Frequency of HLA-G UTR-1/UTR-3/UTR-7 in women with unexplained recurrent spontaneous abortion

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Abstract. Human leukocyte antigen (HLA)-G is an important molecule that maintains maternal-fetal interface tolerance and plays a vital role in a healthy pregnancy. Single-nucleotide polymorphisms in the 3'-untranslated regions (UTR) of the HLA-G gene may differ in women with unexplained recurrent spontaneous abortion (URSA). The present study involved the isolation of genome DNA from peripheral blood leukocytes, Sanger sequencing and analysis of the polymorphism sites in the 3'UTR of the HLA-G gene based on polymerase chain reaction. In total, 261 DNA samples from cases of URSA (n=133), including primary URSA (n=83) and secondary URSA (n=50), and controls (n=128) were evaluated. The present data showed that +3010CC genotype carriers exhibited a higher risk of URSA, while +3187GG genotype carriers exhibited a lower risk. Secondary URSA patients carrying +3010C had a higher risk of URSA, while +3187G carriers exhibited a lower risk of URSA. UTR-1 haplotype carriers may be associated with a reduced risk of primary and secondary URSA. Notably, UTR-3 and UTR-7 could increase the risk of primary and secondary URSA, respectively. The present results showed that HLA-G 3'UTR polymorphisms and haplotypes may be involved in URSA development and be a predictor of pregnancy outcome.

Introduction

Recurrent spontaneous abortion (RSA) is commonly defined as three or more consecutive pregnancy losses prior to 20 weeks of gestation. Nevertheless, some clinicians define RSA as two or more pregnancy losses, and no difference has been determined in the causes of RSA between women with two losses and those who have three or more losses in a large retrospective study (1). The causes of RSA include uterine malformation, vascular and local circulation abnormalities, endocrine disorders, thyroid disease, immune factors, maternal hereditary, acquired thrombotic hemophilia, genital tract infection and parental chromosomal abnormalities (2). However, the underlying cause of RSA can be determined in only 25-50% of couples. The remaining cases are referred to as unexplained RSA (URSA) (3,4). URSA is divided into primary and secondary URSA according to the etiology. Primary URSA is characterized by at least two consecutive losses and no prior successful pregnancy, while secondary URSA is characterized by a normal pregnancy, followed by more than two consecutive spontaneous abortions (5,6).

The human leukocyte antigen (HLA) region is a 3.6-Mb high-density gene region located at 6p21.3, containing >200 genes, accounting for ~2.5% of chromosome 6 (7). HLA-G plays an important role in immunoregulation during pregnancy to prevent the rejection of the fetus (8). Compared with the case of the classical HLA class I loci, which are the most polymorphic loci in the human genome, limited HLA-G variability has been observed around the world (9). According to the IMGT/HLA 3.42.0 database released in 2022, 104 HLA-G alleles, 35 HLA-G proteins and five null alleles have been identified (10).

A high degree of variation is observed in the HLA-G 3'-untranslated region (3'UTR). Although the 3'UTR is a notably short segment, at least eight polymorphisms are regularly identified in this region globally (6). The presence of 14-bp insertion (Ins)/deletion (Del) sites in the HLA-G 3'UTR affects the stability of mRNA (11-13), and the +3142C/G sites increase the affinity of specific microRNAs (miRNAs) for HLA-G mRNA and decrease the expression of HLA-G (14-17), while +3187A/G sites affect the stability of mRNA due to proximal AU-rich modification (18). However, there are few functional studies on single-nucleotide polymorphisms (SNPs) such as the +3001C/T, +3003T/C, +3010G/C, +3027C/A, +3035C/T and +3196C/G SNPs, which are considered to be potential miRNA binding sites (19).

Several HLA-G SNPs are associated with the susceptibility to pregnancy-related diseases. SNPs +3010G/C, +3142C/G and +3187A/G could contribute to recurrent miscarriage (6), while +3187A/G may be associated with preeclampsia (20).

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One study showed that a 14-bp Ins/Del increased the risk of RSA (21); however, another indicated no significant differences for 14-bp Ins/Del and genotype frequencies between RSA and healthy women (13). HLA-G 3'UTR SNP-pair associations, but not individual SNPs, could be useful in a predictive test (6,20,22). A previous study reported that UTR-1(DelTGCCCGC) carriers might be associated with a reduced risk of secondary RSA, but not primary RSA (6). Nevertheless, to the best of our knowledge, there is little information in the literature about the difference between primary and secondary URSA. Overall, the role of the 3'UTR SNPs in URSA has not been fully elucidated and is controversial to date.

