Correlation of Prolactin, Alkaline Phosphatase, VDBP and Calcium in MS and Controls – An Extension of a CYP2J2 Genetic Study

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Introduction and Purpose

The current belief is that a combination of genetic, environmental and infectious factors all play a role in the pathogenesis of MS. Vitamin D has been suggested as the most attractive environmental factor for consideration. There are 3 ways vitamin D can be obtained: as ergocalciferol from plants, cholecalciferol from animal sources or through the conversion of 7-dehydrocholesterol in the skin catalyzed by UV rays from the sun. Regardless of the source, vitamin D bioactivation occurs in 2 steps. First, vitamin D is converted to 25-hydroxyvitaim D (25OHD). Next the 25OHD is converted to 1,25-dihydroxyvitamin D, which is the active form.

There are 6 enzymes from the cytochrome P450 superfamily known to be associated with 25OHD. Casas,et al. (2013) indicated that CYP2J2 is the physiological relevant 25-hydroxylase for the conversion of vitamin D to 25OHD and identified CYP2J2 as a major gene associated with 25OHD in cattle. It was known that the CYP2J2 gene is present in humans but it's affect was unknown. Using a subject population with a known propensity for low vitamin D levels (Multiple Sclerosis) this study was designed to determine the correlation between the CYP2J2 gene and MS. Serum markers thought to play a role in vitamin D conversion and use were identified and tested also. These included: prolactin (PRL), alkaline phosphatase (Alk), vitamin D binding protein (VDBP) and calcium. The information on this poster is data gleaned from the CYP2J2 study.

Prolactin

PRL is a protein hormone and cytokine produced in the lactotrophs of the pituitary gland and in cells of the immune tissue. (Diaz, 2015). It demonstrates both pro and anti-inflammatory effects. PRL acts by stimulating the secretion of other cytokines and the expression of cytokine receptor sites. It is believed to be involved in promoting oligodendrocyte precursor cell (OPC) proliferation and there is a demonstrated positive correlation between white matter volume and PRL in patients with MS. This suggests involvement of PRL in the repair of MS damage (De Giglio 2015). The increase in oligodendrocytes promotes white matter repair and remyelination and has relevance for immune regulation and modulation of T and B cell function. Gregg (2009) discovered that PRL treatments mimicked the regenerative effects of pregnancy. There continues to be a lot of debate on the role of PRL in MS. High levels of prolactin have been identified in thyroid disease which is common in those individuals with MS and as well as diseases affecting the hypothalamus. The rate of hypothalamic involvement is high in MS with implications for fatigue, weight dysregulation and migraine headache occurrence. Disturbed autonomic functions include abnormalities in the regulation of the cardiovascular system, sleep cycles, eating and temperature, the control of bowel and bladder activity and sexual behavior. Along with PRL, plasma levels of luteinizing hormone, follicle stimulating hormone, growth hormone, TSH and testosterone have been reported to be altered during MS. Abnormal synthesis and secretion of PRL could lead to the breakdown of balance in the immune system and could promote autoreactivity or aggravation of the clinical condition in autoimmune diseases as a whole (Diaz, 2013). It increased the number of anti-MOG secreting cells. Levels of PRL correlate inversely with apoptotic B cell percentages, decreasing the B cell receptor-mediated activation threshold (Correale, 2014).

Alkaline Phosphatase

ALK is a class of enzyme that catalyzes the hydrolysis of phosphate ester in a basic environment and has low specificity for what it moves. The major function is transporting these substrates across cell membranes but is now being considered as an immune protein. It is not tissue specific, present in many tissues, including bone, intestines, kidney, liver and white blood cells. Any damage or activity in these tissues cause the release of ALK into the blood stream. It serves as a significant indicator for certain medical conditions, diseases and syndromes. Most often elevation is related to liver disease or bone disorders but can be found in primary hypothyroidism and in secondary hyperparathyroidism. It has been reported that levels of intestinal ALK are elevated in individuals with MS, suggesting that the gut biome may have some role in the disease. Of interest is a relationship to herpes zoster, vitamin D deficiency, rheumatoid arthritis, and Celiac disease. They are being studied as treatment for inflammatory diseases (Kader 2017). There is not a good understanding of the purpose of this enzyme, but it does act as a significant marker for disease activity in general health.

Vitamin D Binding Protein

VDBP is a multifunctional serum protein belonging to the albumin gene family. Is transports Vitamin D and its metabolites between skin, liver and kidney and then on to the various target tissues. The definition of vitamin D deficiency is controversial and may be related to VDBP. The unbound hormones exert biological activity while the bound do not. VDBP will not bind to approximately 1% of the circulating vitamin D leaving that amount active. Vitamin D deficiency should be considered in light of VDBP levels but generally is not. The higher the VDBP, the lower the circulating 25(OH)D or active vitamin D. Current vitamin D status is determined by only the 25 (OH)D levels. These levels vary in different physiologic and disease states along with a difference in race and age and may explain the race related levels of autoimmune disease. It is common for patients with sepsis to have a high prevalence of vitamin D deficiency and a decreased VDBP (Yousefzadeh, 2014). It has recently drawn increased attention related to bone health and immunological regulation.

