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Dysregulation of the Vitamin D Nuclear Receptor may contribute to the higher prevalence of some autoimmune diseases in women

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Abstract

Researchers have noted that the incidence of autoimmune diseases such as Hashimoto's thyroiditis is markedly higher in women than in men, but to date the reason for this disparity has been unclear. The Vitamin D Nuclear Receptor (VDR) is expressed in the human cycling endometrium. Because the VDR controls expression of the Cathelicidin and beta Defensin antimicrobial peptides (AmPs), dysregulation of the receptor greatly compromises the innate immune response. Increasing evidence indicates the presence of a chronic, intraphagocytic metagenomic microbiota in patients with autoimmune disease that may survive by dysregulating the VDR. VDR dysregulation in turn prevents the breakdown of the active vitamin D metabolite 1,25-hydroxyvitamin D (1,25-D) by CYP24. *In silico* data suggest that when 1,25-D rises above its normal range it binds the alpha/beta thyroid receptors, the glucocorticoid receptor (GCR) and the androgen receptor (AR), displacing their native ligands and causing an array of hormonal imbalances. If T3 is displaced from alpha thyroid, thyroiditis may result. Since the VDR, GCR, and AR also express multiple families of AmPs, expression of these natural antibiotics further wanes in response to dysregulation by 1,25-D. The end result is a system-wide drop in AmP expression that may allow pathogens to spread with greater ease. Because women have an extra site

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of VDR expression in the endometrium, the drop in AmP expression associated with nuclear receptor dysregulation may disproportionately affect them. This would cause women to accumulate higher bacterial loads than their male counterparts, particularly during early pregnancy when 1,25-D levels rise by 40%.

Introduction

Systemic lupus erythematosus (SLE) and multiple sclerosis (MS) were first recorded over 100 years ago. Even at that time, it was noted that the diseases affect more women than men.[1] Today it is estimated that autoimmune disease affects approximately 8% of the population, 78% of whom are women.[2] Sex distribution in autoimmune disease such as rheumatoid arthritis (RA), multiple sclerosis (MS) and myasthenia gravis is around 60-70%. The most striking sex differences are observed in Sjogren's syndrome, SLE, and scleroderma, which come from a spectrum of diagnoses in which the patient population is >80% women.[1]

Autoimmune thyroid diseases such as Hashimoto's thyroiditis fall into the latter category. Beeson has reported that approximately 85% of patients with Hashimoto's are women.[3] This rate of incidence is confirmed by data obtained from a retrospective trial in which a VDR agonist and bacteriostatic antibiotics are used to treat patients with various autoimmune diagnoses. While members of both sexes were allowed to participate in the trial, out of 100 subjects with autoimmune disease surveyed, twenty-four had Hashimoto's thyroiditis, and only three of them were men (see Figure 1).[4]

Evidence for important interplays between the endocrine and immune systems has launched the new field of neuroimmunoendocrinology, which has attracted the interest of scientists and clinicians alike.[5] Since autoimmune diseases often show preference for one sex, attention has been given to the possible role of sex hormones in affecting the disease process.[6,7]

The sex hormones activate or repress the activity of specific nuclear receptors, which form homodimers and heterodimers that directly bind DNA in order to regulate the expression of genes. Given the widespread relevance of the superfamily of nuclear receptors to almost all aspects of normal human physiology and the role they play in the etiology in human disease, a detailed understanding of these systems has major implications, not only for human biology but also for the understanding and development of new therapies.[8]

However, the majority of the body's nuclear receptors are not activated by sex hormones. The potential of gender-related differences in the expression of these non-androgenic nuclear receptors to affect the autoimmune disease process has received less attention. This paper focuses on how differential expression of the Vitamin D Nuclear Receptor (VDR) in females may contribute to the higher

prevalence of autoimmune disease in women. It also examines how VDR dysregulation may impact the autoimmune disease process in both sexes.

