The Functional Impact of SLC6 Transporter Genetic Variation

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Key Words

polymorphism, monoamine, creatine, SNP, mutation, ADHD, depression

Abstract

Solute carrier 6 (SLC6) is a gene family of ion-coupled plasma membrane cotransporters, including transporters of neurotransmitters, amino acids, and osmolytes that mediate the movement of their substrates into cells to facilitate or regulate synaptic transmission, neurotransmitter recycling, metabolic function, and fluid homeostasis. Polymorphisms in transporter genes may influence expression and activity of transporters and contribute to behavior, traits, and disease. Determining the relationship between the monoamine transporters and complex psychiatric disorders has been a particular challenge that is being met by evolving approaches. Elucidating the functional consequences of and interactions among polymorphic sites is advancing our understanding of this relationship. Examining the influence of environmental influences, especially early-life events, has helped bridge the gap between genotype and phenotype. Refining phenotypes, through assessment of endophenotypes, specific behavioral tasks, medication response, and brain network properties has also improved detection of the impact of genetic variation on complex behavior and disease.

INTRODUCTION

Solute carrier 6 (SLC6) is a gene family of ion-coupled cotransporters that includes transporters of neurotransmitters [e.g., norepinephrine (NE), dopamine (DA), serotonin (5-HT), γ-amino butyric acid (GABA), and glycine]; amino acids; creatine; and osmolytes, which include betaine and taurine (1). The family consists of four subfamilies that are based on phylogenetic identity: monoamine, GABA, amino acid, and amino acid/orphan (**Figure 1**; 2). These transporters are differentially expressed throughout the CNS in both neurons and glia and in peripheral tissues (**Figure 2**; 1). All are Na^+-dependent transporters that rely on the electrochemical gradient for Na^+ to provide the energy for transport of substrate against a concentration gradient. Some family members also utilize Cl^- or K^+ ions to enhance or support substrate uptake.

SLC transporters share a 12-transmembrane domain (TM) architecture with intracellularly oriented amino and carboxy termini, a structure that has been confirmed

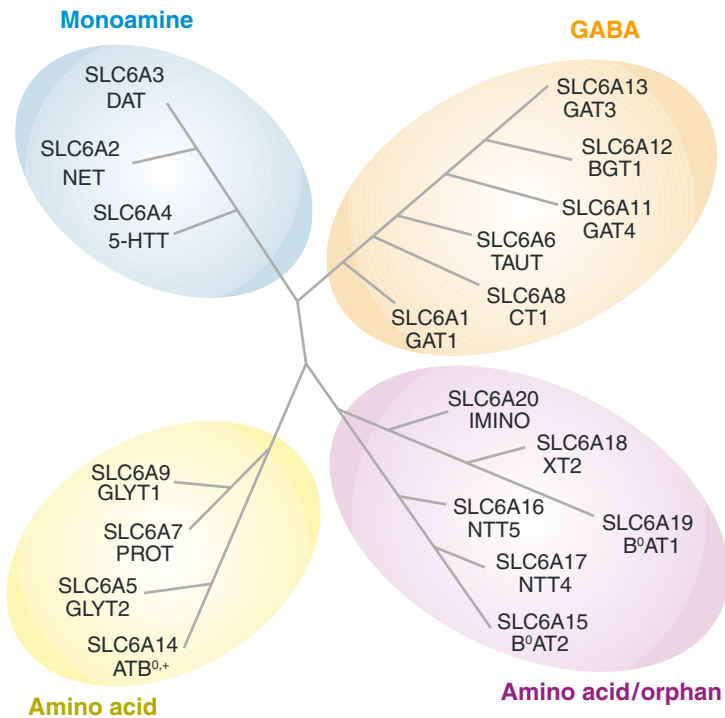

Figure 1

The SLC6 gene family tree divided in four subfamilies: monoamine, GABA, amino acid, and amino acid/orphan. DAT, dopamine transporter; NET, norepinephrine transporter; 5-HTT, serotonin transporter; GAT1, 3, and 4, GABA transporters 1, 3, and 4; BGT1, betaine transporter; TAUT, taurine transporter; CT1, creatine transporter; GLYT 1 and 2, glycine transporter 1 and 2; PROT, proline transporter; $ATB^{0,+}$, neutral and cationic amino acid transporter; B^0AT1 and 2, neutral amino acid transporters; NTT5, substrates unknown; NTT4, substrates unknown; XT2, substrates unknown; IMINO, proline transporter.

