



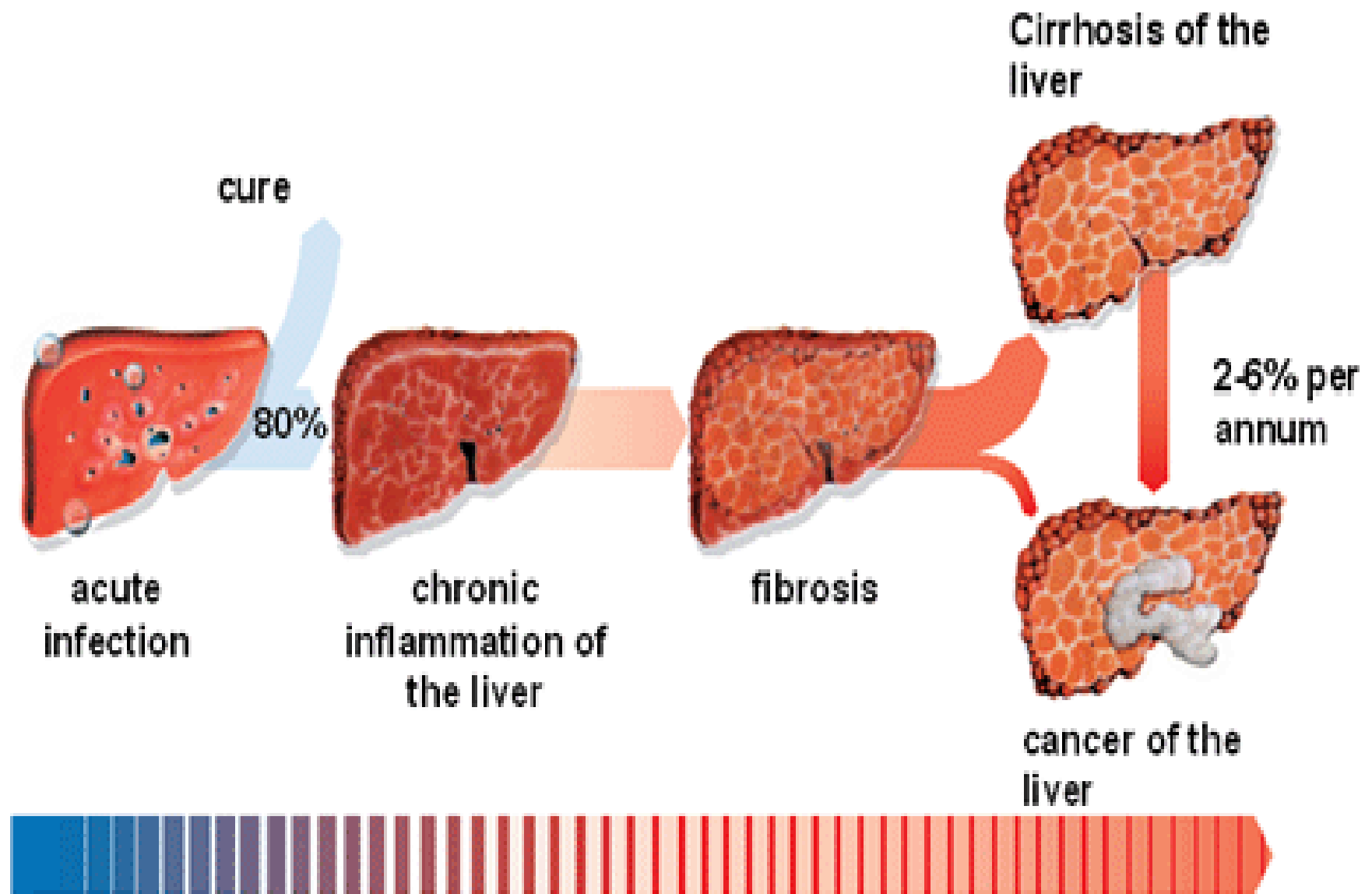
The correlations of activins and follistatin with viral load, liver damage, IL-6 and TNF- α in treatment naïve patients with chronic hepatitis C genotype 1 and 4: A case-control study

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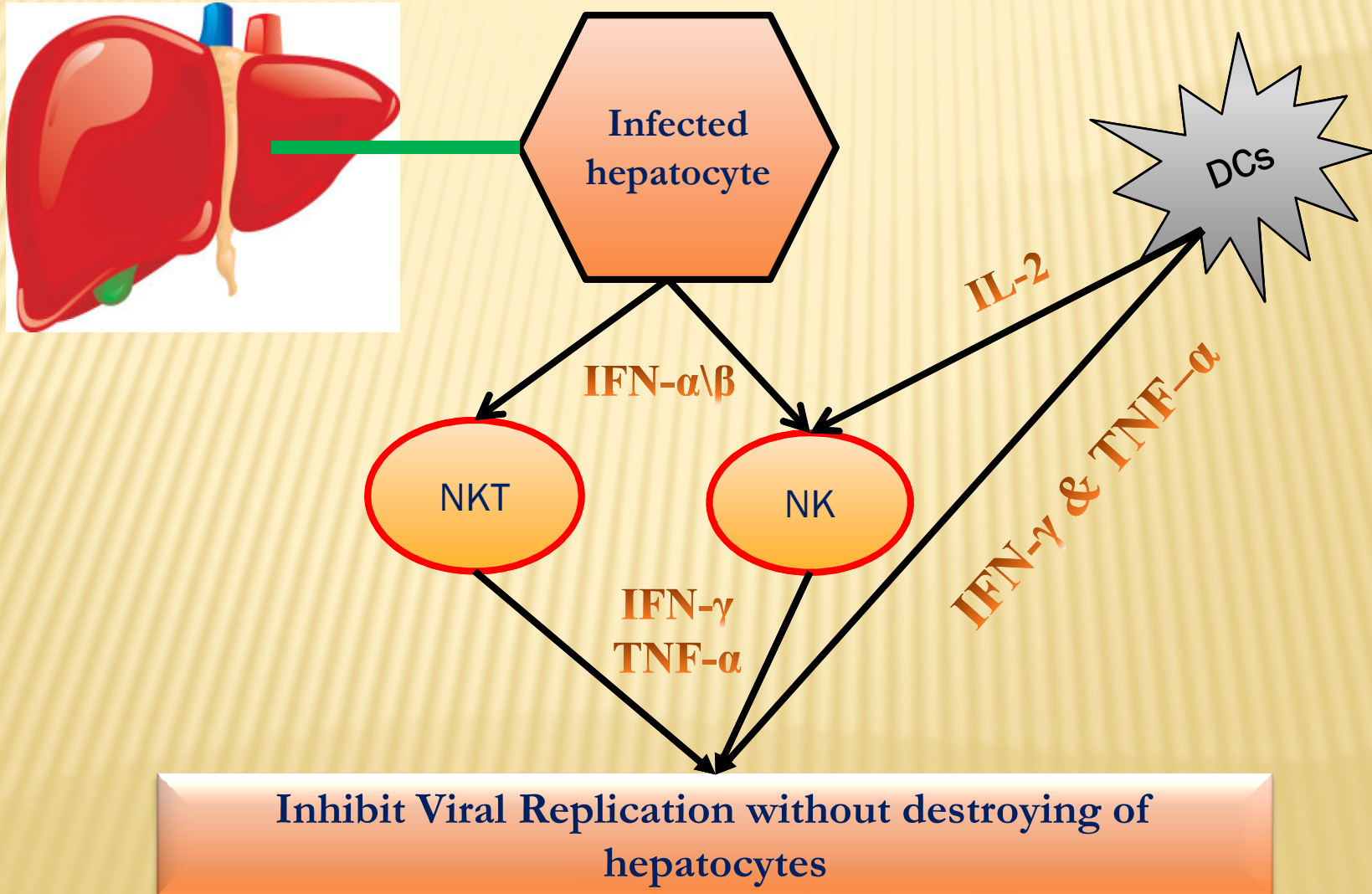
Chronic Hepatitis C (CHC)

- WHO:
 - 170 million infected World Wide
 - 3-4 NEW infections/Year
- It has been classified in to 6 genotypes
 - Genotypes 1 and 4 in Saudi Arabia
- Major cause of liver damage
 - Fibrosis/Cirrhosis
 - Hepatocellular Carcinoma

