Inheritance of HLA-c and pro-inflammatory cytokines polymorphism in association with family history in Type-I psoriasis

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Abstract

Introduction: Psoriasis is a chronic, disfiguring inflammatory skin disease without a single clinical presentation and for which there is no cure, the Pathogenesis is multifactorial and has a bimodal age of onset. It characterized by increase secretion of pro inflammatory cytokines and associated with different HLA alleles in different ethnic groups.

Aim: To evaluate the association of inheritance of either HLA-CW6, CW7 or CW17 allele and polymorphic IL1β, IL6 or TNFα gene with the family history of Iraqi patients with type I psoriasis.

Materials and methods: Current study includes psoriatic group involve 76 patients (type I). After inform consent, a PCR based method (low resolution sequence specific primer. PCR-SSP) used to detect HLA-CW6, CW7 and CW17 allele. And a PCR-SSP for detection of IL1β, IL6 and TNFα polymorphism.

Results: There is none statistical association between either CW6, CW7 or CW17 with a positive family history. IL6-C allele, TNFα A\G genotype significantly associated with family history positive group.

Conclusions: Among the selected parameters, IL6-C allele and TNFα A\G genotype offer a good chance to develop type I psoriasis among family members with positive family history.

Keywords: Psoriasis, type I, CW6, CW7, CW17, IL1β, IL6, TNFα.

Introduction:

Psoriasis is a chronic, disfiguring, painful, disabling and non-communicable disease, without a single typical clinical presentation, and for which there is no cure [1]. The most significant feature of psoriasis is keratinocyte and dermal vascular endothelial cell hyper proliferation and cellular infiltration with subsequent vascular changes and inflammation. About five subtypes of psoriasis have been found include vulgaris (plaque), guttate, pustular, inverse, and erythrodermic. With a plaque psoriasis affects approximately 85%–90% of psoriatic patients [2]. The lesion may appear anywhere on the skin but spared the mucosa characterized by well demarcated erythematous plaque with silvery scale that easily detach, and may aggravated by irritation, trauma, infection, some drugs, seasonality and stress [3]. It has a bimodal distribution with one peak at 20-30 years and other at 50-60 years, with approximately 0.5-1% children affected compared to 2-3% of adults [4]. According to age of onset, type I psoriasis is inherited and associated with Cw6, while type II psoriasis occurs sporadically [5]. Early onset psoriasis (type I) accounting for approximately 75% of patients and occurs before the age of 40 years, Patients have a strong family history and the Majority of them are CW6 positives. late onset psoriasis (type II) presents after the age of 40 years, Patients have no family history and are Cw6 negative [6]. Psoriasis characterized by increase secretion of pro inflammatory cytokines, that create a “cytokine storm” [7]. Dysregulated immunity contributes to the construction of psoriasis by induce the over-production of pro-inflammatory cytokines by keratinocytes [8]. In psoriasis there is over production and persistent secretion of IL- 1, IL-6 and TNF- α [9]. This study conducted to evaluate the association of inheritance of either HLA-CW6, CW7 or CW17 and polymorphic IL1β, IL6 or TNFα gene with the family history of Iraqi patients with type I psoriasis.

Materials and methods:

This is a prospective study comprised seventy-six patients diagnosed by dermatologist, selected from those visited the dermatological clinic in al-Zahra teaching hospital in Al- Kut city from February to august 2017, based on the age of first attach (been less than 40 years) of chronic plaque psoriasis. Informed consent was obtained before venous blood sample (2 ml) toke from each patients in K3-EDTA anticoagulated tube and subjected to a DNA extraction procedure. After that, the extracted DNA that fulfil the required purity and concentration aliquot and stored at -20c° for polymerase chain reaction amplification and detection. The primer sequence employed in current study shown in table (1).

Table 1: Primer Sequence with Their Product Size.

