Presenter Disclosure Information

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“A Genetic Variant of Human Aldosterone Synthase Gene Causes Salt-Dependent High Blood Pressure in Transgenic Mice”

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No relevant financial relationship exists

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None
A Genetic Variant of Human Aldosterone Synthase Gene Causes Salt-Dependent High Blood Pressure in Transgenic Mice

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To
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- Hypertension is a polygenic disease.

- Hypertension is a major risk factor for myocardial infarction, heart failure, stroke and renal disease.

- Hypertension results by the interplay of multiple genetic and environmental factors.

- ~45% of the inter-individual differences in blood pressure can be accounted by the genetic differences.
SNPs in the promoter region may modulate transcription of the gene.

- About 1 million SNPs have already been identified.
- How many of these SNPs are functional is not clear.

Promoter and Enhancer Regions: Quantitative Changes in the Expression of a Protein.

Coding Sequence: Functionally Altered Proteins.

Introns: Function not clear.
Renin-angiotensin-aldosterone system plays an important role in the regulation of the blood pressure.

Exon/intron structure of the CYP11B1 and CYP11B2 genes.
Linkage disequilibrium between the SNPs in 1Kb promoter
Relative luciferase activity after transient transfections in H295R cells

* p<0.05 vs. cells transfected with Hap-II
We have generated transgenic mice with “knocked in” hCYP11B2 gene, containing either haplotype-I or haplotype-II, at the mouse HPRT locus.

The advantage of using this gene targeting system is to selectively target a single copy of the gene at the HPRT locus in the genome.

**Genotyping of hCYP11B2 transgenic mouse**

**hCYP11B2 Genomic DNA copy number quantitation by QRT-PCR**
hCYP11B2 mRNA expression in the adrenal gland and the kidney

* p<0.05 vs. haplotype II
hCYP11B2 protein levels in the adrenal gland and the kidneys of transgenic mice

* p<0.05 vs. haplotype II
Expression of hCYP11B2 in the adrenal gland of the transgenic mice by Immunohistochemistry

ZG=Zona glomerulosa, ZF=Zona fasciculata
Chromatin immunoprecipitation schematic

1. **RNA pol II complex** + **Protein Antibody**
2. **Reverse Cross-link**
3. **DNA bound to the protein of interest**
4. **PCR determines the site specific enrichment of the protein of interest**
Chromatin immunoprecipitation assay shows stronger binding of Pol II to the promoter of hCYP11B2 gene in the TG mice containing hap-I

* p < 0.05 versus haplotype II
Plasma aldosterone levels measurement by ELISA from the transgenic mice fed normal diet

* p < 0.05 versus haplotype II
Mean arterial pressure measurement using radiotelemetry in the transgenic mice fed normal diet

* p<0.05 vs. haplotype II
Mean arterial pressure measurement using radiotelemetry in the transgenic mice fed low or high salt diet

* p<0.05 vs. respective low salt and † p<0.05 vs. haplotype II high salt.
Aldosterone levels measurement by ELISA from the plasma of the transgenic mice fed high salt diet

\* p < 0.05 versus haplotype II
We have identified two distinct haplotype blocks in the hCYP11B2 gene, constituted by three SNPs that are in complete linkage disequilibrium.

Transgenic mice generated via HPRT-targeted gene knock-in strategy show increased expression of the hCYP11B2 in mice with haplotype I.

Haplotype I transgenic mice show elevated MAP and plasma aldosterone levels at baseline.

Inappropriate suppression of plasma aldosterone in transgenic mice with haplotype I of the hCYP11B2 gene contributes to salt-sensitive hypertension in these mice.
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