

PERSPECTIVE

How the Monoamine Transporter Garden Grows

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ABSTRACT

In this issue of *Molecular Pharmacology*, Hahn et al. (p. 457) present a study of previously uncharacterized single amino acid variants of the human norepinephrine transporter. Intracellular trafficking, surface expression, transport properties, interaction with antagonists, and regulation by a protein kinase C-linked regulatory pathway were studied by heterologous expression in COS-7 cells. In recent years, there has been increasing focus

on the natural variations and roles of nonsynonymous single-nucleotide polymorphisms in human membrane transporter genes (and their protein products) in human disease. As this information is assimilated and understood at the molecular and genetic level, the relationship between transporter pharmacogenomics and therapeutics in the age of individualized medicine will be greatly impacted.

Over the past 30 years, the time between basic science discoveries and the application of new information to the treatment of human disease has become increasingly compressed. For example, consider the translational relationship between the purification and characterization of the nicotinic neuromuscular junction acetylcholine receptor (Patrick and Lindstrom, 1973; Berman and Patrick, 1980; Lindstrom, 2000, 2002) and the application of this knowledge to therapeutic treatment regimens for myasthenia gravis. Likewise, consider the discoveries and exhaustive characterization over a few short years related to drug metabolism and exemplified in the P450 field. The dramatic effects that this information has provided to our understanding, not only of drug pharmacodynamics in humans but also of the ethnic genetic variations in world populations, are truly profound (Cascorbi, 2003; Schwarz, 2003). Knowledge of the effects of P450 polymorphisms in drug metabolism is influencing the approaches by which physicians treat human diseases (Dahl, 2002; Roots et al., 2004; Tempfer et al., 2004; Kirchheiner and Brockmoller, 2005). New drugs are now synthesized and screened in the pharmaceutical industry to meet strict requirements dictated by known drug metabolic pathways (Rodrigues and Rushmore, 2002).

Many researchers have been engaged in exciting and highly significant experiments identifying and understanding the consequences of single-nucleotide polymorphisms (SNPs) and other genetically transmitted alterations in neurotransmitter receptors (Michel and Insel, 2003; Small et al., 2003; Kirstein and Insel, 2004; Liggett, 2004), ion channels (Furutani et al., 1999; Zhou et al., 1999; Anson et al., 2004), and enzymes important in cellular and organismal homeostasis.

Membrane transporters play a critical role in maintaining physiological balance in the biological kingdom. Both plasma membrane and intracellular membrane transporters compartmentalize metabolic processes, including the synthesis and sequestration of neurotransmitters and the elimination of toxic waste products and xenobiotics. Many natural variations resulting in SNPs in transporter genes that are drug targets have been identified (Leabman et al., 2003). These transporters include the ATP binding cassette superfamily (e.g., ATP binding cassette transporters, MDR1), which removes xenobiotics from cells, and solute carrier superfamily transporters (e.g., OCT1, DAT, NET, VMAT1), which sequester neurotransmitters and small molecules into and within cells.

The monoamine transporters for norepinephrine (NE), dopamine (DA) and serotonin belong to the SLC6A family of Na⁺/Cl⁻-dependent transporters. Several SNPs for the human norepinephrine transporter (NET), the human dopa-

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ABBREVIATIONS: P450, cytochrome P450; SNP, single-nucleotide polymorphism; NE, norepinephrine; DA, dopamine; hNET, human norepinephrine transporter; NET, norepinephrine transporter.

mine transporter, and the human serotonin transporter have been reported and many have been characterized at the cellular level (Hahn and Blakely, 2002a). Some transporter variants that relate to human phenotypes have been successfully linked within families (Hahn and Blakely, 2002a).

In this issue of *Molecular Pharmacology*, the article titled "Single Nucleotide Polymorphisms in the Human Norepinephrine Transporter Gene Affect Expression, Trafficking, Antidepressant Interaction, and Protein Kinase C Regulation" characterizes several properties of the norepinephrine transporter (NET) with regard to amino acid variants that have not been previously characterized in human populations that include cardiovascular phenotypes. Hahn et al. (2005) have significantly enhanced our knowledge of the characterization of hNET SNPs that influence transporter function at the cellular level. These investigators previously identified an SNP of hNET (A457P) that is directly linked to an autonomic disorder, orthostatic intolerance (Shannon et al., 2000; Hahn et al., 2003). This condition is characterized by elevated heart rate and increased plasma norepinephrine levels in family members. Because NET resequencers most of the NE released in the heart, this reuptake system in the sympathetic neurons serving the heart assumes a singular role, compared with the brain, in which regulation between several neurotransmitter systems (including NE) is shared through interactive regulatory pathways (Hahn and Blakely, 2002a,b).

Most of the hNET variants reported by Hahn et al. (2005) have also been identified in populations having cardiovascular phenotypes that include extremes of blood pressure or long QT syndrome (Halushka et al., 1999; Iwasa et al., 2001) but have not been previously characterized at the cellular level. Figure 1 shows the nonsynonymous SNPs that were characterized in this study (○) in the background of additional nonsynonymous SNPs previously identified (●). Expression levels of each SNP were assessed by Western blots after transient transfection in COS-7 cells. Surface expression of the hNET variants was identified by sensitivity to surface biotinylation. Transporter function was measured by NE and DA uptake.