The Vitamin D Receptor is expressed in the human cycling endometrium

As discussed above, sex hormone expression differs between males and females. But, the active vitamin D metabolite 1,25-hydroxyvitamin D (1,25-D) and its target nuclear receptor, the Vitamin D Receptor, are also expressed in different quantities in males and females. Both sexes express the VDR in the keratinocytes, macrophages, and body tissue.[9] However, Vigano's recent work shows that 1,25-D and the VDR are expressed in the human cycling endometrium, meaning that women possess an extra site of VDR gene expression when compared to their male counterparts.[10] Since the VDR plays a vital role in activating the innate immune response, this gender-based difference may have far-reaching consequences.

The innate immune response serves as the body's first line of defense against infection. The VDR is activated by 1,25-D to directly induce expression of the Cathelicidin and beta Defensin antimicrobial peptides.[11] Furthermore, 1,25-D activates the VDR to transcribe (or repress) at least 913 genes.[12] Several of these genes expressed by the VDR in the endometrium may well play a role in regulating events related to pregnancy or the menstrual cycle. They may also protect the fetus from infection.

Bacteria in autoimmune disease

A recent increase in autoimmune incidence led Rose to express concern over the possible role that infection might play in exacerbating autoimmune disease, particularly in women.[2] Additionally, The Centers for Disease Control and Prevention has written that chronic infectious agents are emerging as notable determinants, not just complications, of chronic disease - stressing that infectious agents likely determine more cancers, immune-mediated syndromes, neurodevelopmental disorders, and other chronic conditions than currently appreciated.[13]

Rook provided evidence that several diseases usually regarded as “autoimmune” or “idiopathic”, including rheumatoid arthritis, Crohn’s disease, ulcerative colitis, sarcoidosis and psoriasis, may be caused by infection with slow-growing bacteria.[14] Similarly, Relman demonstrated evidence of persistent infection in sarcoidosis, various forms of inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, diabetes mellitus, and primary biliary cirrhosis.[15]

Wirostko described persistent bacterial biofilm-like inclusions inside the phagocytes (monocytes, macrophages, neutrophils) of patients with Crohn’s

disease,[16] sarcoidosis,[17] and juvenile rheumatoid arthritis.[18] Serological evidence for bacterial infection has been demonstrated in patients with Hashimoto's thyroiditis.[19,20] Perez showed that 81% of a group of 54 patients representing 20 different autoimmune diagnoses reported continual improvement after treatment durations of 18-53 months with a VDR agonist and antibiotics - further pointing to bacteria as a causative agent in autoimmune disease.[21]

The human microbiome - a metagenome

Recent advances in molecular techniques now allow for the detection of bacterial genomes of organisms that cannot be grown in culture. The scientific community is just beginning to comprehend the full impact of unculturable microbes upon human disease. The global initiative known as the Human Microbiome Project currently estimates that the microorganisms that live inside or on *Homo sapiens* outnumber somatic and germ cells by a factor of ten.[22] To this point, only approximately 1% of this microbiota has been characterized and identified.[23] The combined genetic contributions of these microbes — in excess of 100,000 protein-coding genes — provide traits not encoded in our own genomes.[24] Some of these traits may well lead to autoimmune disease. Researchers affiliated with the Human Microbiome Project aim to use an array of molecular sequencing techniques to characterize the full *Homo sapiens* microbiota over the coming years.[24]

Bacteriologists are increasingly examining how the metagenome of complex microbial communities may contribute to disease. Koch's postulates, which require that a single pathogen cause a single disease state, are being re-examined.[25] This suggests that autoimmune disease results when patients concurrently accumulate a variety of different pathogenic forms, such as those that exist in a persistent metagenomic biofilm or in intracellular communities where they are better protected from the host immune response.[21]

