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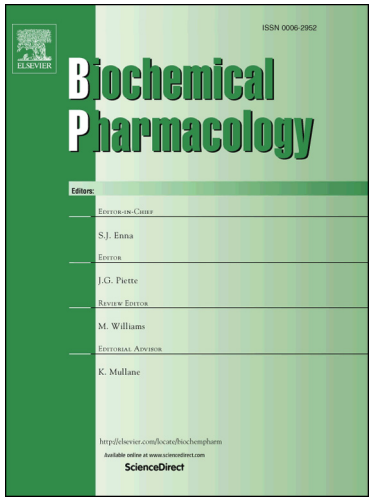

Review

Vitamin D and evolution: pharmacologic implications

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Vitamin D and evolution: pharmacologic implications

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ABSTRACT

Vitamin D_3 is produced non-enzymatically when the cholesterol precursor 7-dehydrocholesterol is exposed to UV-B, *i.e.*, evolutionary the first function of the molecule was that of an UV-B radiation scavenging end product. Vitamin D endocrinology started when some 550 million years ago first species developed a vitamin D receptor (VDR) that binds with high affinity the vitamin D metabolite 1α,25-dihydroxyvitamin D_3. VDR evolved from a subfamily of nuclear receptors sensing the levels of cholesterol derivatives, such as bile acids, and controlling metabolic genes supporting cellular processes, such as innate and adaptive immunity. During vertebrate evolution, the skeletal and adaptive immune system showed in part interesting synchronous development although adaptive immunity is evolutionary older. There are bidirectional osteoimmune interactions between the immune system and bone metabolism, the regulation of both is under control of vitamin D. This diversity of physiological functions explains the pleiotropy of vitamin D signaling and opens the potential for various pharmacological applications of vitamin D as well as of its natural and synthetic derivatives. The overall impact of vitamin D on human health is demonstrated by the fact that the need for its efficient synthesis served in European hunter and gatherers as an evolutionary driver for increased 7-dehydrocholesterol levels, while light skin was established far later *via* populations from Anatolia and the northern Caucasus entering Europe 9000 and 5000 years ago, respectively. The later population settled preferentially in northern Europe and we hypothesize that that the introduction of high vitamin D responsiveness was an essential trait for surviving dark winters without suffering from the detrimental consequences of vitamin D deficiency.

KEYWORDS

Vitamin D; evolution; metabolism; immune system; DHCR7

ABBREVIATIONS

$1,25(OH)_2D_3$	1α,25-dihydroxyvitamin D_3
$25(OH)D_3$	25-hydroxyvitamin D_3
CAMP	cathelicidin
CAR	constitutive androstane receptor, official gene symbol NR1I3
CYP	cytochrome P450
DHCR7	7-dehydrocholesterol reductase
FBP1	fructose-bisphosphatase 1
PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4
FXR	farnesoid X receptor, official gene symbol NR1H4
GC	GC vitamin D binding protein
HLA	human leukocyte antigen
LBD	ligand-binding domain
LXR	liver X receptor, official gene symbols NR1H3 (α) and NR1H2 (β)
OCA2	oculocutaneous albinism type II
PBMC	peripheral blood mononuclear cells
PXR	pregnane X receptor, also called NR1I2
RANKL	receptor activator of nuclear factor-κB ligand, official gene symbol TNFSF11
SLC	solute carrier family
SNP	single nucleotide polymorphism
TLR	toll-like receptor
TNFSF11	TNF superfamily member 11
VDR	vitamin D receptor

Introduction

Evolution is the process of changing heritable traits, such as anthropomorphic properties like height and eye color (1) as well as physiological characteristics like lactose tolerance (2) and risk for developing type 2 diabetes (3), of a population over a larger number of generations. These phenotypic variations are based on the function and expression of genes, *i.e.*, the genotype, that are passed from parents to offspring. In a given population the respective traits often exist in many variations causing different levels of biological fitness, *i.e.* viability, mating success and fertility. The process of natural selection for the biologically most successful offspring, as first formulated by Darwin (4) and Wallace (5), lead to that some traits become more rare and others more common. In parallel, random occurrence of mutation and recombination results in neutral evolution causing genetic drifts (6). For example, in humans there are approximately 50 base pair changes per generation (7). The confrontation with different selection pressures influences which genes persist. On every level of biological organization, such as populations, individual organisms, signaling and metabolic pathways as well as molecules, these evolutionary processes produce a rise of biodiversity. The most favorable traits in relation to given environmental conditions are selected, in order to reach most efficient reproduction and survival. Thus, traits associated with increased fitness are referred to as adaptations to the respective environment. “Nothing in biology makes sense except in the light of evolution” (8) is an important statement sorting all biologically processes by their relevance in the context of evolution.

After the creation of the Earth some 4.6 billion years ago, it took at least 700 million years until first life evolved on the basis of combining the elements information (nucleic acids), metabolism (providing energy and biomolecules) and membranes (separation from environment) (**Fig. 1**). Membranes are very important, since they i) separate metabolic pathways into compartments, ii) enable to build electrochemical gradients and iii) allow information to be individualized. The latter allows the cellular system to become selfish, which is an essential condition allowing natural selection during evolution (9). Some 2.1 billion years ago after the “Great Oxidation Event” the first eukaryotic organisms evolved

(10). The rise of atmospheric oxygen enabled the synthesis of the molecule cholesterol as a special component of eukaryotic membranes as well as a precursor of important biomolecules including vitamin D_3 (10) (**Fig. 2**). Thus, without oxygen there is no cholesterol production and without cholesterol no vitamin D synthesis.

This review focuses on the evolution of vitamin D, its endocrine system and its physiological actions. We will demonstrate that the pleiotropy of vitamin D signaling has an evolutionary origin, the understanding of which will allow a better use of the pharmacological potential of vitamin D and its derivatives.

Pre-endocrine functions of vitamin D

UV radiation is an important motor of evolution, as moderate levels induce DNA mutations that may result in beneficial phenotypic changes, while too much of UV has deleterious effects (11). In this context, it is interesting that the secosteroid vitamin D_3 is created in a non-enzymatic reaction, when the direct precursor of cholesterol, 7-dehydrocholesterol, is exposed to UV-B (290-320 nm) (**Fig. 2**). The activity of the enzyme 7-dehydrocholesterol reductase (DHCR7) determines the levels of its substrate 7-dehydrocholesterol in eukaryotic membranes (12). 7-dehydrocholesterol absorbs the energy of the UV-B radiation *via* the double bound between C7 and C8 leading to the thermodynamically unstable molecule pre-vitamin D_3, which is characterized by an open B ring between C9 and C10 (13). Under the catalysis of heat, pre-vitamin D_3 then rapidly isomerizes into vitamin D_3. The fact that vitamin D_3 synthesis does not need any enzyme suggests that vitamin D_3 already exists as long as species, such as the algae, produce cholesterol (14), *i.e.*, at least some 1.2 billion years.

Continuous UV-B exposure also converts pre-vitamin D_3 into lumisterol, tachysterol and other photoproducts, which, however, do not lead to any compounds having endocrine function (15). In this way, vitamin D_3 precursors and metabolites absorb and dissipate the energy of UV-B radiation by the rearrangement of double bonds. This represents an UV scavenging process, which protects species from excessive DNA mutations (16). Thus,

vitamin D can serve as a sunscreen that protects sensitive molecules, such as DNA and proteins, from mutagenesis and degradation. For this reason, vitamin D accumulates in photosynthetic phytoplankton without having any endocrine function in these species. However, phytoplankton, *i.e.*, primarily algae, is the starting point of the marine food chain (14, 17), which leads to the accumulation of vitamin D in their endpoints, such as in liver of cod ("cod liver oil") (18).

Nuclear receptors as metabolic sensors

Constantly changing environmental conditions have been ever since the main driver of evolution. This gives species that are able to adapt easily to their environment an important selective advantage. Since metabolism in general, and energy metabolism in particular, is a central function of life, it is obviously under tight evolutionary control (19). The well-known example of the lactose operon in prokaryotes (20) demonstrates that the control of gene regulation by transcription factors is commonly driven by the need to sense the level of key nutritional molecules, such as sterols and fatty acids. Accordingly, with nuclear receptors a special type of transcription factors emerged in eukaryotes, the members of which took as their main task to sense the level of macro- and micronutrients and to regulate metabolic pathways. In an adaption process involving a few rounds of gene duplications the large nuclear receptor superfamily evolved, which is represented by 48 members in the human genome (21).

The members of the NR1H subfamily, liver X receptor (LXR) α and β as well as farnesoid X receptor (FXR), have the same ancestor as those of the NR1I subfamily comprising VDR, pregnane X receptor (PXR) and constitutive androstane receptor (CAR) (22) (**Fig. 3**). All six receptors act as sensors for cholesterol derivatives, such as oxysterols, bile acids and 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). The fact that FXR, PXR, CAR and VDR are binding and activated by bile acids (23-26) suggests that their common ancestor acted as a sensor for these cholesterol derivatives. In the course of evolution, the LBD of LXRs adapted to bind oxysterols, while the LBD of VDR specialized for the binding of $1,25(OH)_2D_3$. Since

oxysterols occur in micromolar concentrations, there was no need to provide LXR's LBD with higher affinity for its ligands, while the sub-nanomolar concentration of $1,25(OH)_2D_3$ required a specialized adaption of VDR's LBD. Thus, from the subfamilies NR1H and NR1I only VDR evolved to a typical endocrine nuclear receptor, which is comparable to the nuclear receptors for the sex steroids estrogen, progesterone and testosterone.

An interesting triangular relationship between receptors, their ligands and the enzymes and transporters handling these ligands suggests that the genes encoding for specific metabolic enzymes and transporters control the concentration of metabolites, which act as specific ligands for nuclear receptors regulating these genes (27, 28) (**Fig. 4**). For example, the cytochrome P450 (CYP) 27B1 (*CYP27B1*) gene encodes for an 1α-hydroxylase, which converts 25-hydroxyvitamin D_3 ($25(OH)D_3$) into $1,25(OH)_2D_3$ (**Fig. 2**); this gene is down-regulated by $1,25(OH)_2D_3$-activated VDR. In contrast, the *CYP24A1* gene, which is encoding for a 24-hydroxylase inactivating $1,25(OH)_2D_3$, is a prominent up-regulated VDR target gene (**Fig. 4**). There are many comparable examples and all together they provide strong evidence for a co-evolution of metabolic enzymes, transporters and the transcription factors regulating them. In parallel, the structural comparison of the ligand-binding domains of orphan, adopted orphan and endocrine nuclear receptors (29) in combination with the phylogenetic tree of their evolutionary relationship (30) indicates that probably the evolutionary first function of nuclear receptors was the regulation of metabolism. Thus, many nuclear receptors, including VDR, are still majorly involved in the control of metabolic pathways. This explains why transcriptome-wide analysis of different vitamin D-stimulated tissues revealed many metabolic genes as VDR targets (31-33).