Although studies have confirmed the importance of HLA-G 3'UTR expression in various populations with URSA (6,21-23), this remains unclear in the Han Chinese population. To the best of our knowledge, the current study might be the first attempt to explore the significance of primary and secondary URSA in HLA-G 3'UTR polymorphism and haplotype. Therefore, the present study investigated the HLA-G 3'UTR polymorphism and haplotype structure of the HLA-G gene in the Han Chinese population from Wenzhou, China.

Materials and methods

Samples. The case cohort included 133 Han Chinese women who visited the Obstetrics and Reproductive clinic, Wenzhou Hospital of Traditional Chinese Medicine (Wenzhou, China), who were classified as the URSA group. The inclusion criteria were two or more unexplained continuous abortions with the same partner at <20 weeks of pregnancy. The URSA group was divided into two subgroups: Primary URSA and secondary URSA. The inclusion criterion for the primary URSA group inclusion was no prior successful pregnancy, whereas for the secondary URSA group it was at least one successful pregnancy before experiencing URSA. The clinical characteristics of the women are listed in Table I. Genital tract and thyroid hormone abnormalities, as well as immunological disorders, diabetes, hypertension, preeclampsia, thrombotic diseases, microbial infections and parental chromosomal abnormalities were exclusion criteria for this study.

The control cohort consisted of 128 healthy pregnant Han Chinese women who had given birth to at least one healthy child and had experienced no previous miscarriages, ectopic pregnancies, preterm births or stillbirths and were recruited from the same hospital as the patients with URSA. The mean age of the women in the control group matched that of the patients with URSA. In addition, the control subjects showed no foundational diseases or complications of pregnancy. The recruitment period was between January 2017 and March 2018.

The Ethics Committee of Wenzhou Hospital of Traditional Chinese Medicine (Zhejiang, China) approved this study (approval no. WTCM-H-2018038). Written informed consent was obtained from each participant.

Isolation of genome DNA. A total of 3 ml peripheral blood was collected in EDTA-containing vials from the antecubital fossa vein. A DNA extraction kit (Gentra Systems; TianGen Biotech Co., Ltd.) was used to extract genomic DNA from human peripheral blood mononuclear cells in accordance with the manufacturer's protocol. DNA samples were stored in a freezer at -20˚C.

Amplification and sequencing of the 3'UTR of the HLA-G gene. The DNA isolated from the peripheral blood was used for polymerase chain reaction (PCR) amplification, starting with the 14-bp Ins/Del in the 3'UTR of the HLA-G gene and extending across downstream ~500 bp. The following primer pair was used to amplify the HLA-G 3'UTR fragment: Forward, 5'-GTGGGTTGTTGAGGGG-3' and reverse, 5'-GTCTTCCATTTATTTTGTCTCT-3'. Each 25-μl PCR mixture consisted of 1 μl of each primer (10 μM), 13 μl 2X Taq MasterMix (Sangon Biotech Co., Ltd.), 2 μl of genomic DNA (50 ng/μl) and 8 μl ddH_2O. PCR was performed beginning with a denaturation step at 94˚C for 5 min, followed by 35 cycles of 94˚C for 45 sec, 58˚C for 45 sec and 72˚C for 60 sec, followed by the final extension step at 72˚C for 10 min. The amplification products were detected by electrophoresis on Goldview-stained 1.5% agarose gels. The reverse primer (Sangon Biotech Co., Ltd.) was used to directly sequence the products of PCR amplification. The results were compared with the HLA-G 3'UTR standard sequence (Chromosome 6, NC_000006.12) using CodonCode Aligner 6.0.2 software (CodonCode Corporation). The 14-bp Ins/Del (rs1704), +3003C/T (rs1707), +3010C/G (rs1710), +3027A/C (rs17179101), +3035C/T (rs17179108), +3142C/G (rs1063320), +3187A/G (rs9380142) and +3196C/G (rs1610696) polymorphic sites were individually labeled. Haplotypes and genotypes were assigned according to a previous study (24).

