Impact of Genetic polymorphisms in Vitamin D pathway -related Genes on Vitamin D status in Human Health: Evidences from Local studies in Nepalese population

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By

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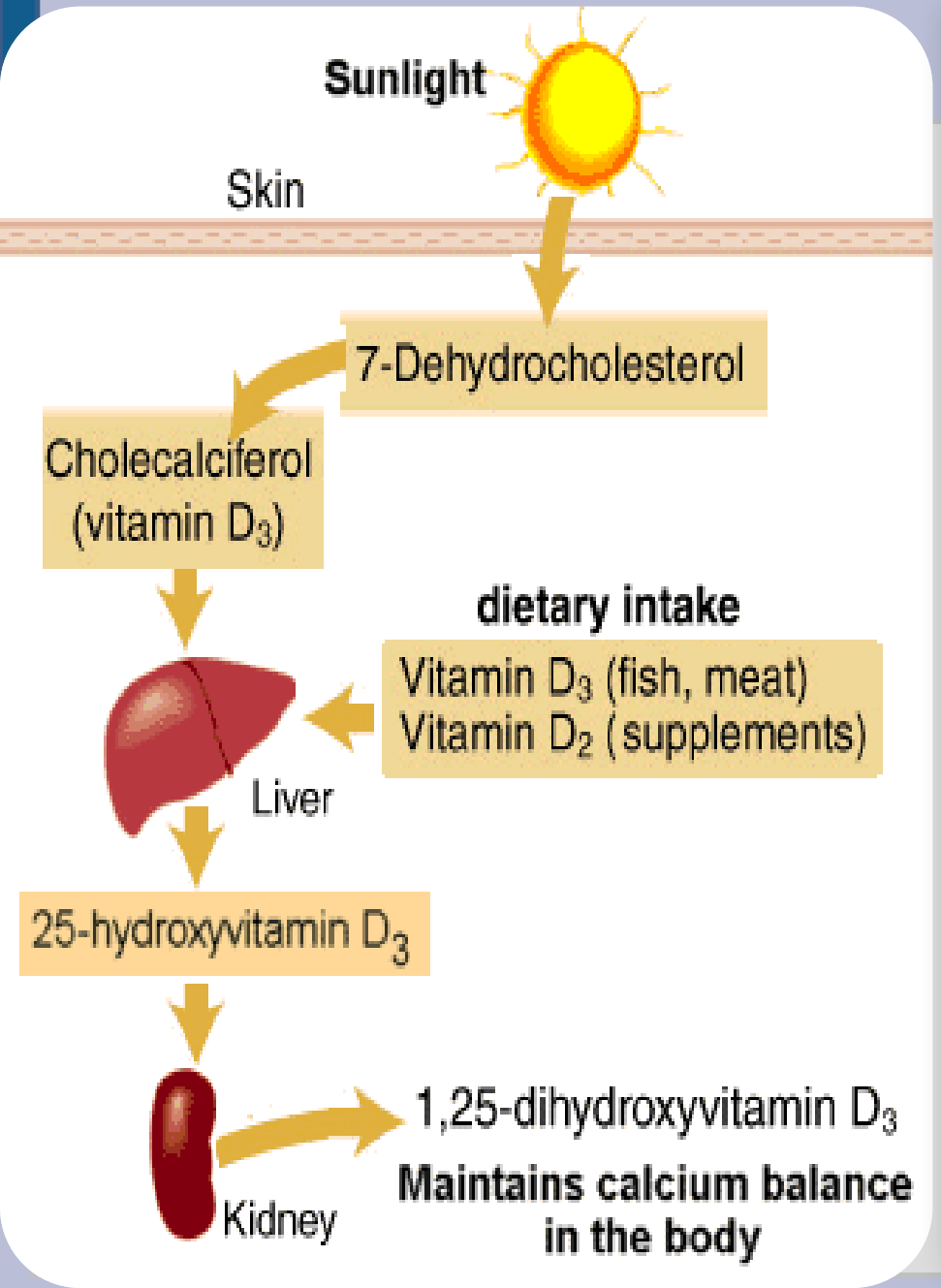
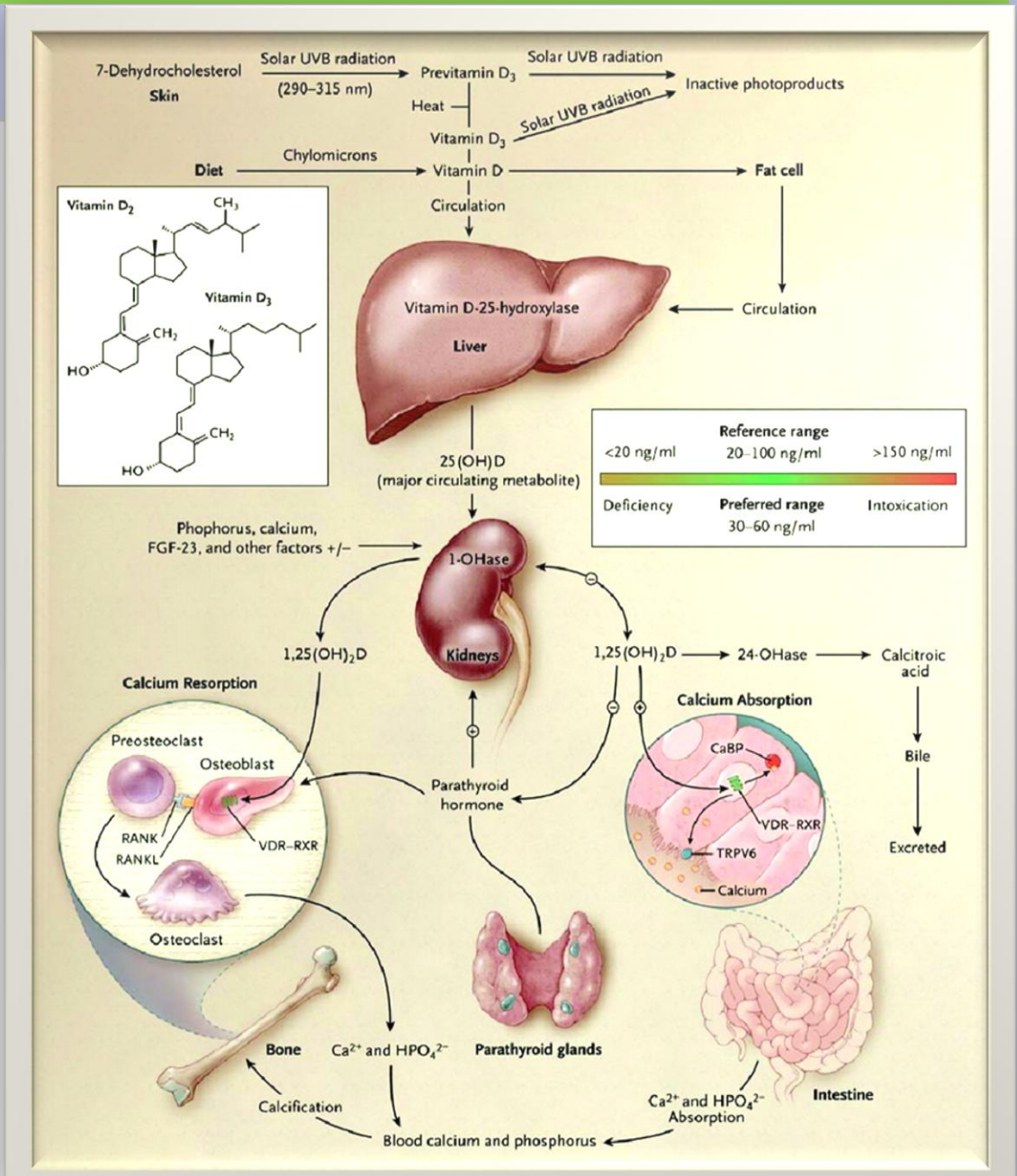
Vitamin D