The human body, once considered to be sterile, exists in symbiosis with the human microbiome. Recent studies show that chronic pathogens persist in the endometrium. Eighteen different taxa of microbes were recently identified in the amniotic fluid of women who gave birth prematurely.[26] *Mycobacterium tuberculosis* and influenza HSN1 have been shown to cross the placental barrier.[27,28] Infection with Shigella has been proposed as an explanation for the etiopathogenesis of endometriosis[29] and invasion of the endometrium by bacteria has been implicated in implantation failure, spontaneous abortion, and preterm birth.[30]

VDR dysregulation by the microbiota

While the expression of the VDR in the endometrium should put a healthy woman at an advantage by strengthening her ability to fight infectious agents, a

dysregulated VDR leads to a state in which women are less able to mount an effective innate immune response. Among other compounds, bacterial ligands are capable of dysregulating the VDR. For example, the sulfonolipid capnine, produced by gliding biofilm bacteria, is a strong VDR antagonist.[31] Because the creation of a VDR dysregulating ligand provides a persistent pathogen with an evolutionary advantage, it's quite possible other bacteria have developed equivalent survival mechanisms. If this is the case, a chronic microbiota capable of dysregulating the VDR may well be perverting what the body intends to be a protective environment during pregnancy and menstruation into one that allows disease to flourish.

The likelihood of a VDR-dysregulating microbiota in autoimmune disease is strengthened by the data collected by Perez, in which subjects were routinely administered the VDR agonist olmesartan in conjunction with bacteriostatic antibiotics. He reported bacterial death resulting from the release of endotoxins and inflammatory cytokines, causing patients to experience an exacerbation in disease symptoms caused by immunopathology, sometimes referred to as the Jarisch-Herxheimer Reaction.[21]

Patients administered the antibiotics without olmesartan experienced weak, sometimes negligible, increases in immmunopathology. By contrast, when the same patients took the antibiotics in conjunction with olmesartan, immunopathology often became so strong that it had to be carefully controlled by palliative measures. That this dramatic change in immunopathology correlates with administration of a VDR agonist adds weight to the hypothesis that VDR dysfunction is central to the pathogenesis of autoimmune disease.

The effects of VDR dysregulation

Not only does VDR dysregulation decrease Cathelicidin and beta Defensin expression, it opens a number of other pathways leading to hormonal imbalance. The activated VDR expresses CYP24, the enzyme primarily responsible for breaking 1,25-D down into the inactive vitamin D metabolites. This exerts a feedback control on the maximum level that 1,25-D will attain.[32] However, CYP24 is suppressed in autoimmune disease, allowing 1,25-D to reach unusually high levels.

In silico modeling demonstrates that besides activating the VDR, 1,25-D also has a strong affinity for several of the body's other nuclear receptors. This indicates that at high concentrations it can displace their native ligands.[32] Table 1 shows, for example, that 1,25-D has a very high affinity for the alpha thyroid receptor (ThRa), suggesting that it can keep triiodothyronine (T3) out of the binding pocket (see Figure 2). Thyroid beta is similarly affected.

If 1,25-D prevents T3 from activating the thyroid receptors, genes with alpha

thyroid promoters will no longer be transcribed. The resulting thyroid disease would explain why increasing levels of exogenous thyroid hormone are necessary to maintain thyroid homeostasis as the disease progresses. Furthermore, since the type 1 nuclear receptors work as a group, if transcription by ThRa is dysregulated, a cascade of metabolic dysfunction will result. It is instructive to note that excessive 1,25-D also potentially interferes with several of the body's other nuclear receptors. Table 1 shows high Kd values for the Glucocorticoid, Androgen, and Progesterone receptors.

Secondary effects of VDR dysregulation on antimicrobial peptide expression

If 1,25-D is able to dysregulate the nuclear receptors it would have detrimental effects on system-wide antimicrobial peptide production. Just as the VDR expresses Cathelicidin and beta Defensin, other nuclear receptors also express AmPs. Brahmachary has shown that the Glucocorticoid Receptor, the Androgen Receptor, and the Vitamin D Receptor, are in control of 20, 17 and 16 families respectively, out of the 22 analyzed.[33] Thus, VDR dysfunction causes flow-on effects via glucocorticoid, thyroid, androgen, and other nuclear receptors, which potentially disable the bulk of the body's production of antimicrobial peptides.