Endocrinology of vitamin D

In the liver the enzymes CYP2R1 and CYP27A1 convert vitamin D_3 into $25(OH)D_3$, which is the most stable and abundant vitamin D metabolite in serum (**Fig. 2**). Kidneys and other human cell types, such as immune cells and keratinocytes, express the enzyme CYP27B1, which mediates further hydroxylation at position C1. The resulting molecule $1,25(OH)_2D_3$

binds with high affinity (K_D = 0.1 nM) to the nuclear receptor VDR. Thus, the enzymes CYP2R1, CYP27A1 and CYP27B1 as well as the transcription factor VDR are essential components of the endocrinology of vitamin D.

Comparative genomics analysis indicated that the boneless early vertebrate sea lamprey (*petromyzon marinus*), which evolved during the "Cambrian explosion" some 550 million years ago (**Fig. 1**), is the first known species expressing a VDR that shows sufficient homology (60%) with the ligand-binding domain (LBD) of human VDR, so that it binds $1,25(OH)_2D_3$ at sub-nanomolar concentrations (34) (**Fig. 5**). From these times on basically all vertebrates, such as bony fish, amphibians, reptiles, birds and mammals, have a VDR with high affinity for $1,25(OH)_2D_3$ (35). Moreover, $1,25(OH)_2D_3$ is found in the plasma of lamprey in concentration similar to that in mammals, *i.e.*, enzymes for its production had also evolved (34). Thus, already some 150 million years before first species left the ocean the core proteins of vitamin D endocrinology, VDR, CYP2R1 and CYP27B1, evolved in a softbody vertebrate without calcified skeleton and teeth. Accordingly, rather than the well-known regulation of calcium homeostasis was the regulation of metabolism, such as the control of detoxification via vitamin D-triggered CYP enzymes, the first task of vitamin D endocrinology (36).

Evolution of the immune system

Life on Earth started approximately 3.9 billion years ago and evolved in the pathogen-rich aquatic environment (**Fig. 1**) (37). Ever since the early history of Earth, organisms were challenged by harming invaders. This created a strong evolutionary pressure for defense mechanisms recognizing between self and non-self. The immune system is subdivided into the evolutionary older innate immunity, which is common to all organisms and involves a series of barriers and non-specific responses to pathogens, and adaptive immunity, which occurs only in vertebrates and shows highly specific reactions to them (38, 39). The "Big bang" emergence of almost all features of human adaptive immunity occurred 500 million years ago in ectothermic (cold-blooded) cartilaginous fishes (**Fig. 6**) (34, 40).

Vitamin D is known to have a large impact on innate immunity, for example, by regulating the expression of antimicrobial peptides, such as cathelicidin (CAMP) (41). Moreover, the critical co-receptor of toll-like receptor (TLR) 4, CD14, is a prominent vitamin D target gene in monocytes (42). These actions of vitamin D on innate immunity had already been established in fish (43). Furthermore, antigen-presenting dendritic cells are another cellular component of innate immunity that is very responsive to vitamin D (44). Dendritic cells interact with T cells, *i.e.*, they modulate the actions of adaptive immunity. Thus, the vitamin D responsiveness of the evolutionary older part of the immune system nowadays still has a major impact on efficient pathogen defense. Interestingly, the human leukocyte antigen (HLA) locus cluster on human chromosome 6 was detected as a "hotspot" of epigenome-wide responsiveness of chromatin accessibility to an oral vitamin D_3 bolus (45). In addition, the proper action of vitamin D on cells of the adaptive immune system is important for the prevention of autoimmune diseases (46).

Evolution of vitamin D immuno-metabolic functions

The proliferation of immune cells as well as their action in defense and tissue repair requires high levels of energy metabolism (47). For example, the imprinting of dendritic cells with tolerogenic properties involves the reprogramming of their glucose metabolism via up-regulation of the vitamin D target gene *PFKFB4* (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4) (48). FBP1 (fructose-bisphosphatase 1) is another metabolic enzyme that is encoded by one of the most responsive primary vitamin D target genes in monocytes (42). Thus, the ability of vitamin D to regulate energy metabolism supports key functions of the immune system, *i.e.*, cellular metabolism and immunity are closer linked as initially assumed.

When 385 million years ago first species moved on land, the high complexity of terrestrial microbes provided additional evolutionary pressure (49), driving the development of an even more sophisticated immune system and the highly energetically costly endothermy (50). At the same time, leaving the calcium-rich ocean required a tight control system for calcium homeostasis and a skeleton adapted for locomotion in the new terrestrial environment (51).

The formation of mineralized tissue was a major leap in vertebrate evolution. Cartilaginous fishes *(Chondrichthyes)* like sharks and rays produce calcified cartilage and dermal bone, such as teeth, fin spines and dermal denticles (40). Interestingly, in this species also the adaptive immune system emerged (38, 40). A next step was the development of the complex system of endochondral ossification, *i.e.*, replacing the cartilage in bony vertebrates (40). Bony fishes *(Osteichthyes)*, which are the largest class of all vertebrates, developed in their aquatic environment a skeleton based on calcium, which is abundant (approximately 10 mM) in the ocean. In contrast, on land animals had to evolve a regulatory system, in order to tightly control the concentration of calcium in intra- and extracellular compartments. Bone serves as an internal reservoir of calcium, which is able to balance variations in nutritional supply of the mineral. Correspondingly, from amphibians onward bone is regulated more and more dynamically. In mammals, $1,25(OH)_2D_3$ antagonizes the peptide hormone PTH (parathyroid hormone) in its function concerning calcium homeostasis and bone turnover. In this context, proteins encoded by vitamin D target genes, such as the calcium channel TRPV6 (transient receptor potential cation channel subfamily V member 6) as well as the calcium binding proteins calbindin 1 and 2, control the uptake and transport of calcium in the intestine and regulate the resorption of the mineral in the kidneys (52).

Osteometabolism and osteoimmunity

The balanced action of bone cells, such as osteoblasts, osteoclasts and osteocytes, is maintaining bone and mineral homeostasis. $1,25(OH)_2D_3$ regulates the activity of bone resorbing osteoclasts, which are bone-specific macrophages. Bone mineralization is under the control of non-collagenous proteins, such as osteopontin and bone sialo protein (also called osteocalcin), which are encoded by vitamin D target genes. To surprise of many, the latter protein can also regulate glucose metabolism and energy expenditure (53), which emphasizes the role of vitamin D in “osteometabolism”, a term created in analogy to “immunometabolism”.

Interestingly, bone and immune systems are also linked on multiple levels leading to the concept of osteoimmunity (49). The interior of large bone, the bone marrow, provides a niche for hematopoietic stem cells, *i.e.*, it is the origin of basically all cells of the immune system. The bone marrow harbors myeloid and lymphoid progenitors as well as mature immune cells, such as B and T cells, neutrophils and macrophages, *i.e.*, it is a place in the body where cells are most effectively shielded from UV and other radiation and in parallel supported by a high calcium concentration.

Hematopoietic stem cells and bone cells are exposed to the same microenvironment and influence each other in their development. For example, bone resorbing osteoclasts relate in their function very much to other tissue repairing macrophages, which derive from monocytes of the innate immune system. Both bone and immune cells rely on the communication via signaling molecules, such as the cytokine RANKL (receptor activator of nuclear factor-κB ligand), which is encoded by the vitamin D target gene *TNFSF11* (TNF superfamily member 11) (54). RANKL and its receptor RANK are important for the genesis of osteoclasts but play also a role in lymph nodes and the medulla of the thymus. Already in cartilaginous fish, in which the first components of adaptive immunity developed (**Fig. 6**), RANKL signaling is used. Interestingly, in bony fish, such as zebrafish (*danio rerio*), vitamin D increases the growth of hematopoietic cells during embryogenesis, *i.e.*, ligand-activated VDR had already rather early in evolution a central role for the production of hematopoietic stem cells (55). Thus, already before species moved on land vitamin D affected the cell cycle, cell polarity and lineage commitment, *i.e.*, it regulates the number of hematopoietic stem cells in the embryo and therefore acts on the foundation of the hematopoietic tree. In this context, the chemokine interleukin 8 (*CXCL8*) is an important vitamin D target gene (56), which is known to stimulate the cell-autonomous survival and proliferation of hematopoietic stem cells (57). In parallel, bone-resorbing osteoclasts, which are tissue-resident macrophages, emerged in the same species. Moreover, transcriptome-wide analysis indicated that a large proportion of the some 1000 vitamin D target genes in zebrafish are related to cellular metabolism (58). Thus, the in part synchronous development of immune and skeletal systems represents a co-

evolution on the basis of the metabolic function of the VDR, at the end of which vitamin D turned out to act as an important regulator on both sides.

Skin lightening in Europe

Like today's chimpanzees, hominids initially had pale skin below their dark fur. When they lost their body hair about 2 million years ago, in order to improve their physical performance *via* more efficient sweating, they developed intensively pigmented skin, probably for protecting from sunburn and UV-induced cancer (59).

Approximately 300,000 years ago anatomically modern humans (*homo sapiens*) developed (60). When some 50-75,000 years ago dark skinned modern humans started to spread all over the planet, some populations turned back to their pale skin, *i.e.*, their reduced again their skin pigmentation (61). Therefore, today's human populations vary a lot concerning the color of their skin, eyes and hair, which is possibly based on adaptive changes after migrating out of Africa.

The key pigment molecule is melanin that is produced within melanosomes of melanocytes located in skin, eyes and hair follicles (62). Polymorphisms in genes encoding for key proteins in melanosome genesis and melanin synthesis correlate with variations of skin, eyes and hair color. For example, northern Europeans often have light hair, light skin and blue eyes, while intense pigmentation is mostly found close to the equator and at high elevations, *i.e.*, in geographic regions of intensive UV-B exposure. Importantly, despite dark pigmentation the intensive sun at the equator allows sufficient vitamin D_3 synthesis, as demonstrated by the world's last traditionally living hunter and gatherers, the Tanzanian Hadza who have $25(OH)D_3$ serum levels of 109 nM in average (63). Lower UV-B exposure at high latitudes could favor reduced skin pigmentation, in order to allow sufficient vitamin D_3 synthesis (64). Accordingly, derived single nucleotide polymorphisms (SNPs) of the gene solute carrier family 24 member 5 (*SLC24A5*), which encodes for a transmembrane protein regulating the calcium concentrate ion in melanosomes, accumulated in Europeans. In particular, the non-

synonymous SNP rs1426654, which causes an alanine to threonine exchange at position 111 of the protein (Ala111Thr), monitors a strong difference between populations (**Fig. 7A**).

