The Egyptian Journal of Hospital Medicine (Jan. 2013) Vol. 50 , Page 141 – 149

The Role of Interleukin-18 Promoter Polymorphisms (-607 C/A and 137 G/C) in Determining HCV Clearance or Persistence.

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Abstract

Objective: Two interleukin (IL-18) Polymorphisms (- 607 C/A and -137 G/C) and their haplotypes are known to affect the IL-18 expression. A number of SNPs (single nucleotide polymorphisms) that influence IL- 18 production are found in the gene promoter region.

Aim of the work: The study will determine HCV clearance or persistence as a result of IL-18 promoter polymorphisms (-607 C/A and 137 G/C) in chronic hepatitis C virus infected patients during interferon and ribavirin treatment.

Patients and methods: Eighty patients with chronic hepatitis C virus infection, their age ranges between (23-57) years, selected from the National Hepatology and Tropical Medicine Research Institute were included in this study, during interferon and ribavirin therapy and fifteen healthy individuals were included to serve as controls. All the patients and controls were subjected to the following history, clinical examination, abdominal ultrasonography and collection of blood samples for routine laboratory investigation, CBCs and serological assay and specific sequence primer polymerase chain reaction (PCR) IL-18-137, 607 SNP.

Results: There was no significant difference in the frequencies of -137 allelic distribution in CHCV infection patients and healthy controls.
The -607 AA allele was higher among controls than in patients with CHCV infection.
The -607 CC allele was higher among the CHCV patients than in the healthy controls.
87.5 % of the studied CHCV patients had response to IFN therapy, the majority of cases had A1F1 biopsy results.

Conclusion: IL-18 promoter polymorphism at -607 position with AA allele is a potential protective marker, as it is higher among healthy controls than the CHCV patients.

Recommendations: that IL-18 could be considered as a target for therapeutics.

Key words: IL-18 promoter polymorphism -607 C / A, - 137 G / C, hepatitis C virus, single nucleotide polymorphisms.

Introduction

IL 18 promoter polymorphism may affect the outcome of HCV infection in certain groups (1). Three single nucleotide polymorphisms (SNPs) are found in the IL-18 gene promoter in positions -656 G-T, -607 C-A, and – 137 G – C. Two of these, -607 C – A and – 137 G – C, are located at the binding sites for CREB transcriptional factors cAMP response-element binding proteins and the H4TFI nuclear factor, respectively, therefore mutation at these two sites could influence IL-18 expression and change the production of the cytokine (2). IL-18 is a pleiotropic proinflammatory cytokine that stimulates production of INF-γ, TNF-α, IL-1, IL-2, adhesion molecules and apoptosis factors, increasing T-lymphocyte prolipheration, and enhancing the lytic activity of NK-cells. It participates in the cellular and humoral immune

response (3), both innate and adaptive (4). IL-18 plays an important dual role in Th1 polarization and viral clearance, as in the development of liver fibrosis. Single-nucleotide promoter polymorphisms influence the transcription of IL-18 mRNA. Promoter polymorphisms are linked to delayed virus clearance and disease susceptibility in many diseases. However, there is no information about their role in hepatitis C virus infection (5). Hepatitis C virus (HCV) infection is one of the most common chronic viral infections in the world. Approximately 80 to 90 % of acutely infected individuals develop persistent infection, a major risk for developing liver cirrhosis and liver cancer, while a small portion of patients (10-20 %) clear the virus (6-8). The immune response is critical in determining the outcome of hepatitis C virus (HCV) infection. Interleukin (IL)-18 is a pivotal mediator of Th1/Th2 driven immune response (1). Interleukin-18 (IL-18) is a potent proinflammatory cytokine, which can promote hepatitis B virus clearance. The latest studies find that genetic polymorphisms near the IL-28B gene are strongly associated with sustained viral response and spontaneous viral clearance in patients with chronically infected hepatitis C and hepatitis B (9).

Patients and Methods:

Eighty patients with chronic hepatitis C virus (CHCV) infection, their age ranges between (23- 57) years, selected from the National Hepatology and Tropical Medicine Rsearch Institute, were included in this study during interferon and ribavirin therapy,

And fifteen healthy individuals were included to serve as controls. All patients have anti-HCV antibodies, HCV RNA in serum, evidence of chronic hepatitis on liver biopsy, elevated levels of aminotransferase above the upper limit, serum albumin, bilirubin, and prothrombine time within normal limit with negative history of drug abuse, non reactive HBsAg, with exclusion of other chronic disease and pregnancy no clinical signs of decompensated liver disease. All the patients were subjected to the following history and through clinical examination, abdominal ultrasonography and collection of blood samples.

