Identifying Genetic Markers for Osteoporosis Among Emirati Females Based on Vitamin D Receptor Variants & Biomechanical Parameters

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U.A.E

modern, dynamic, multicultural with strongly cosmopolitan character
7-Dehydrocholesterol

Solar UVB radiation

Diet → Vitamin D₃

25-OHase

Vascular cells
- Increases smooth muscle cell proliferation
- Reduces inflammation

Parathyroid gland
- 1-OHase
- Decreases PTH synthesis and release

Heart
- Decreases LVH

Bone
- Increases bone mineralization
- Increases osteoclastic differentiation

Intestine
- Increases absorption of calcium and phosphate

Pancreas
- Increases insulin secretion

1,25(OH)₂D₃

Decreases renin expression

Breast, colon, prostate cells
- Inhibits clonal proliferation

Macrophages
- Increases microbicidal activity
- Induces differentiation in immune cells

Red Blood Cells
- Improves hemopoiesis
Other Functions of vitamin D

- Regulation of blood pressure and insulin production (Heart Disease and diabetes prevention)
- Calcium intestinal absorption & skeletal and bone integrity
- Regulation of immune function (prevention of autoimmune diseases such as type 1 diabetes, multiple sclerosis, rheumatoid arthritis)
- Regulation of cell growth (Cancer prevention)
Populations in countries of continuous sunshine are deprived of the sunshine vitamin!!!

Higher risk of developing VTD Deficiency:

- Obesity (less bioavailability) & unhealthy Diet
- Skin pigmentation (Melanin is a natural sunblocker in the skin; reduces efficiency of VTD photosynthesis)
- Avoidance of sunlight exposure (due to heat & extensive clothing)
VTD Deficiency: Policy versus Recommendations
VTD Deficiency in the Gulf

Deficiency in VTD common in young Saudi university students (30% of the males and 73% of the females had low VTD status).

In Kuwait VTD deficiency was common in Kuwaiti women (40% prevalence).

VTD deficiency very prevalent (81%) among adolescent females.

In Oman VTD deficiency was common among women (90% prevalence).

(N ranged between 200-300)
VTD Deficiency in the UAE

VTD deficiency, diabetes & Osteoporosis are highly prevalent among UAE females

Osteoporotic Emirati women suffer from mild to severe VTD deficiency (n= 259, Saadi et al., 2006).

VTD deficiency common (36% frequency) in women of childbearing age (n = 33) in Arab communities in the UAE (Dawodu et al., 2001).

(Al Anouti et al.2011, 2013)
Results

Vitamin D Study

(Al Anouti et al. 2011, 2013)

Abu Dhabi medical awards presented
February 2013
UAE STUDENTS FROM ZAYED UNIVERSITY

% of Students

Vitamin D (nmol/L)

- <25: 68
- 25-49: 26
- 50-75: 5
- >75: 1

Legend:
- <25
- 25-49
- 50-75
- >75
Justin Thomas¹, Fatme Al Anouti¹, Sara Al Hasani¹, Laila Abdel-Wareth² and Afrozul Haq²

¹Zayed University, Department of Natural Science and Public Health, Abu Dhabi, United Arab Emirates
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Sunshine, Sadness and Seasonality: 25-Hydroxyvitamin D, and Depressive Symptoms in the United Arab Emirates (UAE)
Sun avoidance among indoor employees leading to vitamin D deficiency and depression in the United Arab Emirates

Fatme Al-Anouti¹, Sumaya Al-Ameri¹, Justin Thomas¹, Laila Abdel-Wareth², Subashnie Devkaran³, Jaishen Rajah⁴ and Afrozul Haq²*
Follow Ups

Intervention Plans

• Participants referred to medical consultation (supplement regimen)

• Students educated about VTD deficiency (campaigns, lectures, social media)
The Bitter Truth

Young Adults are none-compliant
stop taking supplements after few weeks

VTD intake from diet & fortified foods is variable/inconsistent........

Your VTD levels are still very low! Are you taking your supplements?
Alternative Therapy

• Creative ways to enhance sun exposure

• Sun Enhanced Behavioral Activation Therapy
Is Sunlight Exposure the right solution to improve VTD levels???

Some individuals maintain a low VTD status despite taking supplements!

There is a genetic predisposition to VTD Deficiency among Some individuals
Genes play important role in Predicting [VTD]

- There is evidence of genetic contributions (up to 20%) to both 25 [OH]D and 1,25[OH]2D (population genetics research)

- Candidate Genes for modulating VTD status ???

