Gene 569 (2015) 136–140

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

C-reactive protein gene variants associated with recurrent pregnancy loss independent of CRP serum levels: A case-control study☆,☆☆

Salma K. Ahmed [a], Naeema Mahmood [b], Zainab H. Malalla [a], Fanar M. Alsobyani [a], Israa S. Al-Kiyumi [a], Wassim Y. Almawi [a,*]

[a] Department of Medical Biochemistry, Arabian Gulf University, Manama, Bahrain
[b] Department of Obstetrics and Gynecology, Salmaniya Medical Complex, Manama, Bahrain

ARTICLE INFO

Article history:
Received 19 March 2015
Accepted 21 May 2015
Available online 24 May 2015

Keywords:
Alleles
Inflammation
Polymorphisms
Recurrent pregnancy loss

ABSTRACT

The aim of this study is to investigate the association of recurrent pregnancy loss (RPL) with altered C-reactive protein (CRP) serum levels, and genetic variation in *CRP* gene. This was a retrospective case–control study, involving 275 women with three or more consecutive pregnancy losses, and 290 age-matched control women, who were recruited from outpatient obstetrics/gynecology clinics. CRP serum levels (hs-CRP) were determined by latex-enhanced nephelometry, and *CRP* genotyping was done by allelic discrimination. Mean serum CRP levels were higher in RPL cases than in control women, and carriage of the (minor) T allele of rs2794520 was associated with significant increase in CRP levels ($P = 0.017$). Minor allele frequency (MAF) of rs7553007 was significantly different between RPL cases and control women, and was associated with reduced risk of RPL after adjusting for BMI and menarche. There was a significant enrichment of minor allele-carrying genotypes of rs1130864 and rs1417938 SNPs, and reduced frequency of minor allele-carrying genotypes of rs876537, rs2794520, and rs7553007 in RPL cases, thus assigning RPL-susceptible and -protective nature to these genotypes, respectively. Carriage of (minor) T allele of only rs2794520 was associated with significant increase in CRP levels. *CRP* variants that influenced circulating CRP levels in chronic inflammatory conditions are also associated with RPL, pointing to CRP as RPL candidate gene.

1. Introduction

C-reactive protein (CRP) is an acute phase protein synthesized by hepatocytes and other tissues, including trophoblasts (Malek et al., 2006), and is a documented marker of low-grade, chronic systemic inflammation (Pepys and Hirschfield, 2003). Elevation in the serum CRP levels accompanies inflammatory conditions (Morales et al., 2011), which include cancer, asthma (Morales et al., 2011), type 2 diabetes (Ghaffari et al., 2014), sickle cell disease (Mohammed et al., 2010), cardiovascular diseases (Blake and Ridker, 2002; Wood et al., 2014), and in adverse pregnancy outcomes (Lohsoonthorn et al., 2007; Tjoa et al., 2003). The latter was highlighted by the finding that serum CRP levels are normally elevated during the 4th gestational week onwards (Cohen et al., 2014), and significantly higher in adverse pregnancy outcomes such as preeclampsia (PE) (Best et al., 2013), preterm delivery (Lohsoonthorn et al., 2007), intra-uterine growth restriction (IUGR) (Tjoa et al., 2003), and in neonates with pneumonia aspiration and intrapulmonary sepsis (Morales et al., 2011; Rasmussen et al., 2009; Sunyer et al., 2008). CRP is a stable serum protein, and is detected by measuring high-sensitivity CRP (hs-CRP) levels.

Idiopathic recurrent pregnancy loss (RPL) is a significant obstetric complication, affecting 5% of otherwise healthy women (Gallot et al., 2014; Jeve and Davies, 2014). Despite the identification of several modifiable and non-modifiable factors contributing to increased risk of RPL (Bogdanova and Markoff, 2010; Coulam and Acacio, 2012), most RPL cases remain idiopathic (Jaslow et al., 2010). Evidence supporting the association of inflammatory changes with RPL was based on up-regulation of pro-inflammatory markers, and/or down-regulation of anti-inflammatory mediators in RPL cases than in control women (Banerjee et al., 2013; Garzia et al., 2013; Qaddourah et al., 2014).