Consequently, there is a strong relationship between hormonal dysfunction and autoimmune disease. Indeed, most of the patients with Hashimoto's thyroiditis in the study reported by Perez had also been diagnosed with other inflammatory or autoimmune diseases. Only 8% of subjects with Hashimoto's thyroiditis had Hashimoto's thyroiditis alone.[4] Similarly, autoimmune thyroiditis has been reported in an elevated percentage of fibromyalgia patients.[34] Smith has described a proven association between Hashimoto's thyroiditis and Addison's disease, type 1 diabetes mellitus, pernicious anemia, celiac disease, dermatitis herpetiformis, MS, rheumatoid arthritis, SLE, and systemic sclerosis.[35] Sloka found that in nearly every subject studied, hypothyroidism caused by autoimmune thyroid disease showed a tendency to be more severe and more often present in patients with MS.[36] Both men and women suffering from multiple sclerosis have been shown to manifest low serum T3 concentrations.[37]

Since women have an extra site of VDR gene transcription – the endometrium – it is likely that a greater variety of genes are expressed by the female VDR. Thus, as women age, they may well be disproportionately affected by VDR dysfunction, particularly when it comes to AmP expression. It is likely they would accumulate heavier bacterial loads than their male counterparts. This might contribute to the higher incidence of autoimmune disease among females.

Elevated 1,25-D as a marker for autoimmune disease

When active, transcription of CYP24 by the VDR keeps 1,25-D levels in the normal range.[32] If the VDR is disabled by disease and unable to express CYP24 patients should display higher than normal levels of 1,25-D. Studies on Crohn's disease, ulcerative colitis, RA, Sjogren's, and other autoimmune diagnoses confirm a higher than normal level of 1,25-D among study subjects.[38] Blaney reported 1,25-D levels well above the accepted range in the majority of his cohort of 100 patients with autoimmune disease.[39]

Yet data on 1,25-D levels in autoimmune disease remain relatively scarce because most clinicians test only the inactive vitamin D metabolite 25-hydroxvitamin-D (25-D) when determining vitamin D status. Low levels of 25-D have been tied to a higher incidence of autoimmune disease, leading to the consensus that vitamin D "deficiency" may be a risk factor for autoimmune disease.[9] However, the low levels of 25-D often observed in autoimmune disease must also be viewed in the light of data advanced by Marshall, Blaney and others in which low 25-D levels are the *result* of the autoimmune disease process rather than part of its cause.[32] According to this model, the likely pathway for the downregulation 25-D arises directly from the elevation of 1,25-D. Reduced gene expression by the PXR nuclear receptor inhibits expression of CYP27A1 and thus downregulates conversion of vitamin D into 25-D.

It is clear that both 25-D and 1,25-D must be measured in patients with autoimmune disease, as the presence of inhibited 25-D expression or excessive 1,25-D expression both act as reliable markers of the disease process and are best interpreted in relation to one another.

Pregnancy

In MS and RA, women experience periods of palliation during gestation only to become increasingly symptomatic after giving birth.[40,41] Since 1,25-D production rises by 40% in the early pregnant decidua,[10] its ability to dysregulate the nuclear receptors and the AmPs they express is particularly prevalent during this time. If a woman's VDR expression has already become dysfunctional due to pathogen induced 1,25-D dysregulation, the 40% surge in 1,25-D during pregnancy would result in additional substantial immunosuppression. Under such conditions, immunopathology would decrease, resulting in symptomatic relief. When the surge in 1,25-D disappears after pregnancy, AmP expression and immunopathology should increase, leading to exacerbation of disease symptoms.

Measuring both 25-D and 1,25-D may help resolve the anomalies in symptomatic presentation among MS, RA, and lupus.

