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Original Article

Common *Interleukin-18* Gene Polymorphisms in Women with Recurrent Spontaneous Abortion in Gaza Strip-Palestine

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ABSTRACT

Background: This work was carried out in order to investigate the association between *Interleukin-18* (*IL-18*) gene SNPs (+105A>C, "rs549908"; -137 G>C, "rs187238"; -607 C>A, "rs1946518"; and -656 G>T, "rs1946519") and RSA among Palestinian women residing in Gaza Strip. **Methods:** In this case-control study, samples from 200 women (100 suffering from RSA and 100 control) were examined. All participants were genotyped for *IL-18* SNPs: -137 G>C, -607 C>A, +105A>C, and -656 G>T. Allele specific polymerase chain reaction (AS-PCR) was used to detect *IL-18* -137 G>C and -607 C>A SNPs whereas, restriction fragment length polymorphism (RFLP-PCR) method was used for genotyping *IL-18* +105A>C and -656 G>T polymorphisms. **Results:** The results revealed that there is no significant association between the allele/genotype frequencies of the investigated *IL-18* SNPs and RSA in the study population. Though not significant, the two groups showed remarkable differences in terms of allele/genotype frequencies of SNP -137 G>C. **Conclusion:** Among the examined *IL-18* SNPs the promoter -137 G>C may be important in RSA in the investigated population.

Keywords: *Interleukin-18*, Recurrent spontaneous abortion, polymorphism, SNP, Gaza strip.

1. INTRODUCTION

Recurrent spontaneous abortion (RSA), the occurrence of three or more consecutive abortions before the 20th

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week of gestation, occurs in approximately 1–3% of conceiving women. [1]

Immunological factors are reportedly recognized as important players in establishing a successful pregnancy and, several studies have shown that pro-inflammatory and anti-inflammatory cytokines play a major role in the reproductive phenomena. [2] Failure of fine tuning maternal immune response towards the allogeneic fetus can lead to adverse pregnancy outcomes including abortions. [3]

IL-18 plays a crucial role in regulating both innate and acquired immune responses. [4] This pro-inflammatory cytokine is secreted by a wide range of cells, including T and B lymphocytes, and antigen-presenting cells. [5, 6] IL-18, in synergy with IL-12 or IL-21 enhances IFN-γ (a signature cytokine of the Th1 cells involved in cellular immunity) production in human NK and T Cells. [7, 8]

Research efforts have focused on SNPs in cytokine genes [9] and various SNPs have been shown to be associated with inflammatory conditions, including the risk of pre-labor rupture of the amniotic membranes and preterm labor . [10]

The *IL-18* gene is located on chromosome 11 (11q22.2–22.3), and contains many polymorphisms (SNPs in particular) especially, in the promoter region. [11] SNPs may influence the level of cytokine production and the two polymorphisms (–137 G>C and –607 C>A) in the *IL-18* gene promoter seem to affect the transcription and hence the amount of *IL-18*. [12]

The *IL-18* gene is also expressed in the fetal chorion and the maternal deciduas and is thereby present at the materno-fetal interface. The *IL-18* level has been shown to increase from the first trimester until the onset of labor. Therefore, a role of *IL-18* in pregnancy, labor onset, and pregnancy complications has been suggested. [13]

This study was designed in order to investigate the association between *IL-18* gene polymorphisms (-137 G>C, "rs187238"; -607 C>A, "rs1946518"; +105A>C, "rs549908"; and -656 G>T, "rs1946519") and RSA among Palestinian women residing in Gaza Strip.

2. MATERIALS AND METHODS

Study subjects

The study group (n=100) included women aged 20-35 years who had experienced at least two spontaneous abortion before 20th week of gestation. The control group (n=100) consisted of women who had delivered at least one healthy child and had no previous history of pregnancy loss. Controls were matched with study subjects for all other possible characteristics. None of the individuals included in the study population used oral contraceptives, hormonal, or any serious medication affecting body vital functions.

DNA extraction and SNPs genotyping

About 2.0 ml of venous blood were drawn into sterile EDTA tubes under quality control and safety procedures. Genomic DNA was isolated from blood using Wizard Genomic DNA Purification Kit (Promega, USA) following the manufacturer instructions. The four SNPs were genotyped using either AS-PCR or PCR-RFLP employing published protocols. PCR primers and conditions, restriction enzyme digestion and results interpretation were done essentially as described by the authors indicated in Table 1.

Ethical considerations

Informed consent was obtained from all participants, and approval for conducting the study was obtained from the local ethics committee.

