
بسم الله الرحمن الرحيم

Association of *IL-6* Gene Polymorphism with Cardiovascular Disease in Pakistani Families

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Background

- Cardiovascular disease is the prevailing non-communicable cause of death and disability in the South Asia.

  (Lopez *et al,* Lancet, 2006)

- Pakistan is facing a growing burden of CVD.

- Recent data shows dramatic increase in CVD risk factors.

- A growing understanding that South Asian ethnicity is an independent risk factor for CVD in addition to traditional risk factors.

  (Brister *et al,* J Thorac Cardiovasc Surg , 2007)

- A need for new genetic diagnostic and prognostic markers.

Inflammation and CVD

- Atherosclerosis is an inflammatory disease in which immune mechanisms interact with metabolic risk factors to initiate, propagate and activate lesions in the arterial wall.

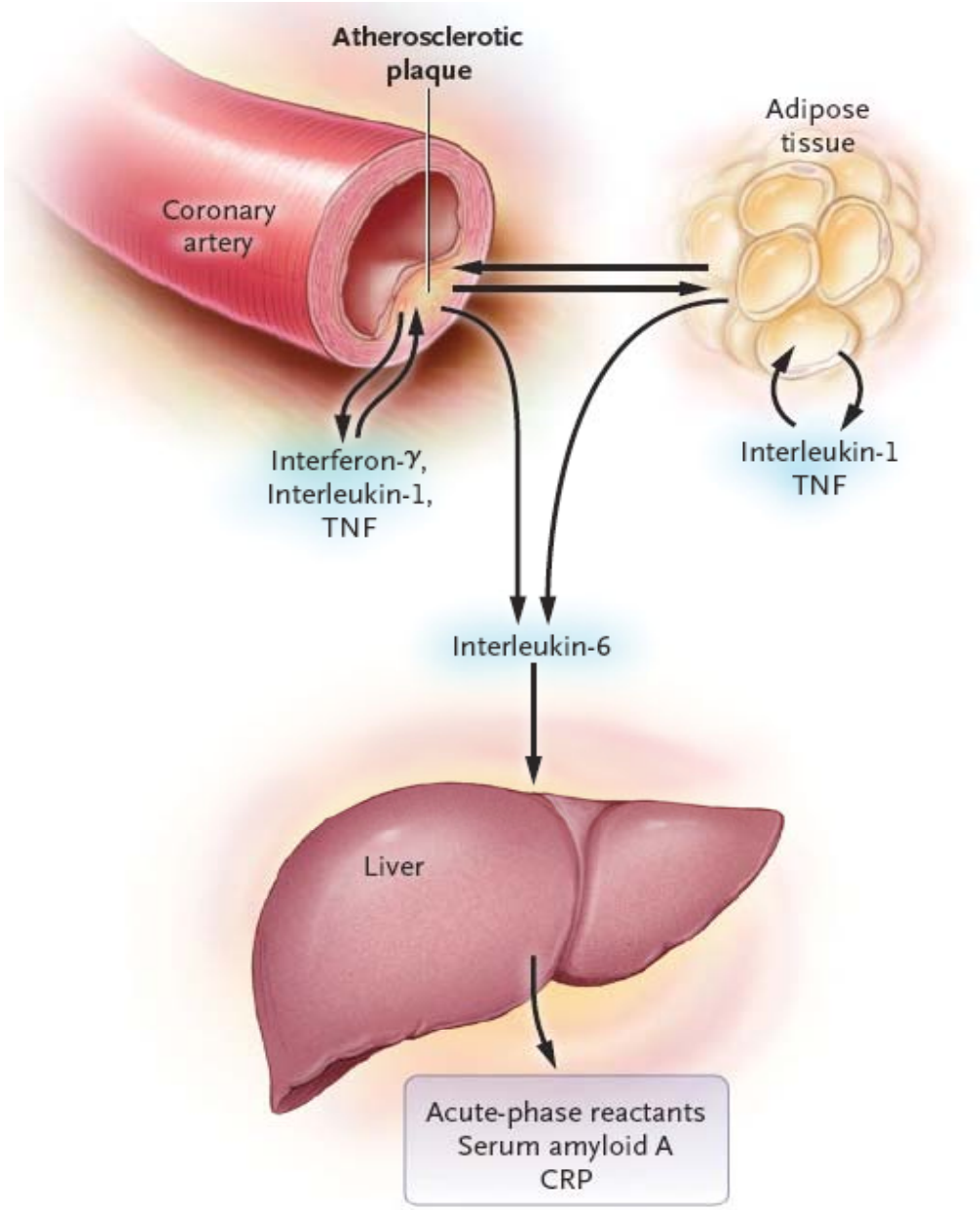
- Inflammatory cells dominate early atheromatic lesions.