No.	Target	Sequence 5'-3'	Product size	References
1	HLA-cw6 F	GGATCAGGACGAAGTCCCAG	170 bp	NCBI/ primer-BLAST
2	HLA-cw6 R	GGGGACGCGTCATGAGTATT		
3	HLA-cw7 F	TTA CAT CGC CCT GAA CGA GG	237 bp	NCBI/ primer-BLAST
4	HLA-cw7 R	GGC CAT CCC GGG AGA TCT AT		
5	HLA-cw17 F	GGA TGA GGG GTC ATG TGT CT	400 bp	NCBI/ primer-BLAST
6	HLA-cw17 R	AGT AAG TGC TGG CAC ACA GG		
7	IL1β F	CTC ATC TGG CAT TGA TCT GG	215 bp	HUNT, et al., 2000 [29]
8	IL1β R1	GGT GCT GTT CTC TGC CTC G		
9	IL1β R2	GGT GCT GTT CTC TGC CTC A		
10	IL6 F	GAG CTT CTC TTT CGT TCC	234 bp	Ahmad, et al., 2015 [28]
11	IL6 R1	CCT AGT TGT GTC TTG CC		
12	IL6 R2	CCC TAG TTG TGT CTT GCG		
13	TNFα F	CTG CAT CCC CGT CTT TCT CC	863 bp	Ahmad, et al., 2015 [28]
14	TNFα R1	ATA GGT TTT GAG GGG CAT CG		
15	TNFαR2	ATA GGT TTT GAG GGG CAT CA		
16	GAPDH F	AGA CCA CAG TCC ATG CCA TC	498 bp	NCBI/ primer-BLAST
17	GAPDH R	CAG GGC CCT TTT TCT GAG CC		

Each reaction contains a pair of primers specific for each allele. A second pairs of primer specific for a house keeping gene (GAPDH) also added to each HLA reaction to make sure that a human target DNA is present in each sample. The obtained results for HLA gel electrophoresis shown in figure (1), while the results for gel electrophoresis of selected cytokines are illustrated in figure (2).

(A)

(B)

(C)

