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The study evaluated the ZBTB40 gene polymorphism and biochemical parameters in women suffering from osteoporosis

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Abstract
Osteoporosis weakens bones. Falling or bumping increases the risk of bone fracture. Due to its lack of symptoms, many people are unaware they have it until they fracture. Bone mineral density testing is a safe, fast, and painless approach to detect osteoporosis or its risk. Spine fractures are diagnosed using traditional X-rays, which cannot measure bone mineral density. Specialized procedures are needed to assess bone mineral density. Multiple bone mineral density tests are available, but the most prevalent and recommended is dual-energy X-ray absorptiometry. DXA X-rays can detect small bone loss percentages. The T-score on the bone density evaluation shows how much bone mass differs from a healthy 20-something. The T-score, measured in standard deviations (SD), indicates whether the bone mass is normal, osteopenia, or osteoporosis. This work examines the genetic variation and function of ZBTB40 rs6426794 G/C, rs 7524102 A/G, and rs 34920465 A/G in osteoporosis etiology using molecular methods. The Baghdad Teaching Hospital Bone Density Unit conducts this investigation from September 2020 to January 2021. The DXA test detected osteoporosis in women. Nested PCR was used to investigate the ZBTB40 rs6426794 G/C polymorphism, and biochemic al methods were used to measure vitamin D3 and PTH levels in serum. Calcium concentration was assessed by digestion.

Keywords: Osteoporosis, polymorphism, parathyroid hormone, calcium

Introduction
Osteoporosis (OP) is a long-lasting and gradually worsening illness. Osteoporosis is a systemic skeletal disorder characterized by reduced bone density and degeneration of the bone tissue's micro-architecture. This leads to increased bone fragility and a significantly higher risk of fractures [1, 2]. Measuring bone mineral density (BMD) is crucial for early detection of osteoporosis, enabling prompt implementation of effective preventative and therapeutic interventions [3-5]. The Human Genome Project is anticipated to uncover early signals of vulnerability to complex diseases using genetic data. Since the Human Genome Project, a lot of genetic data has been collected, thus it's time to assess its usefulness for fracture prediction. Due to environmental and genetic factors, fragility fractures are a promising genetic prediction scenario. Currently, prediction algorithms do not use genetic data. Genetic data may help tailor fracture prevention, treatment, and care. Fragility fracture, the final result of osteoporosis, is common in elderly people and has serious clinical consequences. Women have a 50% lifetime fracture risk after 50, whereas males have 30%. Women's lifetime hip fracture risk is comparable to or larger than invasive breast cancer risk. Men have a 17% lifetime risk of hip and vertebral fractures, similar to prostate cancer [6, 7]. Then, reverse genetic approaches may establish that ZBTB40, an under-characterized gene, influenced osteoblast mineralization ex vivo. This study reveals that these strategies are effective for discovering osteoblast function genes [8]. Parathyroid hormone (PTH) regulates bone remodeling to maintain calcium homeostasis [9]. The vitamin D receptor (VDR) controls cell differentiation and proliferation in many cell types [10]. High calcium content in bone mass. Maintaining adequate calcium intake throughout adolescence can prevent age-related bone loss and increase peak bone mass. Conversely, low calcium consumption induces osteoporosis. The body stores calcium in bone in addition to its structural purpose [11, 12].

Literatures Review

Osteoporosis (OP)

Figure (1) shows that osteoporosis causes low bone mass and micro-architectural degeneration of bone tissue, making bones more fragile and prone to fracture. Osteoporosis has been challenging to define clinically since BMD does not include all fracture risk variables, and fracture-based definitions prevent identification of at-risk groups. They convened in 1994 to define osteoporosis as a combination of BMD and a prior fracture. If the BMD assessed by dual X-ray absorptiometry is more than -2.5 standard deviations below the sex-matched young adult mean and the patient has a history of fragility fractures, the term 'established osteoporosis' is used [13-15]. Systemic skeletal condition Osteoporosis reduces bone mass and causes qualitative changes (macro- and micro-architecture, bone material characteristics) that increase fracture risk. Primary osteoporosis occurs after menopause or with age. Many diseases and medicines induce secondary osteoporosis. Bone densitometry accurately measures bone mass and mineral density (BMD) in g/cm^2 of the predicted bone area. BMD contributes to 60–80% of bone mechanical resistance [16, 17]. Osteoporosis is a "silent epidemic" because of its global prevalence and rising patient numbers, which require interdisciplinary prevention and treatment. Osteoporosis affects 10% of the globe and 30% of post-menopausal women. Broken bones are the primary outcome of osteoporosis and a major cause of elderly death. Several causes contribute to osteoporosis, including hereditary and environmental influences [18, 19].