- Vitamin D is a steroid hormone ,playing important role in calcium mineral metabolism and bone growth and maintenance.

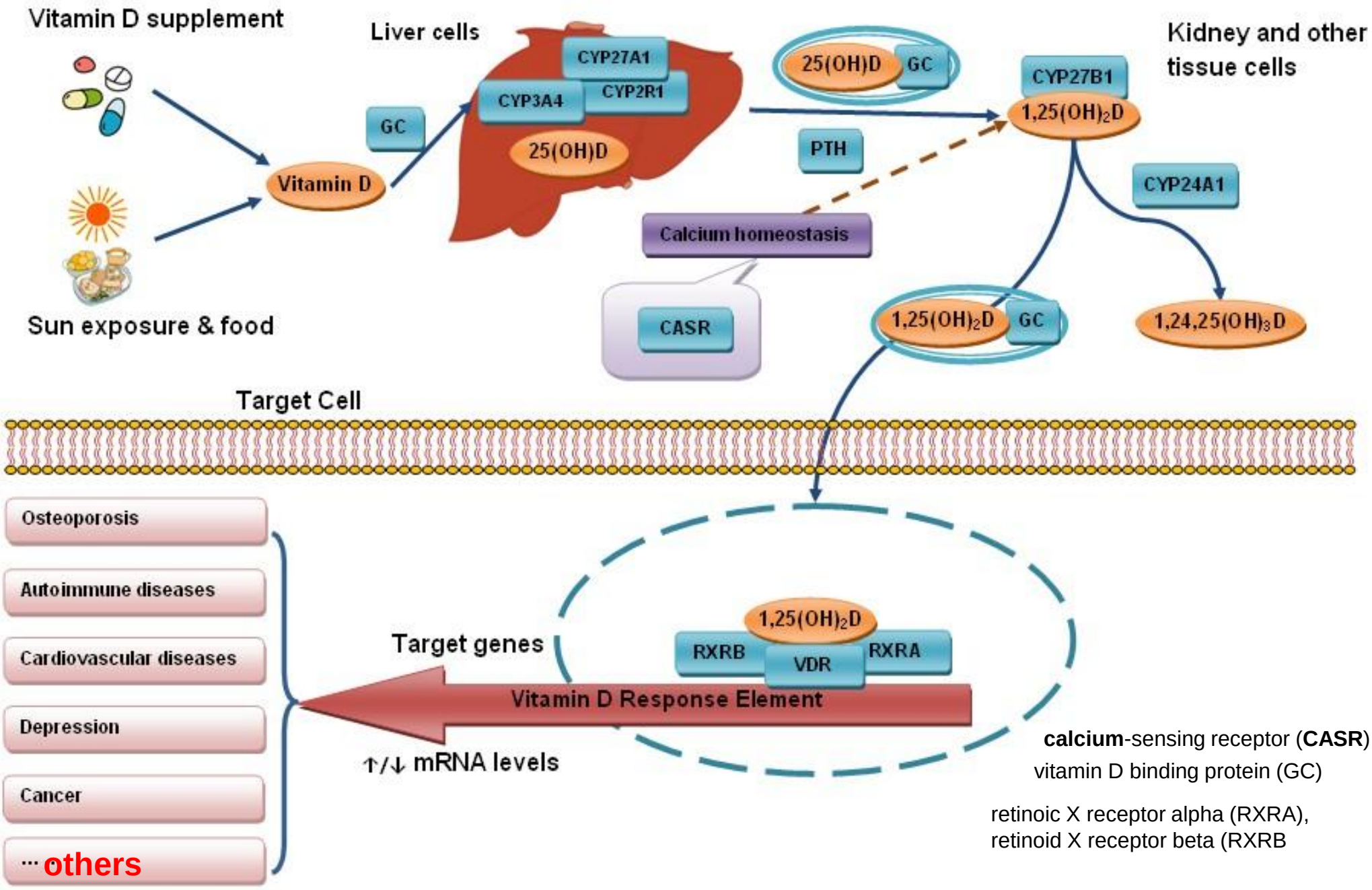
- Most cells in the body have been found to have receptors for vitamin D, and is therefore now seen as an important nutrient in preventing many chronic diseases.