EPIGENETICS
Factors determining [VTD] Homeostasis
### Genes Involved in determining [VTD]

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Vitamin D metabolism</th>
<th>Protein function</th>
<th>Chromosomal location human</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2R1</td>
<td>Activation</td>
<td>25-hydroxylase</td>
<td>11p15.2</td>
</tr>
<tr>
<td>CYP27A1</td>
<td>Activation</td>
<td>25-hydroxylase</td>
<td>2q33-qter</td>
</tr>
<tr>
<td>CYP27B1</td>
<td>Activation</td>
<td>1α-hydroxylase</td>
<td>12q13.1-q13.3</td>
</tr>
<tr>
<td>VDR</td>
<td>Mediator</td>
<td>Receptor</td>
<td>12q13.11</td>
</tr>
<tr>
<td>CYP24A1</td>
<td>Inactivation</td>
<td>24-hydroxylase</td>
<td>20q13</td>
</tr>
</tbody>
</table>

**vitamin D binding protein (GC, DBP)**
The VDR, steroid-receptor gene super-family

Almost **200 polymorphisms** have been identified in VDR.

Genetic variation within **VTD receptor** could alter associations of 25-(OH)D levels with disease outcomes.
AIM of the Study

- Is there an association between Genetic Variants of VTD receptor & Osteoporosis among postmenopausal Emirati women?

Hypothesis of the Study

- Can VTD receptor variants predict BMD?

There will be an association between Genetic Variants of VTD receptor/Metabolism Enzymes & Osteoporosis among Female Nationals
• **Research Idea:** studying the Genetic Polymorphism Of Osteoporosis with Vitamin D in Emirati Females.

• **Where:** Mafraq Hospital, Abu Dhabi

• **Funds:** Zayed University

❖ **Aim:** Focus on the association between VTD genetic variants and osteoporosis in addition to other risk factors like poor diet and lack of exercise in order to develop a better screening tool for specific individuals.

❖ **Develop new techniques in molecular diagnosis for early detection of OSTEOPOROSIS.**
• Worldwide 1 in 3 women and one in five men over 50 will suffer from osteoporotic fractures.

• Study in Abu Dhabi revealed 30% of people over 50 have low bone density.

• 1825 patients with average age of 42, 28 %, had osteopenia and 2.4% had osteoporosis.
Methodological Approach and Work plan

- **Where:** Mafraq Hospital:
- **Sample Size:** 400 samples (Females postmenopausal and matched for controls)
  
  **Inclusion criteria:**
  - UAE National
  - Female/Male
  - >45 years old
  - Osteoporosis

  **Exclusion criteria**
  - Non UAE National
  - Disabled and has serious illness

- **Sample collection:**
  - IOF one minute questionnaire and anthropometry
  - Saliva Sample: for DNA extraction, TaqMan assay for VITD SNPs, known Osteoporosis SNPs and Cyps.
  - Blood Sample: for VitD and Biochemistry test *(results from the medical record)*
Methodological Approach and Work plan

- Biochemical markers: VTD, 1, 25 (OH) D, Calcium Ca and PTH
- Consent Forms

- Name of the researcher: Doctor Fathima Al Anoodi. University of Zayed, Department of Natural Sciences and Public Health, Abu Dhabi, Telephone: 0505873160

- Title of the research: Determination of the genetic markers of osteoporosis disease based on vitamin D receptors in Emirati women.
- Place of research: Health centers of the Ministry of Health in the United Arab Emirates.

You are invited to participate in a scientific research to be conducted in the health centers of the United Arab Emirates. Please take the time to carefully read the information below before you decide whether or not to participate. You can request clarifications or additional information about anything mentioned in this form or this study.

- Project description: The main objective of this project is to propose a health plan to support the health of women through the early detection of women who are more exposed to the disease of osteoporosis and its complications.

The study will investigate the relationship between genetic markers of vitamin D and osteoporosis, as well as other influencing factors such as malnutrition and lack of exercise, in order to develop a better diagnostic tool for the defined individuals.

Methodological Approach and Work plan
Methodological Approach and Work plan

• Bone Densitometry and Fracture Assessment using DEXA
• Biochemical Markers

• Identification of Genetic Variants by Polymerase Chain Reaction (PCR) Assays

• Data Analysis by STATA and SPSS
Genome wide association studies (GWAS) have led to the identification of many SNPs in certain genes (VTD metabolism) & linked them to Osteoporosis and other diseases like T2DM (McGrath et al., 2010 and Ahn et al., 2010).
<table>
<thead>
<tr>
<th>SNPs</th>
<th>Diseases Associated with SNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs731236 (VDR)</td>
<td>Breast cancer risk, susceptibility to type 1 diabetes in Crete, Greece and MS</td>
</tr>
<tr>
<td>*Rs2228570 (VDR)</td>
<td>Breast &amp; colorectal cancer and <strong>T2DM</strong> (Caucasions, North Indians, Saudis)</td>
</tr>
<tr>
<td>Rs1544410 (BsmI) VDR</td>
<td>Breast cancer risk, <strong>Osteoporosis</strong></td>
</tr>
<tr>
<td>*Rs4646536 (CYP27B1)</td>
<td>Autoimmunity and progression to type 1 diabetes, Vitamin D levels determinant in Hispanic and African Americans, rare haplotype protective against diabetic nephropathy.</td>
</tr>
<tr>
<td>Rs2762939 (CYP24A1)</td>
<td>Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes, prostate cancer risk, colon cancer risk</td>
</tr>
<tr>
<td>*rs10877012 (CYP27B1)</td>
<td>Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes.</td>
</tr>
<tr>
<td>*Rs6013897</td>
<td>Associated with the risk of myocardial infarction, diabetes, cancer and mortality, vitamin D insufficiency in Chinese population.</td>
</tr>
<tr>
<td>*Rs6013905 (IVS5-162T)</td>
<td>Pancreas cancer risk</td>
</tr>
<tr>
<td>Rs8176345</td>
<td>MS risk</td>
</tr>
</tbody>
</table>
AIM of the Study