Immune effector molecules accelerate lesion progression .

- Activation of inflammation elicits acute coronary syndromes.

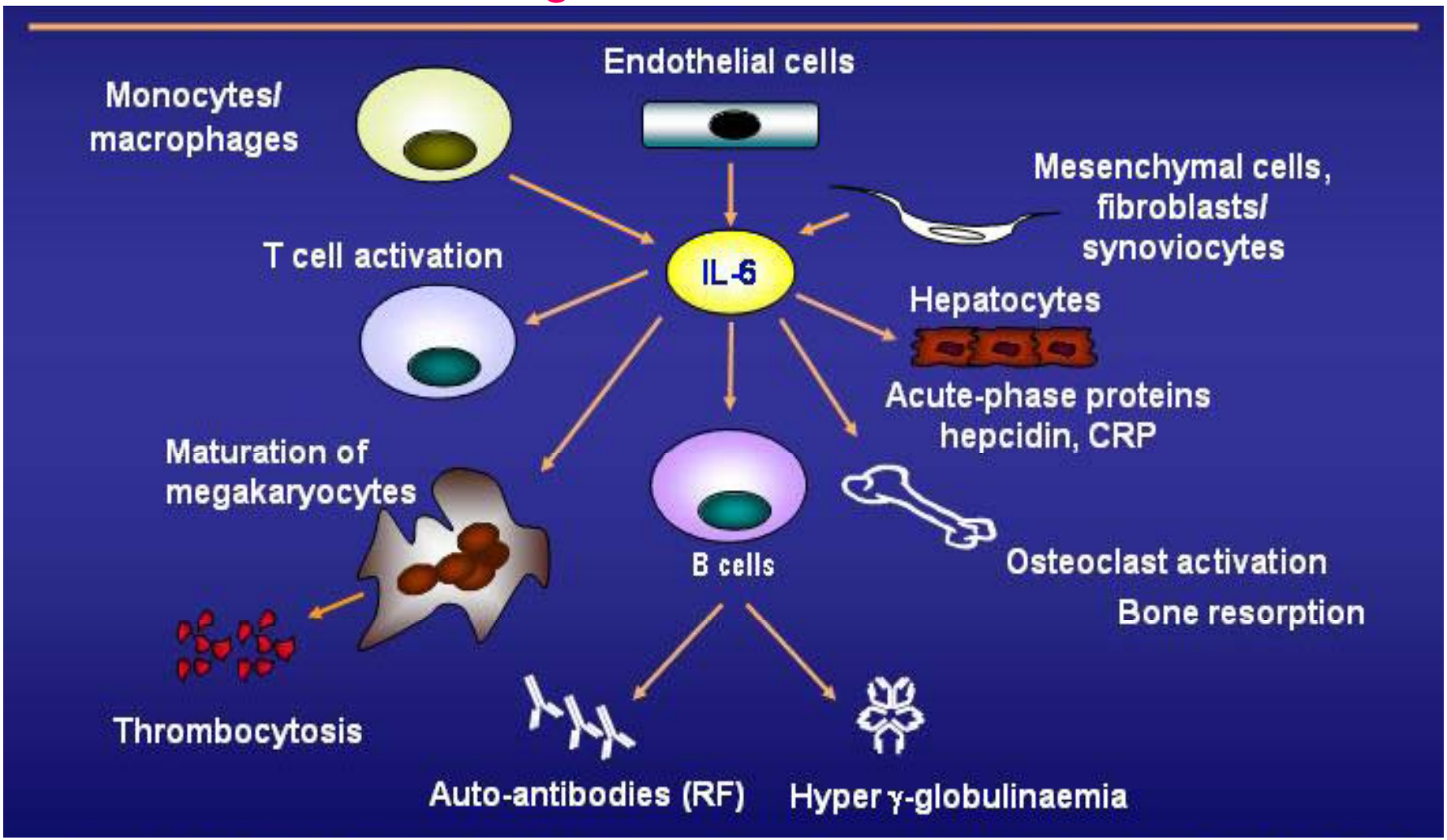
- May lead to increased plasma levels of cytokines and acute phase proteins.