About 5 mL of peripheral venous blood was collected under aseptic conditions into

sodium-citrated tubes and processed within the same day. The genomic DNA: was extracted using a quiagene extraction kit lot no (139289002) according to manufacturer instruction. The purity and concentration of DNA was determined using spectrophotometry. Following deproteinisation, the quality of DNA was reflected by a consistent ratio of 1.8 to 2.0. The coded genomic DNA solution was stored at 4°C.

Specific Sequence Primer Polymerase Chain Reaction (PCR): The –137 SNPs were detected using sequence specific PCR, described by Giedraitis et al. A common reverse primer 5'-AGGAGGGCAAAATG CACTGG-3' and 2 sequence specific forward primers, 5'-CCCCAACTTTTACGGA AGAAAAG-3' and 5' CCCCAACTTTTACGGAAG AAAAC-3', were used. An amplification product of 261-pb was detected. A control forward primer 5'-CCAATAGGACTGATTATTCCGCA-3' was used to amplify a 446-bp fragment covering the polymorphic site to serve as an internal positive amplification control.

Restriction Fragment Length Polymorphism (RFLP): analysis for the -607 SNPs , RFLP analysis was used to genotype the polymorphism. Generally, PCR for genotyping was performed with mutated primers: the forward primer 5'GTTGCAGAAAGTGTAAAAAT TAGTA-3' introduced a restriction site for the RsaI (0281105) enzyme. The reverse primer was: 5'-TAACCTCATTCAGGACT TCC-3'. Biometra T3 thermocycler was used in PCR with the following conditions: 1 cycle of 95°C for 5 minutes followed by 35 cycles denaturing at 94°C for 10 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 30 seconds. The final extension step was done at 72°C for 10 minutes.

30 uL of PCR reaction contained 50 ng genomic DNA, 0.2 uM each of forward and reverse primers; 0.1 mM of dNTPs, 1x reaction buffer and 0.2 units of DyNAzymeTM II DNA polymerase (FinnZymes lot no 139295497). After confirmation of a successful PCR process, 6 uL of PCR product was incubated with RsaI enzyme and the buffer recommended by the supplier for 60 minutes at 37 ℃. Visualisation of RFLP was done in ethidium bromide stained 3 % aragose gel in 0.5x TBE buffer (2).

Statistical consideration: Analysis of data was done by IBM computer using SPSS (Statistical Program for Social Science Version 12). Data were expressed as : Description of quantitative variables: as mean, SD and range. Description of qualitative variables: as number and percentage. Chi- Square test was used to compare qualitative variables between groups. All this was expressed as probability of value (p value) the difference was considered significant if p value < 0.05, (10).

Results

The study included 80 patients of CHCV infection during interferon and ribavirin therapy, and 15 healthy volunters.

We found that the IL-18 Promoter SNPs -137 shows no statistically significant difference between CHCV patients and controls as regarding genotypes by using Chi-Square test ($p > 0.05$) table (1). IL-18 -137 CC = (17.5%),-137 CG = (40%), -137 GG = (44.5%) in patients, and -137CC =26.7 %, CG =33.3 %, GG = 40 % in the control group.

We examined the IL-18 promoter SNPs -607 in two cases and controls . The analysis was performed for the IL-18 -607 AA, AC and CC alleles genotypes frequencies . The IL-18 -607 AA was 15 % for CHCV patients and 40% for controls. The IL-18 -607 AC for cases 27.5 % and 26.7 % for controls while IL-18 -607 CC = 47.5 % for cases and 33.3 % for controls, table (2) and Graph (1) show that ther's significant difference between CHCV infected patients and controls ($p < 0.05$) by using Chi-Square test.

Fig: A

Fig: B

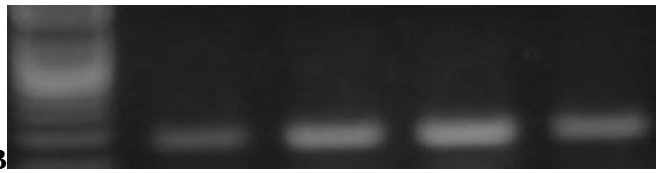


Fig: C

An agarose gel electrophoresis show PCR products of IL-18 (137 site) genotype Genotype G (fig.A) and genotype C (fig.B) genotype G&C (fig.C).