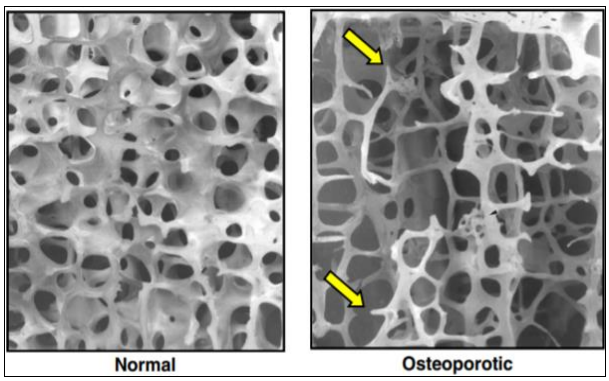

Fig 1: The image shows normal (left) and osteoporotic (right) cancellous bone under a light microscope. Osteoporosis thins trabecular components. The destruction of linking components (arrows) weakens bone far more than bone loss [13].

1.2.2 Classification of Osteoporosis

Both primary and secondary osteoporosis exist; however, primary osteoporosis is more prevalent. Primary osteoporosis is age-related. Secondary osteoporosis occurs when other medical conditions and drugs impede bone remodeling and reformation. There is also "primary type I" and "primary type II" osteoporosis [20, 21].

- **Type I** osteoporosis often develops after menopause when estrogen levels drop rapidly, causing bone loss and being characterized by trabecular (spongy) and hard-cortical bone. Postmenopausal osteoporosis (Type 1) affects 5% to 20% of women, peaking in the 60s and mid-70s. Women lose 2% to 3% of their bones each year during the first 5 years following menopause. Reduced estrogen generation causes women to lose half their trabecular bone and 35% of their cortical bone, shortening their lifespan. Absence of estrogen causes 75% of women's bone difficulty in the first two decades following menopause, not maturation. Bone loss during menopause may begin 1–3 years before amenorrhea [20].
- **Type II:** osteoporosis (senile osteoporosis) often occurs after 70 and causes trabecular and cortical bone loss. [20].