Vitamin D Metabolism





How does Vit-D work

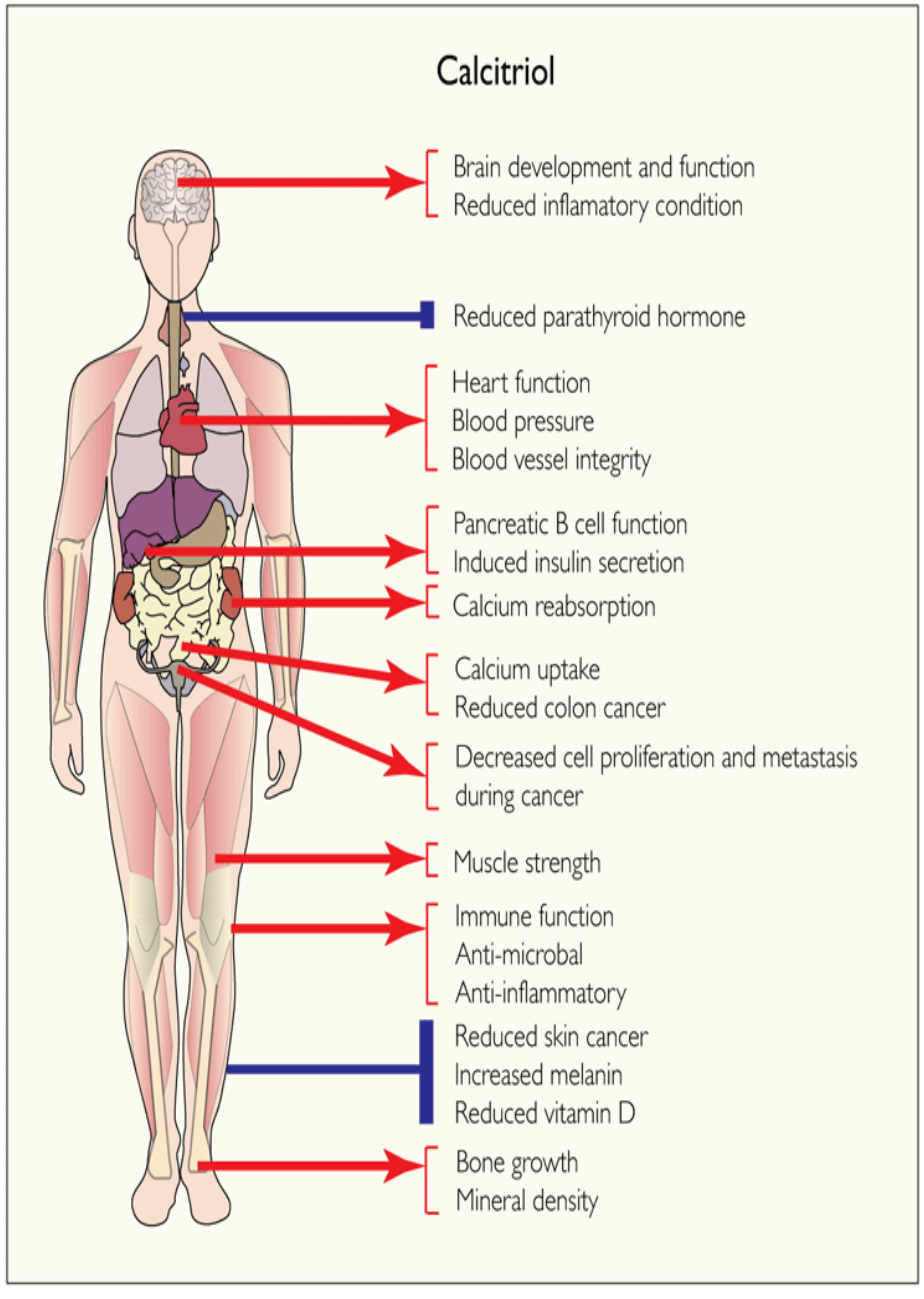


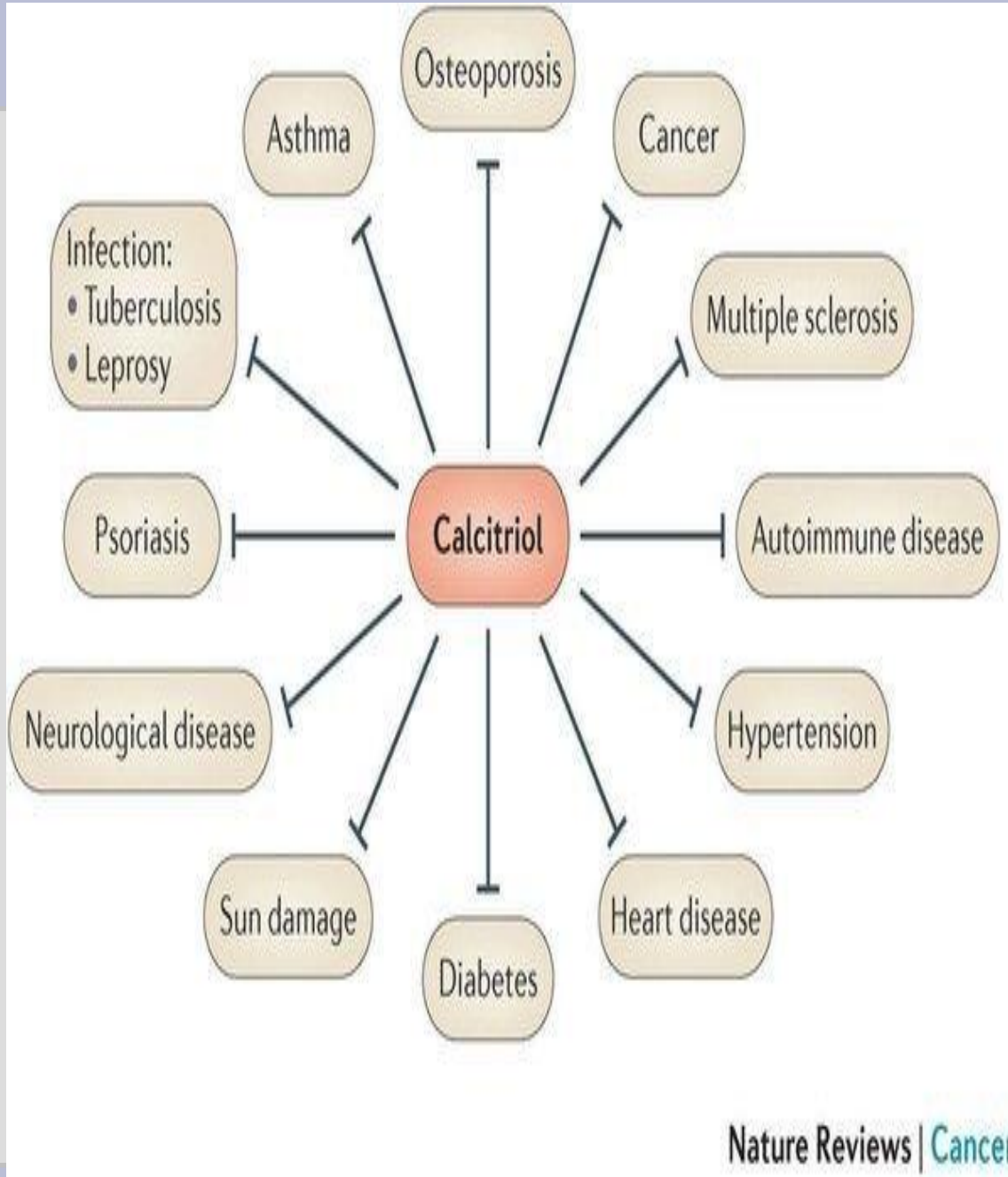
calcium-sensing receptor (**CASR**)

vitamin D binding protein (GC)

retinoic X receptor alpha (RXRA),
retinoid X receptor beta (RXRB

Vitamin D in health and disease





Vitamin D status:

(as per guidelines set by US Endocrine Society)

- **Vitamin D sufficiency: >30 ng/ml**
- **Vitamin D deficiency: <30 ng/ml**
- **Vitamin D insufficiency: 10-29 ng/ml and**
- **Severe vitamin D deficiency: <10 ng/ml**

Prevalence of Vitamin-D Deficiency in Nepal: Some Fact

- Diana Avagyan et al in 2013 in Nepalese children : Hypovitaminosis D identified in 91.1% of children.
- Pokharel BR et al 2015,Vit-D in stroke found 61.92% of stroke pts had Vit-D deficiency
- Study done by Bhatta MP et al (2016) in western region: 73.68% had Vitamin-D deficiency and Female were more deficient than male By 5.29%. (laboratory based data)
- Study done by *Shanti Regmi et al, 2016 at CMC,* 74.1% (80) patients had 25(OH) D levels lower than 30 ng/ml.