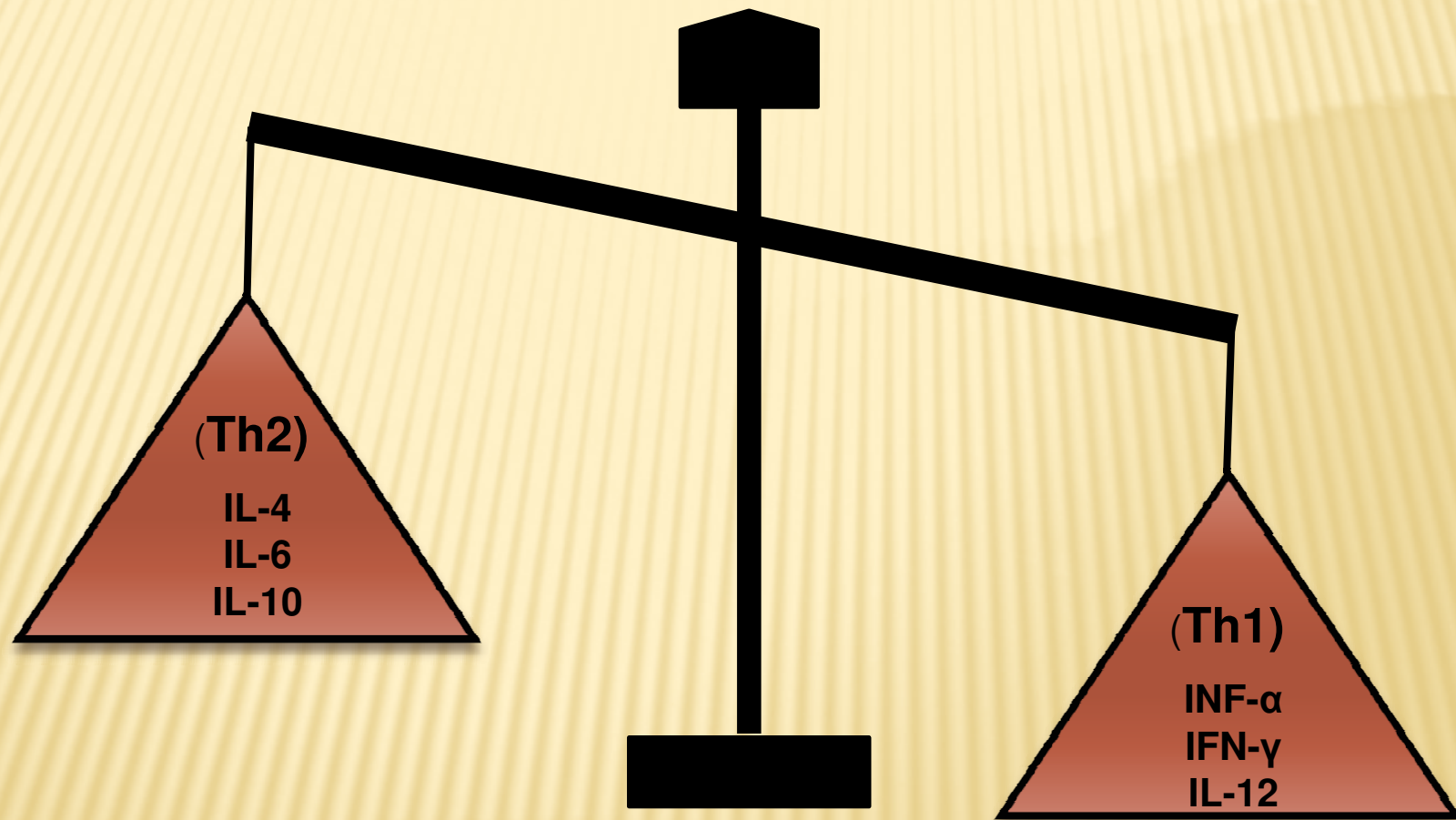
Course of illness with Hepatitis C



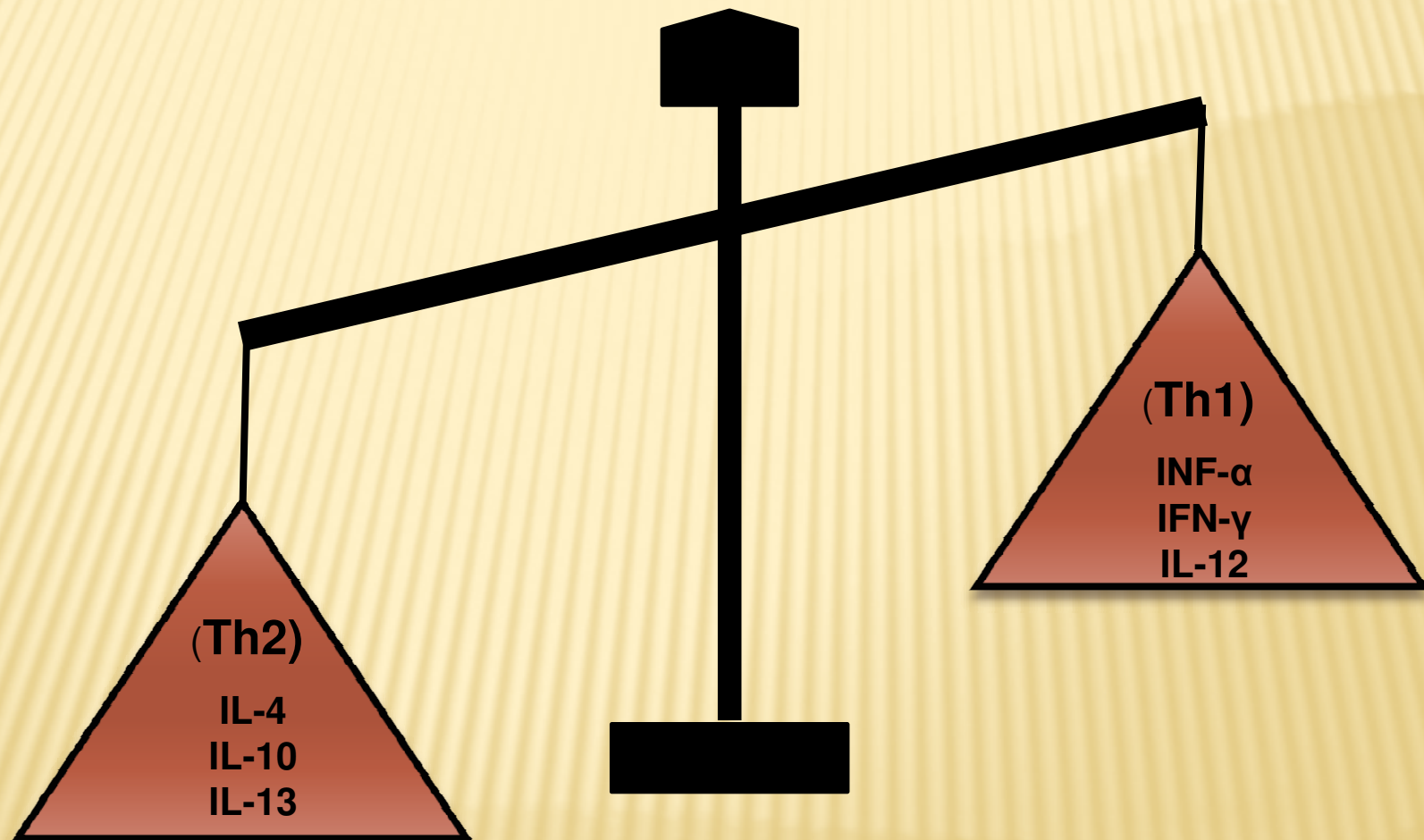
Immune Response to HCV



Acute Phase of HCV Infection



Chronic Phase of HCV Infection

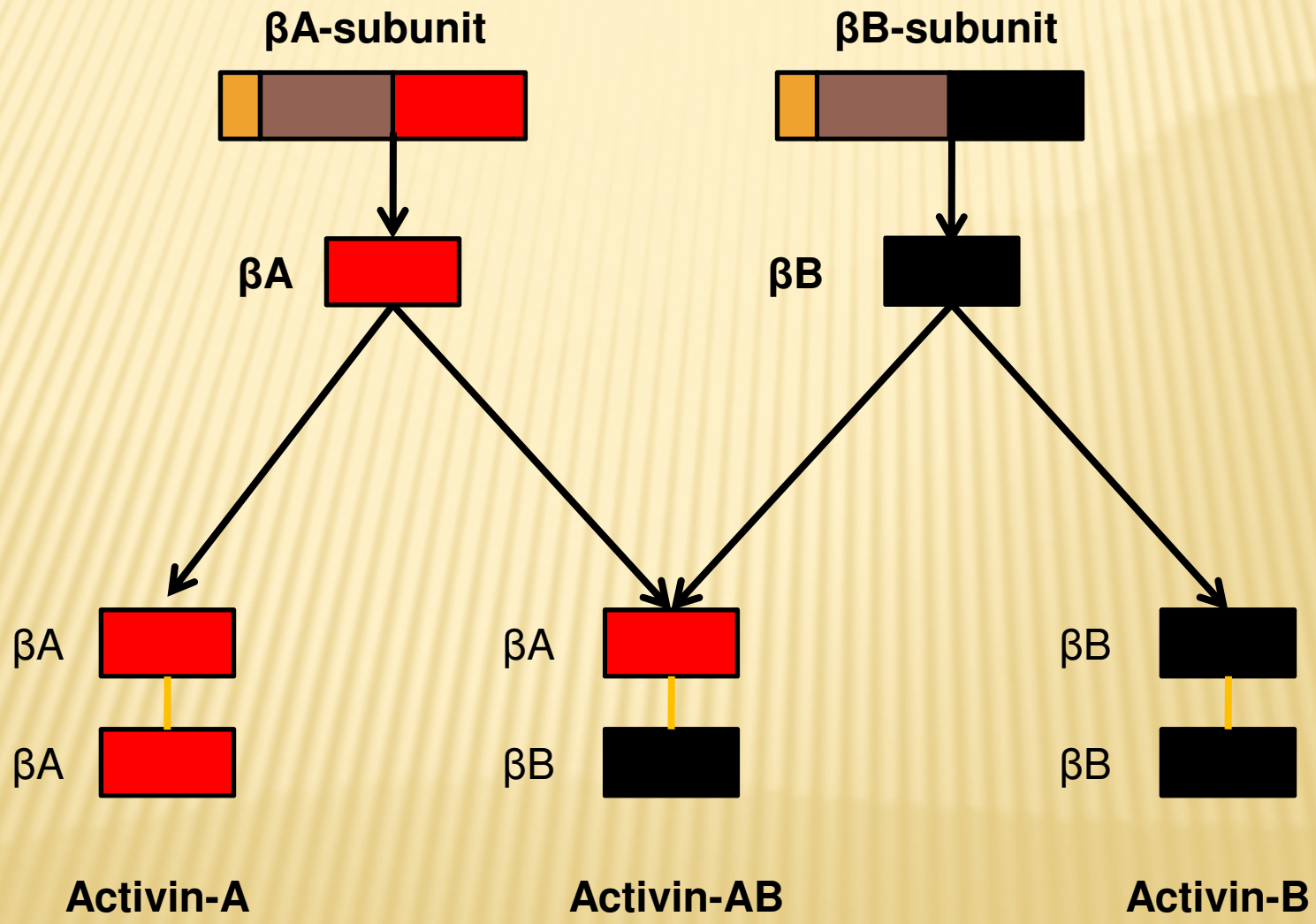


Th2 Response also includes the stimulation of Transforming Growth Factor (TGF)- β