Discussion

The confluence of *in silico*, *in vivo*, and *in vitro* data has elucidated a pathway in the molecular biology that can potentially contribute to an understanding of the higher incidence of autoimmune disease observed among women. Dysregulation of the VDR by a chronic intraphagocytic microbiota would cause significant hormonal disruption by allowing 1,25-D to accumulate and displace native ligands from alpha thyroid, glucocorticoid, androgen, and other nuclear receptors. By reducing the ability of these same nuclear receptors to express AmPs, accumulating 1,25-D would also cause a system-wide drop in AmP expression, allowing pathogens to proliferate. Since 1,25-D is expressed in the human cycling endometrium and rises by 40% during early pregnancy, women are disproportionately affected by the potential drop in AmP expression associated with VDR dysregulation and likely accumulate a more diverse microbiota than their male counterparts.

The advent of highly parallel DNA sequencers, high-throughput mass spectrometers and other molecular techniques is ushering microbiology into a new era - steering focus away from the properties of isolated organisms to the manner in which a microbiota can act as metagenome when causing disease. Researchers affiliated with the Human Microbiome Project are beginning to characterize the milieu of unidentified bacterial organizations that persist in *Homo sapiens*.

Their findings have the potential to greatly expand our understanding of how chronic pathogens contribute to autoimmune disease. That potential role of persistent pathogens in autoimmune disease mandates reconsideration of the use of corticosteroids as a first-line treatment for many autoimmune diseases. Corticosteroids effectively reduce the ability of the immune system to respond to pathogens, including persistent microbiota, which is counterproductive to recovery. Perez's report that antibacterial therapy can induce recovery from a variety of autoimmune diseases further cautions against the over-use of corticosteroids.

Scientists and clinicians should be encouraged to test both 25-D and 1,25-D in their subjects. A low 25-D and a high 1,25-D are both useful markers of the disease process. Low levels of 25-D are more likely a result rather than a cause of disease progression.

While this model provides novel insight into the manner in which pathogens may dysregulate the innate immune response and potentially contribute to the autoimmune disease process, much more research is needed. The relationship between nuclear receptors and the AmPs they express has been sorely under-explored. Potential AmP expression by the estrogen or progesterone receptors has yet to be studied, leaving a gap in our understanding of how fluctuations in estrogen and progesterone during pregnancy and menstruation may also affect the female immune response.

Figures

Figure 1. Comorbidity of Hashimoto's thyroiditis with other autoimmune diagnoses

Both color and black/white images have been uploaded.

Table 1. Affinities of native ligands and 1,25-D for various nuclear receptors

A table has been submitted as a separate Word document.

Figure 2. The Thyroid alpha receptor and its native ligand, T3 [PDB:2H77], with 1,25-D superimposed in the ligand binding pocket. Note how 1,25-D displaces T3 from binding to the key receptor residues. Calculated Kd is 8.41 for 1,25-D and 7.20 for T3.

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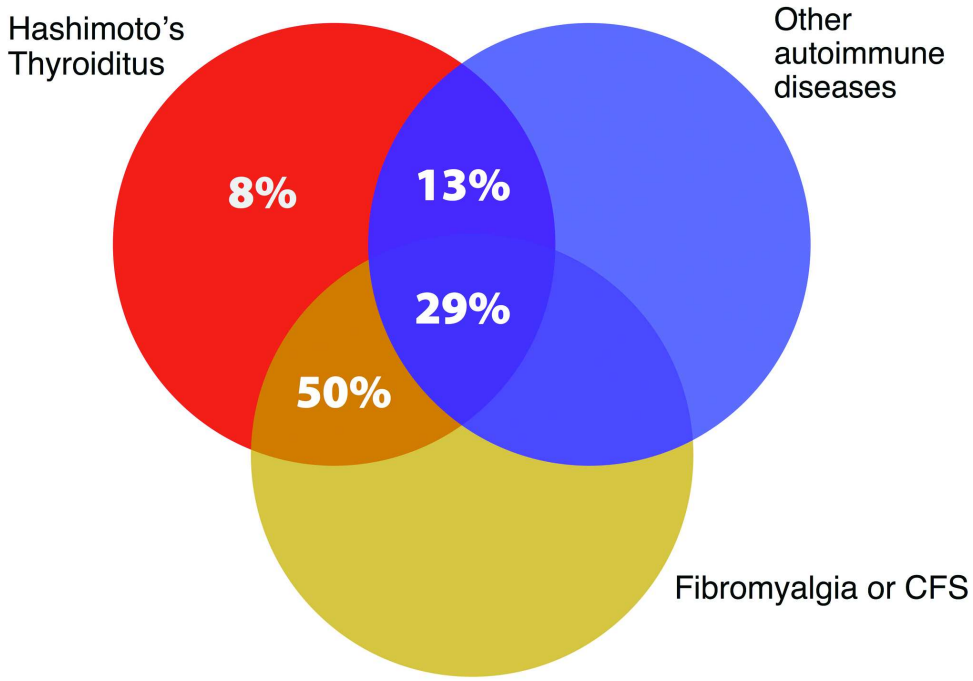