Osteoporosis prevalence

In all, 69 papers were studied. Over the previous 12 years, osteoporosis prevalence increased from 14.94% before 2008 to 27.96% from 2012–2015. The frequency of osteoporosis rose with age and was greater in women (25.41%) than men (15.33%). Rural regions (20.87% vs. 23.92%) and southern areas (23.17% vs. 20.13%) had higher osteoporosis rates. The pooled prevalence of osteoporosis in adults 50 and older was more than double that in 2006 (34.65% vs. 15.7%) [22]. As life expectancy rises, more senior individuals at high risk of fracture will raise the global health and economic burden of osteoporosis. We expect the over-65 population in 15 EU member states to grow from 58 million in 1995 to 108 million in 2040. In particular, the population over 80, where osteoporotic fractures are most common, will expand from 8.9 million women and 4.5 million men in 1995 to 26.4 million and 17.4 million in 2050 [23-25]. Data on the prevalence of osteoporosis in Italy is scarce and limited to specific populations. The sole general population cross-sectional research used quantitative heel ultrasonography to measure osteoporosis prevalence, finding 18.5% and 44.7% of osteopenia in women aged 40–79. The biggest risk variables were smoking (12.9) and parental hip fracture history, according to Bonaccorsi *et al*. [26]. One tertiary care facility reported 11.2% and 10.3% secondary osteoporosis in women aged 50–90 [27, 28]. With a 1:1 male-female ratio in 2009, Mexico's population stood at 111 million, and projections indicate that it will rise to 125 and 148 million by 2020 and 2050. When the numbers are forecast for people 50 and older, they rise more rapidly [29]. Osteoporosis and fractures cause global death and morbidity. Asia is home to 75% of the world's population, as well as a rapidly growing senior population. Experts predict that Asia's elderly population will increase from 5.3% in 1995 to 9.3% by 2025. Asian osteoporosis has gotten little attention until lately, with rare exceptions [30, 31]. In Saudi Arabia, 24, 62, and 74% of women aged 50–59, 60–69, and 70–80 were osteoporotic, whereas 21% of males over 50 were [17, 32]. In women, osteoporosis was 9% in the UK, 15% in France and Germany, 16% in the US, and 38% in Japan, but 1% in males, 4% in Japan, 3% in Canada, and 8% in France, according to a study [33]. Previous research found 7.9% to 22.6% of Caucasian women over 50 have osteoporosis. According to the Taiwan Nutrition and Health Survey, 25.0% of women and 11.6% of men had forearm osteoporosis [34]. In 2000, researchers documented about 9.0 million osteoporotic fractures, including 1.6 million hip fractures, 1.7 million forearm fractures, and 1.4 million clinical vertebral fractures. All the data showed that osteoporosis is frequent, although less is known about its epidemiology in northwestern China than in other nations. [35].

Pathophysiology of Osteoporosis

Postmenopausal osteoporosis can be caused by the failure to attain peak bone density or accelerated bone loss after

menopause as shown in Figure (2). The attainment of an optimal peak bone mass is important in the prevention of osteoporosis. Optimal skeletal health is dependent on genetics, with an appropriate lifelong balance of diet and lifestyle factors, such as weight-bearing exercise and the avoidance of bone-toxic substances. Bone mass acquisition occurs during childhood, adolescence and early adult life.

Low peak bone mass can be determined by genetic factors, inadequate nutrition during growth and development (particularly calcium and protein intake), limited physical activity, concurring diseases (e.g. thyrotoxicosis, Cushing's) or drugs (e.g. corticosteroids, anticonvulsants) during growth, which impair bone mass acquisition [36, 37].

Normally function of osteoclast is bone resorption and osteoblast is osteoid formation and mineralization. After the age of 40 years. bone resorption is more than formation i.e. an increase in osteoblastic activity and/or decrease in osteoblastic activity. If osteoclast penetrate were in trabecular bone, they leave no template for new bone formation causing rapid bone loss. In cortical bone it causes activation of remodeling and ultimately more porous bone, is formed. In women, there is an accelerated phase of bone loss after the menopause due to estrogen deficiency, which causes uncoupling of bone resorption and bone formation, such that the amount of bone removed by osteoclasts the rate of new bone formation by osteoblasts. Age-related bone loss is a distinct process that accounts for the gradual bone loss which occurs with advancing age in both genders. Bone resorption is not particularly increased but bone formation is reduced and fails to keep pace with bone resorption [38, 39].

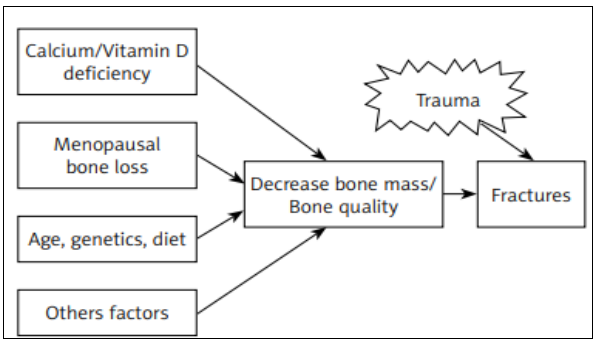

Fig 2: Pathophysiology of osteoporosis-related fractures [36]

