing the sample size might clarify whether CD46 polymorphism in the first intron can be regarded as a risk factor for RSA.

REFERENCES


