VITAMIN D RECEPTOR GENE POLYMORPHISMS AND ITS ROLE IN LEPROSY SPECTRUM

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Introduction

Vitamin D:

• Vitamin, hormone, Immunomodulator.

• Key role in innate and adaptive immune responses.

• Deficiency associated with infectious, autoimmune & metabolic diseases.

• Presently: Epidemic of vitamin D "deficiency"
Vitamin D receptor

- Transcriptional regulatory factor.
- Transcribes many genes inc. antimicrobial peptides, NFκb & regulates cytokines such as IL-10, IL-2, IL-8 & IL-12b.
- “Gatekeeper of the innate immune system”
- Polymorphisms in VDR alters its phenotype.
• Role of VDR polymorphisms & Vitamin D in leprosy?
Objective

➢ To determine the association of three SNPs, Taq I (rs731236), Fok I (rs10735810) & Apa I (rs7975232) of VDR gene in Leprosy disease.

➢ To correlate VDR gene expression with Th1 (IFN-γ), Th2 (IL-10) & IL-17 cytokines in clinical forms of leprosy.
Subjects

Genetic study: (n=404)
• Leprosy Patients (n=222) comprising
  T-Lep (TT & BT) Patients (n=87)
  L-Lep (BL & LL) Patients (n=135)
• Healthy controls (n=182)

Cell culture & Stimulation Study: (n=22)
• Treatment naïve leprosy patients (n=14) comprising
  T-Lep (TT & BT) Patients (n=8)
  L-Lep (BL & LL) Patients (n=6)
• Healthy Controls (n=8) ; HIV seronegative, Asymptomatic with no familial history of leprosy/TB.
Methodology

• Genotyping by PCR-RFLP.
• Cell culture and stimulation with MLSA(*M. leprae* Soluble antigen).
• *VDR* mRNA expression by RT-PCR.
• Cytokine assay (IFN-γ, IL-10 & IL-17) in culture supernatants by ELISA.
• Statistical analysis by Haploview, MPM & Graphpad prism softwares.
# Results

<table>
<thead>
<tr>
<th>Locus</th>
<th>Genotype</th>
<th>Susceptible</th>
<th>Resistance</th>
<th>Susceptible</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Taq I (T/C)</strong></td>
<td>Genotype</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CC</td>
</tr>
<tr>
<td></td>
<td>Allele</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Fok I (F/f)</strong></td>
<td>Genotype</td>
<td>ff</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>Allele</td>
<td>f</td>
<td>F</td>
<td>-</td>
<td>-</td>
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<tr>
<td><strong>ApaI (G/T)</strong></td>
<td>Genotype</td>
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<td>-</td>
<td>GG</td>
<td>TT</td>
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<td>G</td>
<td>T</td>
<td>G</td>
<td>T</td>
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<tr>
<td></td>
<td>Haplotype</td>
<td>T-f-G</td>
<td>-</td>
<td>T-F-G</td>
<td>T-F-T</td>
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Results contd..

VDR Expression

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Relative VDR mRNA expression (Folds)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.0</td>
</tr>
<tr>
<td>T-Lep</td>
<td>2.5</td>
</tr>
<tr>
<td>L-Lep</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* Significance p<0.05, Error bars denote Mean±SE

IFN gamma levels

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>IFN gamma levels (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
</tr>
<tr>
<td>T-Lep</td>
<td>0.5</td>
</tr>
<tr>
<td>L-Lep</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* *** Significance p<0.001
IL-17 levels

Study Groups
Control T- Lep L- Lep

IL-17 levels (pg/ml)

* Significance p<0.05, Error bars denote Mean±SE

IL-10 levels

Study Groups
Control T- Lep L- Lep

IL-10 levels (pg/ml)
<table>
<thead>
<tr>
<th>Expression</th>
<th>T-Leprosy</th>
<th>L-Leprosy</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDR mRNA</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>IFN-γ levels</td>
<td>↓</td>
<td>*</td>
</tr>
<tr>
<td>IL-17 levels</td>
<td>*</td>
<td>↑</td>
</tr>
<tr>
<td>IL-10 levels</td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>

* denotes Significance p<0.05

- VDR expression was in inverse correlation with IL-10 (r= -0.97, p=0.02) levels in L-leprosy.
- IFN-γ and IL-17 levels were in positive correlation (r=0.73, p=0.03) in T-Leprosy.
- No correlation of VDR expression with IFNg & IL-17 levels with either of the leprosy phenotypes.
Conclusion

• ff, GG at Fok & Apa positions in VDR may act as risk markers.
• Increased VDR expression may be associated with higher antibacterial activity limiting the bacterial growth in T-leprosy.
• Inverse Correlation of VDR expression & IL-10 levels in L-leprosy might be due non availability of ligand and/or polymorphisms in VDR gene.
Future Perspective:

✓ Assessment of Vitamin D levels.

✓ Genotype/haplotype based functional study.

✓ Cytokine profiling.

-underway.
Acknowledgements

Thank You