Diagnosis of Osteoporosis

The T- or Z-score, both SD units, typically represent bone mineral density. The T-score calculates the deviation of an individual's BMD from the predicted mean value in young, healthy individuals, measured in SDs. Osteoporosis is characterized by a T-score for BMD at the femoral neck that is 2.5 SD or more below the young female adult mean (T-score − 2.5 SD). The Z-score is the number of SDs that an individual's BMD deviates from the age- and sex-expected mean. Children and teenagers utilize it most [40]. A fragility fracture following a fall or a DXA T-score of less than 2.5 at the lumbar spine, total hip, or femoral neck can diagnose osteoporosis in postmenopausal women and men over 50 (Table 1). This T-score cutoff equates to a BMD 2.5 SD units lower than the "average" young adult female. According to WHO criteria, a T-score above -1.0 is considered normal, but -1.0 to -2.5 without fracture is considered "low bone mass" (osteopenia). The definition of osteoporosis in premenopausal women and men is under 50. [41].

Table 1: Diagnosis of Osteoporosis [41].

	Postmenopausal W and M >50 years	Premenopausal W and M <50 years
Osteoporosis	T-score ≤−2.5 or fragility fracture	Z-score ≤−2.0 and fragility fracture
Low bone mass (osteopenia)	−1.0 to −2.5, no fracture	–
Low bone mass for age	–	Z-score <−2.0 and no fracture
Normal	T-score ≥−1.0, no fracture	Z-score ≥−2.0, no fracture

Dual Energy X-ray Absorptiometry (DXA)

In the late 1980s, postmenopausal women received the DXA. In the 1990s, pediatric software was able to utilize improved algorithms for recognizing bone boundaries in children (Figures 3). We use regional spine and hip measures for bone density assessment. DXA pictures, being two-dimensional, cannot measure volumetric BMD. We calculate Real BMD (a BMD) from DXA by dividing bone mineral content (BMC (g)) by predicted bone area (cm2) and reporting the result in grams/cm2. Total body scans should measure body composition, including BMC. Pediatric bone measurements have debated the BMC or BMD of the head, given the skull's significant contribution to total bone mass in children. Head BMD is lower in young gymnasts than controls and increases in astronauts during space travel, suggesting bone redistribution during loading and unloading [42].

Fig 3: Dual Energy X-ray Absorptiometry (DXA).

Other Techniques

1. Peripheral Dual Energy X-ray Absorptiometry (PDEXA).
2. Single X-ray Absorptiometry (SXA).
3. Quantitative Ultrasound (QUS).
4. Quantitative Computed Tomography (QCT).
5. Radiographic Absorptiometry [43].

Osteoporosis Risk Factors

Much of the research on osteoporotic fracture has focused on risk variables related to BMD fluctuation because of DXA's wide availability, high accuracy, and association with fracture risk. The researcher provides a brief summary of BMD and fracture risk variables in Tables 1–2, with more comprehensive reviews available. Women had lower BMD and greater hip fracture rates than males and Caucasians than non-Caucasians. Higher body weight is consistently associated with higher BMD and lower hip fracture risk, possibly due to higher mechanical loading on skeletal bone, adipokine secretion from fat tissue, and cushioning from falls. Falls cause most non-vertebral fractures, making

fracture prediction challenging [44].

Women's bone loss accelerates after menopause for reproductive and hormonal reasons. High-trabecular bones like the spine and ultra distal forearm are especially impacted by estrogen deprivation. Estradiol is the most significant hormone for bone health in both genders, but growth hormone, testosterone, and insulin are also crucial. Calcium, vitamin D, and protein consumption help build and maintain optimal bone mass. However, methodologic constraints in evaluating dietary intakes and the temporal lag between dietary intake and bone health have hindered a better knowledge of nutritional variables' impacts on BMD and bone health. Physical inactivity, smoking, alcohol use, low sun exposure (for vitamin D production), and some medications, such as glucocorticoids and anticonvulsants, can also increase fracture risk and lower BMD. Due to differences in cortical and trabecular bone composition, several of these osteoporosis risk factors may affect age and site differently. Hip geometry also increases fracture risk, according to research [41, 45].

Table 2: Non-genetic risk factors for osteoporosis [41].