CRP is located on chromosome 1q23.2, and several functional and non-functional variants located within or around the *CRP* gene affecting CRP levels were reported (Carlson et al., 2005; Wessel et al., 2007). Insofar as variation in plasma CRP levels is genetically determined (Wessel et al., 2007), several studies reported an association between single nucleotide polymorphisms (SNPs) in *CRP* gene in blood CRP levels with cardiovascular (Hong et al., 2014) and ischemic vascular disease (Zacho et al., 2008), cancer (Su et al., 2014), and metabolic disorders including diabetes, insulin resistance, and adiposity (Bochud et al., 2009;

Abbreviations: BMI, body mass index; CI, confidence intervals; CRP, C-reactive protein; MAF, minor allele frequency; OR, odds ratios; RPL, recurrent pregnancy loss; SNP, single nucleotide polymorphism.

☆ Financial disclosures: None to declare.

☆☆ Funding disclosures: None to declare.

* Corresponding author at: Department of Medical Biochemistry, College of Medicine & Medical Sciences, Arabian Gulf University, PO Box 22979, Manama, Bahrain.
E-mail address: wassim@agu.edu.bh (W.Y. Almawi).

http://dx.doi.org/10.1016/j.gene.2015.05.052
0378-1119/

Wolford et al., 2003). The objective of this study was to evaluate the association between rs1572970, rs876537, rs2794520, rs2808630, rs1130864, rs1417938, and rs7553007 SNPs in the *CRP* gene with altered CRP serum levels and with risk of RPL in Bahraini women. To the best of our knowledge, this is the first study to investigate this association.

2. Subjects and methods

2.1. Subjects

A total of 565 women were consecutively recruited from January 2012–April 2013 at outpatient OB/GYN clinics in Manama, Bahrain. Of these, 275 women reported three or more unexplained miscarriages with the same partner (mean age 31.6 ± 5.4 years). RPL diagnosis was as per Royal College of Obstetricians and Gynecologists (RCOG) guidelines (http://www.rcog.org.uk/guidelines). These included screening of anti-phospholipid antibodies (lupus anticoagulant or anti-cardiolipin antibodies), karyotyping of both partners, pelvic ultrasound scan for evaluating uterine anatomy (hysteroscopy or sonohysteroscopy), and inherited thrombophilias screening (factor V Leiden, factor II G20210A mutations); all cases had these procedures done. Additional exclusion criteria included older age (>40 years at first pregnancy), Rh blood group incompatibility, preeclampsia (defined as elevated systolic and diastolic blood pressure (BP) >145/95 mm Hg, or rise in systolic/diastolic BP > 30/15 mm Hg on at least two occasions, and proteinuria assessed by 24-h protein excretion of 300 mg, or at least two + 1 dipstick readings in the absence of prior proteinuria), and preclinical miscarriages and/or biochemical pregnancy. Patients were also excluded if they reported systemic autoimmune disease, diabetes mellitus and thyroid dysfunction, anatomical disorders, infections (toxoplasmosis, HCMV, rubella, HIV, Group B streptococci, Chlamydia trachomatis, hepatitis B and C and bacterial vaginosis), elevated liver enzymes, and low plet (HELLP) syndrome.

Controls comprised 290 fertile women with at least two live births, with no personal or family history of miscarriage, preeclampsia, ectopic pregnancy or preterm delivery. Controls comprised university and hospital employees, or volunteers, who were recruited following a routine check-up after an uncomplicated pregnancy, and were matched to cases according to age and self-identified ethnic origin. All women in both groups had conceived naturally, and were self-reported Bahraini Arabs. Blood samples were taken from all participants in EDTA-containing tube for total genomic DNA extraction and in plain tubes (no preservatives) for serum preparation. Since pregnancy influences CRP levels (Belo et al., 2005), similar blood collection was done for cases and control women, which occurred at least 4 months (4–9 months) after the miscarriage (cases) or delivery (controls).

2.2. Ethical approval

Both cases and controls were required to sign a consent form before inclusion in the study, the protocol of which was approved by local research and ethics committees.