Interestingly, the SNP is located within a large (78 kb) haplotype block suggesting that this skin lightening causing variation derives from a single carrier, who may have lived some 10,000 years ago in the Fertile Crescent (65). The second strongest polymorphism associated with low skin pigmentation in Europeans is the SNP rs16891982, which causes a phenylalanine to leucine exchange at position 374 (Phe374Leu) of an ion transport protein encoded by the *SLC45A2* gene (**Fig. 7B**). Thus, the light skin color of Europeans is primarily based on non-synonymous variations of the proteins SLC24A5 and SLC45A2 (66). This raises the question, whether skin lightening in Europeans was driven by lower light conditions at higher latitudes and its deleterious consequences, such as immunosuppression and musculoskeletal diseases caused by vitamin D deficiency.

The genetic history of Europeans

Homo sapiens arrived some 45,000 years ago in Europe, where they got into contact with Neanderthal type *homo erectus* that already had moved out of Africa to Europe at least some 400,000 years earlier (67). The Neanderthal line disappeared shortly after the arrival of the modern humans by interbreeding, *i.e.*, they were simply outnumbered by the new arrivals. The contribution of the Neanderthal's genome to that of modern Europeans decreased from 5 to 2% within the past 40,000 years, a result of natural selection against Neanderthal DNA (67).

Interestingly, the European hunter and gatherers kept for nearly 40,000 years their dark skin but already developed blue eyes due to variations of their *OCA2* (oculocutaneous albinism type II) gene locus causing iris depigmentation (68) (**Fig. 8A**). With the exception of populations living at the coast and eating primarily fish, such as the Inuit, average human diet is a poor source of vitamin D_3. Therefore, the cutaneous synthesis of vitamin D_3 is still the preferred way of supplying the human body with the molecule. It can be assumed that in the past humans exposed larger percentages of their skin far longer to sun than nowadays, *i.e.*,

vitamin D deficiency may not have been as extreme as observed with working class people in England of the 19th century, where rickets was a very common disorder of children, also called the "English disease" (69, 70). Moreover, these European hunter and gatherers carried SNPs in the regulatory regions of their *DHCR7* gene, such as rs7940244, rs7944926 and rs12785878 resulting in reduced protein expression (71, 72) (**Figs. 7C and D**). Reduced DHCR7 enzyme activity leads to increased concentrations of 7-dehydrocholesterol in the skin and more efficient synthesis of vitamin D_3. Genome-wide association studies confirmed that the *DHCR7* gene significantly contributes to 25(OH)D_3 serum levels (73). Moreover, the *DHCR7* gene had been identified as risk locus for the autoimmune disease multiple sclerosis (74), which is highly associated with vitamin D deficiency. Thus, evolutionary adaption of DHCR7 expression is an efficient way avoiding vitamin D deficiency and its medical consequences. In addition to the *DHCR7* gene, the loci of the genes *CYP2R1*, *CYP24A1* and *GC* (GC vitamin D binding protein) also show significant association with the vitamin D status (73). However, the SNPs of none of the three genes are enriched in populations living at higher latitudes, *i.e.*, in contrast to the *DHCR7* gene there is no sign of evolutionary adaption to human migration.

Based on haplotype diversity of microsatellites the skin lightening polymorphisms of the genes *SLC24A5* and *SLC45A2* were estimated to have occurred in Europeans already some 11,000 to 19,000 years ago (75). However, a cutting-edge genome-wide analysis of ancient DNA from 230 West Eurasians who lived between 8500 and 2300 years ago, constructed more precisely the evolution and timing of trait changes within European populations (72). Accordingly, in a period of 8400 to 6000 years ago farmers originating from northwestern Anatolia spread all over Europe (**Fig. 8B**). This was the start of the Neolithic revolution in Europe, which was characterized by the use of polished stone tools and pottery, a more sedentary lifestyle and the domestication of certain animal and plant species, i.e., these Anatolian farmers introduced the concept of agriculture to the hunter and gatherers. By interbreeding with the indigenous hunter and gatherers the Anatolian farmers also brought their *SLC24A5* gene variant for lighter skin into Europe. The Anatolian farmers had rather

short body stature, brown eyes and lived preferentially in southern Europe. Some 5000 years ago with the Yamnaya pastoralists from the Eurasian steppe (northern Caucasus) a second population wave arrived who brought the horse, the wheel and Indo-European languages to Europe (76-79). Yamnaya had high body stature, brown eyes and lighter skin due to a *SLC45A2* SNP in addition to the *SLC24A5* variant. They settled preferentially in northern Europe. Differences in the relative admixture of the three ancient populations (hunter and gatherers, Anatolian farmers and Yamnaya pastoralists, **Fig. 8A**), explain the variation in skin pigmentation, eye color, body stature and many other traits of present Europeans.

The original population of anatomically modern humans solved the problem of inefficient vitamin D_3 synthesis in their sub-Saharan dark skin at higher latitudes by selecting for genetic variants that result in lower DHCR7 activity and respective higher 7-dehydrocholesterol levels in the skin. Also genome-wide studies of ancient bones (76, 79-81) confirmed that the original west European population of *homo sapiens* just got lighter skin when 8800 and 5000 years ago external populations from northwestern Anatolia and the northern Caucasus, respectively, introduced both variants of the ion transport genes that cause reduced skin pigmentation. Thus, in contrast to the common hypothesis (82) there is no evidence of a direct evolutionary effect of vitamin D on skin lightening of Europeans, but it is obvious that they benefited from an elevated vitamin D status.

The *SLC24A5* rs1426654 allele has the single largest effect on skin lightening of the variants identified to date (76). Intriguingly, a negative selection against light pigmentation variants in the high UV environment of South Asia is observed only for the derived *SLC45A2* rs16891982, but not the *SLC24A5* allele (83) (**Fig. 7A**). Similarly, some eastern African and Khoe-San populations of South Africa got their lighter skin just 2000 years ago through this *SLC24A5* allele *via* a back-to-Africa migration event (84). This suggests the lighter skin allele *SLC24A5* rs1426654 and/or its whole haplotype block may carry a beneficial effect in addition to the improved vitamin D synthesis at higher latitudes.

Pharmacological implications

In the course of evolution the biologically active form of vitamin D, $1,25(OH)_2D_3$, became a pleiotropic compound that regulates not only genes involved in cellular metabolism but also in calcium homeostasis and bone mineralization as well as in innate and adaptive immunity (**Fig. 9A**). A pharmacologic application of $1,25(OH)_2D_3$ or its synthetic analogs over a prolonged time period is often limited by the risk of causing hypercalcemia (85). Therefore, the main aim for developing vitamin D analogs is to dissociate their function, *e.g.*, to activate the immune system without increasing calcium absorption. For a few analogs, such as topically applied calcipotriol (marketed as "Dovonex", "Daivonex" and "Psorcutan") against psoriasis, this had been successful (86). However, the molecular basis of the achieved selectivity is not based on any differential activation of the VDR (87) but on the pharmacokinetic profile of the compound leading to rapid degradation (88). Thus, like other important nuclear receptors with pleiotropic function, such as those for estrogen and cortisol, a dissociated activation is difficult to achieve purely on the basis of variations in the receptor-ligand interaction.

The pleiotropic actions of vitamin D also implies that the molecule has a large impact on the proper function of the human body, *i.e.*, health may be compromised when the disease preventive action of vitamin D_3 is not optimal. Before the migration out of Africa, humans were every day exposed to UV-B inducing vitamin D_3 synthesis, *i.e.*, their body had adapted to a constantly high vitamin D status of 100 nM or more (63). The migration toward North changed the environmental conditions and at a latitude above 37 °N significant seasonal changes in sun exposure occurred. Dark skinned European hunter and gatherers adapted to these conditions by reducing their DHCR7 activity, but it is likely that there were additional mechanisms of adaptation. For example, humans can be distinguished into high, mid and low responders to vitamin D (89, 90). Based on this observation, for high vitamin D responders a rather low vitamin D status is still sufficient to activate all vitamin D target genes (91). In contrast, low vitamin D responders will require $25(OH)D_3$ serum concentrations that cannot be reached via sun exposure, in particular not during winter and at high latitude. This suggests

that, in particular in northern Europe, there may have been a selection for high vitamin D responders. So far, it is not known which genomic loci mediate a high or low responsiveness to vitamin D. Therefore, it can only be speculated that the Yamnaya pastoralists, who were themselves admixed with hunter-gatherers from Siberia and had a rather dominant effect on the phenotype of northern Europeans (66, 92), may have brought a higher vitamin D sensitivity to Europe (**Fig. 9B**). In this comparison, the European hunter and gatherers as well as the Anatolian farmers may have been mid and low vitamin D responders, respectively. The better vitamin D responsiveness of individuals of the Yamnaya population may have provided them with a more robust immune system for better resistance against infections and surviving the cold dark winters of northern Europe. Thus, individuals of today's human populations may refer more to their genetic admixture and its phenotypic consequences. Concerning vitamin D this suggests a personalized-nutrition approach by determining the vitamin D response index and supplement with appropriate daily vitamin D_3 doses (10-100 µg) for reaching the individual's optimal vitamin D status or to supplement everyone to 25(OH)D_3 serum levels of the times before the migration out of Africa (more than 100 nM).

Conclusion

The evolutionary story of vitamin D started more than a billion years ago as an inert molecule being an end product of a photochemical reaction. Since more than 500 million years vitamin D gained *via* the nuclear receptor VDR endocrine functions. The role of vitamin D in bone health is well known but represents only one aspect of the pleiotropic functional profile of the molecule. The immune-regulatory function of vitamin D developed even earlier to its role in calcium homeostasis and seems to have a comparable impact. Moreover, as the evolutionary history indicates and also genome-wide experimental data demonstrate, the original and likely still central function of vitamin D is to regulate genes involved in energy metabolism. Due to the since ancient times preferred non-marine diet of humans (81), different genetic admixture and the rather recent indoor lifestyle, vitamin D_3 became the most important supplement to compensate for insufficient sun exposure.