Systemic inflammatory markers in atherosclerosis



INTERLEUKIN-6

Central Role in Progression of Chronic Inflammation



IL-6 in Cardiovascular Disease

- An elevated IL-6 level is a strong and independent predictor of mortality for patients with acute coronary syndromes.

  (Sattar *et al.*, 2009)

- Combined levels of TNF-α and IL-6 were associated with severity of CAD.

  (Israel *et al.*, 2008)

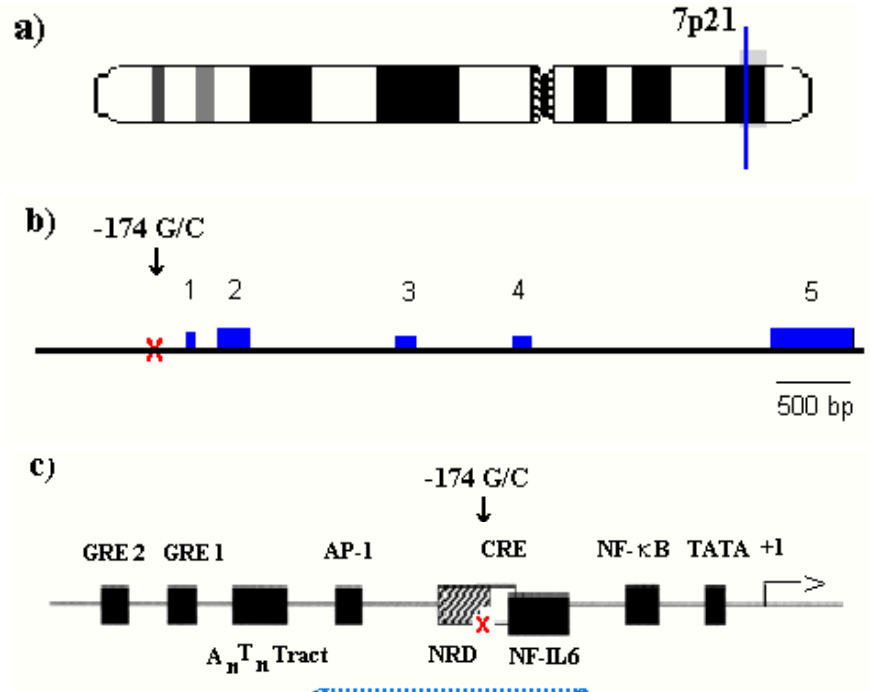
- The IL-6 gene and protein are also expressed in human atherosclerotic lesions and contribute to local plasma concentration of IL-6 in the coronary circulation.

  (Deliargyris *et al.*, 2000)

Polymorphisms

- Common sequence variations that occur at a frequency greater than 1%.
- Single nucleotide Polymorphism (SNP) occur every 500-1000 bases throughout the genome.
- IL-6 gene promoter polymorphisms.
- -174G/C, -572G/C, -597G/A, AnTn tract spanning -392 to -373 position.
- Guanine replaced by Cytosine.
  (-174 locus in the promoter region of IL-6 gene)

-174 G/C Polymorphism in IL-6 Gene Promoter



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CTGGAACGCTAAATTCTAGCCTGTTAATCTGGTCACTGAAAAAAAATTTTTTTTTTTTC
AAAAAACATAGCTTTAGCTTATTTTTTTTCTCTTTGTAAAACTTCGTGCATGACTTCAG
CTTTACTCTTTGTCAAGACATGCCAAAGTGCTGAGTCACTAATAAAAGAAAAAAAGAA
AGTAAAGGAAGAGTGGTTCTGCTTCTTAGCGCTAGCCTCAATGACGACCTAAGCTGC
ACTTTTCCCCCTAGTTGTGTCTTGCCAT**G/C**CTAAAGGACGTCACATTGCACAATCTT
AATAAGGTTTCCAATCAGCCCCACCGCTCTGGCCCCAC**CCTCACCCTCCAACAAAG**
AT-3'

Diseases associated with IL-6 Polymorphism

- Implicated in a wide array of acute and chronic systemic disorders (e.g. juvenile chronic arthritis, cardiac disease, graft versus host disease, chronic liver disease, lupus erythmatosis etc.)