Personal factors	Medications	Diseases
Caucasian	Glucocorticoids	**Monogenic diseases**
Female	Anticoagulants (warfarin, heparin)	OI, OPPG
Age >50 years	Antiepileptics	Muscular dystrophy
Low body weight	Sex hormone suppressants	Connective tissue disorders (eg, Marfan syndrome,
	(eg, aromatase inhibitors, Depo-Provera®)	Ehlers-Danlos syndrome)
Physical inactivity	Proton pump inhibitors	Cystic fibrosis
Falls	Thiazolidinediones	Metabolic (eg, glycogen storage diseases)
Muscle weakness	Antidepressants	**Complex diseases**
Reduced vision		Renal (chronic kidney disease, hypercalciuria)
Smoking		Rheumatoid arthritis
Excess alcohol		Hematologic (eg, mastocytosis)
Elevated homocysteine (females only)		Gastrointestinal (inflammatory bowel disease,
		celiac disease)
		Pulmonary (chronic obstructive pulmonary disease)
		Endocrine (types 1 and 2 diabetes mellitus,
		hyperthyroidism, hyperparathyroidism)

Biochemical parameters and their relationship to Osteoporosis

Vitamin D

When exposed to sunshine or obtained from food, the skin creates Vitamin D, a steroid hormone. It is critical for maintaining healthy bones and regulating body calcium levels [46, 47]. As early as the 1860s, scientists recognized the importance of vitamin D for bone health, and more recently, they discovered its role in promoting overall health beyond the skeletal system. There have been reports of vitamin D insufficiency worldwide. The majority of people develop this condition as a result of insufficient exposure to sunshine and a low intake of vitamin D-rich foods. Fortification was one method of adding vitamin D to regularly consumed food products [48, 49]. Vitamin D primarily serves to regulate calcium metabolism and maintain bone structure. Vitamin D insufficiency is a prevalent health issue that impacts individuals of all ages and is linked to the development of rickets in children and osteoporosis in adults [50, 51]. Below is a brief overview of vitamin D production and metabolism, which have been extensively detailed in many sources [52]. Calcitriol, also known as 1, 25-dihydroxyvitamin D [1, 25(OH)$_2$D], is the biologically active form of vitamin D. Three sources produce it: sunshine, food, and dietary supplements (Figure 1.4). Vitamin D hormones have two precursors: cholecalciferol (vitamin D$_3$) and ergocalciferol (vitamin D$_2$). UVB rays produce Vitamin D$_3$ in the skin. Solar UVB light, with a wavelength range of 290 to 315 nm, enters the skin and transforms 7-dehydrocholesterol into pre-vitamin D$_3$ by photolysis. This pre-vitamin D$_3$ is then quickly transformed into vitamin D$_3$ [53].

Some food sources and dietary supplements can also provide vitamin D$_3$. Only dietary sources can produce Vitamin D$_2$, also known as ergocalciferol, and UVB radiation does not produce it. The bloodstream transports both vitamin D$_3$ and D$_2$, which then attach to the vitamin D-binding protein (VDBP). The bloodstream transports vitamin D to the liver, where vitamin D-25-hydroxylase converts it into 25-hydroxyvitamin D [25(OH)D]. The CYP27A1 enzyme facilitates the first hydroxylation. It is believed that 25(OH)D 1-hydroxylasee in the kidneys transforms this kind of vitamin D, which is not physiologically active, into its active form, 1,25(OH)$_2$D [54]. CYP27B1, an enzyme located in the inner mitochondrial membrane of the proximal tubule cells of the kidneys, facilitates the second hydroxylation process. Two types of vitamin D metabolites are 24-hydroxylated to make 24, 25(OH)D and 1, 24, 25(OH)D. This is the main way that vitamin D metabolites are turned off [55, 56].