VDR gene polymorphisms:
rs731236
rs2228570
rs1544410

Osteoporosis
Related complications
Emirati Population
Osteoporotic patients (n=264)

Healthy controls (n=91)

Blood collected from subjects

Genomic DNA extraction

Genotyping VDR SNPs: rs731236, rs2228570, rs1544410

TaqMan Real-Time PCR assays

- HbA1C
- Triglyceride
- Total cholesterol
- HDL-cholesterol
- LDL-cholesterol
- Vitamin D

Biochemical tests
Results
# RESULTS

Genotype & Allele frequency VDR SNPs in T2DM patients and healthy controls

<table>
<thead>
<tr>
<th>SNPs Allele/Genotype</th>
<th>Osteoporotic patients</th>
<th>Healthy controls</th>
<th>OR [95% CI]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs731236</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>108 (41.22)</td>
<td>37 (40.66)</td>
<td>1 (Reference)</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>111 (42.37)</td>
<td>38 (41.76)</td>
<td>1.001 [0.592-1.691]</td>
<td>0.997</td>
</tr>
<tr>
<td>GG</td>
<td>43 (16.41)</td>
<td>16 (17.58)</td>
<td>0.921 [0.464-1.826]</td>
<td>0.813</td>
</tr>
<tr>
<td>A</td>
<td>327 (62.40)</td>
<td>112 (61.54)</td>
<td>1.037 [0.733-1.468]</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>197 (37.60)</td>
<td>70 (38.46)</td>
<td>0.964 [0.681-1.364]</td>
<td>0.835</td>
</tr>
<tr>
<td>rs2228570</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>20 (7.66)</td>
<td>18 (20.00)</td>
<td>1 (Reference)</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>94 (36.01)</td>
<td>34 (37.78)</td>
<td>2.488 [1.178-5.256]</td>
<td>0.051</td>
</tr>
<tr>
<td>GG</td>
<td>147 (56.33)</td>
<td>38 (42.22)</td>
<td>3.482 [1.678-7.224]</td>
<td><strong>0.0005</strong></td>
</tr>
<tr>
<td>A</td>
<td>134 (25.67)</td>
<td>70 (38.89)</td>
<td>0.543 [0.379-0.777]</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>388 (74.32)</td>
<td>110 (61.11)</td>
<td>1.843 [1.288-2.637]</td>
<td><strong>0.0007</strong></td>
</tr>
<tr>
<td>rs1544410</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>67 (25.47)</td>
<td>33 (36.26)</td>
<td>1 (Reference)</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>118 (44.87)</td>
<td>38 (41.76)</td>
<td>1.529 [0.879-2.663]</td>
<td>0.131</td>
</tr>
<tr>
<td>TT</td>
<td>78 (29.66)</td>
<td>20 (21.98)</td>
<td>1.921 [1.009-3.658]</td>
<td><strong>0.045</strong></td>
</tr>
<tr>
<td>C</td>
<td>252 (47.91)</td>
<td>104 (57.14)</td>
<td>0.69 [0.491-0.969]</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>274 (52.09)</td>
<td>78 (42.86)</td>
<td>1.45 [1.032-2.036]</td>
<td><strong>0.031</strong></td>
</tr>
</tbody>
</table>

OR [95% CI] was calculated by chi-squared-test. *significant p value < 0.05.

Mutant alleles G\textsuperscript{rs2228570} and T\textsuperscript{rs1544410} associated with osteoporosis.
CONCLUSIONS

Emirati Population

Association

Risk factor

Association

VTD and osteoporosis
Future Directions

- Additional Candidate Genes
Acknowledgements

• Research Collaborators Habiba Al Safar, & Kinda Khalaf

• Research office (ZU)

• Efharisto gia tin filoksenia
CHANCES OF SUCCESS:

0% I WON’T
10% I CAN’T
20% I DON’T KNOW HOW
30% I WISH I COULD
40% I WANT TO
50% I THINK I MIGHT

60% I MIGHT
70% I THINK I CAN
80% I CAN
90% I AM
100% I DID