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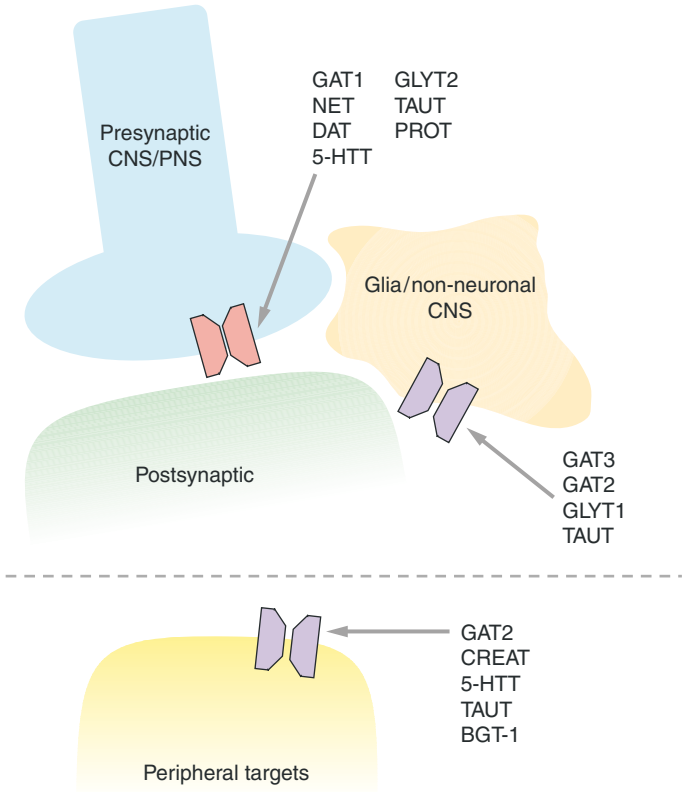

Figure 2

Schematic representation of the distribution of SLC6 transporters in different cell and tissue types, including neurons, glia, and peripheral cells.

with the crystallization of a bacterial SLC6 homologue that is a transporter of leucine (3). The large second extracellular loop contains consensus sites for glycosylation that have been shown to play a role in transporter synthesis, trafficking, and stability (4, 5). Transporters undergo a biosynthetic progression from nonglycosylated to core and higher-order glycosylation, with the most mature form predominating as the plasma membrane resident species responsible for reuptake (4, 6). Studies using biochemical, electrophysiological, and imaging techniques indicate that transporters form oligomeric structures early in biosynthesis and exist as oligomers in the plasma membrane (7). Several transporter TMs have been reported to interact and contribute to oligomer formation (8–10). There are also potential sites for phosphorylation by serine and threonine kinases and tyrosine kinases; these sites are in the amino and carboxy termini and cytoplasmic loops, and evidence supports an important role for protein kinases and phosphatases in the acute modulation of transporter trafficking and activity in response to receptor stimulation, substrates, and psychostimulants (11–16).