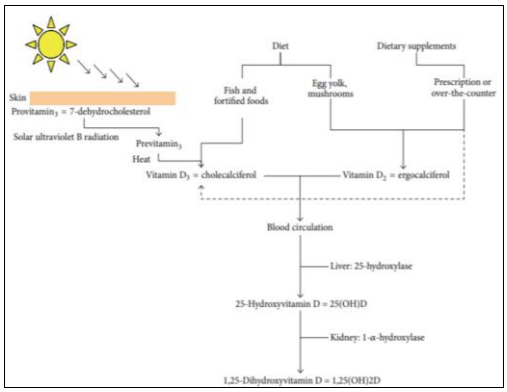

Fig 4: Sources of vitamin D and the first steps of vitamin D synthesis [55]

Parathyroid Hormone

Figure 5 shows four parathyroid glands that release parathyroid hormone. PTH begins as a 115-amino-acid pre-pro-PTH peptide hormone. Double cleavage of pre-pro PTH yields 84 amino acids of PTH. Low serum calcium, or calcitriol, triggers the release of the hormone. PTH raises serum calcium in the kidneys, gut, and bones. Calcium and PTH receptors overlap in the kidneys, so PTH directly affects calcium metabolism. PTH increases kidney glomerular filtrate calcium. In bones, PTH is both catabolic and anabolic. Early action releases calcium from bones into the blood, whereas late action involves reabsorption and bone reconstruction. Among the two bone cell types, osteoblasts interact with PTH. PTH receptors may be absent in osteoclasts. PTH indirectly improves bone calcium absorption through calcitriol [57, 58]. Since various automated immunological tests with outstanding analytical performance are available, regular clinical practice now routinely measures parathyroid hormone (PTH). This measurement is difficult. PTH concentration is crucial for diagnosing calcium/phosphorus metabolism problems and monitoring chronic renal disease patients. We must interpret PTH in conjunction with a serum calcium concentration to determine its suitability for the calcemic state. However, practitioners now recognize that normal calcium and high PTH, or high or low calcium with normal PTH, are common in practice [59]. The anabolic therapies teriparatide (PTH 1-34) and full-length parathyroid hormone (PTH 1-84) were

helpful in treating severe osteoporosis [60, 61]. Teriparatide and PTH, in contrast to the anti-resorptive, promote bone production, allowing bone reconstruction. This component of PTH is fascinating and challenging because its physiological purpose is to maintain blood ionized calcium levels. Teriparatide also contributes to bone formation in a condition known as dynamic bone disease. Dynamic bone disease [62]. Low osteoblast numbers and PTH levels lead to low bone turnover and mineralization deficiencies. Parathyroid cancer, which causes primary hyperparathyroidism, typically leads to bone loss. Research found that PTH vaccination elicits an antibody response against increased PTH levels, lowering blood PTH and calcium levels [63].

Calcium (Ca)

Calcium is the body's most prevalent cation and sixth most frequent inorganic element. The skeleton stores ≈99% of the body's calcium, making it a practically endless storehouse. Extracellular calcium makes up a modest portion of body calcium (Figures 1–5). We ionize about 47% of extracellular calcium, protein-bind about 46%, and complex the remaining calcium with tiny ions. Albumin binds to most protein-binding calcium (75%) [64]. Thus, the extracellular compartment contains fewer than 1% ions. Calcium (Ca) ions are intracellular messengers that contribute to muscle contraction, neurotransmission, enzyme and hormone release, blood coagulation, and other processes [65]. Calcium regulates gene expression, secretion, and muscle contraction. These functions regulate cell development, proliferation, and death. In bones, kidneys, and intestines, parathyroid hormone (PTH) and $1,25(OH)_2D_3$ closely regulate extracellular free Ca. This review includes recent discoveries on Ca homeostasis processes [66].