Prevalence of Vitamin-D Deficiency in Nepal: Some Fact

- **Laxmi RC** et al in 2018 in Lalitpur found 91.2% had Vitamin D deficiency.
- **Tirtha Narayan Shah** et al (2021) in Rupandehi, 14.9% had deficient, 42.8% had insufficient, 35.3% had sufficient and 7% had high level of serum vitamin D.
- **Chandra Yogal** et al in 2022 , Bhaktpur, VDD and VDI were observed in **6.3 and 42.4%** of the participants, respectively, and the prevalence increased by age.

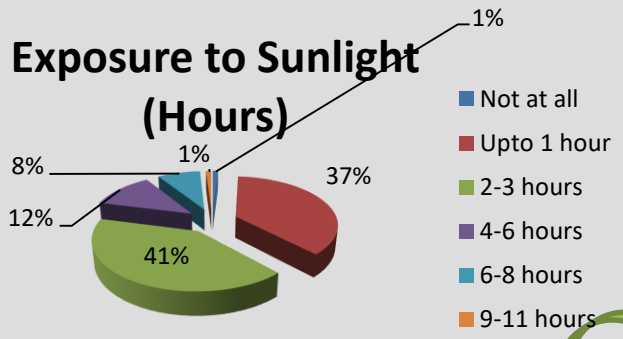
Our Study at TUTH

In Diabetes mellitus by Pradhan S et al , found that around 85% diabetic patients had Vit-D insufficiency

Vitamin D Status in Healthy Population Visiting for General Health Checkup in Tribhuvan University Teaching Hospital found 46.7% had Vit-D deficiency while 13.7 % had severe Vit-D deficiency(2017).

Louis et al (Sweden) at TUTH in 2015

Mean Vitamin D Levels Distribution Across Gender (ng/ml)		
	Mean Serum 25(OH)D Levels	p-value
Male	18.08 ± 6.8	0.029
Female	15.03 ± 6.9	



serum 25(OH)D			
How many hours do you normally spend outside during the day?	Mean	N	Std. Deviation
Not at all	20.9	1	
Upto 1 hour	15.925	37	8.7503
2-3 hours	15.908	41	4.9361
4-6 hours	18.982	12	8.3023
6-8 hours	18.613	8	4.8407

Autoimmune Thyroiditis Among Hypothyroid Individual And Its Association With Vitamin D (Jyoti et al, 2018 at TUTH)

Parameters	Case (Hypothyroid patient with AIT(n = 60)		Control (Apparently healthy euthyroid individual(n = 40)		p-value
	Mean	SD	Mean	SD	
Age	35.22	7.72	36.08	9.15	0.6
TSH	76.2	25.6	2.1	0.8	<0.001 *
FT_4	3.6	2.1	14.4	2.1	<0.001 *
FT_3	3.3	1.3	5.9	0.5	<0.001 *
Vitamin D	13.6	2.8	26.8	2.3	<0.001 *

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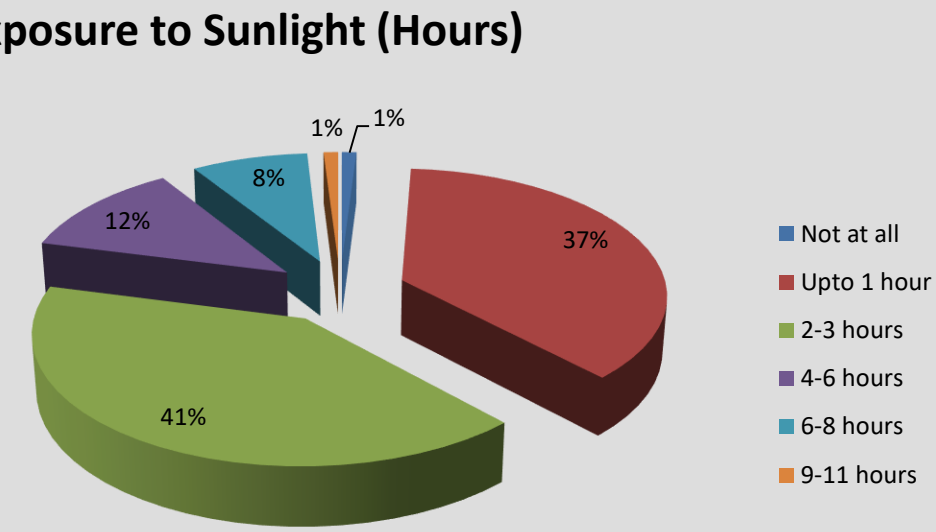

serum 25(OH)D				
How many hours do you normally spend outside during the day?	Mean	N	Std. Deviation	Range
Not at all	20.9	1	.	0
Upto 1 hour	15.925	37	8.7503	35.1
2-3 hours	15.908	41	4.9361	20.8
4-6 hours	18.982	12	8.3023	26.6
6-8 hours	18.613	8	4.8407	16
9-11 hours	8	1	.	0
Total	16.47	100	7.0216	35.1