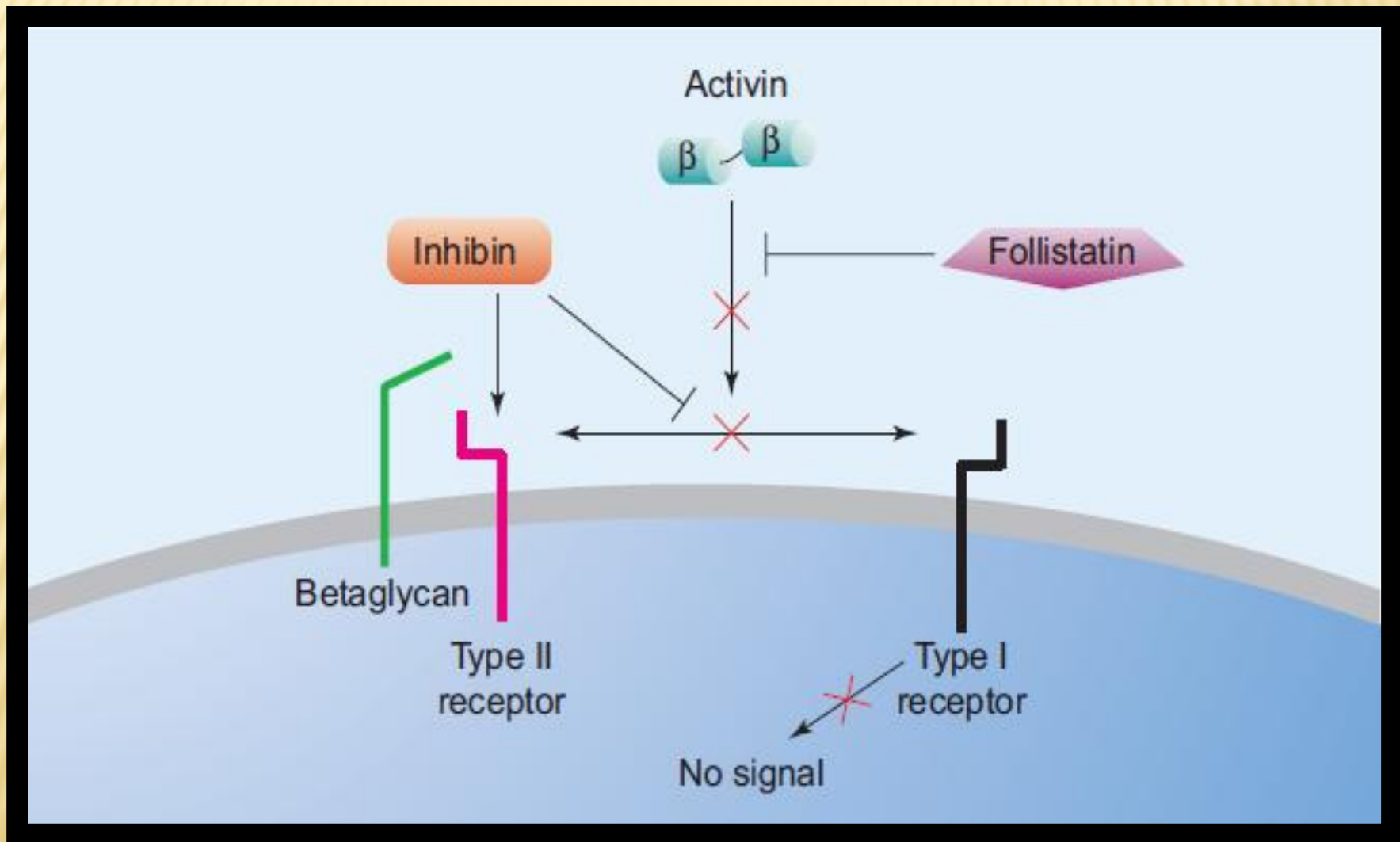
Activins

- ✿ Members of TGF- β superfamily
- ✿ Originally identified as gonadal proteins
- ✿ Recently involved in many systems:
 - Growth & differentiation
 - Inflammation (Pro or Anti depending on cellular context)
 - Fibrotic diseases

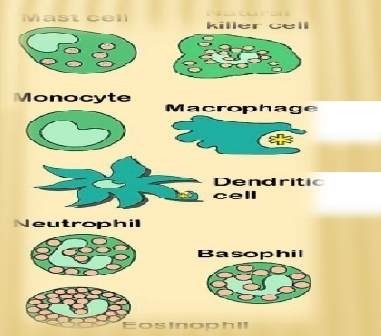
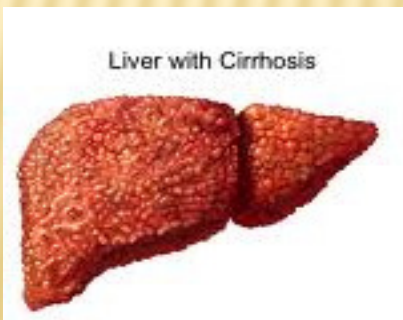
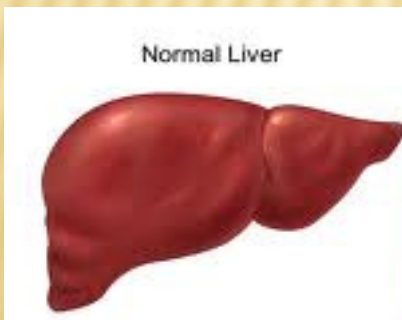
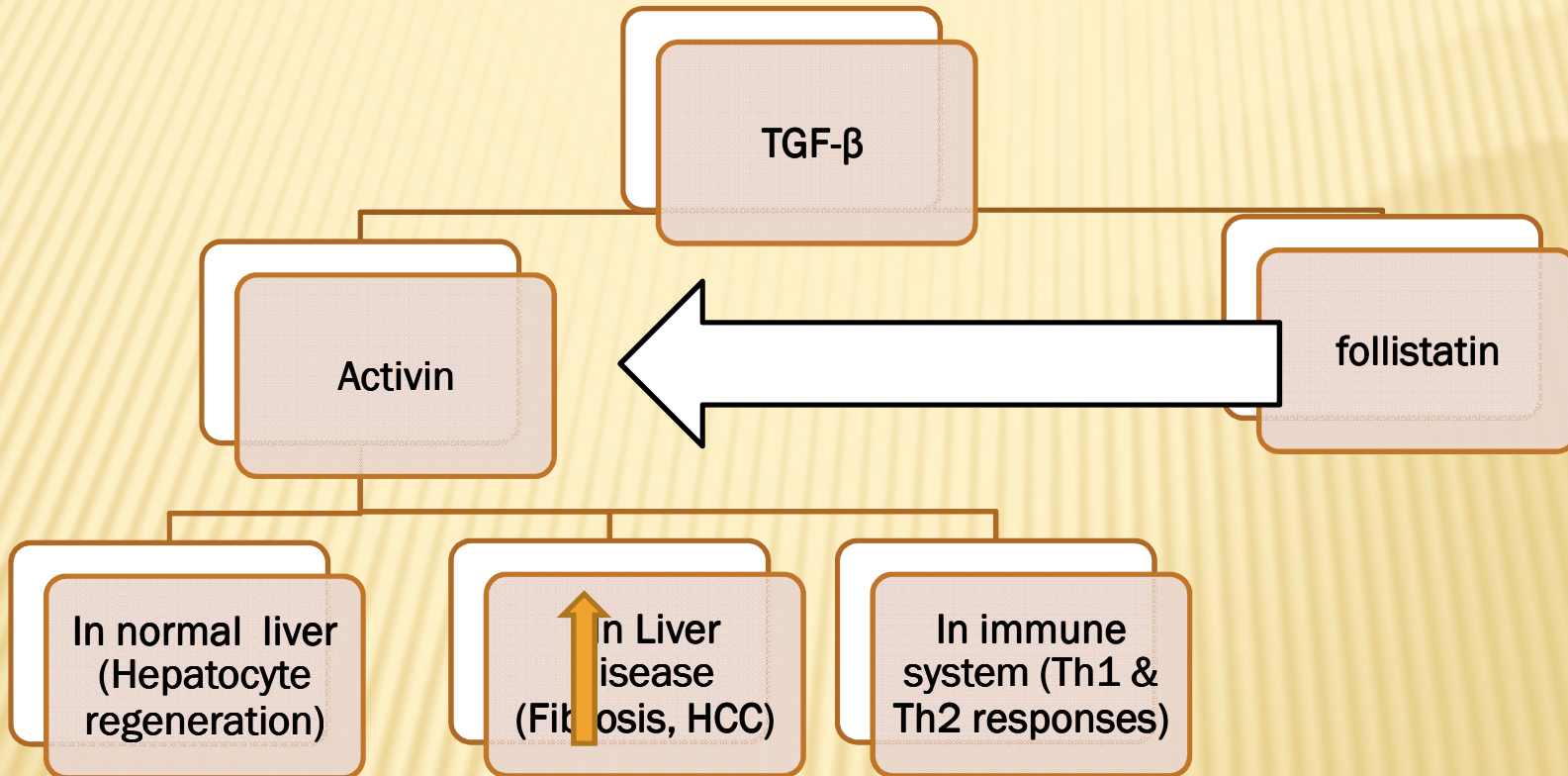
Structure of Activins proteins