Comorbidity of Hashimoto's thyroiditis with other autoimmune diagnoses
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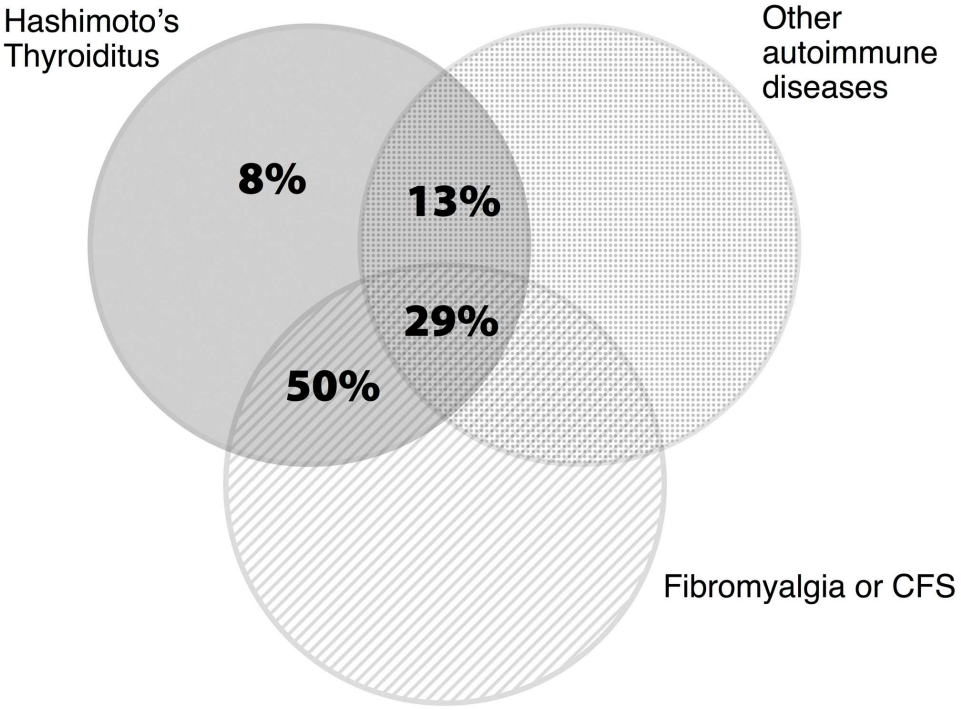

