

## Dopamine D<sub>2</sub> Receptor is Associated with Inverse Salt Sensitivity

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Our previous studies of salt sensitivity of blood pressure have demonstrated that approximately 11% of study participants have a paradoxical increase in blood pressure ( $>$  or  $=$  to 7-mm Hg) on a low NaCl diet (defined as inverse salt sensitivity (ISS)). However the mechanisms responsible for this effect are not known. We demonstrated that single nucleotide polymorphisms (SNPs) in the dopamine type 2 receptor (D<sub>2</sub>R) (RS6276 and 6267) are highly associated with ISS ( $P$  values of  $1.0 \times 10^{-2}$  and  $3.8 \times 10^{-2}$  with odds ratios of 0.32 and 0.48 in unadjusted regression models, respectively). The C allele at both sites confers protection. The D<sub>2</sub>R is strongly expressed throughout the cytoplasm of proximal tubule cells in human kidney tissue slices. We also cultured RPTC from the urine from 4 salt resistant (SR) and 3 ISS participants enrolled in our clinical salt sensitivity studies. We hypothesize that D<sub>2</sub>R containing SNPs have altered receptor expression, and altered signaling compared to wild type controls. ISS participants were homozygous variant for the two D<sub>2</sub>R alleles and showed more D<sub>2</sub>R expression than SR RPTC heterozygous variant (HV) for the two alleles (ISS:  $1.166 \pm 0.059$   $n=3$  vs SR:  $0.969 \pm 0.024$   $n=4$ ,  $P < 0.05$ , t-test). D<sub>2</sub>R expression was increased when the ISS cells were stimulated by a non-selective D<sub>2</sub>R agonist bromocriptine to a greater extent in the D<sub>2</sub>R SNP cell lines (ISS: VEH  $1.166 \pm 0.059$ , vs bromocriptine  $1.474 \pm 0.040$ ,  $n=3$ ,  $P < 0.05$ , t-test). Using the ROS reagent assay, dihydroethidium, there was found to be more ROS products in ISS cells than SR cells when stimulated under low salt (ISS:  $1.145 \pm 0.053$ ,  $n=3$  vs SR:  $0.722 \pm 0.101$ ,  $n=4$ ,  $P < 0.05$ , t-test). We used a highly selective D<sub>2</sub>R agonist (sumanirole) to stimulate wild-type and SNPed cells, and the results demonstrated no effect in the cells with wild type D<sub>2</sub>R but an increase in ROS in cells heterozygous for the D<sub>2</sub>R SNPs (SNP: VEH  $38,364 \pm 1,266$ , sumanirole  $50,926 \pm 3,310$ , VS WT: VEH  $34,562 \pm 1,831$  sumanirole  $34,435 \pm 1,614$  RFU  $n=12$ ,  $P < 0.05$ , t-test) consistent with the higher expression of D<sub>2</sub>R found in ISS urine cells. We hypothesize that SNPs in the D<sub>2</sub>R lead to increased reactive oxygen species which has previously been associated with renal fibrosis and hypertension.

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