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RESEARCH INTERESTS. Our laboratory pursues inter-related themes in basic and genetic approaches to autonomic and renal function, as precursors to the disease states of hypertension and renal failure:

Human twin studies of autonomic and renal function.

The overall goal of this project is to use the power of twin and sibling pairs, coupled with a dense microsatellite genome scan, to position loci, allelic variation at which influences autonomic function in either the sympathetic or parasympathetic branches.

The autonomic nervous system is the key second-to-second regulator of the circulation, and hence of blood pressure. Autonomic activity is deranged not only in patients with established hypertension, but also in their still-normotensive offspring; thus, autonomic traits are valuable as "intermediate phenotypes" in hypertension, a common disease with non-Mendelian (complex) inheritance.

Our specific aims are threefold:

1. Intermediate phenotypes. Phenotypic heritability (h^2). Using twin pairs, establish the heritability of early, pathogenic autonomic/sympathetic phenotypes underlying the later development of hypertension.

2. Allelic association. At candidate loci, establish whether particular SNP alleles (or SNP haplotypes) associate with the intermediate phenotype, and later with the ultimate disease trait.

3. Genome-wide approaches (linkage). In sibling pairs derived from nuclear families ascertained on the basis of twins, couple intermediate phenotypes with genome-wide linkage approaches to position novel, previously unsuspected genetic loci that contribute to trait variation in the intermediate phenotypes.

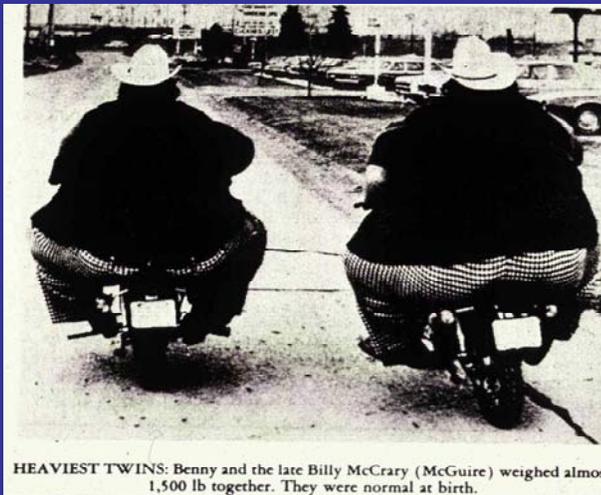
Cell biology of catecholamine storage vesicles: Implications for hypertension.

The sympathoadrenal efferent branch of the autonomic nervous system plays a key minute-to-minute role in regulation of blood pressure, and excessive sympathoadrenal activity is clearly implicated in the pathogenesis of hypertension, both primary (genetic, essential) and secondary (acquired), in both humans and experimental animals.

This system acts by co-release through exocytosis (all-or-none discharge) of co-transmitters from secretory vesicles of postganglionic sympathetic axons and chromaffin cells, into the bloodstream or neuroeffector junctions (synaptic clefts), wherein co-transmitters impinge on cardiovascular target cells, such as vascular smooth muscle, myocardiocytes, and endothelial cells, thereby regulating blood pressure.

While the best-studied sympathoadrenal co-transmitters are the catecholamines (norepinephrine and epinephrine), a complex "cocktail" of substances is co-released by exocytosis from storage vesicles, including not just the catecholamines themselves, but also neuropeptides such as several large acidic proteins, the chromogranins / secretogranins, which are cleaved to biologically active peptides which modulate both neurosecretion and vascular smooth muscle relaxation.

Twins: window into **heritability (h^2)** of any phenotype



HEAVIEST TWINS: Benny and the late Billy McCrary (McGuire) weighed almost 1,500 lb together. They were normal at birth.

Monozygotic (MZ, identical) twins: Billy and Benny . . .

$$V_P = V_G + V_E$$

$$h^2 = V_G/V_P = 2(R_{MZ} - R_{DZ})$$

Source: Guinness Book of World Records.

It has become clear that co-transmitters other than the catecholamines themselves also participate in the vascular responses to sympathoadrenal activation. Evidence in support of this principle includes the observation that the vasoconstriction of sympathetic activation is only partially reversed by α - (even in combination with β -) adrenergic blockade. Both pre- and postsynaptic function may be disturbed in hypertension.

Increasingly, we have turned to the tools of genome technology and statistical genetics in order to understand how heredity shapes human functional responses in the sympathetic neuroeffector junction. We have already discovered substantial polymorphism at “candidate” genetic loci whose products maintain the activity of the neuroeffector junction (CHGA, CHGB, PNMT), and have found functional differences among allelic variants that predict autonomic (including stress blood pressure) responses in humans. A central theme of this program is discovery (by human genomic DNA resequencing) of the spectrum of human allelic variation at “candidate” genetic loci encoding proteins governing sympathetic neuroeffector function, and then subjecting these variants to functional testing, both by allelic and haplotype associations *in vivo*, and by expression studies *in vitro*. These complementary approaches allow us to determine how such variants might influence autonomic activity, both in human responses and at the level of their actions in isolated cells. Incorporation of newly emerging proteomic tools further allows us to understand how protein products of the target (candidate) genes finally undergo post-translational modification to accomplish their tasks in the junction.

Sympathoadrenal catecholamine secretion is exocytotic (all-or-none), releasing not just catecholamines but also the acidic proteins with which catecholamines are stored: chromogranins/secretogranins, quantitatively major components being chromogranin A (CHGA) and chromogranin B (CHGB). Both CHGA and CHGB seem to be necessary factors (“on/off switches”) in the biogenesis of catecholamine secretory vesicles. CHGA is cleaved to biologically active fragments, including the endogenously formed “catestatin” that inhibits catecholamine release; its specific inhibitory mechanism seems to be nicotinic cholinergic antagonism.