- Carriers of IL-6 -174 C allele had inferior graft survival with RR of 3.7 for graft loss

  (Muller-Steinhardt, Kidney Int, 2002)

- Chronic GVHD independently associated with IL-6 gene polymorphism from recipient (RR=4.2, P=0.02)

  (Socie, Transplantation, 2001)

-174 G/C Polymorphism and CVD

- -174CC has been associated with elevated plasma levels in CABG patients

  (Brull, Arterioscler Thromb Vasc Biol, 2002)

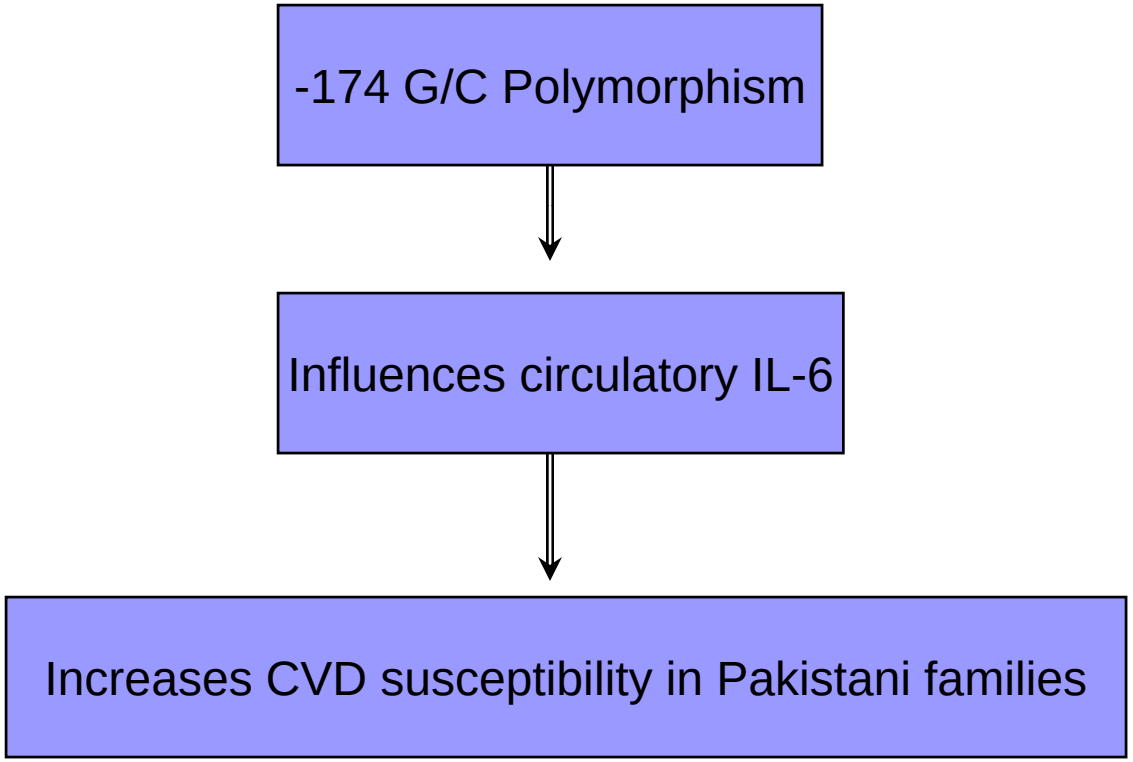
- -174CC genotype is more responsive to statins

  (Basso, Arterioscler Thromb Vasc Biol, 2002)

- -174C allele associated with higher CRP, fibrinogen and IL-6 levels in CVD

  (Jenny, Arterioscler Throm Vasc Biol, 2002)

HYPOTHESIS


-174 G/C Polymorphism
Influences circulatory IL-6
Increases CVD susceptibility in Pakistani families

AIMS and OBJECTIVES

- Genotyping of -174 G/C polymorphism.
- Allelic frequency of variant allele “C”.
- Correlation of IL-6 genotype with circulating levels.
- Association of IL-6 genotype with CVD.