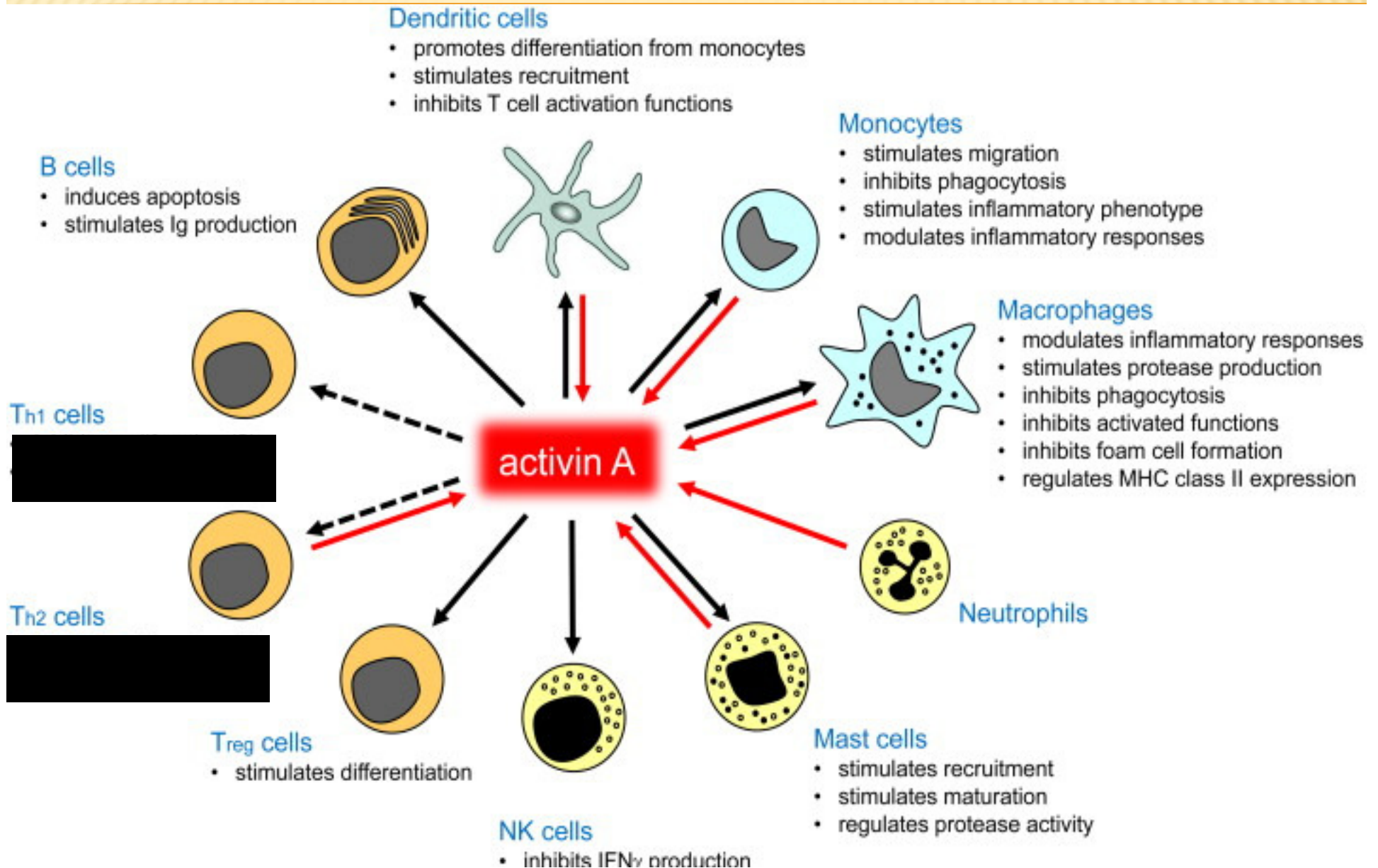
Follistatin (Activin binding protein)



Activins & Follistatin



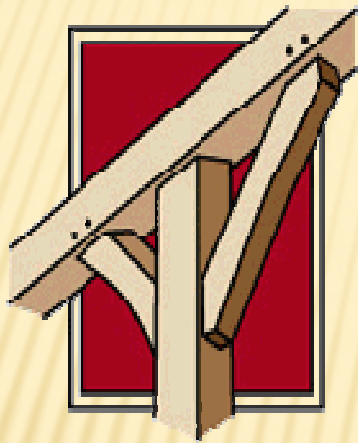
Activins and the Immune system



Research Questions

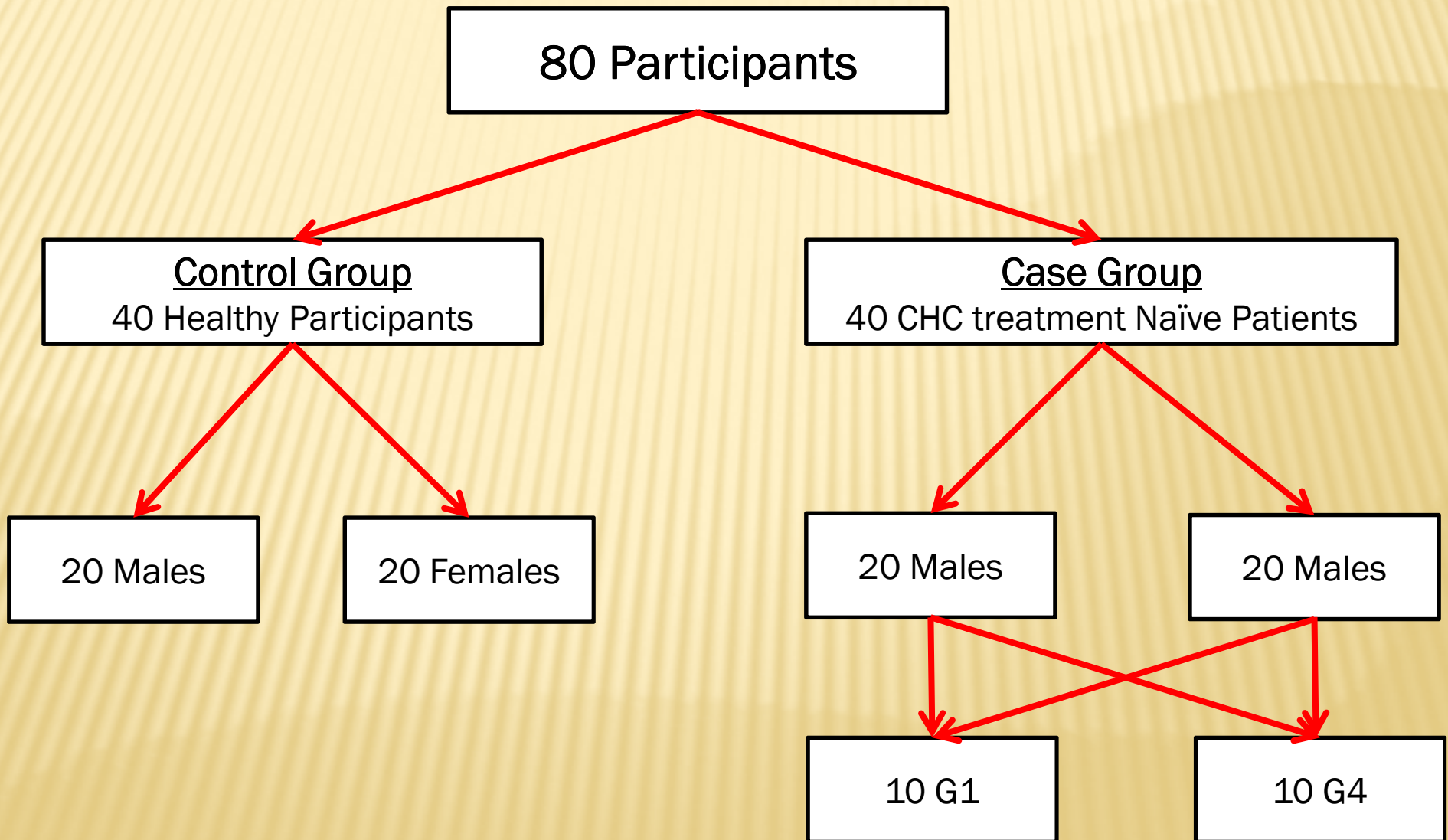
What are the effects of CHC genotype 1&4 on serum activin-A & B and follistatin

- ✿ Is there a difference between:
 - ✿ Male and Female patients?
 - ✿ Genotype 1 & 4?
- ✿ Do activins & follistatin correlate with:
 - ✿ Pro-inflammatory cytokines (TNF- α and IL-6)?
 - ✿ Liver enzymes?
 - ✿ Liver fibrosis?