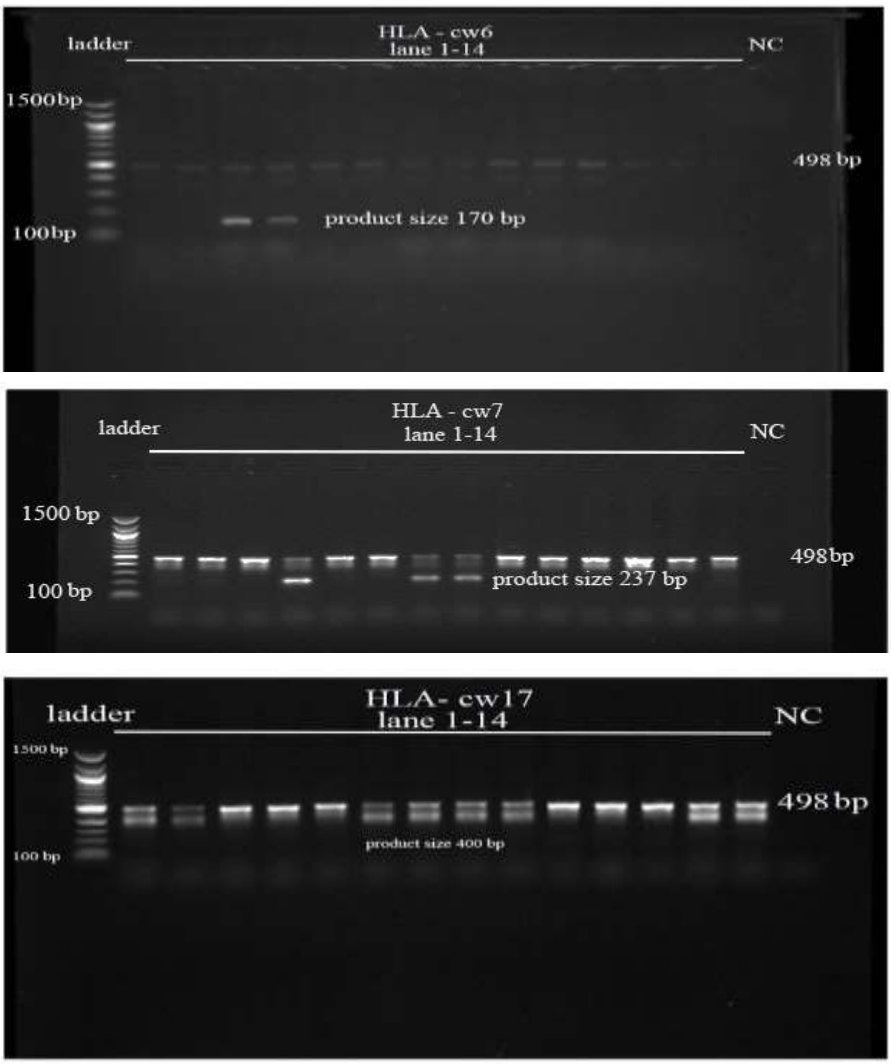
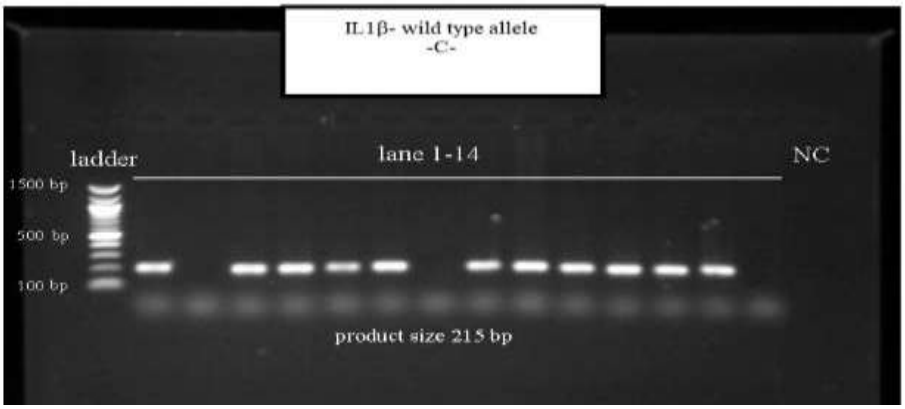
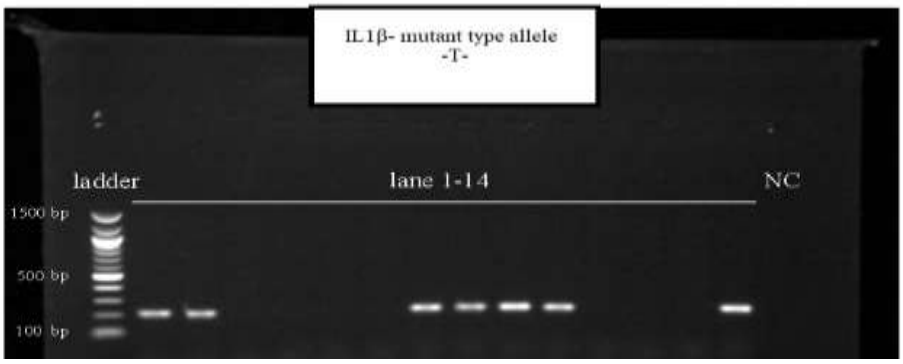


Figure 1: Gel Electrophoresis for PCR Products (HLA). All three procedures include control primer with 498 bp molecular size. (A) *CW6* with molecular size 170 bp. (B) *CW7* with molecular size 237 bp. (c) *CW17* with molecular size 400 bp. The desire molecular size validated by comparing to 100 bp molecular ladder.