References

1. Sturm RA, Duffy DL. Human pigmentation genes under environmental selection. Genome Biol 2012;13:248.
2. Gerbault P, Liebert A, Itan Y, Powell A, Currat M, Burger J, et al. Evolution of lactase persistence: an example of human niche construction. Philos Trans R Soc Lond B Biol Sci 2011;366:863-77.
3. Wells JCK. Body composition and susceptibility to type 2 diabetes: an evolutionary perspective. Eur J Clin Nutr 2017;71:881-9.
4. Darvin C. On the orgin of species. 1859.
5. Wallace R. On the tendency of varieties to depart indefinitely from the original type. 1858.
6. Kimura M. The neutral theory of molecular evolution: a review of recent evidence. Jpn J Genet 1991;66:367-86.
7. Campbell CD, Eichler EE. Properties and rates of germline mutations in humans. Trends Genet 2013;29:575-84.
8. Dobzhansky T. Nothing in biology makes sense except in the light of evolution. The American Biology Teacher 1973;35:125-9.
9. Melendez-Hevia E, Montero-Gomez N, Montero F. From prebiotic chemistry to cellular metabolism--the chemical evolution of metabolism before Darwinian natural selection. J Theor Biol 2008;252:505-19.
10. Sessions AL, Doughty DM, Welander PV, Summons RE, Newman DK. The continuing puzzle of the Great Oxidation Event. Current Biology 2009;19:R567-R74.
11. Hessen DO. Solar radiation and the evolution of life. Solar Radiation and Human Health 2008.
12. Prabhu AV, Luu W, Li D, Sharpe LJ, Brown AJ. DHCR7: A vital enzyme switch between cholesterol and vitamin D production. Prog Lipid Res 2016;64:138-51.
13. Holick MF. The cutaneous photosynthesis of previtamin D_3: a unique photoendocrine system. J Invest Dermatol 1981;77:51-8.
14. Martin-Creuzburg D, Merkel P. Sterols of freshwater microalgae: potential implications for zooplankton nutrition. Journal of Plankton Research 2016;38:865-77.
15. Holick MF, MacLaughlin JA, Doppelt SH. Regulation of cutaneous previtamin D3 photosynthesis in man: skin pigment is not an essential regulator. Science 1981;211:590-3.
16. Wacker M, Holick MF. Sunlight and Vitamin D: A global perspective for health. Dermatoendocrinol 2013;5:51-108.
17. Boyce DG, Lewis MR, Worm B. Global phytoplankton decline over the past century. Nature 2010;466:591-6.
18. Rajakumar K. Vitamin D, cod-liver oil, sunlight, and rickets: a historical perspective. Pediatrics 2003;112:e132-5.
19. Muller M, Mentel M, van Hellemond JJ, Henze K, Woehle C, Gould SB, et al. Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. Microbiol Mol Biol Rev 2012;76:444-95.
20. Monod J, Changeux JP, Jacob F. Allosteric proteins and cellular control systems. J Mol Biol 1963;6:306-29.
21. Escriva H, Bertrand S, Laudet V. The evolution of the nuclear receptor superfamily. Essays Biochem 2004;40:11-26.

22. Krasowski MD, Ni A, Hagey LR, Ekins S. Evolution of promiscuous nuclear hormone receptors: LXR, FXR, VDR, PXR, and CAR. Mol Cell Endocrinol 2011;334:39-48.
23. Makishima M, Lu TT, Xie W, Whitfield GK, Domoto H, Evans RM, et al. Vitamin D receptor as an intestinal bile acid sensor. Science 2002;296:1313-6.
24. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, et al. Identification of a nuclear receptor for bile acids. Science 1999;284:1362-5.
25. Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, et al. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. Proc Natl Acad Sci U S A 2001;98:3369-74.
26. Guo GL, Lambert G, Negishi M, Ward JM, Brewer HB, Jr., Kliewer SA, et al. Complementary roles of farnesoid X receptor, pregnane X receptor, and constitutive androstane receptor in protection against bile acid toxicity. J Biol Chem 2003;278:45062-71.
27. Honkakoski P, Negishi M. Regulation of cytochrome P450 (CYP) genes by nuclear receptors. Biochem J 2000;347:321-37.
28. Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. Nuclear receptors and lipid physiology: opening the X-files. Science 2001;294:1866-70.
29. Wurtz JM, Bourguet W, Renaud JP, Vivat V, Chambon P, Moras D, et al. A canonical structure for the ligand-binding domain of nuclear receptors. Nat Struct Biol 1996;3:87-94.
30. Robinson-Rechavi M, Garcia HE, Laudet V. The nuclear receptor superfamily. J Cell Sci 2003;116:585-6.
31. Seuter S, Neme A, Carlberg C. Epigenome-wide effects of vitamin D and their impact on the transcriptome of human monocytes involve CTCF. Nucleic Acids Res 2016;44:4090-104.
32. Pasing Y, Fenton CG, Jorde R, Paulssen RH. Changes in the human transcriptome upon vitamin D supplementation. J Steroid Biochem Mol Biol 2017;173:93-9.
33. Ferreira GB, Vanherwegen AS, Eelen G, Gutierrez AC, Van Lommel L, Marchal K, et al. Vitamin D_3 induces tolerance in human dendritic cells by activation of intracellular metabolic pathways. Cell Rep 2015.
34. Whitfield GK, Dang HT, Schluter SF, Bernstein RM, Bunag T, Manzon LA, et al. Cloning of a functional vitamin D receptor from the lamprey (Petromyzon marinus), an ancient vertebrate lacking a calcified skeleton and teeth. Endocrinology 2003;144:2704-16.
35. Kollitz EM, Zhang G, Hawkins MB, Whitfield GK, Reif DM, Kullman SW. Molecular cloning, functional characterization, and evolutionary analysis of vitamin D receptors isolated from basal vertebrates. PLoS One 2015;10:e0122853.
36. Bouillon R, Carmeliet G, Lieben L, Watanabe M, Perino A, Auwerx J, et al. Vitamin D and energy homeostasis-of mice and men. Nat Rev Endocrinol 2014;10:79-87.
37. Esteban MA. An overview of the immunological defenses in fish skin. ISRN Immunology 2012;2012:853470.
38. Flajnik MF. A cold-blooded view of adaptive immunity. Nat Rev Immunol 2018;18:438-53.
39. Cooper MD, Alder MN. The evolution of adaptive immune systems. Cell 2006;124:815-22.
40. Venkatesh B, Lee AP, Ravi V, Maurya AK, Lian MM, Swann JB, et al. Elephant shark genome provides unique insights into gnathostome evolution. Nature 2014;505:174-9.
41. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D_3. Faseb J 2005;19:1067-77.

42. Heikkinen S, Väisänen S, Pehkonen P, Seuter S, Benes V, Carlberg C. Nuclear hormone 1α,25-dihydroxyvitamin D_3 elicits a genome-wide shift in the locations of VDR chromatin occupancy. Nucleic Acids Res 2011;39:9181-93.
43. Dioguardi M, Guardiola FA, Vazzana M, Cuesta A, Esteban MA, Cammarata M. Vitamin D_3 affects innate immune status of European sea bass (Dicentrarchus labrax L.). Fish Physiol Biochem 2017;43:1161-74.
44. Bscheider M, Butcher EC. Vitamin D immunoregulation through dendritic cells. Immunology 2016;148:227-36.
45. Carlberg C, Seuter S, Nurmi T, Tuomainen TP, Virtanen JK, Neme A. In vivo response of the human epigenome to vitamin D: a proof-of-principle study. J Steroid Biochem Mol Biol 2018;180:142-8.
46. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 2006;296:2832-8.
47. Ganeshan K, Nikkanen J, Man K, Leong YA, Sogawa Y, Maschek JA, et al. Energetic trade-offs and hypometabolic states promote disease tolerance. Cell 2019;177:399-413 e12.
48. Vanherwegen AS, Eelen G, Ferreira GB, Ghesquiere B, Cook DP, Nikolic T, et al. Vitamin D controls the capacity of human dendritic cells to induce functional regulatory T cells by regulation of glucose metabolism. J Steroid Biochem Mol Biol 2019;187:134–45.
49. Tsukasaki M, Takayanagi H. Osteoimmunology: evolving concepts in bone-immune interactions in health and disease. Nat Rev Immunol 2019.
50. Wang A, Medzhitov R. Counting calories: the cost of inflammation. Cell 2019;177:223-4.
51. Bouillon R, Suda T. Vitamin D: calcium and bone homeostasis during evolution. BoneKEy Reports 2014;3:480.
52. van de Peppel J, van Leeuwen JP. Vitamin D and gene networks in human osteoblasts. Front Physiol 2014;5:137.
53. Ponzetti M, Rucci N. Updates on osteoimmunology: what's new on the cross-talk between bone and immune system. Front Endocrinol 2019;10:236.
54. Kim S, Yamazaki M, Zella LA, Meyer MB, Fretz JA, Shevde NK, et al. Multiple enhancer regions located at significant distances upstream of the transcriptional start site mediate RANKL gene expression in response to 1,25-dihydroxyvitamin D_3. J Steroid Biochem Mol Biol 2007;103:430-4.
55. Cortes M, Chen MJ, Stachura DL, Liu SY, Kwan W, Wright F, et al. Developmental vitamin D availability impacts hematopoietic stem cell production. Cell Rep 2016;17:458-68.
56. Ryynänen J, Carlberg C. Primary 1,25-dihydroxyvitamin D_3 response of the interleukin 8 gene cluster in human monocyte- and macrophage-like cells. PLoS One 2013;8:e78170.
57. Jing L, Tamplin OJ, Chen MJ, Deng Q, Patterson S, Kim PG, et al. Adenosine signaling promotes hematopoietic stem and progenitor cell emergence. J Exp Med 2015;212:649-63.
58. Craig TA, Zhang Y, McNulty MS, Middha S, Ketha H, Singh RJ, et al. Research resource: whole transcriptome RNA sequencing detects multiple 1α,25-dihydroxyvitamin D_3-sensitive metabolic pathways in developing zebrafish. Mol Endocrinol 2012;26:1630-42.
59. Greaves M. Was skin cancer a selective force for black pigmentation in early hominin evolution? Proc Biol Sci 2014;281:20132955.