Fig: A1

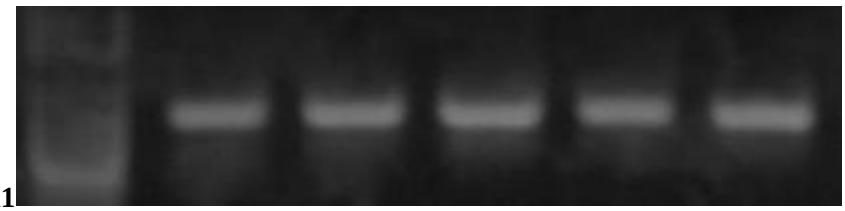


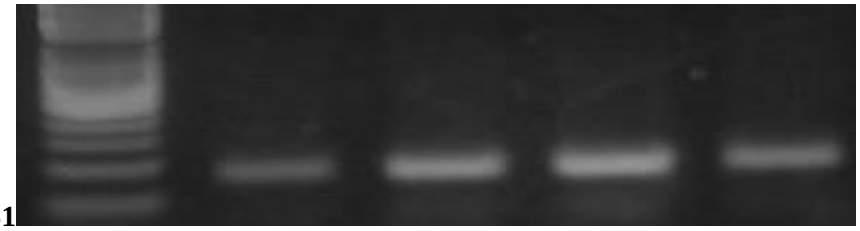
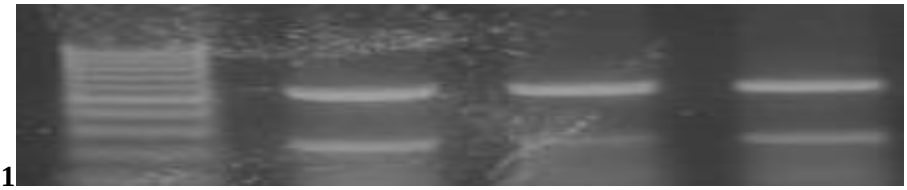
Fig: B1 

Fig: C1 

An agarose gel electrophoresis show PCR products of IL-18 (607 site) genotype Genotype A (fig.A1) and genotype C (fig.B1) genotype A&C (fig.C1).

Schematic representation of IL-18 gene with promoter regions analyzed for promoter polymorphism. Genotyping of IL-18 promoter region at -137 and -607 positions was performed with PCR products were visualized by agarose gel electrophoresis.

Homozygous formed by variants -607 C/C and -137 G/G table (3) show highly statistically significant difference in CHCV patients between both alleles by using Chi-Square test.
The analysis was performed with results: IL-18-607 AA (15 %) , -607 AC (27.5 %), 607-CC (47.5 %) , while the IL-18-137 CC (17.5 %) , -137 CG (40 %) , -137 GG (42.5 %) table (3) and Graph (2) illustrated the variant polymorphism in CHCV patients as regarding IL-18 gene promoter region at -607, -137 alleles genotypes.
There's transversion from C to A at position -607 and from G to C at position -137.

Table (4), Graph (3) illustrated that IL-18 PBMCs from healthy controls carrying the AA Genotype was significantly higher than those carrying the AC and CC genotype at the -607 position.
IL-18 PBMCs from healthy controls carrying the GG genotype was significantly higher than those carrying the CG and CC genotype at the -137 position.

Table (1) Distribution of the studied CHCV patients group as regard IL-18 gene polymorphism of 137 alleles genotypes.

Variables	**Cases** N=80	**Controls** N=15	X^2	**P**
AA	0	0	0.7	>0.05 NS
AC	0	0		
CC	14(17.5%)	4(26.7%)		
CG	32(40%)	5(33.3%)		
GG	34(42.5%)	6(40%)		

This table shows no statistically significant difference between both groups as regard different alleles genotypes of IL-18 SNPs 137 by using chi-square test.

Table (2) Distribution of the studied CHCV patients as regard IL-18 gene polymorphism of 607 alleles genotypes.