Materials and Methods

- Pedigree Analysis
- Anthropometric Data Collection
- Biochemical Profile
- IL-6 Immunoassay
- Genotyping by PCR-RFLP

Study Subjects

- 10 families: (05 consanguineous + 05 nuclear)
- Total Subjects: (84)
- Males (50) : Females (34)
- Patients (32) : Controls (52)

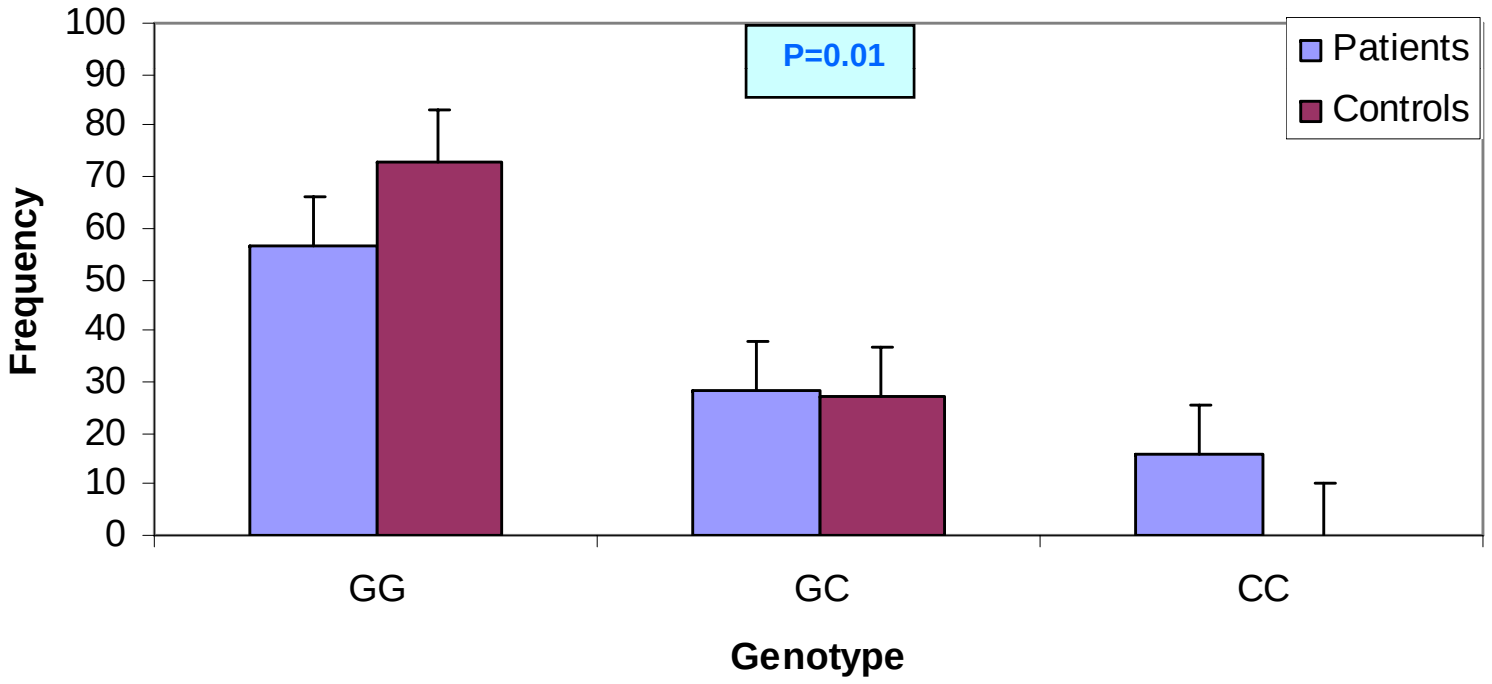
RESULTS
(Baseline Parameters)

