# Haptoglobin 2-2 genotype is associated with decreased levels of active vitamin D and accelerated renal apoptosis in Diabetic Nephropathy mice and patients

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## Background and Aim

Diabetic mice with different Haptoglobin (Hp) genotype (1-1, 2-2) have a different susceptibility to developed Diabetic Nephropathy (DN). Hp 2-2 diabetic mice have impaired hemoglobin clearance and increased iron depositions in kidney proximal tubules (PCT) ,leading to increased renal oxidative stress. Patients and mice with chronic kidney disease (CKD) have decreased renal expression of the anti-oxidant, klotho protein and low plasma levels of active 1,25-dihydroxy vitamin D. It is known that DN includes proximal tubular injury but the precise mechanism behind it remains elusive and need to be determined. We are exploring the influence of the Hp genotype and klotho expression on PCT injury in DN mice and patients. We further exploring the role of active vitamin D supplementation in preventing the initiation and progression of DN in mice with different Hp genotype.

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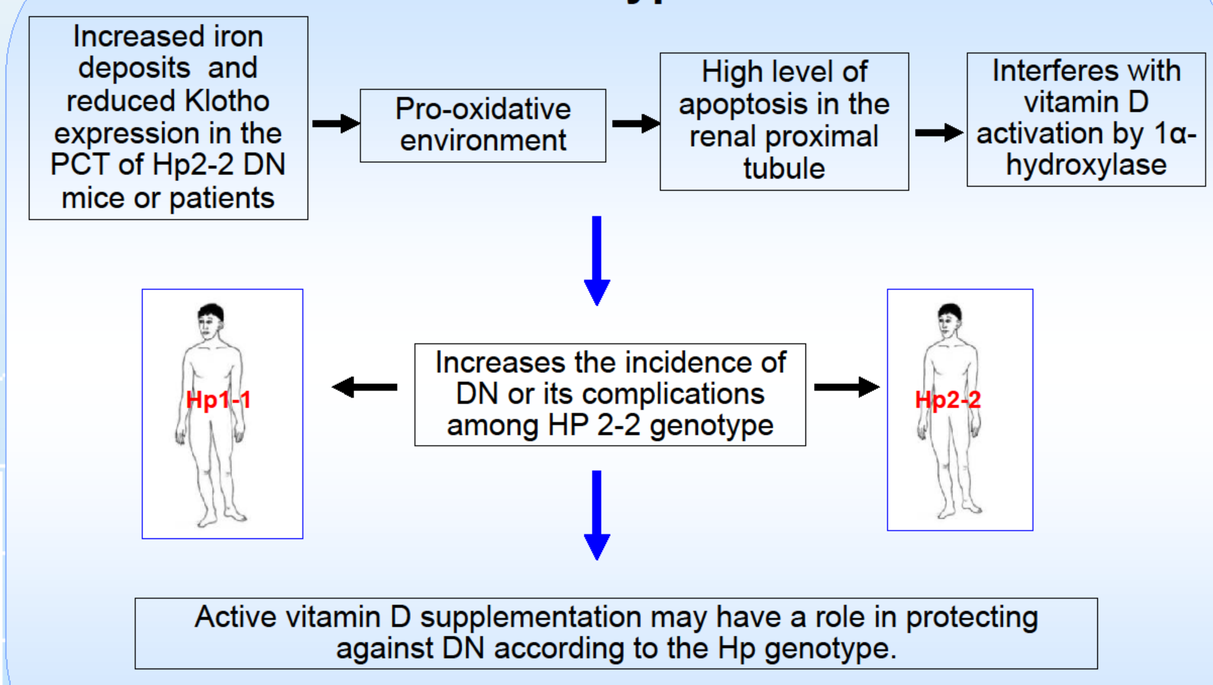
## Research Hypothesis

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## Methods

**The major model systems of the research:**

1. Our genetically engineered mice model of DN with the Hp 2-2 genotype that represents the human disease.
2. DN patients with different Hp genotype.

**Methods:**

- ❖Iron staining and Transmission Electron Microscopy.
- ❖Immunohistochemistry (IHC) of Oxidation-specific epitopes (OSEs) by Oxidative Stress Detection Kit.
- ❖IHC of active caspase 3.
- ❖Tunnel assay(Terminal deoxyuride-5- triphosphate biotin nick-end labeling assay).
- ❖IHC of Vitamin D receptor (VDR) and 1α 25OHD hydroxylase.
- ❖Double staining of active caspase 3 and 1α 25OHD hydroxylase.
- ❖Structural and functional changes of the renal proximal tubule: Hyperfiltration, Glomerular hypertrophy, Albuminuria, Collagen type IV and smooth muscle cell actin)