Variables	**Cases** N=80	**Controls** N=15	**X^2**	**P**
AA	12(15%)	6(40%)	**5.5**	**<0.05 S**
AC	22(27.5%)	4(26.7%)		
CC	46(47.5%)	5(33.3%)		
CG	0	0		
GG	0	0		

This table shows that AA was higher among controls, while CC was higher among cases with statistically significant difference between by using chi-square test .

Fig (1): Illustrates the variants polymorphism in CHCV patients as regarding IL-18 gene promoter region at 607 alleles genotypes.

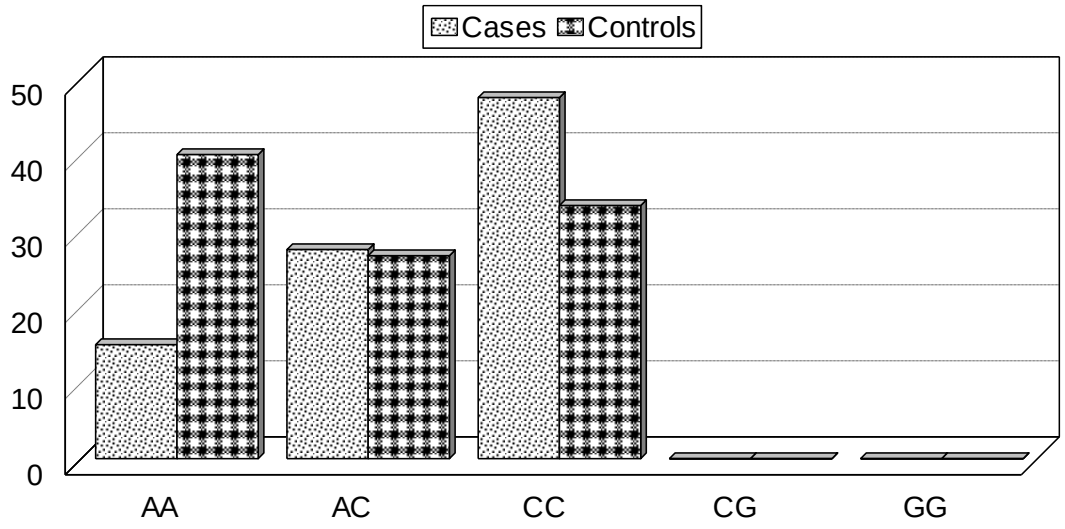
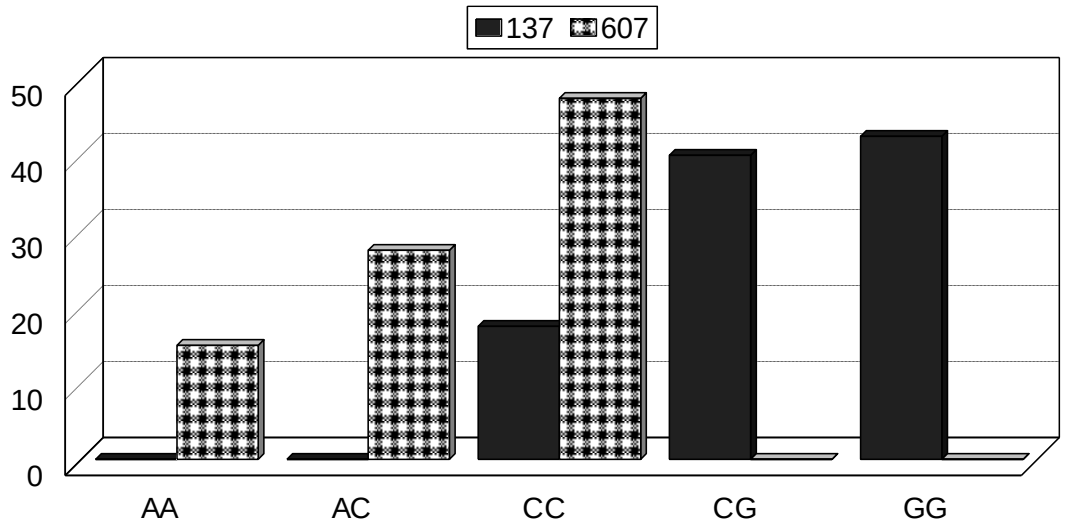


Table (3) Relation between IL-18 gene alleles 607 versus 137 among cases of CHCV infection.