METHODS & MATERIALS

Study Design (Case-Control)



Inclusion criteria

Control Group	Case Group
Patient age $\geq 18 \leq 45$ years.	Patient age $\geq 18 \leq 45$ years.
No concurrent acute/chronic disease	HCV RNA positive
Proven fertility	No concurrent infection with HBV or HIV
Not taking exogenous hormones/oral contraceptive pills for at least 3 months prior to enrolment	Proven fertility
No history of hospitalisation and no medication for significant clinical disease	Not taking exogenous hormones/oral contraceptive pills for at least 3 months prior to enrolment
laboratory results for their haematological, biochemical and metabolic parameters were within normal range	Treatment naïve patients
	Compensated liver disease (e.g. no liver cirrhosis, failure or cancer) & APRI ≤ 1.2
	Acceptable haematological and biochemical indices
	No or controlled type 2 diabetes mellitus and hypertension

Methods

- ✘ Liver function parameters & Viral load

- ✘ AST/Platelet Ratio Index (APRI) to assess liver fibrosis

- ✘ ELISA to measure serum:
 - + Activin-A
 - + Activin-B
 - + Follistatin
 - + IL-6
 - + TNF- α

A green rectangular sign with rounded corners and a white border, mounted on two wooden posts. The sign features the word "Results" in a white, sans-serif font. The background is a bright blue sky filled with fluffy white clouds.

Results

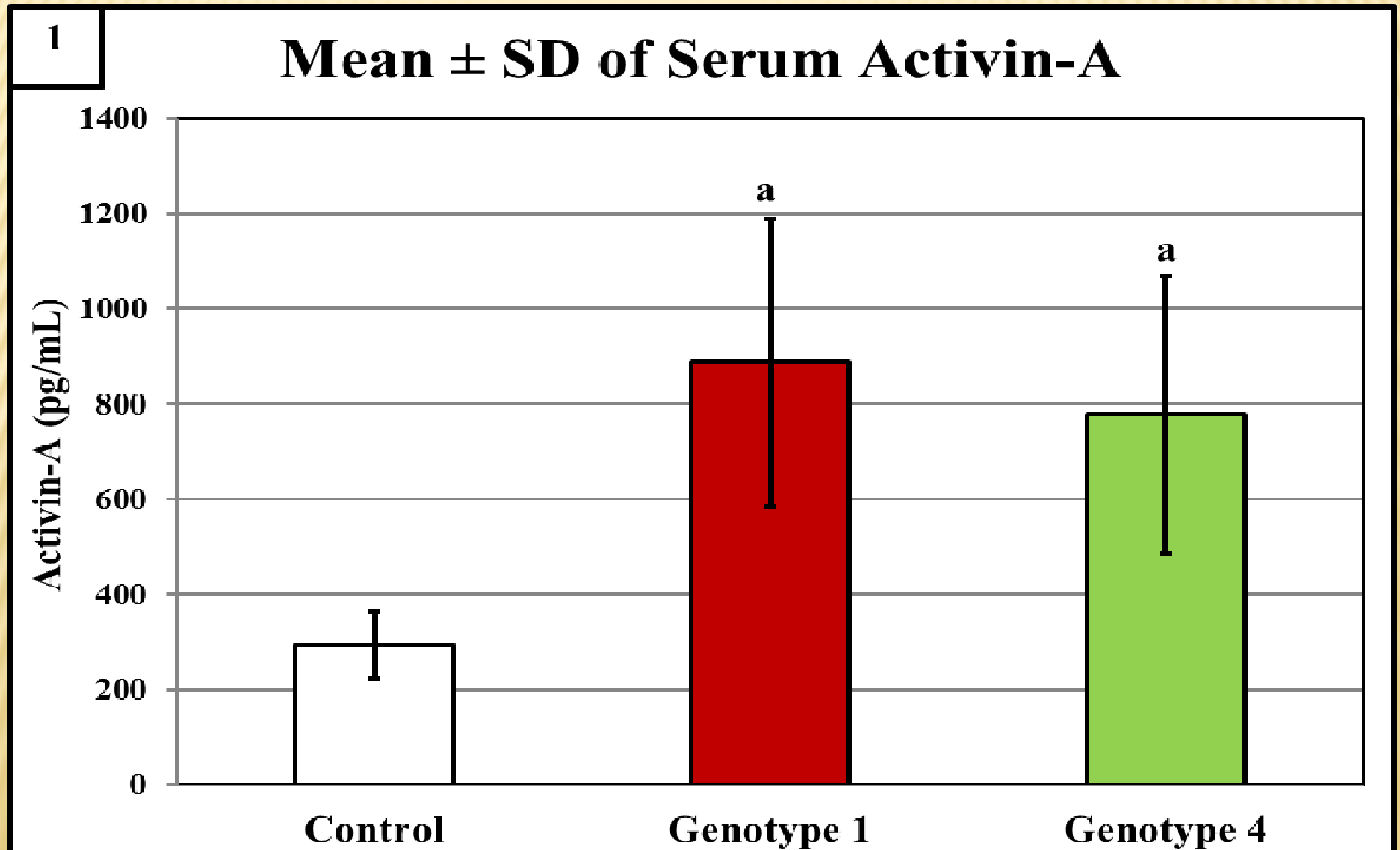
1- Demographic and laboratory characteristics of the patients according to viral genotype and gender of the participants (ND = not done; a = p < 0.05 compared to CM and b = p < 0.05 compared to CF groups).

	Control Male (CM) (n = 20)	Control Female (CF) (n = 20)	Male G1 (MG1) (n= 10)	Male G 4 (MG4) (n= 10)	Female G1 (FG1) (n= 10)	Female G4 (FG4) (n= 10)
Age (years)	39 ± 5.4	36 ± 8.3	40.3 ± 4	39.2 ± 5.7	37.9 ± 6.2	34.8 ± 8.8
Viral load at diagnosis (IU/mL)	ND	ND	866784.8 ± 237056.2	945148.7 ± 381181.2	1007458.5 ± 318742.4	988447.8 ± 372327.8
ALP (IU/L)	79.4 ± 21.6	69.2 ± 16.1	125 ± 45.8 ^{a,b}	133.3 ± 35.4 ^{a,b}	130.6 ± 63 ^{a,b}	124 ± 32.3 ^{a,b}
ALT (IU/L)	28 ± 11.2	22.2 ± 5.6	89.3 ± 18.1 ^{a,b}	81.6 ± 19 ^{a,b}	71.9 ± 22.7 ^{a,b}	72.8 ± 20.1 ^{a,b}
AST (IU/L)	21 ± 4.6	22.1 ± 3.1	50.5 ± 14.3 ^{a,b}	54.6 ± 12.5 ^{a,b}	57.8 ± 13.4 ^{a,b}	51.9 ± 15.5 ^{a,b}
Albumin (g/dL)	4.4 ± 0.24	4.5 ± 0.27	3.7 ± 0.5 ^{a,b}	3.6 ± 0.4 ^{a,b}	3.6 ± 0.4 ^{a,b}	3.7 ± 0.5 ^{a,b}
APRI	0.37 ± 0.07	0.36 ± 0.06	0.78 ± 0.3 ^{a,b}	0.84 ± 0.25 ^{a,b}	0.85 ± 0.29 ^{a,b}	0.89 ± 0.32 ^{a,b}

2- Mean \pm standard deviation of IL-6 and TNF- α in the different study groups (a = p < 0.05 compared to CM, b = p < 0.05 compared to CF; c = p < 0.05 compared to MG1; d = p < 0.05 compared to MG4 and e = p < 0.05 compared to FG4 groups).