Characteristics	History of CVD		P value †
	Patients N(%)	Controls N(%)	
	32 (38)	52 (62)	
Male Female	20 (62.5) 12 (37.5)	30 (57.7) 22 (42.3)	0.66
Hypertension Normal	22 (68.8) 10 (31.2)	07 (13.5) 45 (86.5)	**< 0.0001**
Smoker Non smoker	08 (25) 24 (75)	05 (9.6) 47 (90.4)	0.07
Sedentary Active	19 (59.4) 13 (40.6)	09 (17.3) 43 (82.7)	**< 0.0001**
Stress Normal	23 (72) 09 (28)	16 (30.8) 36 (69.2)	**0.0002**

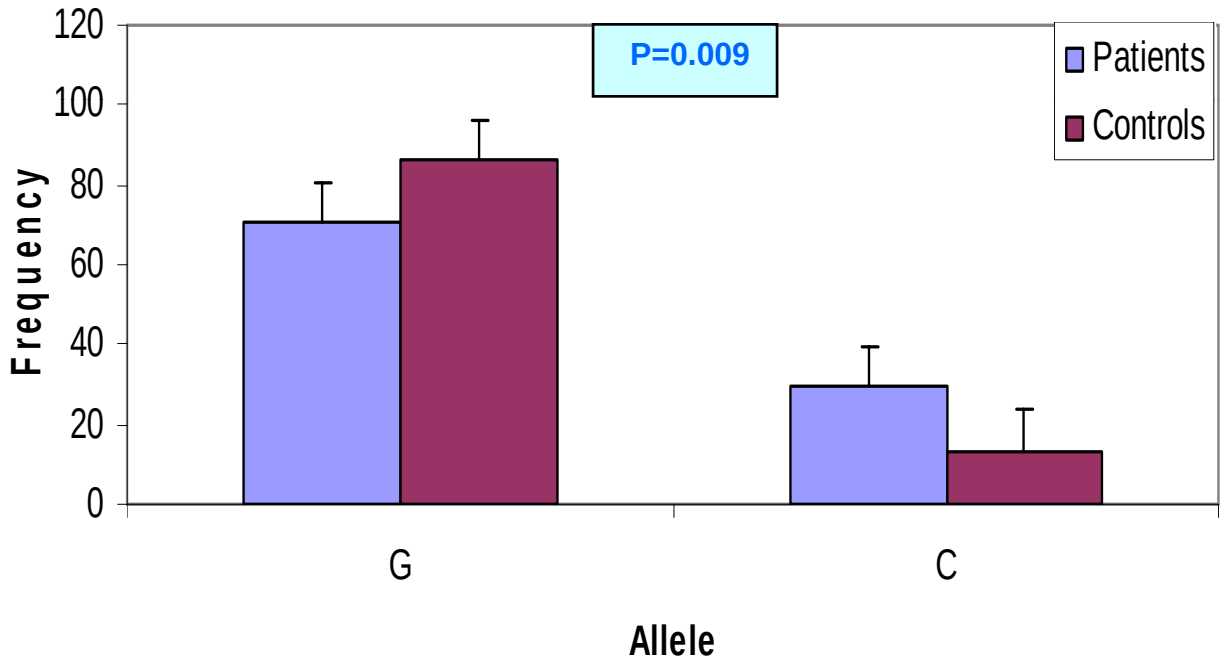
RESULTS
(Clinical variables)

Characteristics	History of CVD		P value
	Patients (n=32)	Controls (n=52)	
Age	48±19	35±17	**0.0017**
BMI (kg/m^2)	25.7±3.7	25.2±3.5	0.54
BP systolic (mm of Hg)	146±26	127±15	**< 0.0001**
BP diastolic (mm of Hg)	93±13	83±8	**< 0.0001**
Cholesterol (mg/dL)	176±39	180±38	0.64
IL-6 (log)	3.25±0.6	3.03±0.6	0.11