Statistical analysis. Student's t-test was performed for the comparison of the median age between cases and control subjects. The χ^2 test was adjusted using odds ratios (ORs) and 95% confidence intervals (CIs) to evaluate the association between genetic markers and prognosis. To rule out the possibility of selecting a certain genotype in the present group, the SHEsis web-based platform (http://shesisplus.bio-x.cn/SHEsis.html) was used to evaluate the Hardy-Weinberg equilibrium of the HLA-G 3'UTR genotypes and haplotypes between the case and control groups. The results were analyzed using SPSS 20.0 (IBM Corp.). When the P-value of the association test was significant, Bonferroni correction was applied for multiple comparisons, and the Bonferroni result was reported (P_{Bonf}=P-value x number of comparisons of one test). Due to linkage disequilibrium, the eight SNPs may be linked to each other (i.e. their distribution is not completely independent); therefore, the P_{Bonf} value was adjusted by 8-fold to make the most conservative P-value threshold, avoiding false positive results. In all cases, a P-value (two-tailed) of <0.05 was considered to indicate a statistically significant difference.

Results

Baseline characteristics of subjects. The baseline characteristics of 133 patients with URSA and 128 uneventful pregnant women in the Han Chinese population are shown in Table I. A similar median age was observed at the first pregnancy

Table I. Clinical characteristics of the URSA and control groups.

Characteristics	Control	URSA	Primary URSA	Secondary URSA	P-value[a]
Number of patients	128	133	83 (62.4%)	50 (37.6%)	
Age, years					0.482
Median	29.7	30.0	28.4	33.1	
Range	25.6-33.8	25.3-34.7	24.3-32.5	28.7-37.5	
Ethnicity	Han Chinese	Han Chinese	Han Chinese	Han Chinese	
Number of spontaneous abortions	0	≥2	≥2	≥2	
Number of successful pregnancies	≥1	≥0	0	≥1	
Number of chromosome abnormalities	0	0	0	0	

[a]Comparison between the control and URSA groups using Student's t-test. URSA, unexplained recurrent spontaneous abortion.

between URSA women and the control group (P=0.482). In the case group, 83 women (62.4%) had primary recurrent miscarriage and 50 women (37.6%) had secondary recurrent miscarriage. Furthermore, dysthyroidism, chromosomal abnormalities, immunological disorders, microbial infections and uterine abnormalities were excluded in both the control and URSA groups.

Genotype analysis. A comprehensive analysis of the frequency of eight major polymorphic sites (14-bp Ins/Del, +3003C/T, +3010C/G, +3027A/C, +3035C/T, +3142C/G, +3187A/G and +3196C/G) between the groups was conducted (Table II).

In the control group, all genotypes of the eight SNPs in the 3'UTR of HLA-G were in accordance with the Hardy-Weinberg equilibrium (Table SI). The results indicated that the difference in the frequency of the +3010C allele between the URSA and control groups was statistically significant (P_{Bonf}=0.008) and that the URSA risk associated with the +3010CC genotype was higher than that associated with the GG genotype (OR, 2.813; 95% CI, 1.435-5.513; P_{Bonf}=0.016; Table SII]. Additionally, the difference in the +3187G allele frequency between the two groups was statistically significant (P_{Bonf}=0.008). Compared with the AA genotype, the +3187GG genotype conferred a reduced risk of URSA (OR, 0.345; 95% CI, 0.171-0.698; P_{Bonf}=0.024; Table SII). There were no significant differences observed for the +3142G and +3196G alleles after the Bonferroni correction(P_{Bonf}=0.248 and P_{Bonf}=0.384).

The eight SNPs also showed different results between the primary URSA and secondary URSA groups compared with the control group (Table II). In the primary URSA group, +3010C allele frequency (OR, 1.628; 95% CI, 1.095-2.421; P=0.016) increased the risk of URSA and the +3010CC genotype conferred a 2.589-fold higher risk of URSA compared with the GG genotype (P=0.017; Table SIII). However, there were no significant differences found after comparisons following Bonferroni correction (P_{Bonf}=0.128 and P_{Bonf}=0.136, respectively). Similarly, the +3187G allele frequency (OR, 1.551; 95% CI, 1.041-2.311; P=0.031) decreased the risk of URSA, while the +3187GG genotype conferred a 0.419-fold lower risk of URSA compared with the AA genotype (P=0.033; Table SIII), but the statistical significance was lost after Bonferroni correction (P_{Bonf}=0.248 and $P_{Bonf=}$0.264, respectively). The +3196G allele showed a significant difference (P=0.013; Table SIII), but there was no significant difference found after multiple comparisons following Bonferroni correction (P_{Bonf}=0.104). After Bonferroni correction for multiple comparisons, the +3010C, +3027A and +3187G alleles presented significant differences (OR, 2.128; P_{Bonf}=0.016; OR, 2.219; P_{Bonf}=0.048; and OR, 0.442; P_{Bonf}=0.008, respectively; Table SIV), whereas the 14-bp ins, +3035T and +3142G alleles did not (P_{Bonf}=0.320, $P_{Bonf=}$0.112 and $P_{Bonf=}$0.384, respectively).