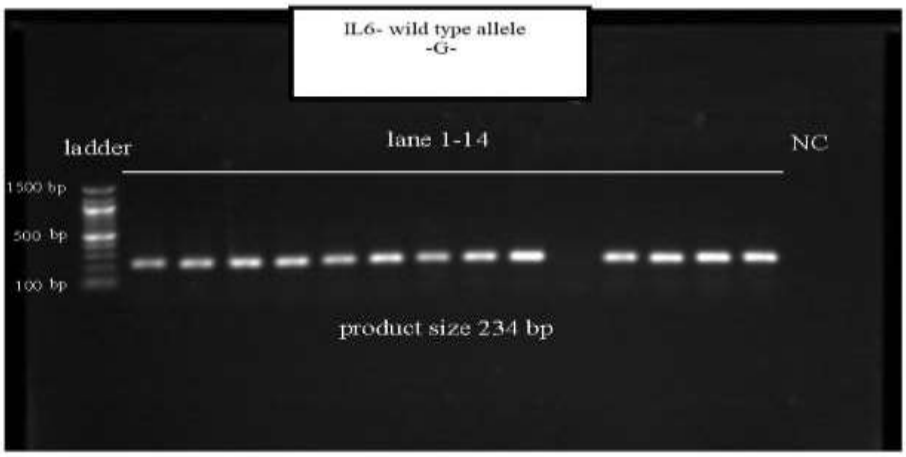
(A)


IL1β- wild type allele
-C-
ladder
lane 1-14
NC
1500 bp
500 bp
100 bp
product size 215 bp

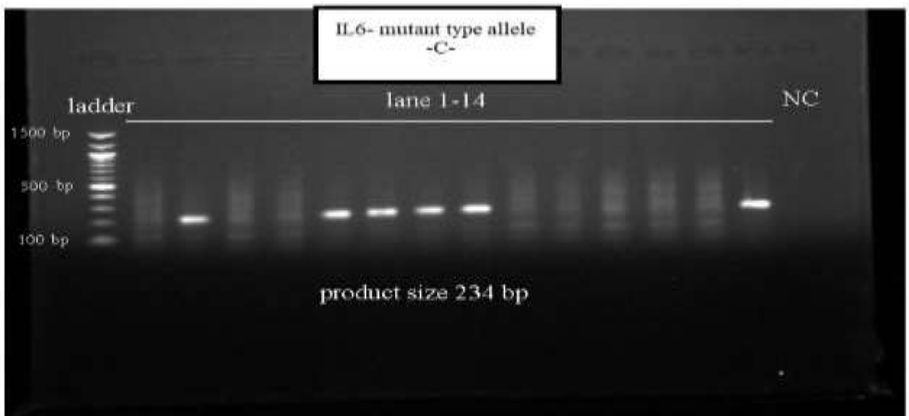
(B)


IL1β- mutant type allele
-T-
ladder
lane 1-14
NC
1500 bp
500 bp
100 bp

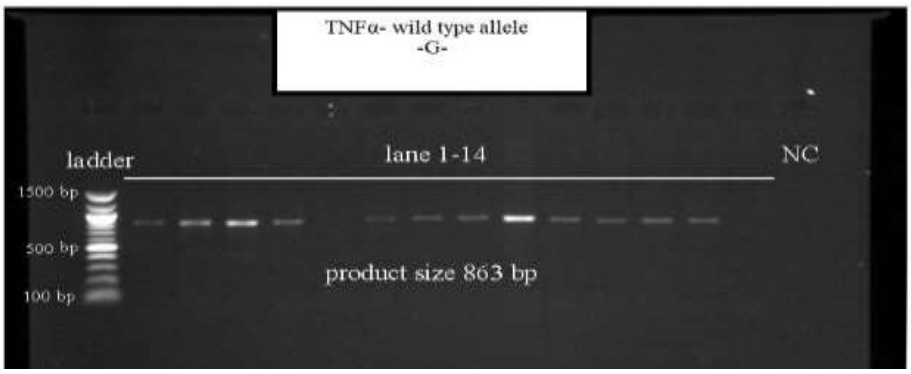
(c)


IL6- wild type allele
-G-
ladder
lane 1-14
NC
1500 bp
500 bp
100 bp
product size 234 bp

(D)



(E)



(F)