60. Hublin JJ, Ben-Ncer A, Bailey SE, Freidline SE, Neubauer S, Skinner MM, et al. New fossils from Jebel Irhoud, Morocco and the pan-African origin of Homo sapiens. Nature 2017;546:289-92.
61. Sabeti PC, Schaffner SF, Fry B, Lohmueller J, Varilly P, Shamovsky O, et al. Positive natural selection in the human lineage. Science 2006;312:1614-20.
62. Pavan WJ, Sturm RA. The genetics of human skin and hair pigmentation. Annu Rev Genomics Hum Genet 2019.
63. Luxwolda MF, Kuipers RS, Kema IP, Dijck-Brouwer DA, Muskiet FA. Traditionally living populations in East Africa have a mean serum 25-hydroxyvitamin D concentration of 115 nmol/l. Br J Nutr 2012;108:1557-61.
64. Loomis WF. Skin-pigment regulation of vitamin-D biosynthesis in man. Science 1967;157:501-6.
65. Canfield VA, Berg A, Peckins S, Wentzel SM, Ang KC, Oppenheimer S, et al. Molecular phylogeography of a human autosomal skin color locus under natural selection. G3 (Bethesda) 2013;3:2059-67.
66. Skoglund P, Mathieson I. Ancient genomics of modern humans: the first decade. Annu Rev Genomics Hum Genet 2018;19:381-404.
67. Fu Q, Posth C, Hajdinjak M, Petr M, Mallick S, Fernandes D, et al. The genetic history of Ice Age Europe. Nature 2016;534:200-5.
68. Donnelly MP, Paschou P, Grigorenko E, Gurwitz D, Barta C, Lu RB, et al. A global view of the OCA2-HERC2 region and pigmentation. Hum Genet 2012;131:683-96.
69. Hardy A. Commentary: bread and alum, syphilis and sunlight: rickets in the nineteenth century. Int J Epidemiol 2003;32:337-40.
70. Zhang M, Shen F, Petryk A, Tang J, X. C, Sergi C. "English Disease": historical notes on rickets, the bone–lung link and child neglect issues. Nutrients 2016;8:722.
71. Kuan V, Martineau AR, Griffiths CJ, Hypponen E, Walton R. DHCR7 mutations linked to higher vitamin D status allowed early human migration to northern latitudes. BMC Evol Biol 2013;13:144.
72. Mathieson I, Lazaridis I, Rohland N, Mallick S, Patterson N, Roodenberg SA, et al. Genome-wide patterns of selection in 230 ancient Eurasians. Nature 2015;528:499-503.
73. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 2010;376:180-8.
74. Alloza I, Otaegui D, de Lapuente AL, Antiguedad A, Varade J, Nunez C, et al. ANKRD55 and DHCR7 are novel multiple sclerosis risk loci. Genes Immun 2012;13:253-7.
75. Beleza S, Santos AM, McEvoy B, Alves I, Martinho C, Cameron E, et al. The timing of pigmentation lightening in Europeans. Mol Biol Evol 2013;30:24-35.
76. Mathieson I, Alpaslan-Roodenberg S, Posth C, Szecsenyi-Nagy A, Rohland N, Mallick S, et al. The genomic history of southeastern Europe. Nature 2018;555:197-203.
77. Haak W, Lazaridis I, Patterson N, Rohland N, Mallick S, Llamas B, et al. Massive migration from the steppe was a source for Indo-European languages in Europe. Nature 2015.
78. Damgaard PB, Marchi N, Rasmussen S, Peyrot M, Renaud G, Korneliussen T, et al. 137 ancient human genomes from across the Eurasian steppes. Nature 2018;557:369-74.
79. Reich D. Who we are and how we got here. Oxford University Press 2018.
80. Marciniak S, Perry GH. Harnessing ancient genomes to study the history of human adaptation. Nat Rev Genet 2017;18:659-74.

81. Brace S, Diekmann Y, Booth TJ, van Dorp L, Faltyskova Z, Rohland N, et al. Ancient genomes indicate population replacement in Early Neolithic Britain. Nat Ecol Evol 2019;3:765-71.
82. Jablonski NG, Chaplin G. The evolution of human skin coloration. J Hum Evol 2000;39:57-106.
83. Yelmen B, Mondal M, Marnetto D, Pathak AK, Montinaro F, Gallego Romero I, et al. Ancestry-specific analyses reveal differential demographic histories and opposite selective pressures in modern South Asian populations. Mol Biol Evol 2019.
84. Lin M, Siford RL, Martin AR, Nakagome S, Moller M, Hoal EG, et al. Rapid evolution of a skin-lightening allele in southern African KhoeSan. Proc Natl Acad Sci U S A 2018;115:13324-9.
85. Maestro MA, Molnar F, Carlberg C. Vitamin D and is synthetic analogs. J Med Chem 2019.
86. Maestro MA, Molnar F, Mourino A, Carlberg C. Vitamin D receptor 2016: novel ligands and structural insights. Expert Opin Ther Pat 2016;26:1291-306.
87. Tocchini-Valentini G, Rochel N, Wurtz JM, Mitschler A, Moras D. Crystal structures of the vitamin D receptor complexed to superagonist 20-epi ligands. Proc Natl Acad Sci USA 2001;98:5491-6.
88. Kissmeyer AM, Binderup L. Calcipotriol (MC 903): pharmacokinetics in rats and biological activities of metabolites. A comparative study with $1,25(OH)_2D_3$. Biochem Pharmacol 1991;41:1601-6.
89. Carlberg C. Molecular approaches for optimizing vitamin D supplementation. Vitam Horm 2016;100:255-71.
90. Carlberg C, Haq A. The concept of the personal vitamin D response index. J Steroid Biochem Mol Biol 2018;175:12-7.
91. Neme A, Seuter S, Malinen M, Nurmi T, Tuomainen TP, Virtanen JK, et al. *In vivo* transcriptome changes of human white blood cells in response to vitamin D. J Steroid Biochem Mol Biol 2019;188:71-6.
92. Lazaridis I. The evolutionary history of human populations in Europe. Curr Opin Genet Dev 2018;53:21-7.
93. Grillner S, Robertson B. The basal ganglia over 500 million years. Curr Biol 2016;26:R1088-R100.

Figure legends

Figure 1: Evolutionary time axis. The axes display the evolutionary history of the past 4.6 billion years (**left**), 1 billion years (**center**) and 700,000 years (**right**). The different eras of Earth history are based on the International Chronostratigraphic Chart (www.stratigraphy.org/index.php/ics-chart-timescale) and are color-coded (**bottom**). Important events are indicated and the time of their approximate occurrence is marked in bold.

Figure 2: Vitamin D_3 and its metabolites. Vitamin D_3 is produced when the cholesterol precursor 7-dehydrocholesterol is exposed to UV-B radiation. This non-enzymatic reaction produces pre-vitamin D_3 (not shown) that rapidly isomerizes to vitamin D_3. However, at continuous UV-B exposure most of pre-vitamin D_3 converts to lumisterol and tachysterol (not shown), which have no known endocrine function. In the liver the enzyme CYP2R1 converts vitamin D_3 into $25(OH)D_3$ and in the kidneys (and other cell types) CYP27B1 produces $1,25(OH)_2D_3$ which is the high-affinity ligand to the nuclear receptor VDR. Important carbon atoms are marked. The activity of the enzyme DHCR7 regulates the levels of 7-dehydrocholesterol and by this the substrate for vitamin D synthesis. Further important cholesterol derivatives with endocrine functions are bile acids and oxysterols.

Figure 3: Nuclear receptor subfamilies. A cladogram illustrates the evolutionary relationship of the metabolic nuclear receptors LXRα/β FXR, VDR, PXR and CAR. The activation of the receptors by oxysterols, bile acids and $1,25(OH)_2D_3$ is indicated.

Figure 4: The metabolic triangle relationship. A triangular relationship is displayed indicating that enzymes and transporters control the concentration of metabolites, which act as ligands for (metabolic) nuclear receptors regulating their target genes encoding for these enzymes and transporters. The example of the VDR target gene *CYP24A1* is depicted, which encodes for an enzyme, degrading $1,25(OH)_2D_3$.

Figure 5: Evolutionary history of VDR. Anatomically modern human (*homo sapiens*) is compared with ten species (representing important steps in evolution (38, 40, 93)) for the homology of the respective VDR LBD. Axes on the left indicate, which of the species have bone metabolism, adaptive and innate immunology as well as energy metabolism. Please note that zebrafish (*danio rerio*) has two genes for VDR. The jawless fish sea lamprey (*petromyzon marinus*) exists since 550 million years and is the oldest known species expressing a VDR, which binds with high affinity $1,25(OH)_2D_3$.

Figure 6: Evolutionary history of the immune system. Anatomically modern human (*homo sapiens*) is compared with ten representative species (see **Fig. 5**) indicating the evolution of cellular and molecular elements of innate and adaptive immunity. * = lymphocyte-like cells that carry variable lymphocyte receptors.

Figure 7: Genetic variants determining skin color and vitamin D status. Data from the 1000 Genome Project were used to illustrate genetic adaption of the genes *SLC24A5* (**A**), *SLC45A2* (**B**) and *DHCR7* (**C**, **D**) representing either skin lightening (**A**, **B**) or reduced DHCR7 enzyme activity (**C**, **D**). Maps were created by using the Geography of Genetic Variants Browser (https://popgen.uchicago.edu/ggv/).

Figure 8: Genetic history of western European populations. All European populations derived from European hunter and gatherers, Anatolian farmers and Yamnaya pastoralists (77). Pie charts indicate the respective percentages of the founding populations (**A**). A time axis of the past 10,000 years indicates the arrival of the different founding populations and schematically illustrates changes in the average percentage of SNPs rs12785878 and rs7940244 representing DHCR7 enzyme activity and skin lightening rs1426654 and rs16891982 (*SLC24A5* and *SLC24A2*), respectively, in the European population (72, 76) (**B**).

Figure 9: Pharmacological implications. Schematic triangle illustration of the physiological actions of vitamin D (**A**) and the contribution of the three European founder populations determining the vitamin D response index (**B**).