	Control Male (CM) (n = 20)	Control Female (CF) (n = 20)	Male G1 (MG1) (n= 10)	Male G 4 (MG4) (n= 10)	Female G1 (FG1) (n= 10)	Female G4 (FG4) (n= 10)
TNF- α (pg/mL)	6.1 \pm 1.5	5.8 \pm 2.1	12..1 \pm 2.4 ^{a,b}	12.5 \pm 1.8 ^{a,b}	11.9 \pm 1.6 ^{a,b}	12.3. \pm 2.1 ^{a,b}
IL-6 (pg/mL)	4.1 \pm 1.1	3.6 \pm 1.01	10.6 \pm 1.6 ^{a,b}	11 \pm 1.9 ^{a,b}	9.9 \pm 1.4 ^{a,b}	10.8.2 \pm 1.7 ^a

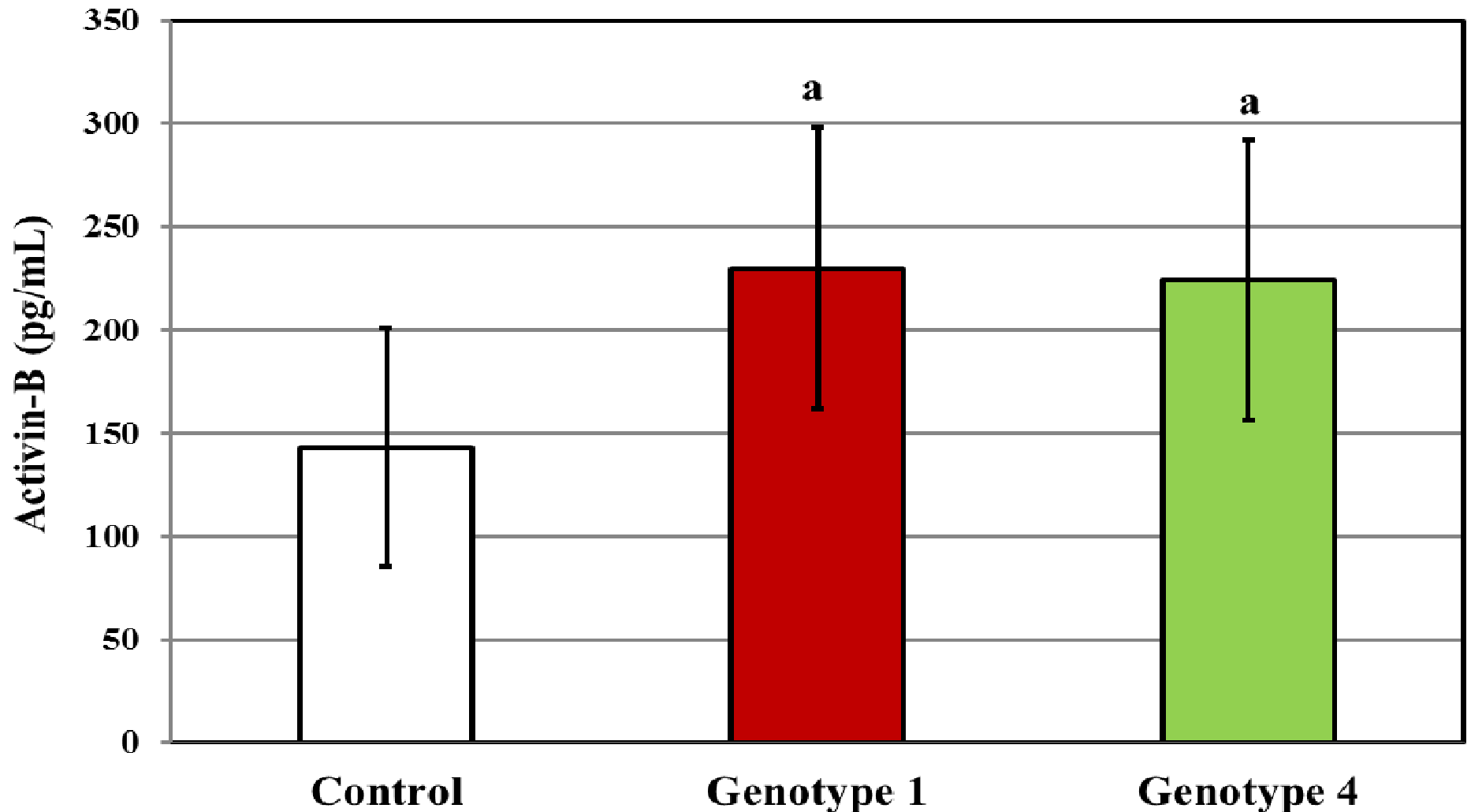
3- Serum Activin-A according to viral genotype (a = $p < 0.05$ compared to control)



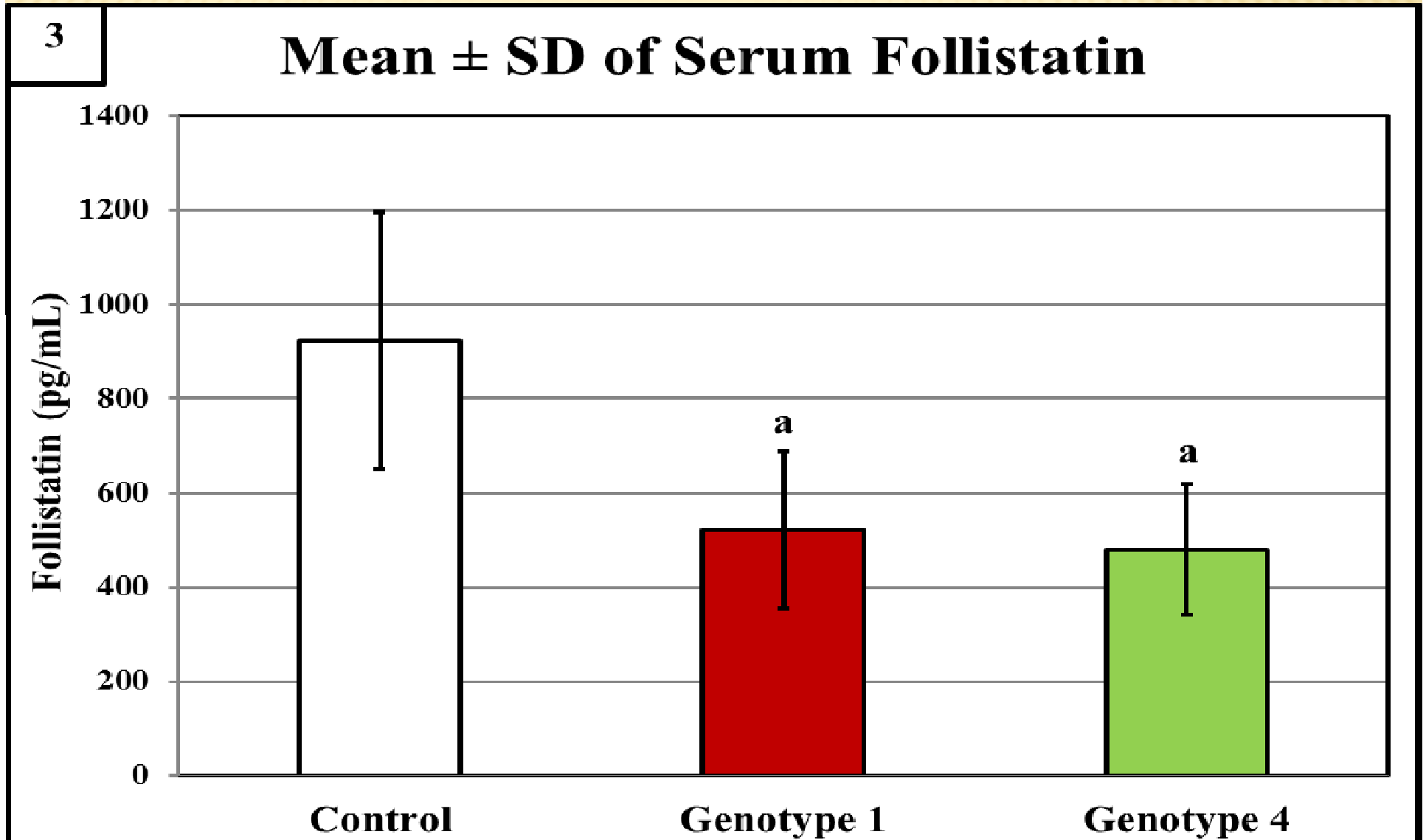
4- Serum Activin-B according to viral genotype (a = $p < 0.05$ compared to control)

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Mean \pm SD of Serum Activin-B