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Parameters	Case (Hypothyroid patient with AIT) n = 60		Control (Apparently healthy euthyroid individual) n = 40		p-value
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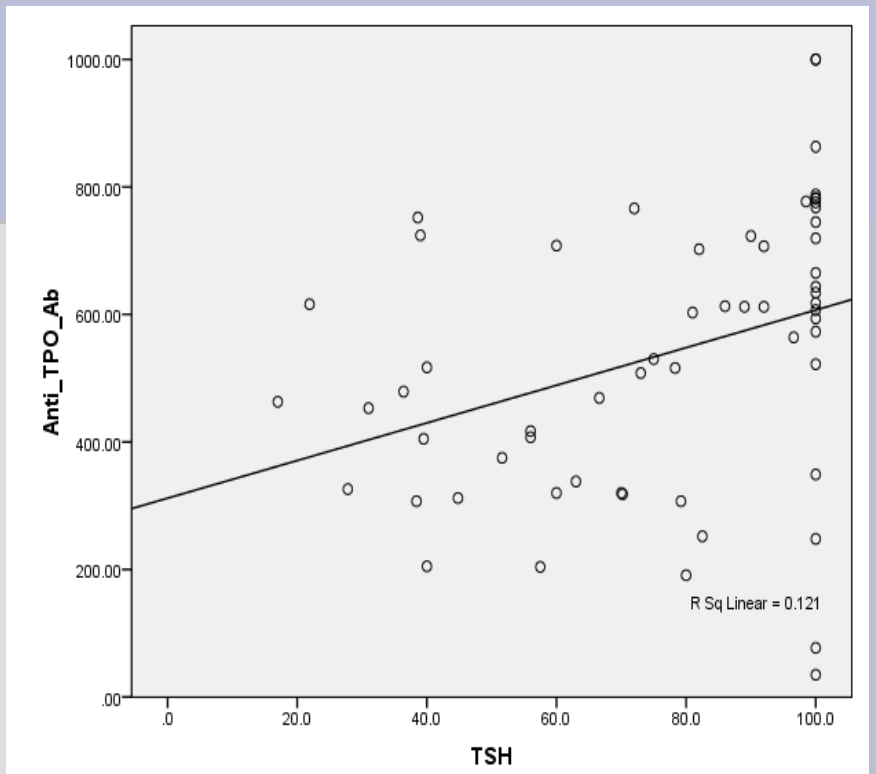
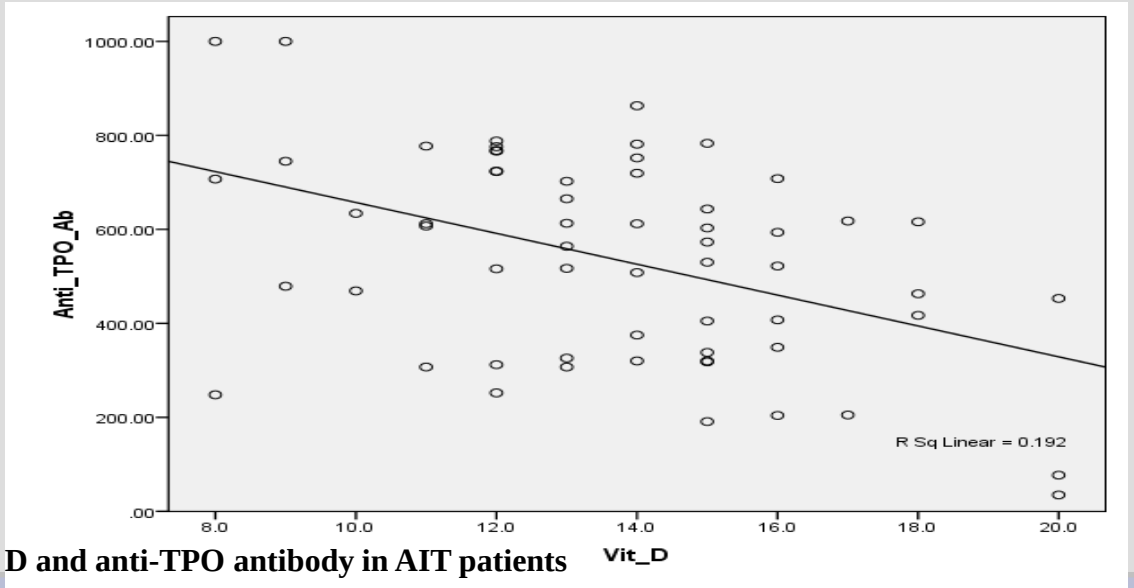


Correlation between TSH and anti-TPO antibody in AIT patients

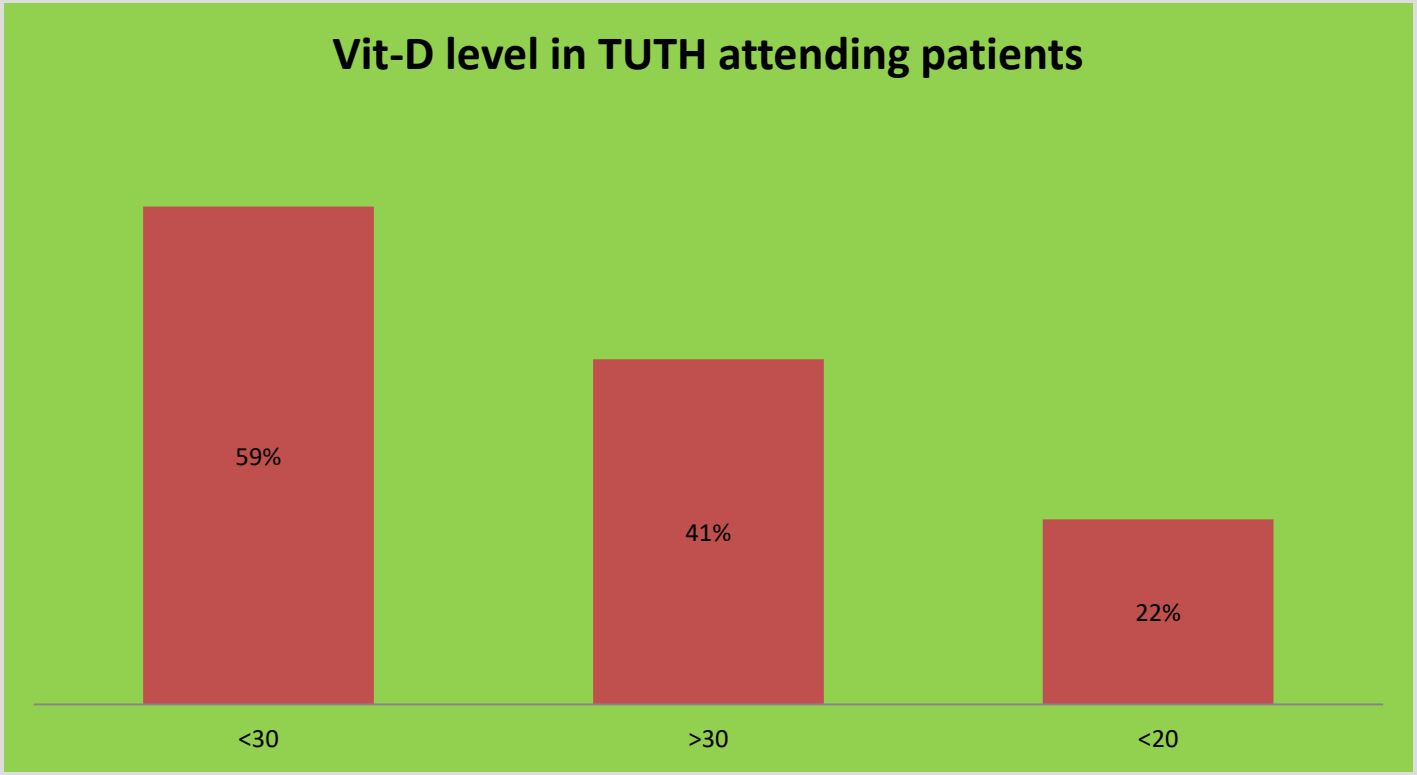


Correlation between vitamin D and anti-TPO antibody in AIT patients

Vit-D level in TUTH attending patients

Binita et al, 2017, TUTH

Vitamin-D	Mean	Std. Deviation
Female(1460)	31.05	31.10
Male(930)	35.45	35.71



Introductory Conclusion

From theses several study,

- Nepal is also suffering from hypovitaminosis-D, instead of having a very good amount of sunlight exposure.