The role of monoamine neurotransmitter systems in mental illness has been a focus of psychiatry for decades. The NE, DA, and 5-HT transporters (NET, DAT and 5-HTT), localized to the presynaptic membranes of NE, DA, and 5-HT neurons,

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respectively, regulate the temporal and spatial influence of released neurotransmitter. Thus, understanding the genetic variation in the NET, DAT, and 5-HTT genes has the potential to elucidate both the etiologies of and therapies for psychiatric disorders. Shortly after the cloning of many of the SLC6 family members (17, 18), the search for transporter polymorphisms began in earnest in an attempt to ascertain whether the differences and uniqueness among human individuals, with respect to behavioral traits, drug sensitivity, and susceptibility to disease, are associated with genetic variation in transporters. Whereas the creatine transporter is a unique example of a SLC6 family gene found to have a Mendelian, one gene–one disease relationship, with a significant fraction of X-linked mental retardation (248), the influence of genetic variation in monoamine neurotransmitter transporters on disease has proven to be more complex. Much work has centered around identification of single nucleotide polymorphisms (SNPs) and variable number of tandem repeats (VNTRs), followed by attempts to determine their association with CNS disorders using case-control and family-based studies. A major challenge to the identification of polymorphisms that contribute to phenotypes is the complexity of many human afflictions, where contributions by multiple genes each contributing a small amount of variation are likely (19). For example, the contribution of a gene to a phenotype may be to a select trait that exists within and across broad diagnostic categories, rather than being a strong predictor of a likely heterogenous disorder. Additionally, markers employed may be in linkage disequilibrium (LD) with another site or exist as part of a haplotype that impacts transporter expression and association with a phenotype. This is complicated by a lack of knowledge of the identity of most of the functional polymorphisms in these genes, that is, those sites of genetic variation that produce changes in transporter activity, via changes in transcriptional activity, splice site usage, mRNA translation and stability, or protein activity. The identification of functional polymorphisms is a challenge owing to several factors. When examined in vivo, the impact of polymorphisms is exacerbated or masked by differing genetic backgrounds, highly individualized histories of environmental exposures, substance abuse, and stressors that contribute to brain development, function, and susceptibility to disease. Studies performed in vitro may elucidate function without the interference of these factors, but such analyses are hindered by both lack of recapitulation of the native environment and failure to engineer gene constructs containing all of the determinants necessary to elicit faithful tissue-specific expression and mRNA regulation. Studies of transporter protein function can also be hampered by artifactual findings caused by overexpression in in vitro systems. This review discusses the role of SLC6 family polymorphisms in disease, with an emphasis on recent findings that tackle these challenges by incorporating multiple markers and genes, functional polymorphisms, haplotype analyses, endophenotypes, and analyses of interactions between genes and environment.

Polymorphism: DNA sequence variation occurring at a gene locus

Single nucleotide polymorphism (SNP): DNA sequence variation occurring when a single nucleotide varies at a gene locus

Variable number of tandem repeats (VNTR): a short nucleotide sequence organized into clusters of tandem repeats

Linkage disequilibrium (LD): nonrandom association of alleles at two or more loci such that the observed frequencies do not agree with that expected from a random formation of haplotypes

Haplotype: a set of alleles located on a single chromosome likely to be inherited as a unit

HUMAN NOREPINEPHRINE TRANSPORTER (SLC6A4, NET)

It has long been hypothesized that depression involves the noradrenergic system, as evidenced by the mood-altering effects of compounds that impact catecholamine

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levels and by studies of noradrenergic metabolite levels and receptor binding sites in depression (20, 21). Furthermore, NET levels measured by [^{3}H]nisoxetine binding are decreased in the brains of patients with major depression, the majority of whom died by suicide (22). NE also plays an important role in attention, vigilance, learning, and memory and is hypothesized to contribute to attention deficit/hyperactivity disorder (ADHD) (23). Stimulant drugs used to treat ADHD, such as amphetamine and methylphenidate, act on both the NET and DAT, whereas atomoxetine, which selectively targets NET, is also effective in treating ADHD (23). The activation and sensitization of NE systems in response to stress suggest that NE may play a role in disorders triggered by early life trauma, including depression and posttraumatic stress disorder (24). NE is also the major neurotransmitter in postganglionic sympathetic synapses, and NE uptake sites and activity are compromised in cardiomyopathy, heart failure, hypertension, and ischemia (25).