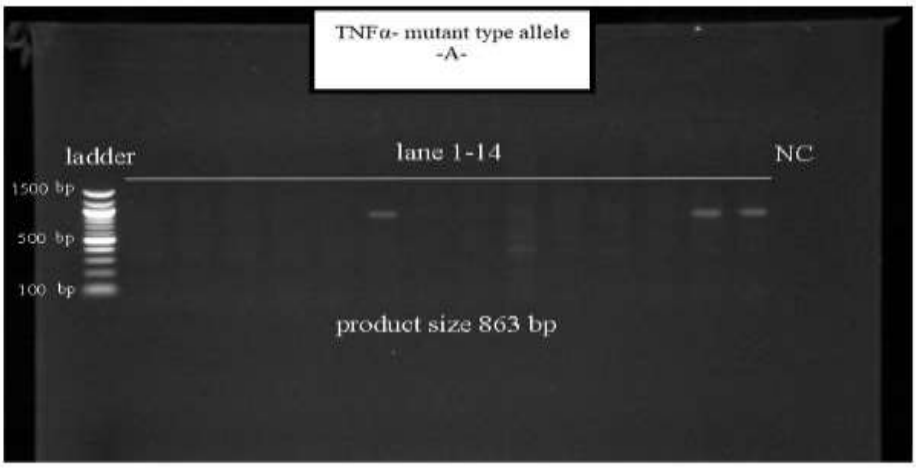


Figure 2: Gel Electrophoresis for PCR Products (cytokines). A- allele C for *IL1β* -511 C/T. B- allele T for IL1β -511 C/T. C- allele G for *IL6* -174 G/C. D- allele C for IL6 -174 G/C. E- allele G for *TNFα* -308 G/A. F- allele A for TNFα -308 G/A. The desire molecular size validated by comparing to 100 bp molecular ladder.

Results:

The patients in this study divided into two group. Patients with family history of psoriasis (40) and patients without family history (36). The mean age is 26.2 years, about 47.4% of them is male. About 33% of those without family history have CW17 allele compared to 30% in those with family history. The CW7 allele distribution (22% vs 30%) and CW6 (22% vs 15%) with no statistical significance difference (p-value >0.05). As shown in table (2).

Table 2: Association of HLA-C Allele with Family History.

Patients	HLA		*OR	**Sig
	Present (no.) %	*Absent (no.) %*		
CW17				
Without family history n=36	(12) 33.3	(24) 66.7	0.9	0.5
With family history n=40	(12) 30.0	(28) 70.0		
Total n=76				
CW07				
Without family history n=36	(8) 22.2	(28) 77.8	1.5	0.3
With family history n=40	(12) 30.0	(28) 70.0		
Total n=76				
CW06				
Without family history n=36	(8) 22.2	(28) 77.8	0.6	0.3
With family history n=40	(6) 15.0	(34) 85.0		
Total n=76				
* Odds Ratio, Chi-square test, **p-value				

IL1β allele and genotypes show non-statistical significance differences between patients with family history and patients without family history (p-value >0.05). Regarding IL6, the C allele significantly increase in those with family history (p-value 0.05 and OR 2.1). On the other hand, the G allele significantly increase in those without family history (p-value 0.05 and OR 0.4).

the GG genotype of IL6 is significantly increase in patients without family history (p-value 0.03 and OR 0.3). The A and G allele of TNFα show non statistical significance between patients with family history from those without family history (p-value >0.05). The AG genotype significantly increase in patients with family history (p-value 0.05 and OR 3.7) while the GG seen in significantly higher

frequency in patients without family history (p-value 0.03 and OR 0.2). These finding shown in table (3).

Table 3: Association of cytokines allele and genotypes with Family History.

	IL1 β	Family history–(36)	Family	**p-value	*OR
Genotypes (no.)%	CC	(16) 44.4	(19) 47.5	0.5	0.8
	CT	(12) 33.3	(14) 35.0	0.5	0.9
	TT	(8) 22.2	(7) 17.5	0.4	1.4
Allele (no.)%	C	(44) 61.1	(52) 67.5	0.4	0.8
	T	(28) 38.8	(25) 32.5	0.4	1.2
	IL6				
Genotypes (no.)%	CC	(3) 8.3	(5) 12.5	0.4	1.6
	CG	(5) 13.8	(12) 30	0.07	2.7
	GG	(28) 77.7	(23) 57.5	0.03	0.3
Allele (no.)%	C	(11) 15.2	(22) 27.5	0.05	2.1
	G	(61) 84.7	(58) 72.5	0.05	0.4
	TNFα				
Genotypes (no.)%	AA	(1) 2.7	(1) 2.5	0.7	0.9
	AG	(3) 8.3	(10) 25.0	0.05	3.7
	GG	(32) 88.8	(29) 72.5	0.03	0.2
Allele (no.)%	A	(5) 6.9	(12) 15.0	0.09	2.4
	G	(67) 93.0	(68) 85.0	0.09	0.4