- More research are needed to rule out the major cause for the same.

- **Probable cause for hypovitaminosis are:**
  - Nutritional deficiency
  - Food habit
  - **Genetics**

Population based study has not been done yet so it will be better to study Vitamin-D level in general population and factors responsible for the same .

Two Population based study in Nepal

- **Bajrachray A and Yadav BK et al 2023 in Bagmati Pradesh (NAST Academician Grant)**

The values of reference interval obtained for age-groups 18 – 30 years, 31 – 50 years and 51 – 65 years were (13.97 – 39.79)ng/ml, (15.18 – 39.68)ng/ml and (15.48 – 51.13)ng/ml, respectively.
The overall value of reference interval for vitamin D obtained in this study was **15 ng/ml to 40 ng/ml.**

- **Yadav BK and Pandit R et al , 2023 in Madhesh Pradesh (NHRC Provincial Grant)**
  - The reference values for age-groups 18 – 30 years, 31 – 50 years and 51 – 65 years were (12.71 – 46.38) ng/ml, (13.45 – 52.61) ng/ml and (14.50 – 55.00) ng/ml, respectively.
  - The overall reference interval values for vitamin D obtained in this study ranges from **13 ng/ml to 50 ng/ml.**

Age grp(yrs)	Bagmati Pradesh	Madhesh Pradesh
18-30	13.97 – 39.79 ng/ml	12.71 – 46.38ng/m
31-50	15.18 – 39.68 ng/ml	13.45 – 52.61ng/ml
51-65	15.48 – 51.13 ng/ml	14.50 – 55.00 ng/ml
Overall	**15 - 40 ng/ml.**	**13 -50 ng/ml.**

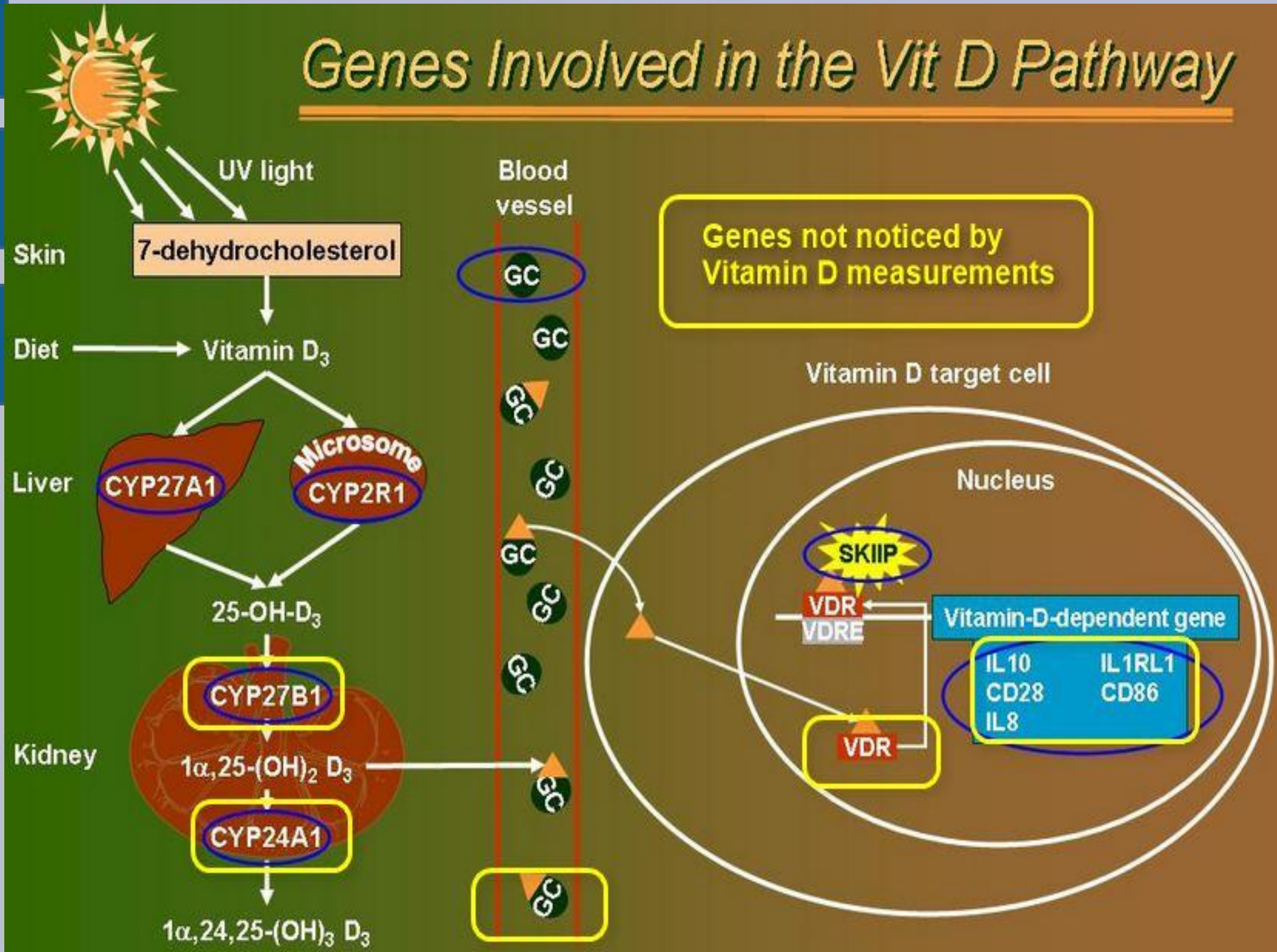
Introductory Conclusion

- From these several study, Nepal is also suffering from hypovitaminosis-D, having a very good amount of sunlight exposure.
- More research are needed to rule out the major cause for the same.
- Population based study has not been done yet so it will be better to study Vitamin-D level in general population and factors responsible for the same .

What may be the cause of Hypovitaminosis -D???????

Probable cause for hypovitaminosis are:

- **Nutritional deficiency**
- **Food habit**
- **Genetics**


Genes Involved in the Vit D Pathway
UV light
Skin
7-dehydrocholesterol
Diet
Vitamin D_3
Liver
CYP27A1
Microsome
CYP2R1
25-OH-D_3
CYP27B1
Kidney
1α,25-$(OH)_2$ D_3
CYP24A1
1α,24,25-$(OH)_3$ D_3
Blood vessel
GC
Genes not noticed by Vitamin D measurements
Vitamin D target cell
Nucleus
SKIIP
VDR
VDRE
Vitamin-D-dependent gene
IL10
IL1RL1
CD28
CD86
IL8
VDR

Research Questions:

Is there a association between genetic polymorphism of Vitamin D pathway -related Genes and vitamin D in individual visiting TUTH for general Health Checkup?