Haplotype analysis. The haplotypes composed of these polymorphic sites are referred to as UTRs (25), the most common of which are UTR-1 to UTR-7 (24). The present study investigated the UTRs from UTR-1 to UTR-7, the most common 3'UTR haplotypes in the URSA and control groups. According to statistical analysis, UTR-1 (DTGCCCGC), which was the most representative haplotype in the present subjects, and UTR-7 (ITCATGAC) showed a significant difference between the control and URSA groups (P<0.001 and P=0.039, respectively; Table III). UTR-1 carriers showed a 0.501-fold reduction in URSA risk (P<0.001), and UTR-7 carriers showed a 1.675-fold increase in URSA risk (P=0.039; Table SV). Compared with the control group, UTR-1 and UTR-3 showed a significant difference in the primary URSA group (P=0.017 and P=0.041). Additionally, in the primary URSA group, UTR-3 was the most frequent (42.6%) and conferred a 1.539-fold increase in the primary URSA risk (P=0.041; Table SVI). UTR-1 conferred a 0.605-fold decrease in primary URSA risk (P=0.017; Table SVI). Furthermore, compared with the results for the control group, the haplotypes of UTR-1 and UTR-7 in the secondary URSA group were significantly different (OR, 0.376; P<0.001; and OR, 2.452; P=0.003, respectively; Table SVII).

Table II. Allele and genotype frequencies observed at human leukocyte antigen-G 3'-untranslated region polymorphic sites in the URSA and control groups.

	Control		URSA		Primary URSA		Secondary URSA		URSA vs. control[a]		Primary URSA vs. control[b]		Secondary URSA vs. control[c]	
Polymorphisms	Cases (n=128)	Freq, %	Cases (n=133)	Freq, %	Cases (n=83)	Freq, %	Cases (n=50)	Freq, %	P-value	P_{bonf}	P-value	P_{bonf}	P-value	P_{bonf}
14-bp Ins/Del														
Del	216	84.400	213	80.100	138	83.100	75	75.000						
Ins	40	15.600	53	19.900	28	16.900	25	25.000	0.199		0.734		0.040[d]	0.320
Del/Del	90	70.300	86	64.700	57	68.700	29	58.000						
Ins/Del	36	28.100	41	30.800	24	28.900	17	34.000	0.521		0.870		0.292	
Ins/Ins	2	1.600	6	4.500	2	2.400	4	0.800	0.278		0.650		0.034[d]	0.272
+3003C/T														
C	3	1.200	3	1.100	3	1.800	0	0.000						
T	253	98.800	263	98.900	163	98.200	100	1.000	0.962		0.906		0.277	
CC	0	0.000	0	0.000	0	0.000	0	0.000	-		-			
CT	3	2.300	3	2.300	3	3.600	0	0.000						
TT	125	97.700	130	97.700	80	96.400	50	1.000	0.962		0.906		0.275	
+3010G/C														
G	131	51.200	98	36.800	65	39.200	33	33.000						
C	125	48.800	168	63.200	101	60.800	67	67.000	0.001[d]	0.008[d]	0.016[d]	0.128	0.002[d]	0.016[d]
GG	38	29.700	22	16.500	13	15.700	9	18.000						
GC	55	43.000	54	40.600	39	47.000	15	30.000	0.107		0.055		0.765	
CC	35	27.300	57	42.900	31	37.300	26	52.000	0.002[d]	0.016[d]	0.017[d]	0.136	0.010[d]	0.080
+3027C/A														
C	221	86.300	216	81.200	142	85.500	74	74.000						
A	35	13.700	50	18.800	24	14.500	26	26.000	0.113		0.820		0.006[d]	0.048[d]
CC	94	73.400	87	65.400	59	71.100	28	56.000						
CA	33	25.800	42	31.600	24	28.900	18	36.000	0.248		0.640		0.094	
AA	1	0.800	4	3.000	0	0.000	4	8.000	0.159		1.000		0.019[d]	0.152
+3035C/T														
C	218	85.200	216	81.200	142	85.500	74	74.000						
T	38	14.800	50	18.800	24	14.500	26	26.000	0.228		0.913		0.014[d]	0.112
CC	92	71.900	87	65.400	59	71.100	28	56.000						
CT	34	26.600	42	31.600	24	28.900	18	36.000	0.331		0.760		0.125	
TT	2	1.600	4	3.000	0	0.000	4	8.000	0.649		0.692		0.058	

Table II. Continued.