Statistical analysis

The genotype, allele frequency in RSA patients and the controls were analyzed by standard Chi-square test and odds ratio (OR) for risk of RSA at 95% confidence intervals (CI). All statistical analyses were performed

using the SPSS 17.0 software package (SPSS, Chicago, I/l, USA). Hardy-Weinberg equilibrium (HWE) was tested using a freely available software: (http://www.oege.org/software/hwe-mr-calc.shtml).

Table 1: Nucleotide sequence of the PCR primers used for genotyping *IL-18* SNPs

IL-18 SNP	Primer sequence (5'→3')	Reference	Method
-607 C>A	F(C): GTTGCAGAAAGTGTAAAAATTATTAC F(A): GTTGCAGAAAGTGTAAAAATTATTAA Common: TAACCTCATTCAGGACTTCC	Naeimi et al., 2006	AS-PCR
-137 G>C	F(G): CCCCAACTTTTACGGAAGAAAAG F(C): CCCCAACTTTTACGGAAGAAAAC Common: AGGAGGGCAAAATGCACTGG	Al-Khateeb et al., 2011	AS-PCR
-656 G>T	F: AGGTCAGTCTTTGCTATCATTCCAGG R: CTGCAACAGAAAGTAAGCTTGCGGAGAGG	Moravej et al., 2012	PCR-RFLP
+105 A>C	F: AGATTTAATGTTTATTGTAGAAAACCTGGACTC R: CAGTCATATCTTCAAATAGAGGCCG	Moravej et al., 2012	PCR-RFLP

3. RESULTS

Allele frequencies of the investigated SNPs

Table 2 illustrates alleles frequency, odds ratio, 95% confidence intervals and P values for the four investigated SNPs among RSA patients and controls. The statistical analyses of the allele frequencies showed that the differences are not significant (all p-values > 0.05) between the two groups. However, the frequency of the minor C-allele of SNP -137 G>C is notably higher in the RSA group.

Genotype frequencies of the investigated SNPs

Table 3 indicates the genotypes' frequencies, odds ratios (ORs), 95% confidence intervals (CI) and P values for the examined *IL-18* SNPs. Statistical analyses showed that the frequency differences among RSA patients and controls were not significant (all p-values > 0.05). The GG genotype of SNP -137 G>C is higher in the control group whereas, the genotypes containing the C-allele (i.e., CC and GC) are elevated in the patient group. This indicates that the C-allele might have a dominant effect in RSA.

Hardy-Weinberg equilibrium for investigated SNPs

The distribution of the genotypes of the four SNPs in the control group conformed with Hardy–Weinberg equilibrium as there was no significant difference between the expected and the observed genotypes.

4. DISCUSSION

RSA is a heterogenous ailment where determining its cause(s) can be extremely difficult and indeed, the cause(s) in about 50% of the cases remains undefined. The causes of RSA can be related to factors associated with genetics, immune response, endocrine abnormalities, infection and anatomic uterine defects. [14]

Table 2: Alleles frequencies of the four *IL-18*" SNPs among RSA patients and controls

SNP	Allele	Patients n= 100	Controls n=100	OR (95 % CI)	P- value
-607C>A	**C**	113 (56.5%)	105 (52%)	1.17(0.79 to 1.74)	0.42
	A	87 (43.5%)	95 (47.5%)		
-137G>C	**G**	122 (61%)	139 (69.5%)	0.68(0.45 to 1.03)	0.07
	C	78 (39%)	61 (30.5%)		
-656G>T	**G**	112 (56%)	99 (49.5%)	1.29(0.87 to 1.92)	0.19
	T	88 (44%)	101 (50.5%)		
+105A>C	**A**	138 (69%)	133 (66.5%)	1.12(0.73 to 1.70)	0.59
	C	62 (31%)	67 (33.5%)		

Table 3: Genotypes frequencies of the four *IL-18* SNPs among RSA patients and controls

SNP	genotype	Patients n= 100	Controls n=100	OR (95 % CI)	P-value
	CC	31 (31%)	28 (28%)	1.15 (0.62 to 2.12)	0.64
-607C>A	**CA**	51 (51%)	49 (49%)	1.08 (0.62 to 1.88)	0.77
	AA	18 (18%)	23 (23%)	0.73 (0.36 to 1.46)	0.38
	GG	38 (38%)	50 (50)%	0.61(0.34 to 1.07)	0.08
-137G>C	**GC**	46 (46%)	39 (39%)	1.33(0.75 to 2.33)	0.31
	CC	16 (16%)	11 (11%)	1.54(0.67 to 3.51)	0.30
	GG	37 (37%)	26 (26%)	1.67(0.91 to 3.05)	0.09
-656G>T	**GT**	38 (38%)	47 (47%)	0.69(0.39 to 1.21)	0.19
	TT	25 (25%)	27 (27%)	0.90 (0.47 to 1.69)	0.74
	AA	47 (47%)	40 (40%)	1.33 (0.75 to 2.33)	0.31
+105A>C	**AC**	44 (44%)	53 (53%)	0.69 (0.39 to 1.21)	0.20
	CC	9 (9%)	7 (7%)	1.31(0.46 to 3.67)	0.60