Variables	**137**	**607**	**X^2**	**P**
AA	0	12(15%)	**17**	**<0.00 HS**
AC	0	22(27.5%)		
CC	14(17.5%)	46(47.5%)		
CG	32(40%)	0		
GG	34(42.5%)	0		

This table shows highly statistically significant difference in CHCV patients between both IL-137, -607 alleles by using chi-square test.



Graph (2): Illustrates the variants polymorphism in CHCV patients as regarding IL-18 gene promoter region at 607 and 137 alleles genotypes.

Table (4) Relation between 607 versus 137 among controls

Variables	**137**	**607**	**X^2**	**P**
AA	0	6(40%)	**14.5**	**<0.00 HS**
AC	0	4(26.7%)		
CC	4(26.7%)	5(33.3%)		
CG	5(33.3%)	0		
GG	6(40%)	0		

This table shows highly statistically significant difference between both alleles by using chi-square test.

Graph (3): Illustrates the variants polymorphism in the controls as regarding IL-18 gene promoter region at 607 and 137 alleles genotypes.

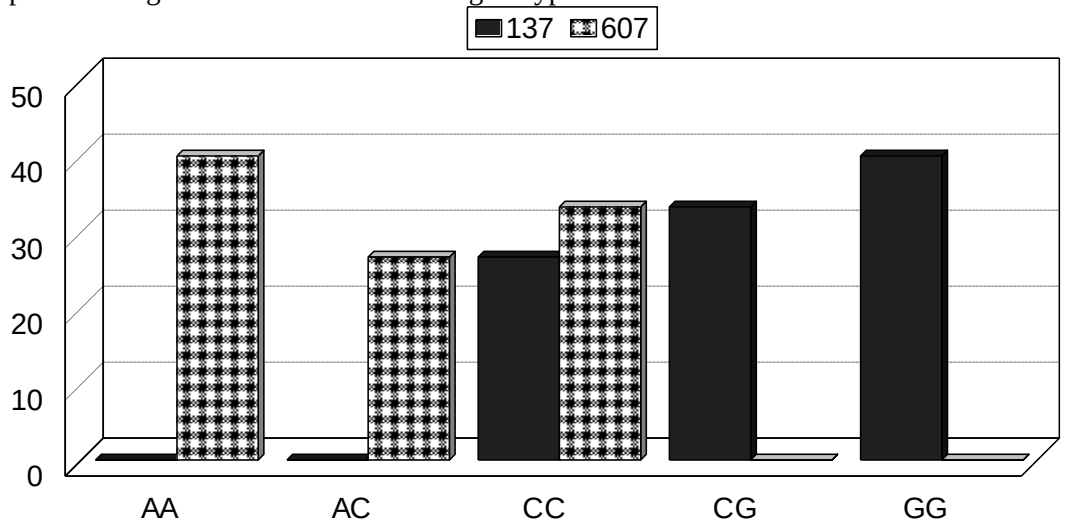


Table (5): Illustrates the response to IFN therapy correlated with IL-18 gene alleles 607 versus 137.

Variable	Differential no of IL-18 -607 patients.			Total No (80)	Differential no of IL-18-137 patients.			%
IFN responder	AA	AC	CC	70	CC	CG	GG	87.5 %
	10	18	42		8	30	32	
IFN non responder	2	4	4	10	6	2	2	12.5 %

This table shows that 87.5 % of the studied CHCV cases had response to IFN therapy, while 12.5 % had no response to IFN therapy.

Table (6): Illustrates the liver biopsy results correlated with IL-18 gene alleles -607 versus 137.

Variable	No of IL-18 -607 patients.			No of patients	No of IL-18 – 137 patients.			%
	AA	AC	CC		CC	CG	GG	
A1F1	4	10	36	50	10	18	22	62.5 %
A1F2	4	8	4	16	0	6	10	20 %
A2F1	0	2	0	2	0	0	2	2.5 %
A2f2	0	2	2	4	2	2	0	5 %
A2F3	4	0	2	6	2	4	0	7.5 %
A3F2	0	0	2	2	0	0	2	2.5 %

This table shows that majority of cases had A1F1 biopsy results and A1F2, while A3F2 was found among 2.5 % of the studied cases.