Polymorphisms	Control		URSA		Primary URSA		Secondary URSA		URSA vs. control[a]		Primary URSA vs. control[b]		Secondary URSA vs. control[c]	
	Cases (n=128)	Freq, %	Cases (n=133)	Freq, %	Cases (n=83)	Freq, %	Cases (n=50)	Freq, %	P-value	P_{bonf}	P-value	P_{bonf}	P-value	P_{bonf}
+3142C/G														
C	127	49.600	107	40.200	69	41.600	38	38.000						
G	129	50.400	159	59.800	97	58.400	62	62.000	0.031[d]	0.248	0.106		0.048[d]	0.384
CC	35	27.300	27	20.300	13	15.700	14	28.000						
CG	57	44.500	53	39.800	43	51.800	10	20.000	0.559		0.062		0.073	
GG	36	28.100	53	39.800	27	32.500	26	52.000	0.053		0.086		0.145	
+3187A/G														
A	133	52.000	175	65.800	104	62.700	71	71.000						
G	123	48.000	91	34.200	62	37.300	29	29.000	0.001[d]	0.008[d]	0.031[d]	0.248	0.001[d]	0.008[d]
AA	38	29.700	60	45.100	33	39.800	27	54.000						
AG	57	44.500	55	41.400	38	45.800	17	34.000	0.078		0.404		0.019[d]	0.152
GG	33	25.800	18	13.500	12	14.500	6	12.000	0.003[d]	0.024[d]	0.033[d]	0.264	0.006[d]	0.048[d]
+3196C/G														
C	256	1.000	262	98.500	162	97.600	100	1.000						
G	0	0.000	4	1.500	4	2.400	0	0.000	0.048[d]	0.384	0.013[d]	0.104	-	
CC	128	1.000	131	98.500	81	97.600	50	1.000						
CG	0	0.000	0	0.000	0	0.000	0	0.000						
GG	0	0.000	2	1.500	2	2.400	0	0.000	0.164		0.078		-	

[a]URSA vs. control group using the χ^2 test; [b]Primary URSA vs. control group using the χ^2 test; [c]Secondary URSA vs. control group using the χ^2 test; [d]Statistically significant. P_{Bonf}, P-value after Bonferroni correction for multiple comparisons; URSA, unexplained recurrent spontaneous abortion; Ins, insertion; Del, deletion.

Table III. Analyzed haplotype frequencies in the HLA-G 3'UTR among patients with URSA and healthy controls.

Haplotypes	Control, %	URSA, %	Primary URSA, %	Secondary URSA, %	URSA vs. control P-value[a]	Primary URSA vs. control P-value[b]	Secondary URSA vs. control P-value[c]
UTR-1 (DelTGCCCGC)	46.8	31.5	34.6	26.0	<0.001[d]	0.017[d]	<0.001[d]
UTR-2 (InsTCCCGAG)	0.0	1.5	2.4	0.0			
UTR-3 (DelTCCCGAC)	33.8	36.4	42.6	32.0	0.534	0.041[d]	0.683
UTR-4 (DelCGCCCAC)	1.2	1.1	1.6	0.0			
UTR-5 (InsTCCTGAC)	0.8	0.0	0.0	0.0			
UTR-6 (DelTGCCCAC)	0.6	1.1	0.0	3.0			
UTR-7 (InsTCATGAC)	11.9	18.4	10.8	25.0	0.039[d]	0.773	0.003[d]

[a]URSA vs. control group using the χ^2 test; [b]primary URSA vs. control group using the χ^2 test; [c]secondary URSA vs. control group using the χ^2 test; [d]statistically significant. URSA, unexplained recurrent spontaneous abortion; UTR, untranslated region; Ins, insertion; Del, deletion.