Data collected in the past few years include discovery of substantial and functional human genetic diversity (polymorphism) at the CHGA and CHGB loci, including 3 novel variants of catestatin with differential potency as nicotinic antagonists, and 8 common CHGA proximal promoter SNPs, giving rise to common CHGA promoter haplotypes with different transcriptional activities in chromaffin cells. Common CHGA promoter polymorphisms predict plasma CHGA concentration. Common 3'-UTR polymorphisms in both CHGA and CHGB predict heritable variation in stress blood pressure responses in human twins. CHGB expression cosegregated with a novel locus on chromosome 11q24-q25, suggesting the presence of a previously uncharacterized major gene regulating human sympathetic outflow.

Lab environment and potential laboratory rotations for graduate students.

We currently have 3 postdoctoral fellows (two MD/PhD, one MD) and 3 graduate (PhD) students. We would welcome students who would like to explore how genetic polymorphism influences human phenotypic variation. *In vitro* approaches, such as expression/transfection of polymorphic variants in cultured chromaffin cells, with subsequent tests of biochemical or transcriptional function, are excellent (very tractable) projects for graduate students or postdoctoral fellows.

Recent pertinent publications.

20. L Zhang, F Rao, J Wessel, BP Kennedy, BK Rana, L Taupenot, EO Lillie, NJ Schork, MG Ziegler, **DT O'Connor**. Functional allelic heterogeneity & pleiotropy of a repeat polymorphism in the tyrosine hydroxylase locus: Prediction of catecholamine secretion & cardiovascular response to stress in twins. *Physiol Genomics* 2004;19:277-291.
34. F Rao, G Wen, SK Mahata, BP Kennedy, M Stridsberg, K Abel, DW Smith, E Eskin, NJ Schork, BA Hamilton, MG Ziegler, **DT O'Connor**. The Catecholamine Release Inhibitory Peptide Catestatin (Chromogranin A [CHGA]₃₅₂₋₃₇₂): A Common, Naturally Occurring Amino Acid Variant (Gly364Ser) Causes Profound Alterations In Human Autonomic Activity. *Circulation* 115:2271-2281, 2007.
40. TA Greenwood, F Rao, M Stridsberg, NR Mahapatra, M Mahata, EO Lillie, SK Mahata, L Taupenot, NJ Schork, **DT O'Connor**. Pleiotropic effects of novel trans-acting loci influencing human sympathochromaffin secretion. *Physiol Genomics* 2006;25:470-9.
41. J Wessel, G Moratorio, F Rao, M Mahata, L Zhang, W Greene, BK Rana, BP Kennedy, S Khandrika, P Huang, EO Lillie, DW Smith, G Wen, BA Hamilton, MG Ziegler, JL Witzum, NJ Schork, GW

- Schmid-Schönbein, **DT O'Connor**. C reactive protein, an "intermediate phenotype" for inflammation: Human twin studies reveal heritability, association with blood pressure and the metabolic syndrome, and the influence of common polymorphism at catecholaminergic/ -adrenergic pathway loci. *J Hypertension* 25:329-43, 2007 (topic of editorial: *J Hypertension* 25:281-3, 2007).
42. TM Seasholtz, J Wessel, F Rao, BK Rana, S Khandrika, BP Kennedy, EO Lillie, MG Ziegler, DW Smith, NJ Schork, JH Brown, **DT O'Connor**. Rho kinase Polymorphism Influences Blood Pressure and Systemic Vascular Resistance in Human Twins: Role of Heredity. *Hypertension* 2006;47:937-47.
 43. RM Salem, PE Cadman, L Taupenot, F Rao, G Wen, BA Hamilton, BK Rana, DW Smith, E Eskin, M Stridsberg, HJ Ward, NJ Schork, **DT O'Connor**. Polymorphisms at Catecholamine Storage Vesicle Biosynthetic Locus CHGA are Risk Factors for Hypertensive ESRD in African Americans. *J Amer Soc Nephrol*, accepted, 2007.
 44. F Rao, L Zhang, BP Kennedy, J Wessel, L Taupenot, G Wen, BK Rana, DW Smith, EO Lillie, PE Cadman, RM Salem, NJ Schork, MG Ziegler, **DT O'Connor**. Tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis: Discovery of common human genetic variants governing transcription, human autonomic activity and blood pressure *in vivo*. *Circulation* 116:993-1006, 2007. (topic of editorial)
 45. F Rao, J Wessel, G Wen, L Zhang, BK Rana, BP Kennedy, EO Lillie, RM Salem, BA Hamilton, DW Smith, N-H Holstein-Rathlou, MG Ziegler, NJ Schork, **DT O'Connor**. Renal albumin excretion, an "intermediate phenotype" for nephropathy: Human twin studies identify influences of heredity, environment, & the interactive role of polymorphism at adrenergic pathway loci. *Hypertension* 2007;49:1015-1031.
 47. BK Rana, PA Insel, S Payne, K Abel, E Beutler, MG Ziegler, NJ Schork, **DT O'Connor**. Population-Based Sample Reveals Gene-Gender Interactions in Blood Pressure in White Americans. *Hypert* 49:96-106, '07.
 48. EO Lillie, M Mahata, S Khandrika, F Rao, RA Bunday, G Wen, Y Chen, L Taupenot, DW Smith, SK Mahata, MG Ziegler, M Cockburn, NJ Schork, **DT O'Connor**. Heredity of endothelin secretion: Human twin studies reveal the influence of polymorphism at the chromogranin A locus, a novel determinant of endothelial function. *Circulation* 115:2282-2291, 2007.
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