Objectives:

General: To find out the association between genetic polymorphism and vitamin-D- level in individual visiting TUTH hospital

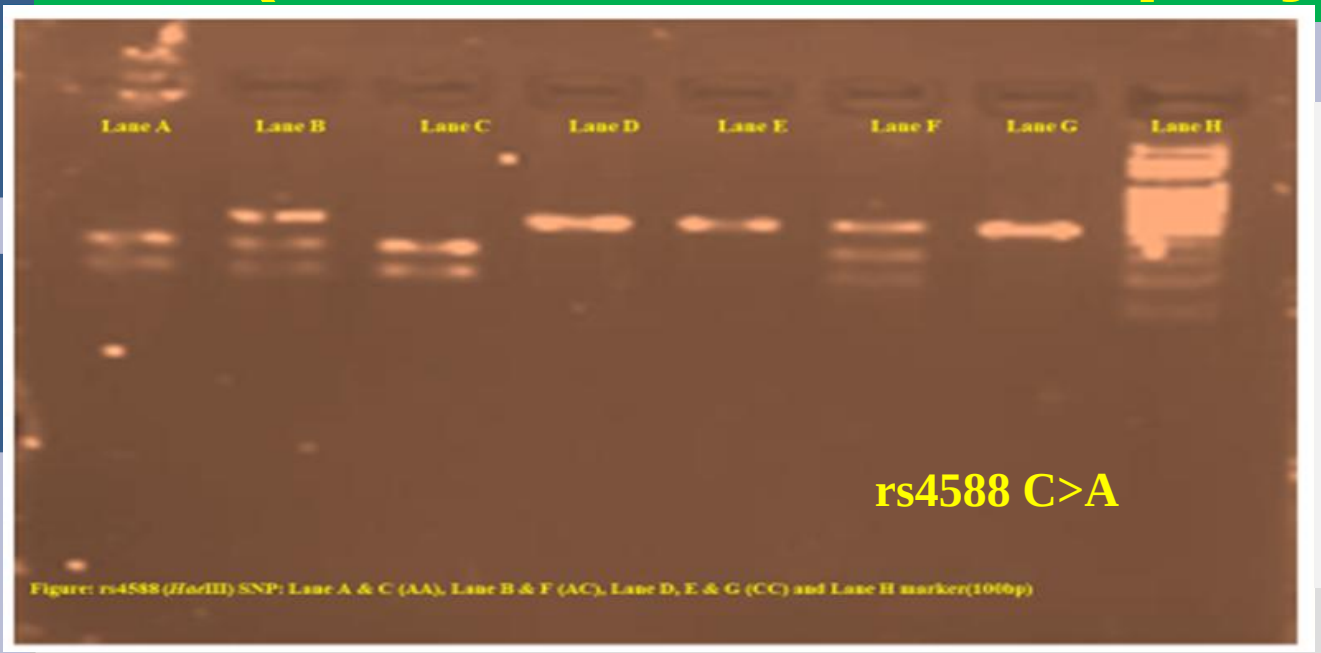
Specific:

1. **To screen Nepalese individuals for polymorphism of vitamin D-binding protein (VDBP)/Group Specific component (GC) gene associated single nucleotide polymorphisms (rs4588 and rs7041) related to Vitamin D metabolism**
2. To study the association of genetic polymorphisms and hypovitaminosis D in the study group.

Gene (SNP ID), Base change, PCR products, Restriction sites and RFLP fragment

Gene (SNP ID)	Base change	PCR product (bp)	Restriction enzyme (Temp.)	Allele: RFLP fragment (bp)
GC (rs4588)	C/A	483	HaeIII, 37˚C	C: 483 A: 297 + 186
GC (rs7041)	T/G	483	StyI, 37˚C	T: 483 G: 305 + 178

The polymorphisms of GC (rs4588 and rs7041 polymorphisms)



rs4588 (*Hae*III) SNP: Lane A & C (AA), Lane B & F (AC), Lane D, E & G (CC) and Lane H marker(100bp)

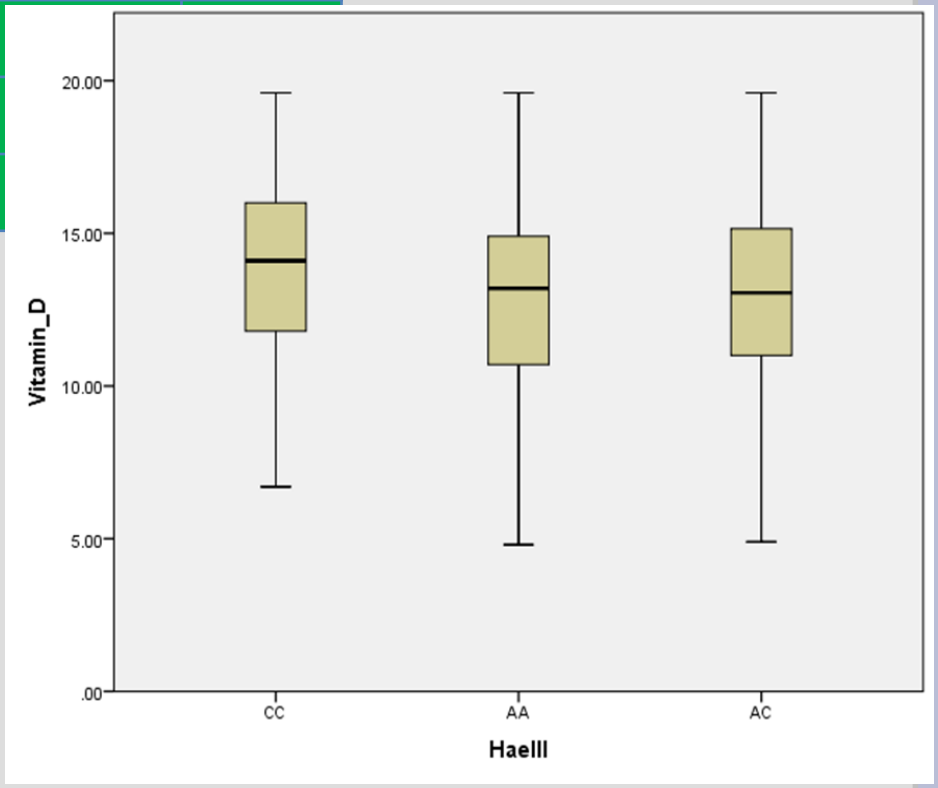
RFLP products for rs4588 C>A (Thr436Lys) were 297 and 186 bp for wildtype homozygous genotype and no restriction (483 bp) for the homozygous mutant genotype.

rs7041 (*Sty*I) SNP: Lane A& F (TT), Lane B, C, D, E & G (TG)

RFLP products for rs7041 T>G (Asp432Glu) SNP were 305 and 178 bp for the mutant homozygous and no restriction for the wild-type homozygous genotype.