Discussion

The focus of the present study was the SNPs in the 3'UTR region of the HLA-G gene. The data were analyzed for genotypes and haplotypes. It was found that the +3010CC genotype was a susceptibility factor to the development of URSA and the +3187GG genotype might be a protective factor for URSA. Research teams from the Netherlands (22) and Southern Brazil (6) reported an association between RSA and +3003C/T, +3010G/C, +3142C/G and +3187A/G, respectively. In the present study, +3142G and +3196G showed a significant difference originally; however, the difference was eliminated by the Bonferroni correction. Notably, these sites cannot be ruled out for future studies due to the rigorous statistical methods performed in the present study. The +3010G/C and +3187A/G sites were previously considered putative miRNA binding sites according to *in silico* analysis (16). miRNA plays an important role in HLA-G gene expression (15). The mutation of the site leads to the change of the miRNA binding site and the imbalance of post-transcriptional regulation leads to the occurrence of diseases (14,19). In addition, it has been reported that the risk of RSA is reduced among +3187AG carriers, as +3187A is close to the AU-rich element associated with mRNA degradation, which reduces the expression of HLA-G (18). The decrease in HLA-G is one of the factors leading to pregnancy complications; therefore, the mutation of the site is beneficial to the progress of pregnancy (6).

Several studies have been performed on the 14-bp Ins/Del polymorphism (9,12,13,21). The present results showed that there was no significant difference in the frequency of the 14-bp Ins/Del polymorphism between the URSA and control groups; however, 14-bp Ins/Ins was significant before Bonferroni correction between the secondary URSA and control groups. Koc *et al* (26) found that the deletion of 14-bp was more common in the examined abortion group, but the difference was not significant. By contrast, Hashemi *et al* (21) and Sipak *et al* (27) showed that the HLA-G 14-bp Ins/Del polymorphism may confer RSA risk. Although other studies have evaluated the role of 14-bp Ins/Del polymorphisms in RSA, the results remain controversial (6,13,22-23).

The UTRs of genes have been proven to be important regulatory elements in the post-transcriptional regulation of gene expression and they may play a key role in individual susceptibility to disease (28). Therefore, we hypothesized that UTR-1 is a protective factor for URSA and that UTR-7 is a risk factor for URSA. The present study found that the haplotype outcome was the same in secondary URSA. However, in primary URSA, UTR-1 was a protective factor and UTR-3 was a risk factor. UTR-1 is the only haplotype carrying +3010G/+3187G, whereas UTR-3 and UTR-7 are haplotypes carrying +3010C/+3187A. Accordingly, all the variant sites of the UTR-1 haplotype were related to the production of high soluble HLA-G (29), which seems to be protective against URSA (6). Considering all these data, it is reasonable to suggest that UTR-1 maintains the high mRNA expression and upregulates the expression of sHLA-G to promote the maintenance of pregnancy, while UTR-3 and UTR-7 are related to a low mRNA structural stability and low levels of HLA-G. However, Amodio *et al* (23) analyzed the frequency of UTR-3 in women experiencing RSA and found that it was considerably lower than that in control women. Therefore, haplotypes could differ in different populations and ethnic groups.

In summary, the present study analyzed all the individual SNPs of the 3'UTR of HLA-G to distinguish eight different haplotypes in the Han population of Wenzhou, China. The data showed that +3010CC genotype is a susceptibility factor to the

development of URSA and that the +3187GG genotype might be a protective factor for URSA. Additionally, the UTR-1 haplotype may reduce the risk of URSA, while the UTR-3 and UTR-7 haplotypes may be associated with high incidences of primary and secondary URSA, respectively. Thus, the overall distribution of different genotypes and haplotypes of HLA-G in pregnancy disorders is now being characterized and further studies will help to establish the importance of their roles. Obviously, there are still a number of deficiencies in this study. In the present study, the samples were only obtained from Wenzhou; therefore, it is necessary to expand the sample size to perfect the research results. To obtain a more reliable result, paternal and chorionic villi should also be examined; however, they were not evaluated in the present study. In addition, the present study did not investigate HLA-G protein expression.

In conclusion, the polymorphism and haplotype of the HLA-G 3'UTR are associated with URSA and pregnancy outcome, and whether it can be used as a predictor of subsequent pregnancy outcome will be investigated in future studies. Future studies will also be performed to extend the research on the regulatory mechanism of the HLA-G 3'UTR gene on URSA, and to detect mRNA or miRNA expression levels, providing a basis for the clinical diagnosis and treatment of URSA.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

WB, ZL and WC conceived the study. WB, ZL and JY conducted the experiments. WB, LZ and JX analyzed the data. ZL, WB and LZ wrote the manuscript. WC and JX contributed to critical revisions for important intellectual content and approved the final manuscript to be published. WB and ZL confirmed the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures performed that involved human participants were in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards and the ethical standards of the National Research Committee (Wenzhou Hospital of Traditional Chinese Medicine Ethics Committee), and were approved by this committee (approval no. WTCM-H-2018038). Written informed consent was obtained from all individual participants included in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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