Calcium

Calcium is the most abundant and one of the most important minerals in the body. It is essential for cell signaling and the proper functioning of muscles, nerves, and the heart as well as the transmission of nerve impulses. Calcium is needed for blood clotting and is crucial for the formation, density, and maintenance of bones. It is stored and released by the bone during periods of stress/distress to maintain an even level of the mineral in the blood and allow normal body functioning. Hypercalcemia can cause inexplicable exhaustion or lethargy, trouble concentrating, urinary frequency, weight loss and bone pain. These symptoms are often seen in MS but may not be linked to changes in calcium. Excess glutamate in MS binds with glutamate receptors, causing an increased intracellular calcium, decreasing the mitochondrial potential and results in an increase in reactive oxygen species and activates many calcium dependent enzymes that promote cell death via apoptosis. (Ciccarelli, 2014)

Methods

- Serum samples were collected between October 2014 and December 2016 from 458 subjects, 220 with MS and 238 unaffected controls. Subject demographics are listed in the table below.
- Informed consent was obtained, questionnaires were administered, and blood was drawn by venipuncture.
- Questionnaires collected subject's demographics, vitamin D supplementation, subject and family history of other autoimmune diseases as well as if the subject exercises and whether that exercise is inside or outside.
- Serum levels of vitamin D, vitamin D binding protein, alkaline phosphatase, calcium and prolactin were determined by accepted laboratory methods in a CLIA certified lab. Vitamin D levels were obtained but not used due to the number of individuals on supplementation.
- Association analyses were performed using SPSS. Outliers were excluded from statistical analyses.

Subject Demographics

	MS	Control	Total
Male	55	87	141
Female	165	151	317
Total	220	238	458
Caucasian	92.5%	96.7%	95.4%
Age ± SD	51.25 ± 12.44	48.9 ± 14.1	49.9 ± 13.73
Average Age at Diagnosis	37.43 ± 11.47		

Results

Multiple sclerosis influences the expression of calcium and VDBP. Affected subjects had lower levels of calcium compared to non-affected (P=0.0001) and higher levels of VDBP (p=0.001). ALK, VDBP and PRL demonstrate a relationship with sex of the subject. Increases in VDBP (p=0.023) and PRL (p=0.004) were found in the female and an increase in ALK (p=0.009) in the male subjects, regardless of disease state. There is a 0.9 mcg/ml decrease in VDBP levels for every year of age and a decrease in PRL of 0.05 ng/ml for each year of age. (See Chart)

Correlations among ALK, Calcium, VDBP and PRL indicated that each are independent from each other.

Serum Statistics

Variable	N	Mean ± SD	Range
Vitamin D (ng/mL)	457	35.90 ± 17.23	4.8 - 130.0
Alkaline Phosphatse (mcg/L)	457	11.95 ± 4.27	3.36 - 29.6
Calcium (mg/dL)	457	10.07 ± 0.66	7.2 - 12.6
Vitamin D Binding Protein (mcg/mL)	457	352.71 ± 77.38	163.9 - 661.1
Prolactin (ng/mL)	454	10.84 ± 6.87	0.2 - 81.4

Association with Sex

Trait	N	P-value	Female	Std err	Male	Std err
Alkaline Phosphatase	457	0.0086	11.6	0.24	12.75	0.36
Calcium	457	0.5863	10.05	0.04	10.1	0.06
Vitamin D Binding Protein	457	0.023	359	4	341	6
Prolactin	454	0.0044	11.4	0.4	9.4	0.6

Association with Multiple Sclerosis Status

Trait	N	P-value	Nonaffected	Std err	Affected	Std err
Alkaline Phosphatase	457	0.7308	12.1	0.28	12.2	0.31
Calcium	457	0.0001	10.19	0.04	9.95	0.05
Vitamin D Binding Pritein	457	0.0013	338	5	361	5
PRL	454	0.6326	10.6	0.5	10.3	0.5

Effect of Age

Trait	N	P-value	Estimate	Explanation on age
Alk	457	0.744	0.005	No change due to age
Ca	457	0.3141	-0.002	No change due to age
VDBP	457	0.0003	-0.9	For each year older, VDBP decreases 0.9 mcg/mL
PRL	454	0.038	-0.05	For each year older, prolactin decreases by 0.05ng/mL

Conclusions

Multiple sclerosis influences the expression of VDBP and calcium, which are known to be integral parts of vitamin function. Vitamin D regulates metabolism of calcium in the organism, while VDBP transports the active form of vitamin D to its target organ. Calcium and VDBP have an inverse relationship when comparing subjects affected and non-affected with multiple sclerosis.

Sex influences the expression of alkaline phosphatase, VDBP and prolactin. Females express higher amounts of VDBP and prolactin and males express higher amounts of alkaline phosphatase.

Age also plays a role in the expression of VDBP and prolactin. For each year older, expression decreases by 0.9mcg/mL and 0.05 ng/mL, respectively.

Clinical Implications

MS is an inflammatory autoimmune disease with traditionally lower Vitamin D levels than non-affected individuals. Serum levels of 45-50ng/ml seem to decrease the rate and severity of MS attacks but is hard to reach and maintain in certain individuals.

PRL is intimately involved in autoimmunity, acting as both inflammatory and anti-inflammatory agent. It should be explored further as an adjunct treatment option. The increase levels in females may explain why MS progression is often easier and better controlled in females.

VDBP plays an important role in serum vitamin D levels and should be given regular consideration when evaluating and treating Vitamin D deficiencies.

Lower calcium levels in MS affected individuals may reflect an increase in release of calcium from bone to address the body's need for calcium during inflammatory attacks of MS, therefore being a contributor to the increase osteopenia and osteoporosis in MS.

ALK remains a main marker for inflammation in numerous disease conditions.

While vitamin D levels may be affected by a malabsorption gene, there are many lines of research that continue to need exploration related to serum proteins, not merely during the disease state of MS, but also for all autoimmunity.

Future Directions

Further research is needed to determine if these changes are specific for MS or are shared by other autoimmune diseases.

It is possible that the associations seen in this population would not be seen in other populations due to the homogeneity of the study population. Future studies would need to include subjects from outside the Midwest and of different ethnic backgrounds.

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