Discussion

Psoriasis is highly heritable disease with a heritability estimate of 68% [10]. In current study, about 47% of psoriatic patients have at least one family member affected. Several study in Dutch, Singaporean, Australian and USA found nearly same finding with minor differences due to variation in genetic background of each population [11]. In a study conducted on Iraqi children, about 37% of them have a positive family history of psoriasis [12]. Psoriasis considered as a heritable disease, but not 100% as a genetic disease based on twin studies, so psoriasis is a complex disease that not simply explained by Mendelian inheritance rules [13]. Although the inheritance pattern is still unclear, patients with childhood psoriasis have a positive family history, Siblings and first-degree relatives of patients show a more increased risk in developing psoriasis [14]. The risk for developing psoriasis is 41% if both parents psoriatic; 14% if one parent is psoriatic, 6% if one sibling is psoriatic and only 2% when no parent or sibling is psoriatic [15]. A positive family history defined as psoriatic person with at least one first degree family member affected with psoriasis [16]. Study on families with multiple affected members found several susceptibility loci with the most strongly associated locus is PSOR1, the family-based studies have confirmed that HLA-C is directly involved in susceptibility to psoriatic [17]. Our results indicate that there is none statistical association between either CW6, CW7 or CW17 with a positive family history. HLA Cw6 revealed to confers susceptibility to PsV and to be the PSORS1 risk variant that has been associated with early onset of psoriasis and higher prevalence of family history [18]. This association also proved by Ruben et al., 2003 [19]. Other study by Annet et al., 2014 [20] could not find any association between CW6 and family history and attribute this discrepancy to the fact that similar proportions of both group with a positive family history and a negative family history were found to have similar proportions of CW6 positivity, many patients with early onset have negative family history while patients with adult onset have a positive family history. Unfortunately, the absence of other study that discuss the association of CW7 and CW17 with family history make comparison and discussion of our results very difficult, but by looking to Iraq community as closed one, closed mating in the family and that several generation residents the same place, we can conclude that the inheritance of these HLA alleles was the same for both group. This conclusion enforced by CW6 finding. At the allele and genotype level, IL6 -174 C allele significantly associated with family history positive group compare to G allele in family history negative group. The G\G genotype of both IL6 and TNFα significantly associated with those negative for family history, but the TNFα A\G genotype significantly associated with those positive for family history. There is no available date about IL1β polymorphism association with family history either for psoriasis or other diseases to support or antagonized our result. Monika et al., 2015 [21] Evaluate IL6 gene polymorphism (30-60 years old patients) and reveal that there is no significant difference

between the IL6 −174G>C genotype and a family history of psoriasis. Such Lack of association also seen in rheumatoid arthritis in adults [22], febrile seizures or epilepsy in children [23]. These data suggest that inheritance of IL6 −174G>C polymorphism offer a better chance for initiation of type I but not type II PS within family, while have no effect with other diseases. There is no other study involve TNFα polymorphism in association with family history of psoriasis to make agreement or not with it, Sangeeta et al., 2016 [24] cannot found an association between TNFα level and family history. TNFα polymorphism association with family history proved in other disease like type 2 diabetes mellitus [25], coronary heart disease [26] but not in alopecia areata [27].

Conclusions and recommendations

Inheritance of HLA allele may not increase the risk of type I psoriasis with in family as it may inherit equally between family members, but certain pro inflammatory allele and genotype my increase the risk, including IL6-C allele and TNFα A/G genotype which can be used as predictive tool and assign as therapy targets for family members with positive family history.

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Conflict of interest:

The authors declare that there is no Conflict of interest.

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