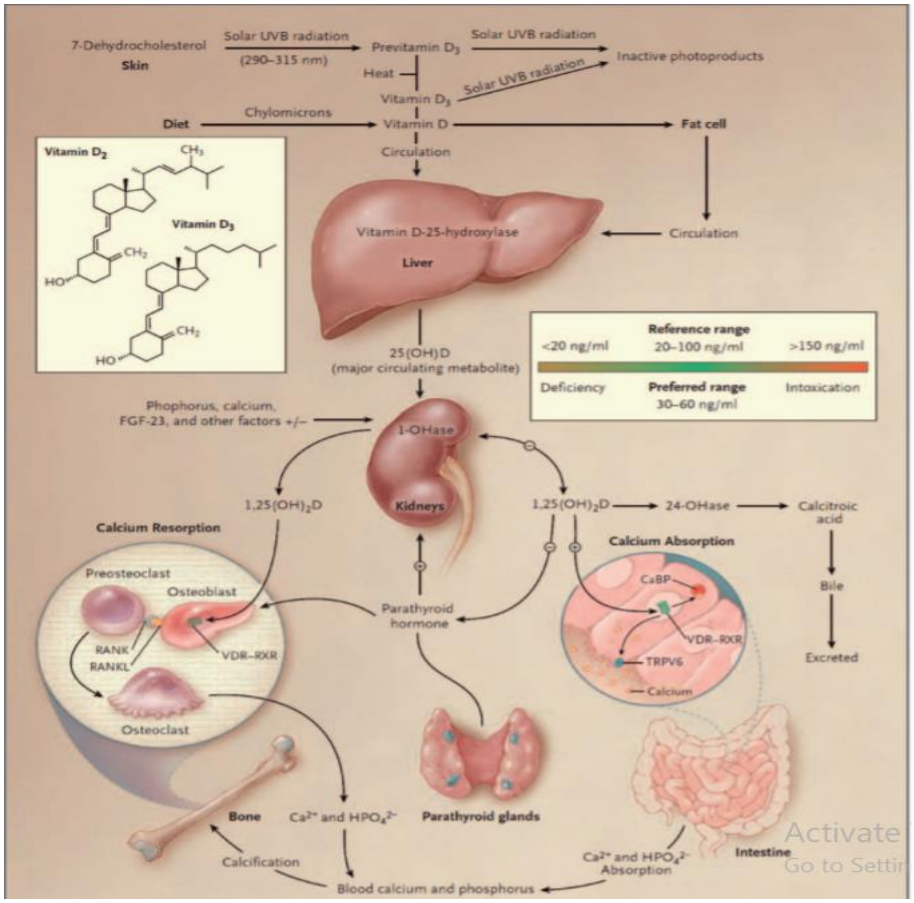

Fig 5: Calcium and vitamin D homeostasis is traditional. The metabolism of vitamin D. The skin's UV Blight converts 7-dehydroxycholesterol to vitamin D3. In the liver, 25-hydroxylase converts vitamin D3 into 25-OHD. The renal 1a-hydroxylase converts 25-hydroxyvitamin D3 (25-OHD) to its hormonal metabolite, 1α, 25-dihydroxyvitamin D3 (1, 25(OH)2D3). 1α-hydroxylase. Parathyroid hormone stimulates the rate-limiting enzyme, while 1, 25(OH)2D3 inhibits it. 1-OH2D3 accelerates the catabolism of 25-OHD and 1, 25(OH)2D3 by metabolizing them through 24-OHase. 1,25(OH)2D3 regulates calcium and phosphate in the kidney, gut, and bone. The hormone performs a variety of non-calcemic functions. The hormone performs several non-calcemic actions [67].

Genetic predisposition of Osteoporosis

Genetic predisposition, old age, gender, immobility, and other risk factors. In twin and family investigations, genome-wide association studies have searched for a link between BMD and osteoporosis. A meta-analysis of genome-wide association studies found 56 low-BMD loci and 14 fracture-risk loci [20, 22].

ZBTB40 (Zinc Finger and BTB Domain Containing 40) Gene: The goal of this study was to identify genetic regulators of osteoblast mineralization, a component of bone remodeling. The researcher did the first cell-specific genome-wide association study of osteoblast function using this trait after showing that osteoblast mineralization is inherited. The research aimed to minimize environmental variation and eliminate extrinsic physiological influences that affect osteoblast function [68]. Then, the researcher used reverse genetic methods to confirm that the previously under-characterized gene, Zbtb40, played a role in osteoblast mineralization ex vivo. Their work shows that the

methods are efficient for finding genes that impact osteoblast function [8]. Additionally, studies show that the osteoporosis GWAS lead SNPs rs34920465, rs6426749, and rs7524102 at 1p36 can modulate ZBTB40 expression through differential binding of bone metabolism-related transcription [69].

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