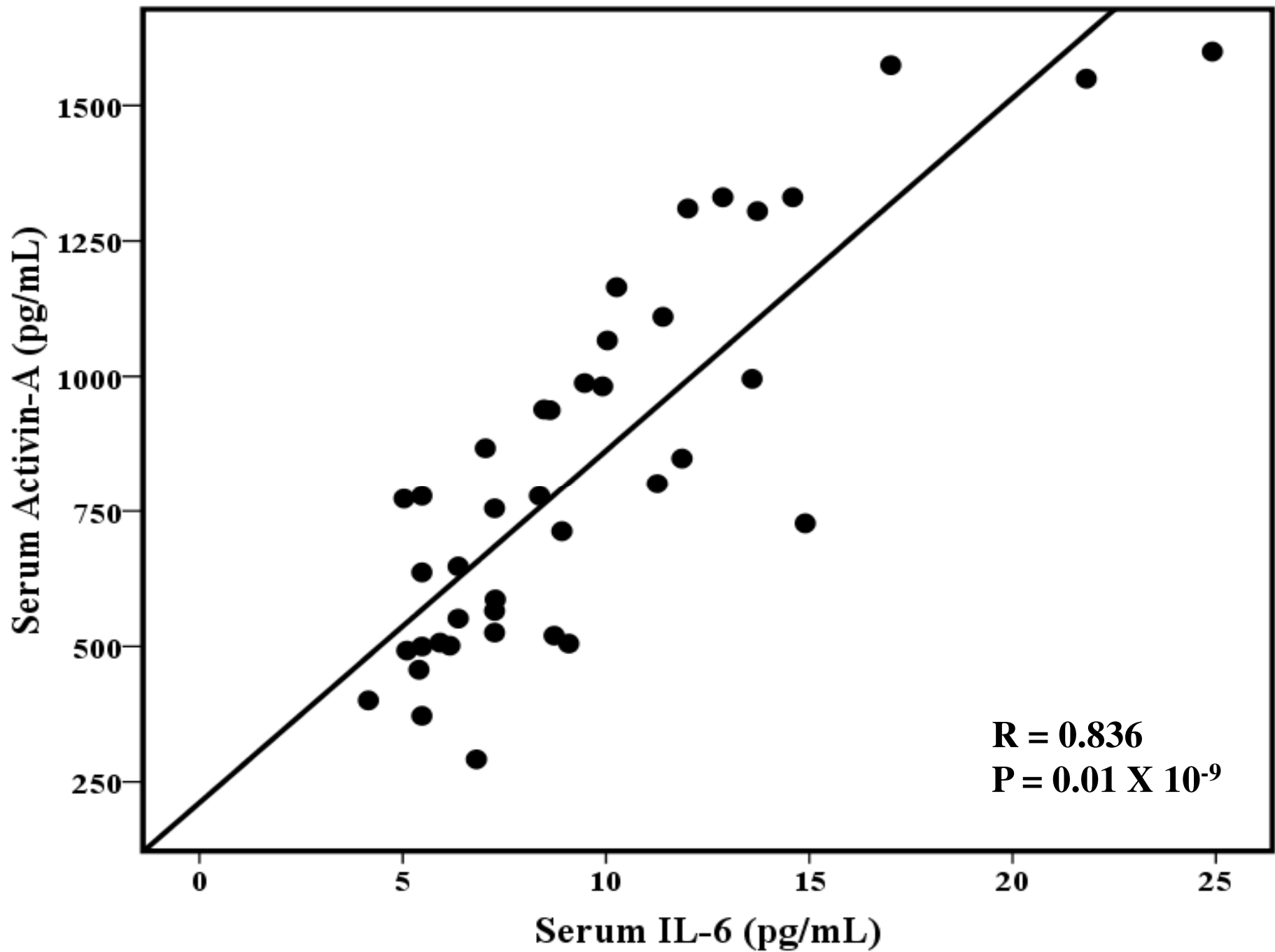


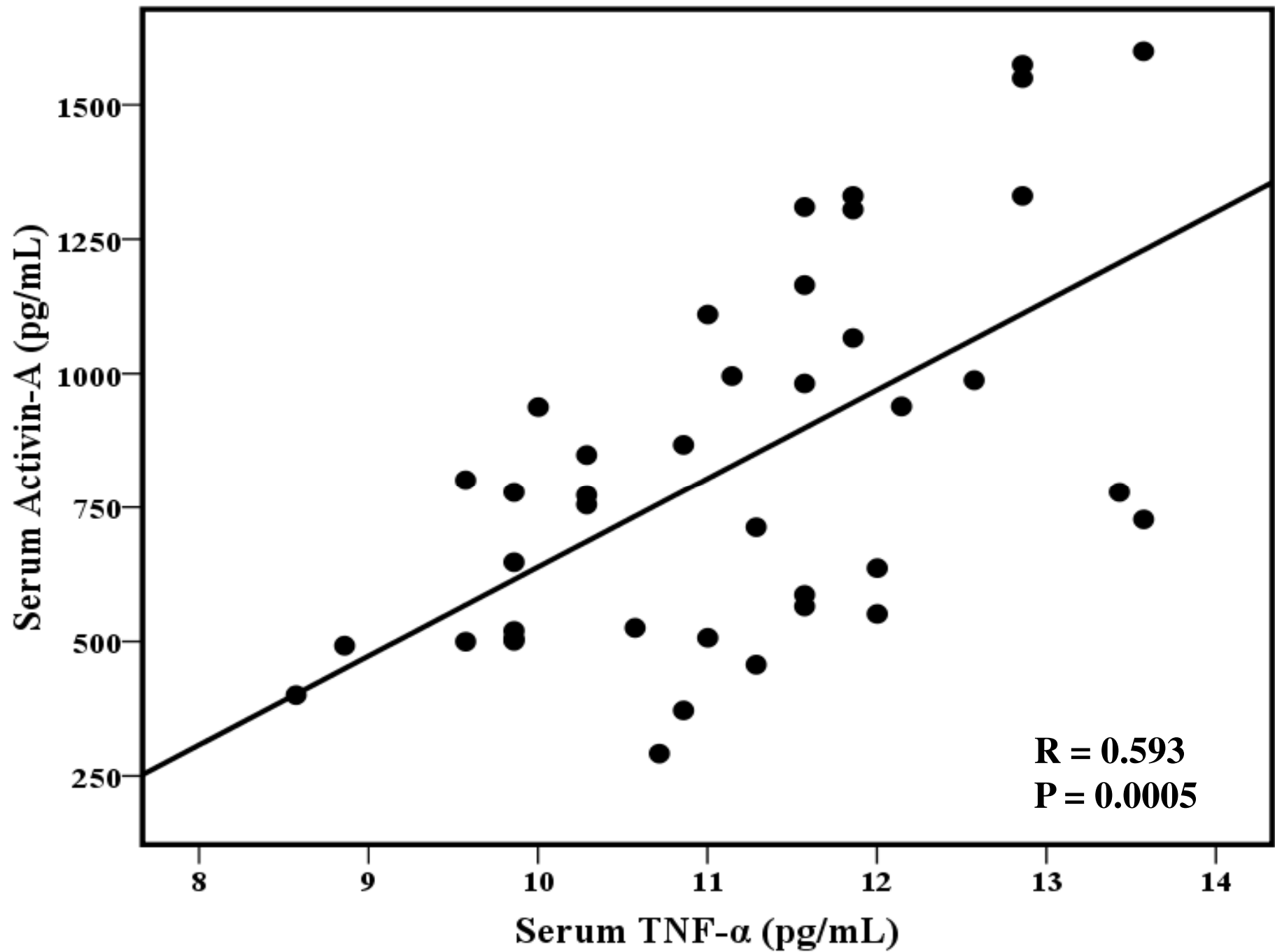
5- Serum follistatin according to viral genotype (a = $p < 0.05$ compared to control)