Production of cytokines, including the pro-inflammatory IL-18 and the distribution of the various

types of immune cells during pregnancy are increasingly recognized critical in affecting the pregnancy outcome. [15-17]

Studies from different populations have been carried out to assess the association between *IL-18* gene polymorphisms in RSA and other immune system-related diseases such as, Rheumatoid arthritis and systemic lupus erythematosus . *IL-18* SNPs have been implicated as potential risk factors in certain populations. [18, 19] Results of this work showed that the allele/genotype frequencies of *IL-18* -607 C>A polymorphism are not significantly different between the RSA patients and the controls. Similar findings were reported by Naeimi *et al*., (2006) and Ostojić *et al*., (2007). [20, 21] Likewise, the *IL-18* -656 G>T allele/genotype distribution did not show significant difference between the two study groups (Tables 2 and 3). To the best of our knowledge the association between this latter SNP and RSA has not been investigated by other authors.

Regarding *IL-18* +105A>C, the allele/genotype frequencies of this polymorphism were comparable between the control women and the RSA patients (Tables 2 and 3) and therefore, this SNP may not be important in modulating the risk of RSA in our population. As with *IL-18* -656 G>T polymorphism no published work has been found regarding the relation between RSA and this polymorphism to compare our results with.

For *IL-18* -137 G>C, though the difference was not significant, the GG genotype is less prevalent in the RSA women (38%) as compared to the control patients (50%), and the genotypes containing the C allele (GC + CC) are more prevalent in the RSA than in the controls (62% vs. 50%). This result indicates that the C-allele may be of dominant effect in RSA and larger sample should be tested to confirm this finding. Interestingly, this SNP has been linked to altered serum levels of IL-18. [22]

Conflicting results have been reported, while Messaoudi *et al*., (2012), Al-Khateeb *et al*., (2011), Ostojić *et al*., (2007) and Naeimi *et al*., (2006) reported lack of association between RSA and *IL-18* -137 G>C, Wang *et al*., 2014 observed a significant association between this SNP and RSA. [24]

Discrepancy between results of genetic association studies like those encountered here could be due to many reasons including population genetic variation (background) unrelated to the investigated alleles, selection criteria of patients, presence of nucleotide polymorphism somewhere else in the examined gene, epigenetic alterations and linkage disequilibrium to other sequence variants in the vicinity of the studied locus.

5. CONCLUSION

In conclusion, this study showed that there is no significant association between the four investigated *IL-18* gene polymorphisms and the risk of RSA in the study population. Still, the C-allele of -137 G>C may have some influence in RSA and this particular SNP should be examined in a larger number of RSA patients

6. REFERENCES

1. Al-Khateeb GM, Sater MS, Finan RR, Mustafa FE, Al-Busaidi AS, Al-Sulaiti MA, Almawi WY. Analysis of interleukin-18 promoter polymorphisms and changes in interleukin-18 serum levels underscores the involvement of interleukin-18 in recurrent spontaneous miscarriage. Fertil Steril 2011; 96(4): 921-6.
2. Baxevanis CN, Gritzapis AD, Papamichail M. In vivo antitumor activity of NKT cells activated by the combination of IL-12 and IL-18. J Immunol 2003; 171: 2953-2959.

3. Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, McNicholl J, Pociot F, Hardt C, D'Alfonso S. Cytokine gene polymorphism in human disease On-line databases, supplement 1. Genes Immunol 2001; 2: 61-70.
4. Chaouat G, Le´de´e-Bataille N, Zourbas S, Ostojic S, Dubanchet S, Martal J, Frydman R. Cytokines, implantation and early abortion: re-examining the Th1 / Th2 paradigm leads to question the single pathway, single therapy concept. Am J Reprod Immunol 2003; 50:177–186.
5. Chen S, Jiang F, Ren J, Liu J and Meng W. Association of IL-18 polymorphisms with rheumatoid arthritis and systemic lupus erythematosus in Asian populations: a meta-analysis BMC Medical Genetics 2012, 13:107
6. Dinarello C A. Interleukin-18 and the pathogenesis of inflammatory diseases. Semin Nephrol 2007; 27: 98–114.
7. Giedraitis V, He B, Huang W X, Hillert J. Cloning and mutation analysis of the human IL-18 promoter a possible role of polymorphisms in expression regulation. J Neuroimmunol 2001; 112: 146–152.
8. Guerin LR, Prins JR, Robertson SA: Regulatory T-cells and immune tolerance in pregnancy: a new target for infertility treatment? Hum Reprod Update 2009; 15:517–535.
9. Laird SM., Tuckerman EM., Cork BA., Linjawi S., Blakemore AI and Li TC. A review of immune cells and molecules in women with recurrent miscarriage. Hum Reprod Update 2003; 9: 163–74.
10. Lee RM, Silver RM. Recurrent pregnancy loss: Summary and clinical recommendations. Sem Reprod Med 2000; 18: 433-40.
11. Li TC, Makris M, Tomsu M, Tuckerman E, Laird S. Recurrent miscarriage aetiology, management and Prognosis. Hum Reprod Updates 2002; 8(5): 463-481.
12. Liang XH, Cheung W, Heng CK, Wang DY: Reduced transcriptional activity in individuals with IL-18 gene variants detected from functional but not association study. Biochem Biophys Res Commun 2005; 338: 736–741.
13. Messaoudi S, Dandana M, Magdoud K, Meddeb S, Ben Slama N, Hizem S, Mahjoub T. Interleukin-18 promoter polymorphisms and risk of idiopathic recurrent pregnancy loss in a Tunisian population. J Reprod Immunol 2012; 93(2): 109-13.
14. Moravej A, Rasouli M, Kalani M, Asaei S, Kiany S., Najafipour S., Koohpayeh A., Abdollahi A. IL-1b (-511T/C) gene polymorphism not IL-1b (+3953T/C) and LT-a (-252A/G) gene variants confers susceptibility to visceral leishmaniasis. Mol Biol Reprod 2012; 39(6): 6907–6914.
15. Naeimi S, Ghiam Al F, Mojtahedi Z, Dehaghani Al. S., Amani D. and Ghaderi A. Interleukin-18 gene promoter polymorphisms and recurrent spontaneous abortion. Eur. J. Obstet. Gynecol. Reprod Biol 2006; 128: 5–9.
16. Ostojić S, Volk M, Medica I, Kapović M, Meden-Vrtovec H., Peterlin B. Polymorphisms in the interleukin-12/18 genes and recurrent spontaneous abortion. Am J Reprod Immunol 2007; 58(5): 403-8.
17. Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M, Farhat R. Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. Hum Reprod 2000; 15 713-718.

18. Schneider B E, Korbel D, Hagens K, Koch M, Raupach B, Enders J, Kaufmann SH, Mittrucker H. W,Schaible U E. A role for IL-18 in protective immunity againstMycobacterium tuberculosis. Eur J Immunol 2010; 40: 396–405.
19. Shigehara K, Shijubo N, Ohmichi M, Takahashi R, Kon S, Okamura H, Kurimoto M, Hiraga ,Tatsuno, T, Abe S, Sato N. IL-12 and IL-18 are increased and stimulate IFN-gamma production in sarcoid lungs J Immunol 2001; 166, 642–649.
20. Strengell M, Matikainen S Sirén J, Lehtonen A, Foster D, Julkunen I, Sareneva T. IL-21 in synergy with IL-15 or IL-18 enhances IFN-gamma production in human NK and T cells. J Immunol 2003; 170(11):5464-9.
21. Smith AJ, Humphries SE. Cytokine and cytokine receptor gene polymorphisms and their functionality. Cytokine Growth Factor Rev 2009; 20: 43–59.
22. Tschoeke SK, Oberholzer A, Moldawer LL. Interleukin-18 a novel prognostic cytokine in bacteria-induced sepsis. Crit Care.Med 2006; 34: 1225-1233.
23. Wang D, Wang C, Zheng LZ, Zhu M, Zhu YM. Relationship between IL-18 gene polymorphism and unexplained recurrent spontaneous abortion. Zhejiang Da Xue Xue Bao Yi Xue Ban 2014; 43(4): 448-52.
24. Wilson R, Moor J, Jenkins C, Miller H, Walker JJ, McLean MA, Norman J, bMcInnes IB. Abnormal first trimester serum interleukin 18 levels are associated with a poor outcome in women with a history of recurrent miscarriage. Am J Reprod Immunol 2004; 51: 156–9.

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