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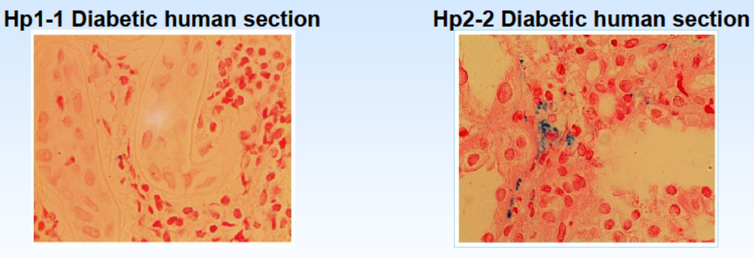
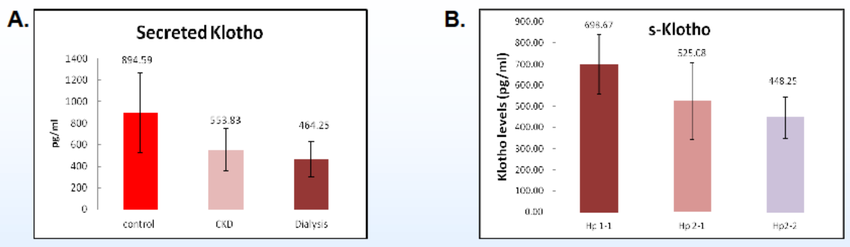
## Results

**Increased iron-rich deposits in the PCT of Hp2-2 DN human sections:**




*Iron staining was used to localize iron in paraffin-embedded kidney sections of mice with different Hp genotype*. Representative images of Hp 1-1 and Hp 2-2 human sections of Diabetic Mellitus patients. There was a significant increase in iron staining (blue) in the renal tissue of Hp 2-2 DM human section compared with Hp 1-1 section.

**Decreased levels of secreted Klotho in DN patients:**




The differences in secreted Klotho levels between healthy control people and DN patients were evaluated by using Elisa assay. There was a significantly decrease in s-Klotho levels in DN patients compared with the control group . As renal injury increase (CKD patients compared with Dialysis patients) the levels of s-Klotho decrease (A). Sorting the DN patients based on their Hp type demonstrate that Hp2-2 genotype correlated with extensive decrease in s-Klptho levels compared with Hp 2-1 or Hp1-1 genotype (B).

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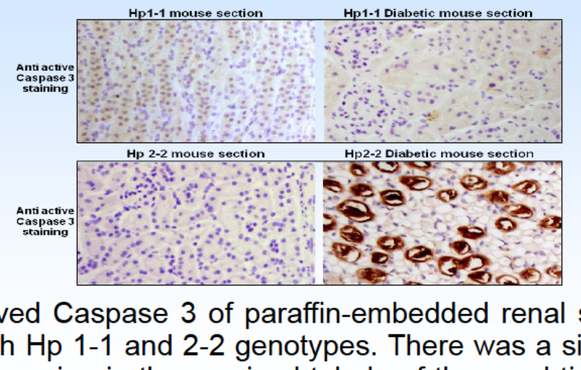
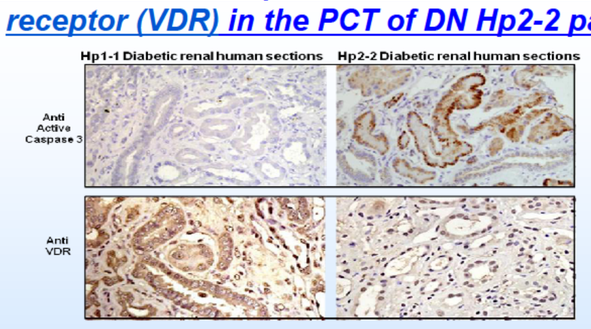
## Results

***Increased expression of active caspase-3 in the PCT of DN Hp2-2 mice:***




IHC staining of Cleaved Caspase 3 of paraffin-embedded renal sections of diabetic and non-diabetic mice with Hp 1-1 and 2-2 genotypes. There was a significant increase in the active caspase-3 expression in the proximal tubule of the renal tissue of Hp 2-2 DN mice compared with Hp 1-1 DN genotype.

***Increased expression of active caspase-3 and Decreased expression of Vitamin D receptor (VDR) in the PCT of DN Hp2-2 patients:***




Slides from human biopsy were subjected to cleaved caspase 3 or VDR IHC. In Hp 2-2 renal human section there was a significant increase in the active caspase-3 expression in the PCT of Hp 2-2 DN patients compared with Hp 1-1 DN genotype. The increase expression of cleaved caspase 3 was correlates with significant reduction in the renal VDR expression.

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## Conclusions

- ❖Hp 2-2 genotype associated with increased iron deposits and high levels of PCT apoptosis.
- ❖It further associated with decrease levels of the anti oxidant klotho and vitamin D receptor in the renal PCT.
- ❖Based on our observations we propose a molecular mechanism explaining the influence of Hp genotype and klotho expression on renal PCT injury in DN patients.
- ❖These results provide insights into genetic predisposition to develop active vitamin D deficiency in DN patients that correlates with sever renal damage.
- ❖We hope that our observations will lead to developing a pharmacogenomics clinical approach to deal with DN complications by selective administration of vitamin D.

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