Fig. 1

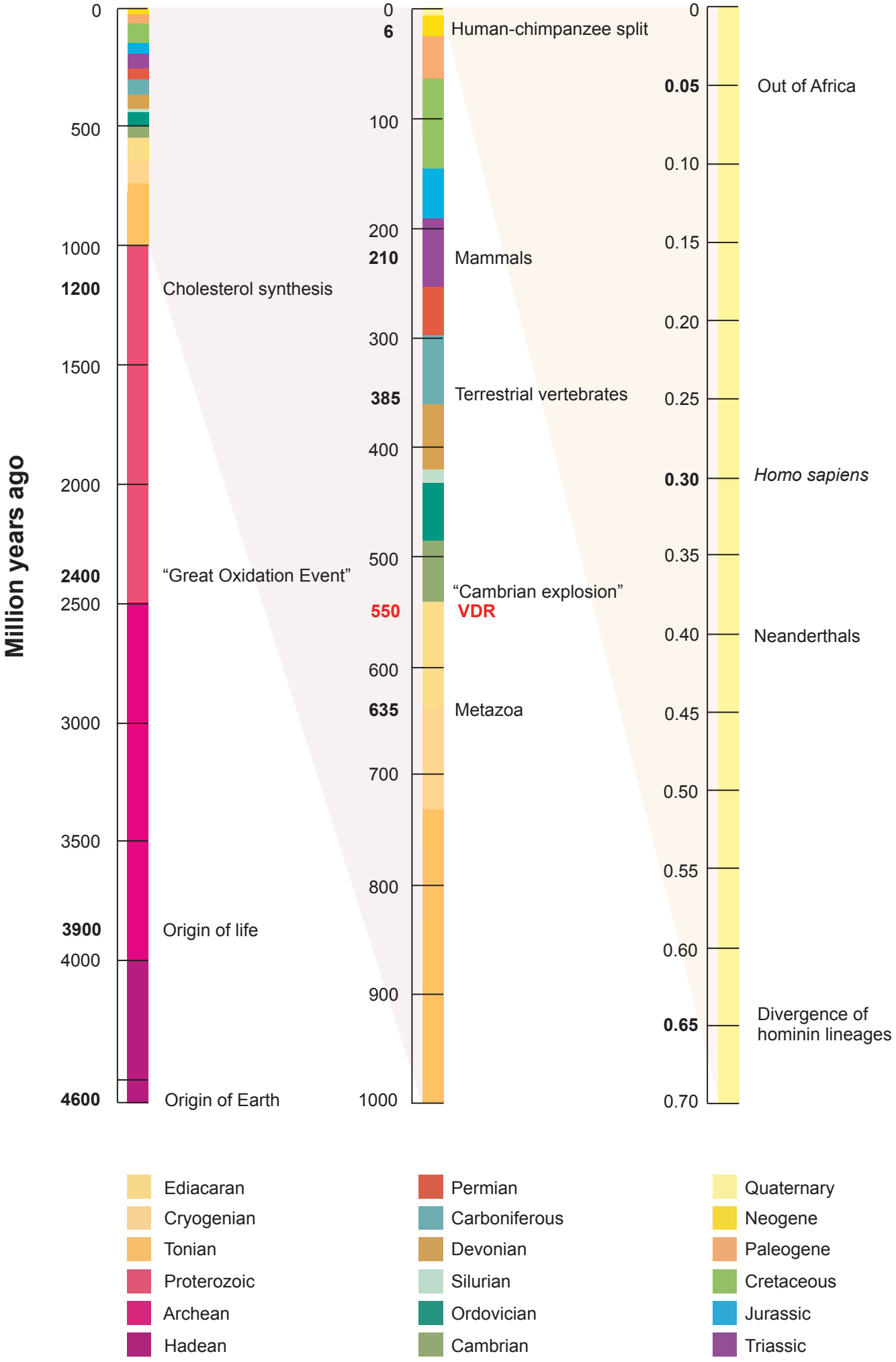

Million years ago
0
500
1000
1200
1500
2000
2400
2500
3000
3500
3900
4000
4600
Cholesterol synthesis
"Great Oxidation Event"
Origin of life
Origin of Earth
0
6
100
200
210
300
385
400
500
550
600
635
700
800
900
1000
Human-chimpanzee split
Mammals
Terrestrial vertebrates
"Cambrian explosion"
VDR
Metazoa
0
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
Out of Africa
Homo sapiens
Neanderthals
Divergence of hominin lineages
Ediacaran
Cryogenian
Tonian
Proterozoic
Archean
Hadean
Permian
Carboniferous
Devonian
Silurian
Ordovician
Cambrian
Quaternary
Neogene
Paleogene
Cretaceous
Jurassic
Triassic

Fig. 2

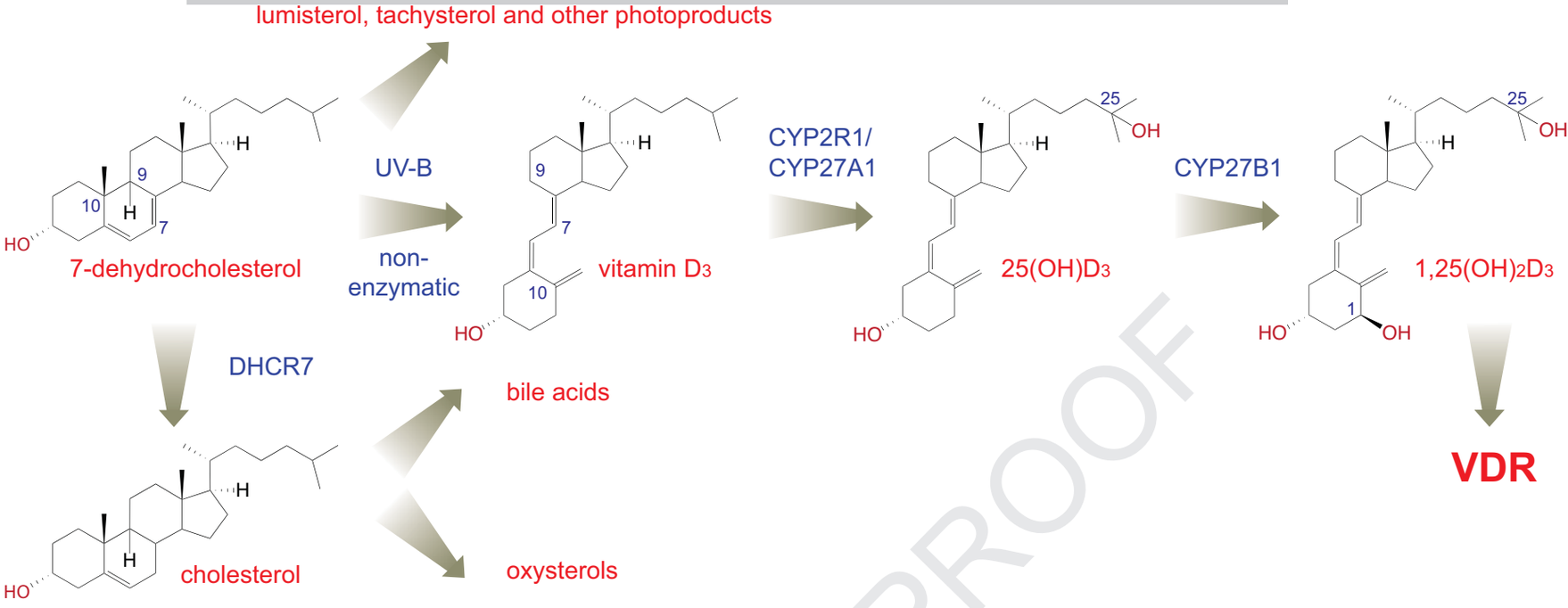


Fig. 3

Cholesterol metabolite	Metabolic receptors				
Oxysterols [μM]	+	-	-	-	-
Bile acids [μM]	-	+	+	+	+
$1,25(OH)_2D_3$ [<nM]	-	-	+	-	-
	LXR α/β (NR1H3/2)	**FXR** (NR1H4)	**VDR** (NR1I1)	**PXR** (NR1I2)	**CAR** (NR1I3)