Discussion

IL-18, a proinflammatory cytokine, is an important regulator of innate and acquired immune response IL-18 is involved in both T-helper type 1 (Th1) and Th2 immune responses, depending on the context of the immunological milieu. In the presence of IL-12, IL-18 stimulates IFNG expression, promoting Th1-mediated immune response, whereas, without IL-12, IL-18 stimulates Th2 responses. IL-18 plays a critical role in the host defense against infection with intracelleular microbes, and on the other hand, in inducing autoimmune diseases and propagating inflammatory process (3), (11). There is growing evidence suggesting that interleukin-18 (IL-18) plays a crucial role in viral clearance and disease pathogenesis, and that single nucleotide polymorphisms (SNPs) within the gene may influence its production (12).

In the present study, we found that IL-18 promoter SNPs -137 show no statistically significant difference between CHCV patients and controls as regarding genotypes. Manohar et al. (5) showed that there was no significant difference in frequencies of – 607 and – 137 allelic distribution of IL-18 in CHCV patients and controls, these finding are in agreement with our results of IL-18 at -137 position but against – 607 position.

Khripko et al. (13) explained that IL-18 production by LPS-stimulated PBMC was significantly less in persons carrying CC genotype than in those with the GG genotype at the -137 position, this finding is in agreement with our results, that IL-18 -

137 CC =17.5 %, CG = 40 %, GG = 42.5 % in CHCV patients.

Khripko et al. (13) found that the C allele frequency is greater in the group with a low level of stimulated IL-18 production, this finding is in agreement with our results that the IL-18 CC genotyping was 26.7 % in the controls and 17.5 % in CHCV patients, CG genotyping was 33.3 % in the controls and 40 % in CHCV patients.

In our work results the IL-18-607 show that there's significant difference between cases and controls, and the AA alleles was higher among controls than the patients group.

Khripko et al. (13) explained that PBMCs from donors carrying allele 607A showed significant increases in spontaneous and stimulated IL-18 production compared to wild type, these finding are in correlation with our results. Also Khripko et al. showed that IL-18 production by LPS-stimulated PBMC was significantly greater in healthy donors carrying the CA genotype than in those with the CC genotype at the -607 position of the promoter, this finding is against our results that AA alleles was higher among controls than the patients.

In the present study there's transversion from G (42.5 %) to C (17.5 %) at the position- -137-IL-18 , and transversion from C (47.5 %) to A (15 %) at position -607-IL-18 both are a conditions which affect pathogenesis involving a significant role of IL-18 .

Giedraitis et al. (1) postulated that single-nucleotide polymorphisms of IL-18, transversion C to A at position -607, and a transversion G to C at position -137 modify two transcription binding sites that influence the quality of transcribed IL-18 mRNA, these finding are in agreement with our results .

Montminy (14) found that promoter SNPs -607 and -137 modulate IL-18 gene expression through alteration of nuclear factor binding site. A change from C to A at site – 607 disrupts a binding site of cAMP-responsible element (CRE), which mediates transcriptional activation in response to cAMP.

Our work, showed an increased spontaneous production of IL-18 associated with -137 GG (42.5 %) and -607 CC (47.5 %).

The capacity to produce IL-18 by mononuclear cells is associated with IL-18 polymorphism (13).Two Polymorphisms-137 G/C and -607 C/A, were in strong linkage disequilibrium (15). Interindividual variations in cytokine production, and polymorphisms in cytokine and / or their receptor genes, may directly influence the outcome of cytokine – based immunotherapy. Genetic variation exerts a major influence on susceptibility and progression of infectious diseases (16), these findings are in correlation with our results that showed the presence of significant difference between IL-18-173, 607 in controls group. In our study the results of Interferon and Ribavirin therapy after 48 weeks (87.5 %) 70 CHCV patients responder, the variant polymorphisms in the these patients as regarding IL-18 gene promoter region at -607, -137 genotypes

-607 AA was 10 patients, AC was 18 patients, CC was 42 patients, -137 CC was 8 patients, CG was 30 patients, GG was 32 patients and (12.5 %)10 patients non responder.

Our work showed the liver biopsy results correlated with IL-18 gene alleles -607, versus

-137 (62.5 %) 50 patients A1F1, - 607 AA was 4 patients, AC was 10 patients and CC was 36 patients, -137 CC was 10 patients, CG was 18 patients, and GG was 22 patients, A1F2 (20 %) was 16 patients, 4 was 607 AA, AC was 8 patients, CC was 4 patients, -137 CG was 6 patients, CG was 10 patients, 2.5 % A2F1, 5 % A2F2, 7.5 % A2F3, 2.5 % A3F2.

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