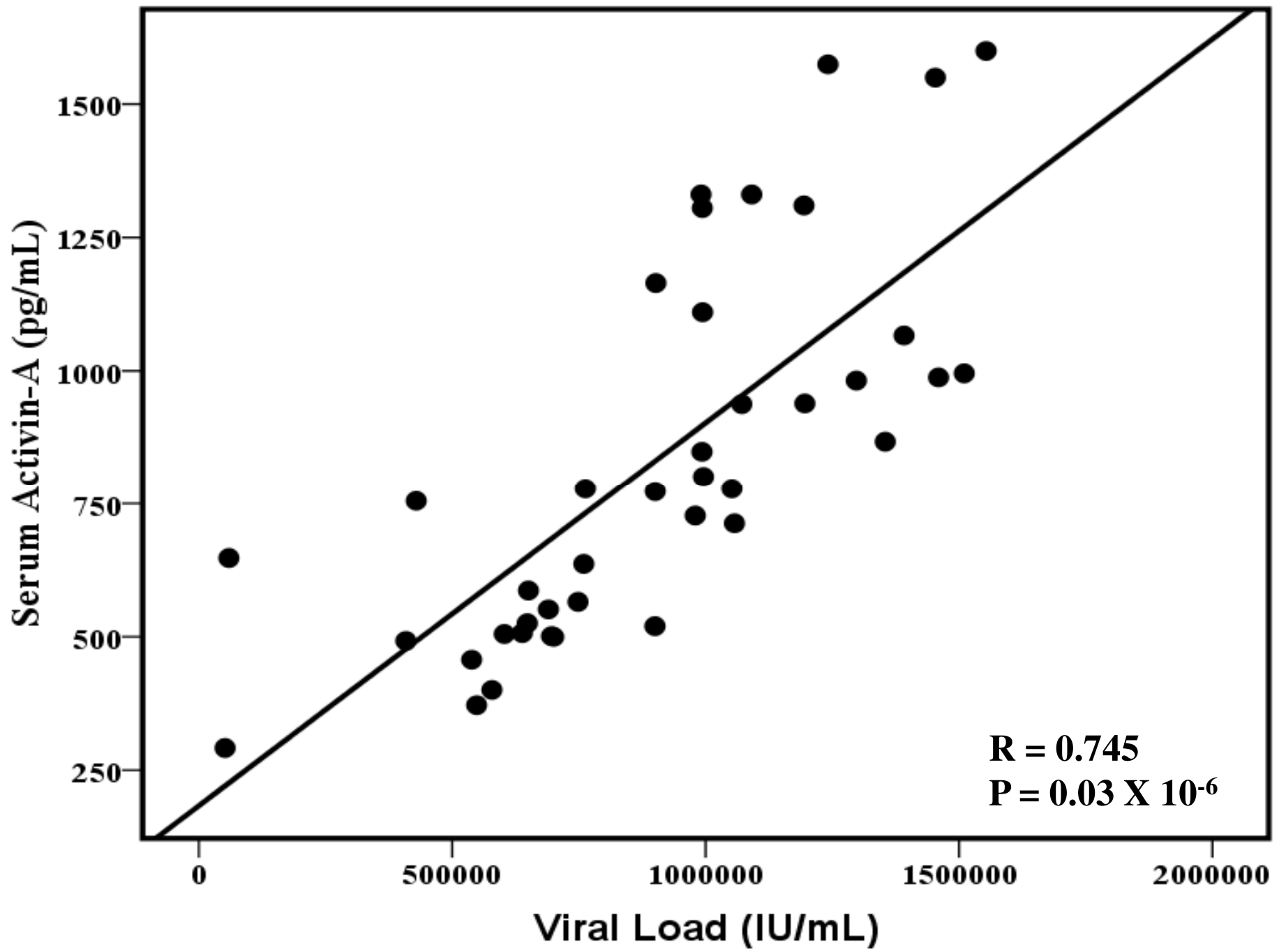


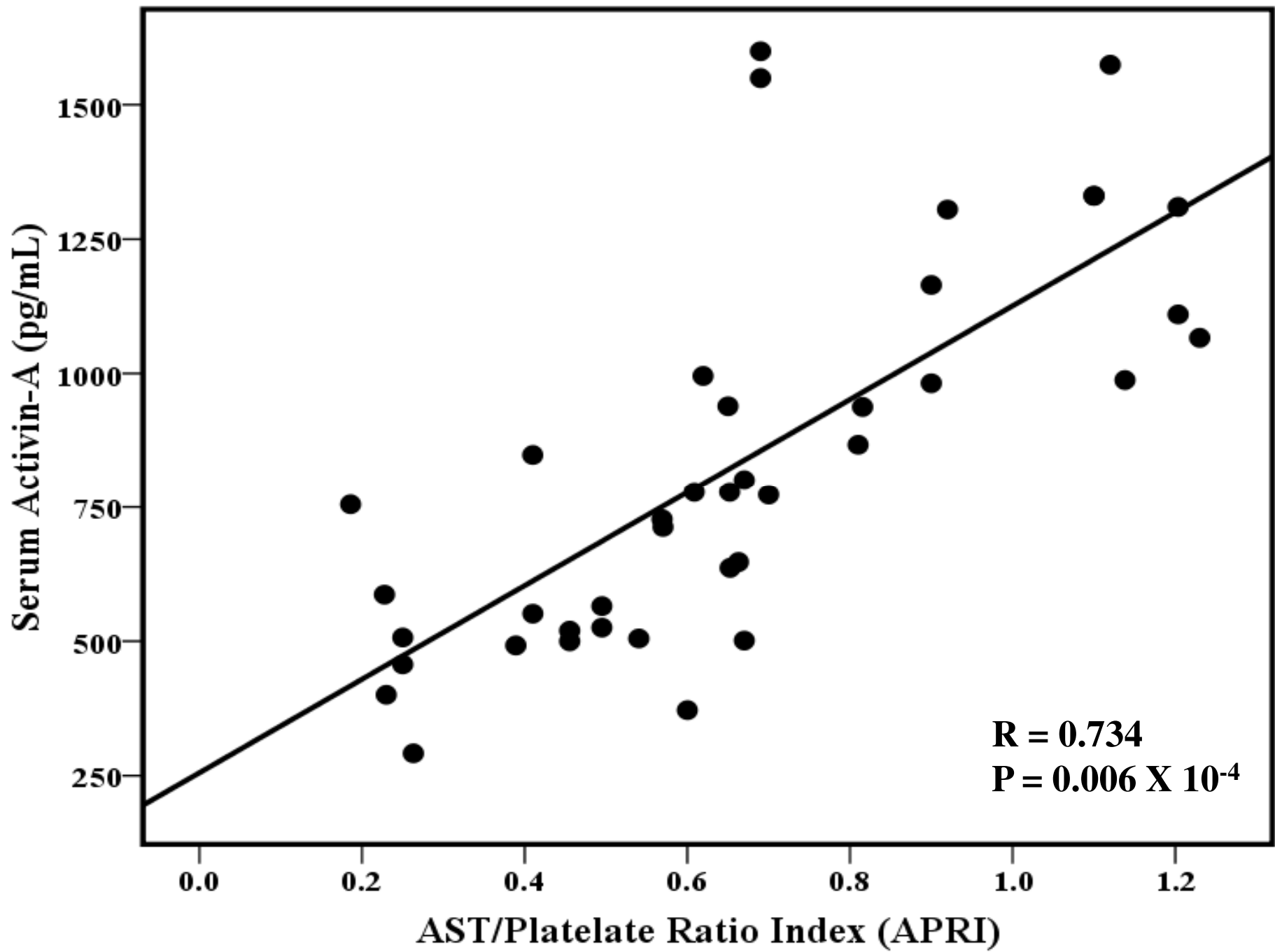
6- Correlation of serum activins and follistatin with pro-inflammatory cytokines, liver function parameters and viral load

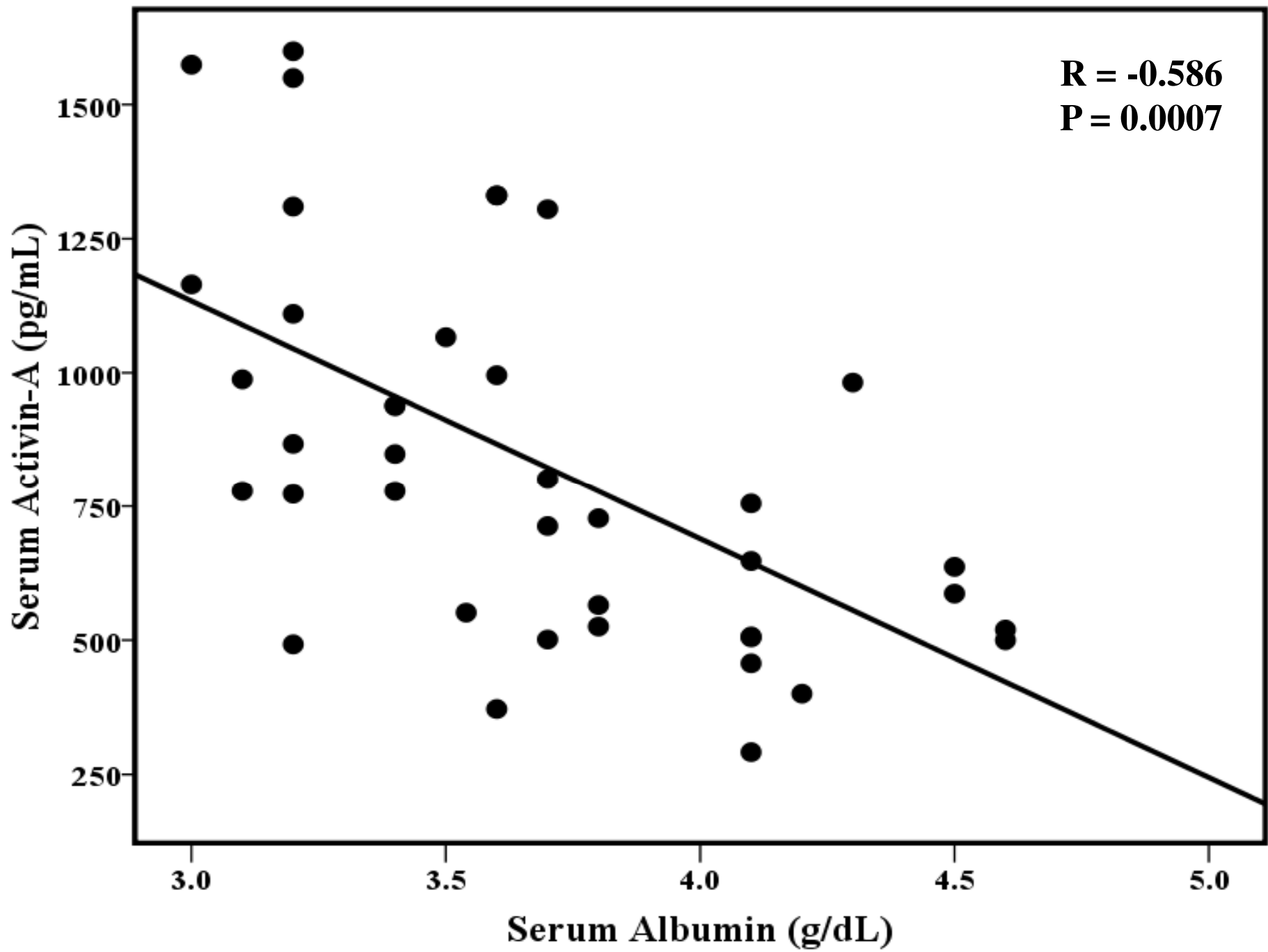
- ✿ Activin-A was strongly and positively correlated with:
 - ✿ viral load
 - ✿ APRI
 - ✿ IL-6 and TNF- α
 - ✿ negatively with albumin
- ✿ Activin-B showed similar correlations to activin-A but only in CHC genotype 1 and it was weaker
- ✿ No correlation was detected for follistatin











Conclusions

- ✿ CHC genotype 1 and 4 significantly altered serum activins and follistatin
- ✿ The observed significant correlations with viral load, IL-6 & TNF- α suggests that activins are involved in the host immune response to HC
- ✿ The correlation of activins with liver enzymes and APRI suggest that the dysregulation of activins is associated with liver injury

Future Work

- ✿ Could serum activins and follistatin be used as non-invasive markers for the diagnosis/staging of liver fibrosis?
- ✿ Could serum activins and follistatin be used as prognostic markers for the prediction of CHC treatment outcome?

Thank God