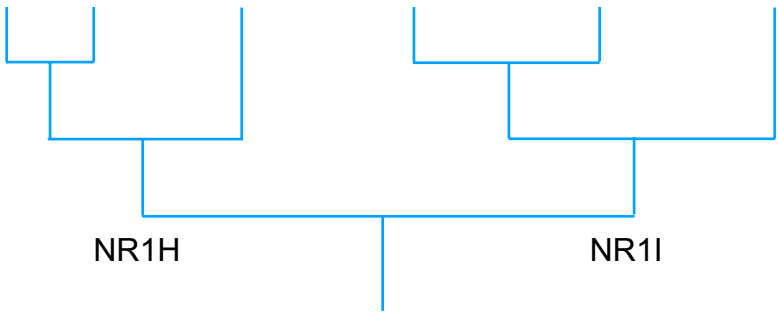


Fig. 4

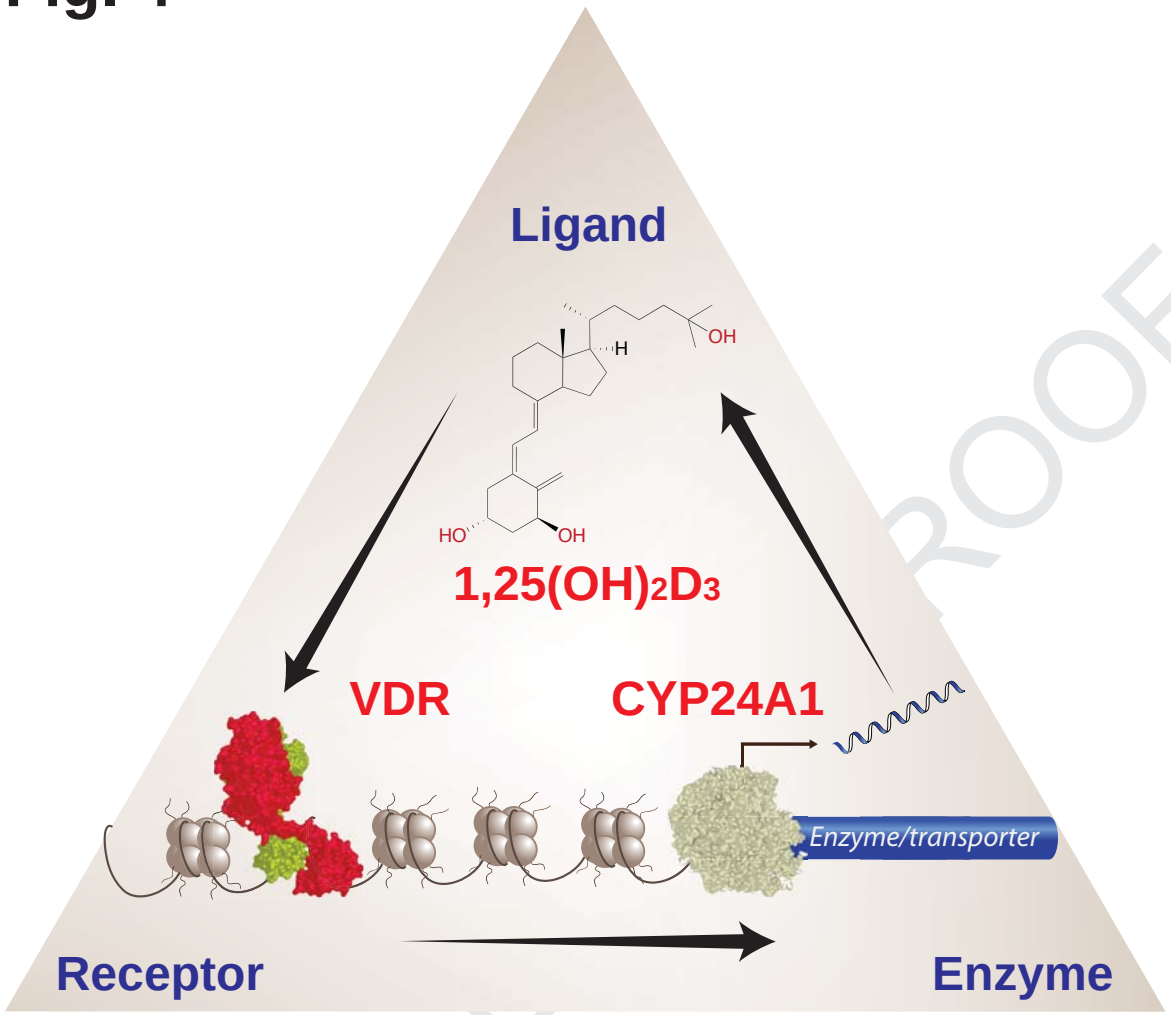


Fig. 5

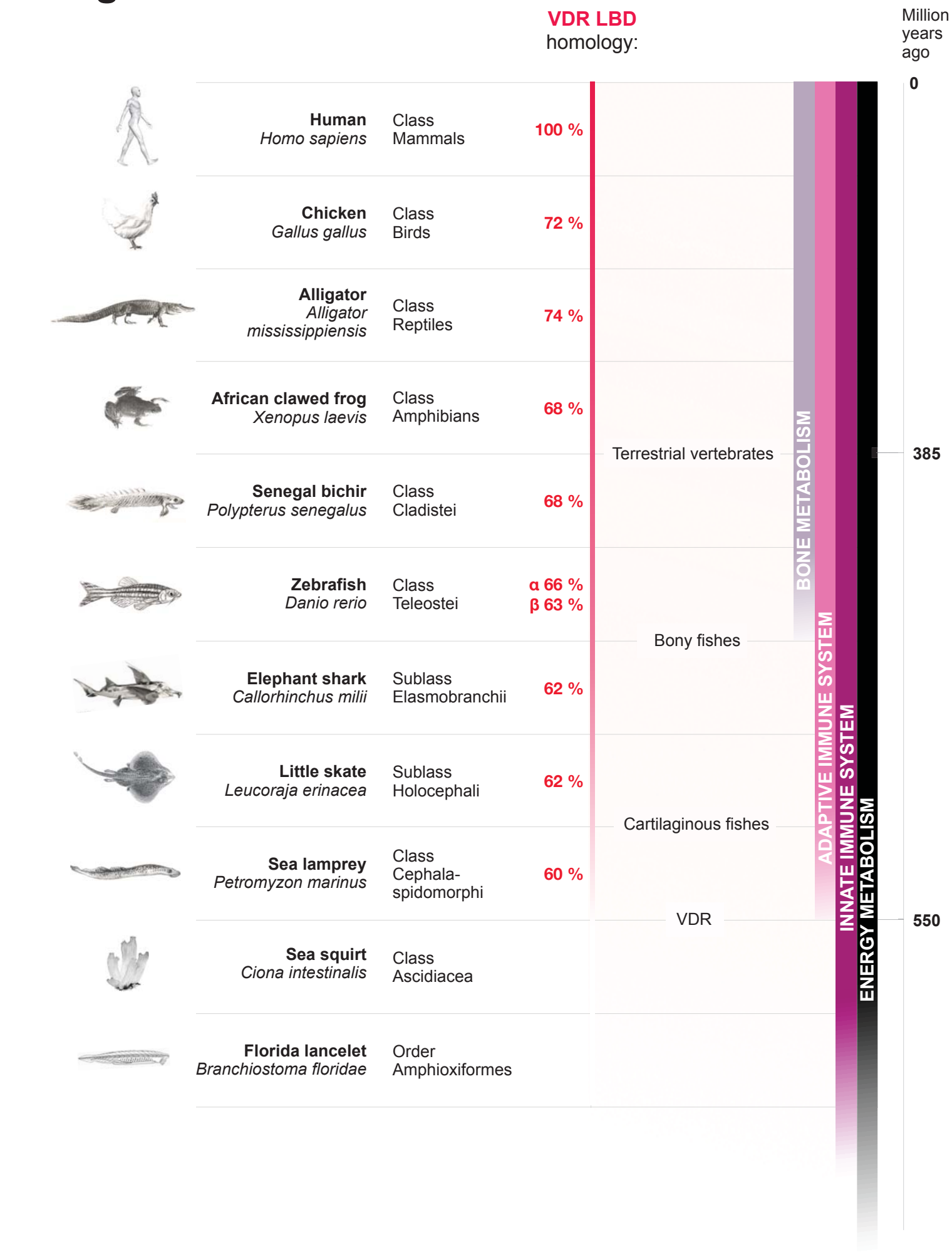

VDR LBD homology:
Million years ago
0
385
550
Human
Homo sapiens
Class Mammals
100 %
Chicken
Gallus gallus
Class Birds
72 %
Alligator
Alligator mississippiensis
Class Reptiles
74 %
African clawed frog
Xenopus laevis
Class Amphibians
68 %
Terrestrial vertebrates
Senegal bichir
Polypterus senegalus
Class Cladistei
68 %
Zebrafish
Danio rerio
Class Teleostei
α 66 %
β 63 %
Bony fishes
Elephant shark
Callorhinchus milii
Sublass Elasmobranchii
62 %
Little skate
Leucoraja erinacea
Sublass Holocephali
62 %
Cartilaginous fishes
Sea lamprey
Petromyzon marinus
Class Cephala-spidomorphi
60 %
VDR
Sea squirt
Ciona intestinalis
Class Ascidiacea
Florida lancelet
Branchiostoma floridae
Order Amphioxiformes
BONE METABOLISM
ADAPTIVE IMMUNE SYSTEM
INNATE IMMUNE SYSTEM
ENERGY METABOLISM

Fig. 6

			Homeothermy	Thymus	Bone marrow	Lymph node	Monocytes/Macrophages	Dendritic cells	Osteoclasts	Tdß/B- Lymphocytes	MHC I/II	Memory
	**Human** *Homo sapiens*	Class Mammals	+	+	+	+	+	+	+	+	+	+
	**Chicken** *Gallus gallus*	Class Birds	+	+	+	?	+	+	+	+	+	+
	**Alligator** *Alligator mississippiensis*	Class Reptiles	-	+	+	?	+	+	+	+	+	+
	**African clawed frog** *Xenopus laevis*	Class Amphibians	-	+	+	-	+	+	+	+	+	+
	**Senegal bichir** *Polypterus senegalus*	Class Cladistei	-	+	-	-	+	+	+	+	+	+
	**Zebrafish** *Danio rerio*	Class Teleostei	-	+	-	-	+	+	+	+	+	+
	**Elephant shark** *Callorhinchus milii*	Sublass Elasmobranchii	-	+	-	-	+	+	-	+	+	+
	**Little skate** *Leucoraja erinacea*	Sublass Holocephali	-	+	-	-	+	+	-	+	+	+
	**Sea lamprey** *Petromyzon marinus*	Class Cephala-spidomorphi	-	+	-	-	+	?	-	*	-	?
	**Sea squirt** *Ciona intestinalis*	Class Ascidiacea	-	-	-	-	+	-	-	-	-	-
	**Florida lancelet** *Branchiostoma floridae*	Order Amphioxiformes	-	+	-	-	+	-	-	-	-	-