Table 1: Allelic frequency and vitamin D levels in rs4588 polymorphism

HaeIII (rs4588) genotype (C>A) Thr436Lys	Genotype	Frequency n(%)	Vitamin D nmol/L	p-value
483(CC)	Wild-type	86 (21.5)	13.73 ± 3.04	0.063
297+186(AA)	Mutant	134 (33.5)	12.92 ± 3.09	
483+297+186(AC)	Mutant	180 (45.0)	12.72 ± 3.34	
Total		400		
Allele		Frequency (%)		
C		44.0		
A		56.0		

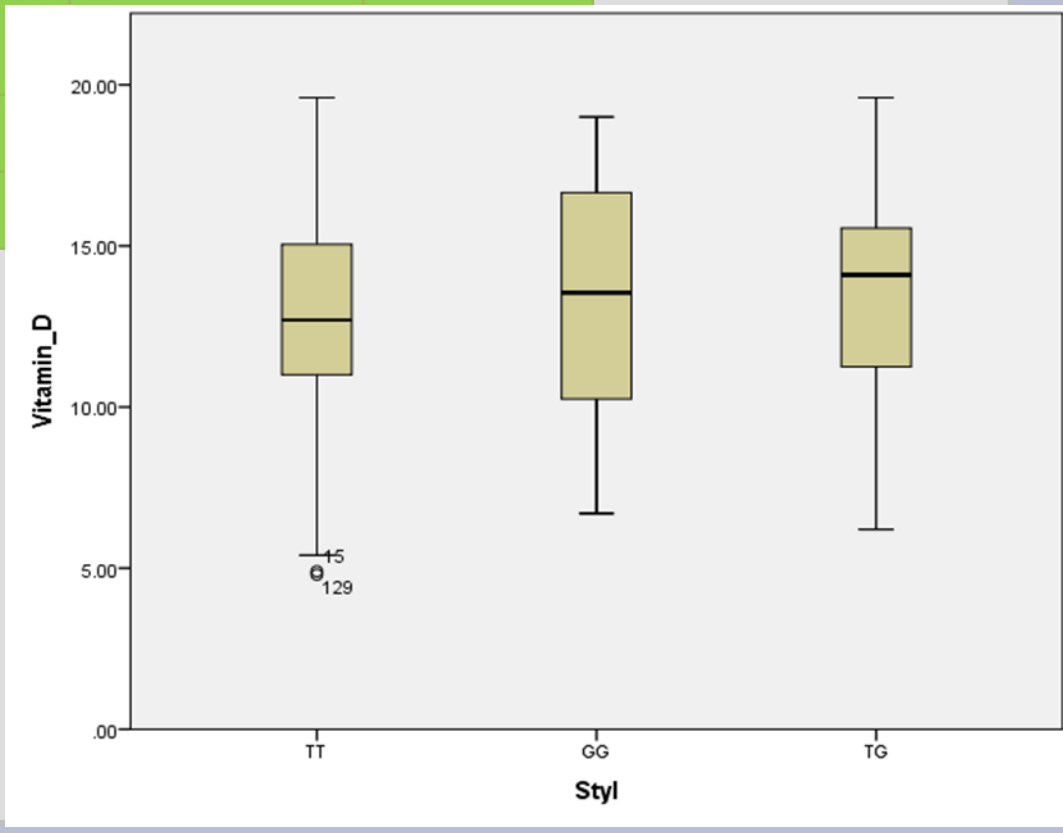


Box-plot showing vitamin D levels in different allelic distribution of rs4588 polymorphism

Table 2: Allelic frequency and vitamin D levels in rs7041 polymorphism

StyI (rs7041) genotype (T>G) Asp432Glu	Genotype	Frequency, n (%)	Vitamin D nmol/L	p-value
483(TT)	Wild-type	232 (58.0)	12.8 ± 3.15	0.181
305+178(GG)	Mutant	12 (3.0)	13.28 ± 3.76	
483+305+178(TG)	Mutant	156 (39.0)	13.29 ± 3.25	
Total		400		
Allele		Frequency (%)		
T		77.5		
G		22.5		

Box-plot showing vitamin D levels in different allelic distribution of rs7041 polymorphism



Discussion

- In a study by Dogan et al., individuals with **rs4588-A** allele in GC gene had lower vitamin D levels(26). It coincides with the findings of our study in which individuals with A allele had comparatively lower vitamin D. In concordance to Lafi et al.(21), the levels of vitamin D was higher in wild-type genotypes (GG and CC), respectively **for rs4588 and rs7041**.
- The allele frequency for rs2238136 in our study was similar to that reported by Xiong et al. in American population with A allele 28.1% while G allele accounting for 71.9%.
- Our findings were in concord with those of Das et al. (28) in which vitamin D deficiency was comparatively higher in individuals with TT genotype **(rs7041)** and AA genotype **(rs4588)** respectively though, statistically insignificant in our case.
- In contrast to the findings of Lafi et al(21) showing higher vitamin D levels among the genotype containing A allele in rs10743657, our study iterate comparatively lower vitamin D levels in the individuals with A allele.

Conclusion: Takeaway messages

- This study recommends the use of local finding of Vitamin D level in healthy individuals and concept of genetic polymorphism of gene related to Vitamin D related metabolism while interpreting the value of Vitamin D and taking any clinical decision.

- Because of some limitations, this study also strongly recommend a nation-wide study in large number of population covering wide geographical area in correlation with the parathyroid hormone level, calcium level and bone mineral density status for the better result of Association of genetic polymorphism of gene related to vitamin D metabolism.

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Short biography highlighting on your academic acheivements, expereinces, and affiliations. If possible with particular focus with editorial experiences. (150 words maximum)

Prof, Dr. Binod Kumar Yadav is M.S. (Medical Biochemistry), Ph.D. (Medical Science) and Postdoctoral fellowship (Neuroscience) and currently working as Registrar and Professor (Biochemistry) at Madhesh Institute of Health sciences (MIHS), Janakpurdham, Madhesh Pradesh. He is the permanent faculty of Biochemistry at Maharajgunj Medical Campus, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal since 2005.His area of interest in laboratory medicine, Clinical Chemistry, Endocrinology, Vitamin D, Genetics (especially genetic polymorphism), Metabolism. He has published more than 60 research articles in reputed international and national journal having **citation 1175, H-index (20) and i10 index (30).** He has worked as member of editorial team member of Journal of Nepalese Association of Laboratory Sciences (2010-2012), an official journal of Nepalese Association of Laboratory Sciences (JNAMLS) and currently in Annals of Clinical Chemistry and Laboratory Medicine (ACCLM), an official journal of Nepalese Association for Clinical Chemistry (NACC) and in the Meds Alliance Journal of Medicine and Medical Sciences(MJMMS), an official Peer-reviewed journal of center for clinical Research and community.