Comorbidity of Hashimoto's thyroiditis with other autoimmune diagnoses
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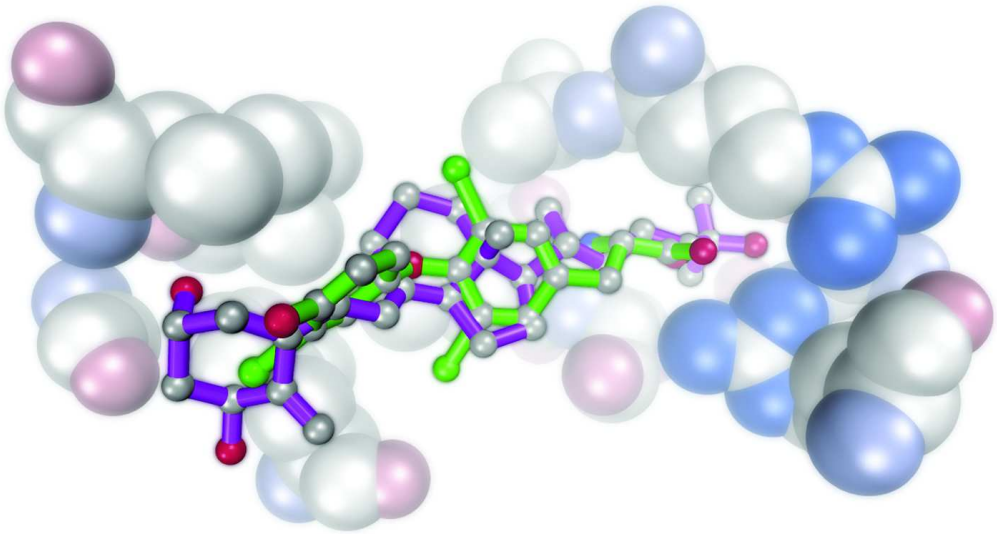
Table 1. Affinities of native ligands and 1,25-D for various nuclear receptors

Nuclear receptor	Native ligand	Native ligand (Kd)	1,25-D (Kd)
Thyroid alpha	T3	7.20	8.41
Thyroid beta	T3	7.18	8.44
Glucocorticoid	Cortisol	7.36	8.12
Androgen	Testosterone	7.38	8.05
Progesterone	Progesterone	7.53	8.09



The Thyroid alpha receptor and its native ligand, T3 [PDB:2H77], with 1,25-D superimposed in the ligand binding pocket. Note how 1,25-D displaces T3 from binding to the key receptor residues. Calculated Kd is 8.41 for 1,25-D and 7.20 for T3.
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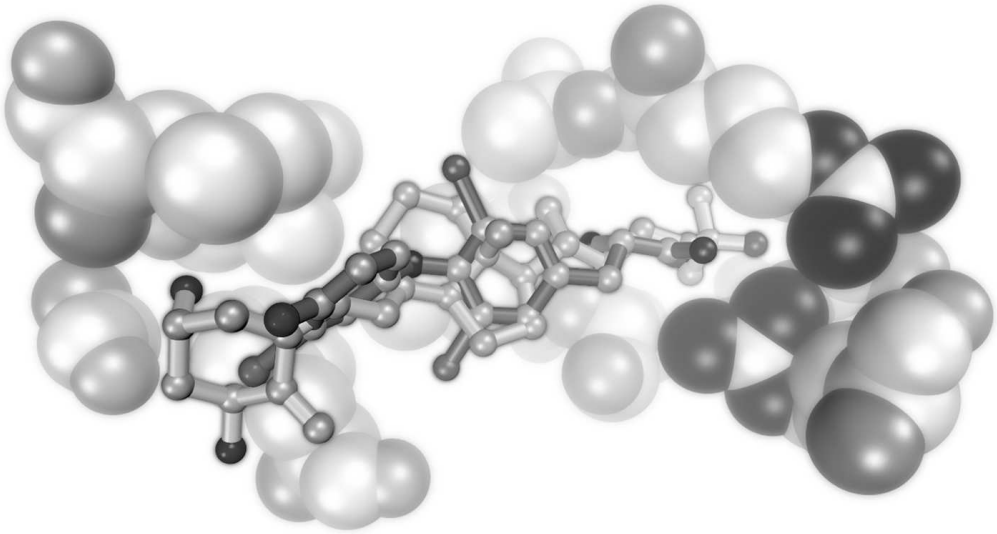

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