Fig. 7

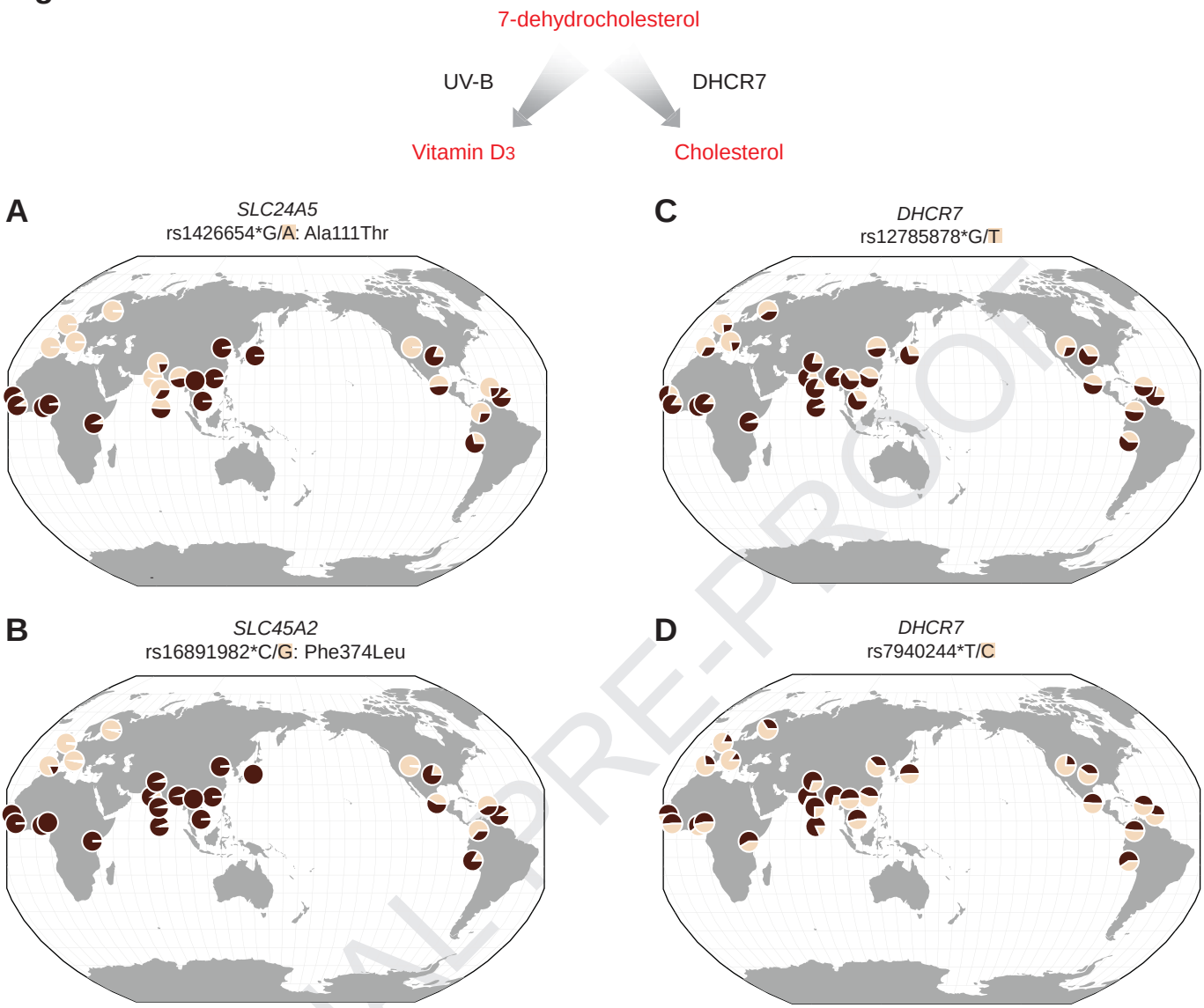


Fig. 8

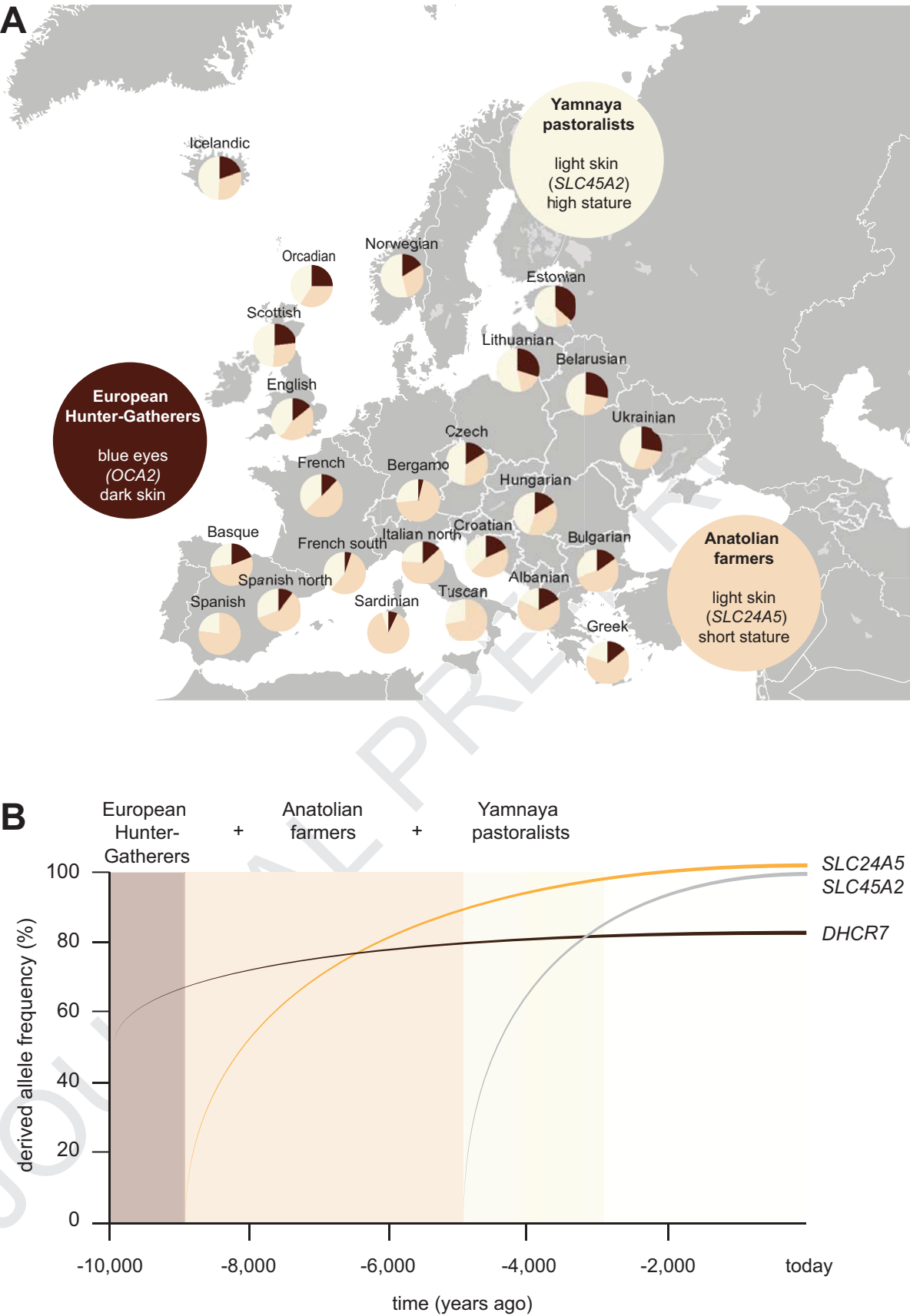
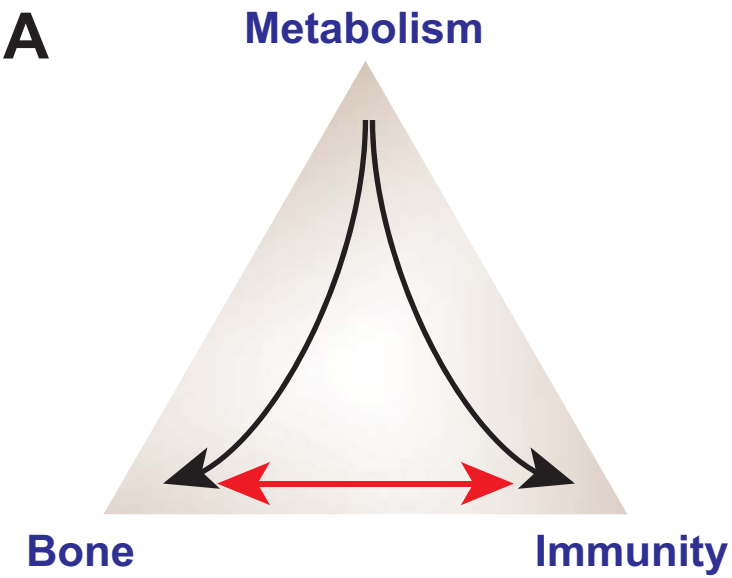
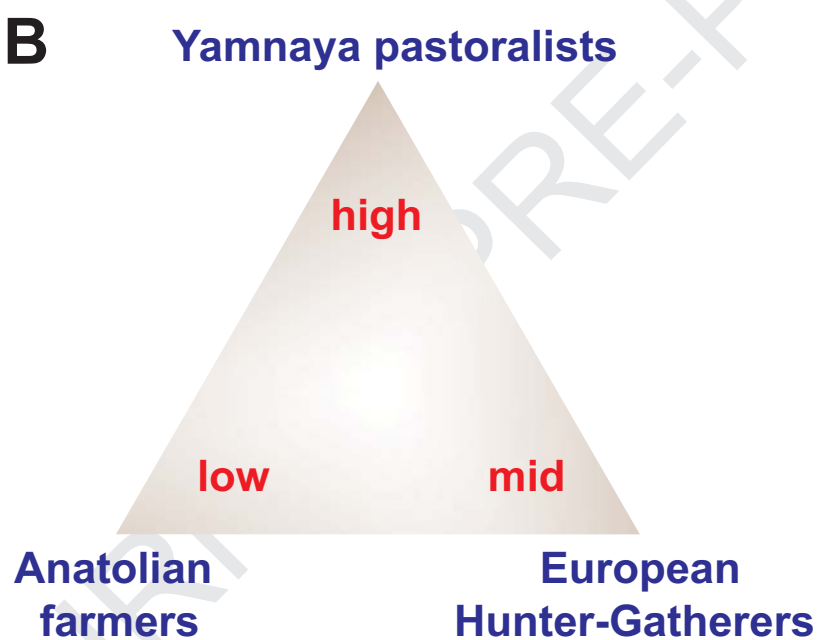
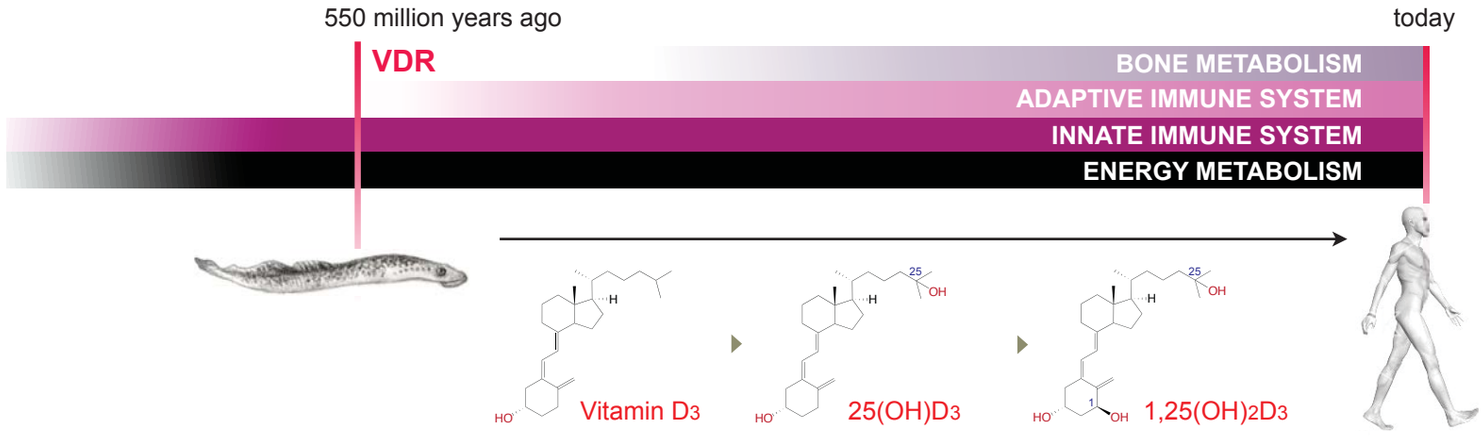


Fig. 9






550 million years ago
today
VDR
BONE METABOLISM
ADAPTIVE IMMUNE SYSTEM
INNATE IMMUNE SYSTEM
ENERGY METABOLISM
Vitamin D3
25(OH)D3
1,25(OH)2D3