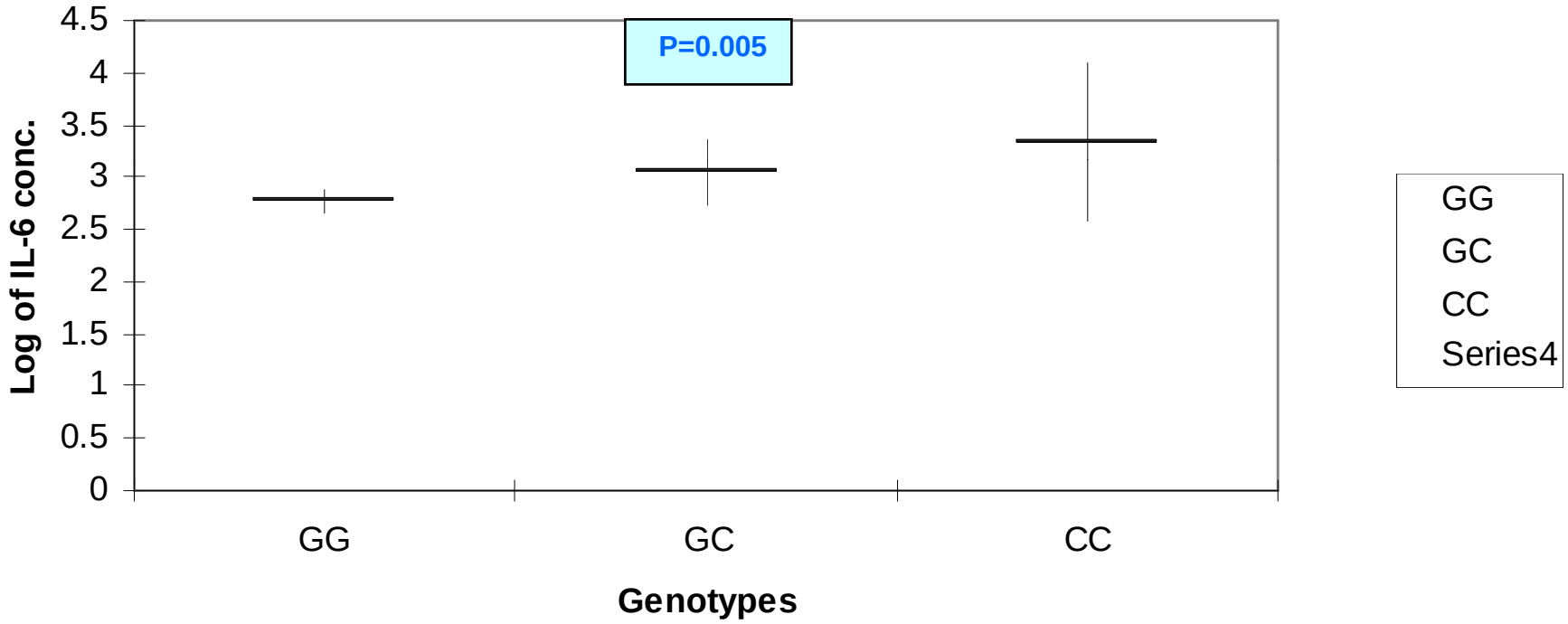
Comparison of -174 G/C genotype frequency (Patients vs Controls)



Comparison of IL-6 G and C allele frequencies



Genotype wise concentration of IL-6 in patients (Mean±SD)



Association of IL-6 genotype with CVD in presence of selected variables

(CVD as Reference Variable)

Parameter	95% Confidence Interval (Lower and Upper Limit)	P-value
CVD		
Age	(1.01 – 1.09)	0.027
Systolic	(0.91 – 1.04)	**0.356**
Diastolic	(1.07 – 1.41)	0.004
IL-6 serum Conc.	(0.7 – 4.29)	**0.23**
-174G/C genotype	(1.16 – 4.51)	0.017

Key Findings

- IL-6 Genotype remained significantly associated with CVD even after adjusting for covariates.

  (OR=2.29, 95% CI= 1.16 – 4.51, P=0.017)

- The serum level of IL-6 varied significantly within patients group in a genotype-dependant manner. (CC genotype showing highest level).

  (P=0.005)

- The variant “C” allele was also more abundant in patients group as compared to control group.

  (OR=2.71, 95% CI= 1.25 – 5.91, P=0.009)

CONCLUSION

- Our findings suggest that:

  -174G/C genotype is significantly associated with serum IL-6 level in CVD patients. (CC genotype showing highest levels)

  IL-6 genotype is independently associated with CVD susceptibility in native Pakistani families


Thank You