The hNET variants V244I (TM4), V356L (TM7), N375S (ECL4), and K463R (TM9/ECL5) had little effect on hNET expression or transport of NE and DA. These data indicate

that the relatively conservative amino acid substitutions at these positions are stably tolerated, as further evidenced by comparison of monoamine transporter sequences from several species in which various amino acid substitutions at these analogous positions are allowed.

On the other hand, several SNPs were demonstrated to result in unfavorable substitutions. The most dramatic substitution involved A369P (TM7/ECL4), which resulted in the total loss of surface expression of the mature 110-kDa transporter and loss of NE and DA transport of the surface-expressed 54-kDa molecules. In addition, this hNET variant, as previously reported for the A457P allele as well (Hahn et al., 2003), decreased total and surface levels of hNET, demonstrating an important dominant-negative suppression of wild-type transporter expression and function. The potential synergism of diminished wild-type neurotransmitter transporter activity through dominant-negative oligomerization in individuals carrying variant alleles is particularly significant and is likely to further exacerbate cardiovascular deficiencies.

Three additional striking ideas that were generated in the current study could have profound consequences: 1) The hNET F528C (TM2) allele showed a selective change in transport for NE over DA. This analogous region of the serotonin transporter has been identified as extremely important for determining the potencies of substrates and antidepressants and similarly for functional activity of the dopamine transporter. The notion that variant alleles in the TM2 region (including hNET) could impact drug responses in these individuals is especially significant. 2) In addition, as the authors correctly point out, variants in the Phe528 region of hNET could well alter the balance of monoamine neurotransmission in areas of the brain (e.g., the prefrontal cortex) where free DA is cleared by NET (Gresch et al., 1995), with resultant elevation of both NE and DA and significant global consequences after use of selective NET antagonists. 3) Finally, altered functional responses of hNET variants (expressed separately or in concert with wild-type hNET) to protein kinase C regulation raises additional significant concerns related to hNET activity when variant alleles are present. Further experimental work is needed to fully understand the consequence of SNPs on PKC-mediated regulation of hNET. Certainly it is reasonable to conclude that PKC

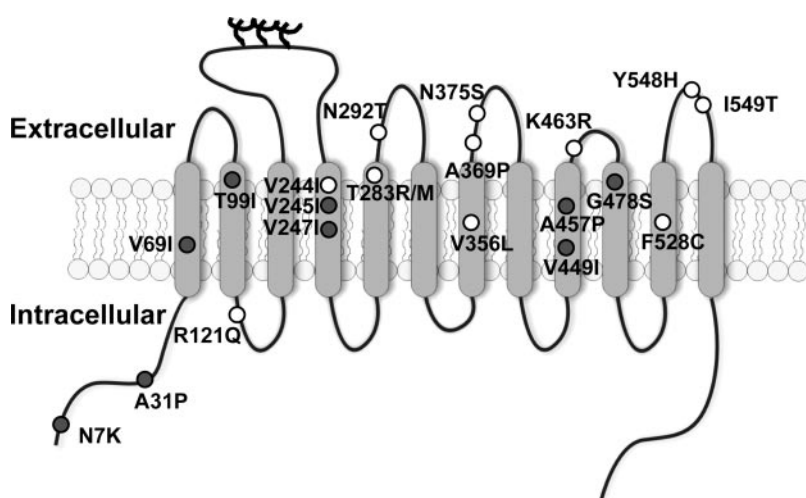


Fig. 1. Schematic representation of hNET depicting the amino acid variants generated by nonsynonymous SNPs that have been identified. SNPs characterized in this study (Hahn et al., 2005) are in open circles, and those previously identified are in closed circles.

regulation of variant transporters will be affected during both the basal and regulated "state" (compare F528C with R121Q), but the complete elucidation of functional interactions and PKC regulation must await further experimental investigation.

Taken together, these data add significantly to our knowledge regarding the properties of SNPs in hNET. The authors do an excellent job of correlating in a broader sense the significance of these mutations with regard to function and regulation and the correlative changes that have been observed in other monoamine transporters such as the serotonin transporter (Lesch et al., 1996; Hahn and Blakely, 2002a; Kilic et al., 2003). Eventually, a thorough database will be needed to fully understand human conditions that are affected by SNPs in transporters and other drug targets. This process is already under development by the National Institutes of General Medical Sciences (NIGMS) through the Pharmacogenomics Network (<http://www.PharmGKB.org>) as a repository of pharmacologically relevant genes (Hewett et al., 2002; Klein and Altman, 2004). Several entries are currently present from the solute carrier transporter superfamily, including the hNET SNP A457P, which is linked to orthostatic intolerance. As additional relevant linkages of genotypes and phenotypes for transporters are discovered, this pharmacogenomic knowledge base will be invaluable.

Although there is a long and exciting journey ahead to further define the role of genetic polymorphisms in many drug targets, the transporter families are already well seeded and nourished. The substantial significance of the work represented in this article (Hahn et al., 2005) and analogous experiments performed in many other laboratories are worthy of our notice and support. Individualized medicine, with a molecular mechanism basis, long a dream of many, is one step closer to reality.

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