2.3. hs-CRP measurement

Measurement of hs-CRP in plasma samples was done by latex-enhanced nephelometry on a BN II Nephelometer (Dade Behring, Milan, Italy). Samples were assayed in duplicate in each analytical run; the lower limit of detection was 0.15 mg/L, and the assay range was 0.175–11.0 mg/L (initial dilution). Serial serum dilutions were made in measuring high hs-CRP (>10 mg/L) levels. Percentile hs-CRP values were estimated for comparison purposes.

2.4. CRP genotyping

The *CRP* polymorphisms included in the study were selected using SNPbrowser software (version 4.0, Applied Biosystems, Foster City, CA, USA), based on their minor allele frequency (MAF) of >5% in Caucasians. *CRP* genotyping was done by the allelic (VIC- and FAM-labeled) discrimination method. TaqMan assays for the following CRP SNPs: rs1572970, rs876537, rs2794520, rs2808630, rs1130864, rs1417938, and rs7553007 were ordered from Applied Biosystems (ABI, NJ). The reaction was performed in 6 μl volume on StepOne real-time PCR system, according to manufacturer's instructions (Applied Biosystems). Replicate blinded quality control samples were included to assess reproducibility of the genotyping procedure; concordance was >99%.

2.5. Statistical analysis

Statistical analysis was performed on SPSS v. 22.0 (IBM, New York, NY). Data were expressed as percentages of total (categorical variables) or as mean ± SD (continuous variables). Student's *t*-test was used to determine differences in means, and Pearson χ^2 or Fisher's exact test was used to assess inter-group significance. Allele frequencies were calculated by the gene-counting method, and SNP genotypes were tested for departures from Hardy–Weinberg equilibrium (HWE) in both control women and RSM cases using Haploview version 4.2 (http://www.broad.mit.edu/mpg/haploview).

All analyses were conducted under additive genetic effect (which assumes that the risk conferred by a given allele increases r-fold for heterozygotes and r^2-fold for homozygotes in a disease association study), as it is the conservative model, using SNPStats software (bioinfo.iconcologia.net/snpstats/). Logistic regression analysis was performed in order to determine the odds ratios (OR) and 95% confidence intervals (95% CI) associated with the RPL risk, taking the control as the reference group. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Study participants

Initially 305 women with RPL and 310 control women were recruited. However, 25 women in the RPL group and 8 control women were later excluded due to sampling problems (wrong anticoagulant, low sample volume), or due to ambiguous screening results, leaving 280 women with RPL, and 302 age-matched multiparous fertile control women available for genotyping association analysis. The baseline characteristics of the study subjects are shown in Table 1. Higher BMI ($P = 0.006$), and prevalence of obesity ($P = 0.020$), and lower menarche ($P < 0.001$) was seen in the RPL group. Mean systolic and diastolic blood pressure reading was comparable between the two groups.

Table 1
Demographics and clinical characteristics of study subjects.

	RPL Cases[1]	Controls[1]	*P*[2]
Age at inclusion in study[3]	31.6 ± 5.4	31.6 ± 4.9	0.878
Body-mass index (kg/m^2)[3]	26.3 ± 5.4	25.2 ± 4.3	0.006
Obesity [n (%)]	58 (19.6)	37 (12.1)	0.020
Menarche (years)	12.2 ± 1.1	12.8 ± 1.0	5.3×10^{-4}
Fasting glucose (mmol/L)	5.1 ± 0.9	5.2 ± 0.7	0.549
Systolic blood pressure (mm Hg)	114.1 ± 11.9	112.0 ± 12.0	0.245
Diastolic blood pressure (mm Hg)	72.0 ± 8.4	72.8 ± 9.1	0.284
Number of pregnancies[3]	4.2 ± 1.5	4.0 ± 1.1	0.11
Number of children[3]	0.8 ± 1.1	4.0 ± 1.1	<0.001
Miscarriages[3]	3.6 ± 1.0	0.0 ± 0.1	<0.001
Serum hs-CRP (pg/ml)[3]	6.1 ± 1.2	5.3 ± 1.8	0.002

[1] A total of 275 RPL cases and 290 control women were included.
[2] Student's *t*-test for continuous variables, Pearson's Chi square test for categorical variables.
[3] Mean ± SD.