Allele: DNA coding occupying a position in a chromosome. For diploid organisms, two alleles comprise the genotype

NET Polymorphisms and Linkage and Association with Disease

The NET gene exhibits multiple polymorphisms in the promoter, coding and noncoding regions. Several synonymous or noncoding SNPs have been examined in association studies. Studies of the synonymous SNP 1287 G/A (rs5569), located in exon 10,[1] but not affecting protein sequence, have resulted in mixed or negative findings in relation to depression, ADHD, personality traits, alcohol dependence, panic disorder, schizophrenia, and bipolar disorder (26–32). Two studies suggest a connection between 1287 G/A and drug response, whereby the A allele may support poorer responses to both antidepressants in major depressive disorder (MDD) and the effects of methyphenidate to ameliorate hyperactive impulsive symptoms in ADHD (33, 34). It is unknown if 1287 G/A is a functional SNP that impacts mRNA expression or stability, for example, or alternatively, is in LD with a nearby, functional SNP. Recent work has begun to characterize polymorphisms in the NET promoter region. A 4-bp deletion of a GGAA sequence was identified within a repeat region located approximately 4 kb 5′ to the translational start site that abolishes a potential binding site for the transcription factor, Elk-1 (35). The insertion of this sequence, or long allele, was preferentially transmitted to patients with anorexia nervosa. −182 T/C (rs2242446) has shown positive, negative, or no association with depression and antidepressant response (34, 36, 37). Our laboratory has been utilizing endophenotypes of MDD to refine the search for associated SNPs in multiple genes and this has revealed a highly significant association of −182 T/C with recurrence of depression and a strong trend for association with chronicity of depression (M.K. Hahn, R.C. Shelton, R.D. Blakely, unpublished data). Recently, screening of a large extent of the NET 5′ promoter region identified an A/T SNP 3081 bp upstream of the translational start site (38). The presence of the minor allele, T, introduces a *cis*-acting element that binds the transcriptional repressor, Slug (38). This provides the first

[1]This nomenclature defines exon 1 to be the novel exon, identified 5′ to the originally described exon 1, now termed exon 2, and all other exons and introns are named accordingly.

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functional promoter SNP in NET with an identified mechanism of transcriptional regulation. This work suggests that further studies of Slug as a risk factor for noradrenergic dysfunction are warranted and highlights the importance of identifying functional SNPs to reveal genes, such as Slug, that are not intuitively thought of as contributing factors but may also harbor genetic variability.

Wild-type: sequence of DNA or protein considered normal, as opposed to a mutation

NET Protein Variants

To date, approximately 20 SNPs in the NET coding region (cSNPs) that alter protein sequence (nonsynonymous cSNPs) have been reported. Stöber and coworkers were the first to identify a group of NET protein variants: V69I, T99I, V245I, V449I, G478S. All were observed at frequencies of <0.02 and were not associated with bipolar disorder, schizophrenia, or Tourette's syndrome (TS) (39, 40). G478S demonstrated an increase in the K_M value for NE transport in transfected cells (41). Although this initial work did not yield strong evidence of contribution of NET variation to disease, the proposition that protein variants that impact transporter function would be enriched in selective phenotypes that implicate noradrenergic systems fueled subsequent NET SNP discovery studies.

One disorder suggestive of NET dysfunction is orthostatic intolerance (OI), characterized by an increase in standing heart rate of at least 30 bpm that is not accompanied by hypotension (42). A proband with OI was identified who demonstrated standing-induced increased NE spillover and decreased NE clearance, as well as decreased intraneuronal metabolism of NE, as measured by decreased dihydroxyphenylglycol (DHPG) to NE ratios (43). The proband and other family members harbored a heterozygous nonsynonymous SNP in exon 10 that produced the amino acid substitution A457P in TM9 (44). When assessed across the proband's immediate family, a significant correlation between the presence of A457P and elevations in standing-induced heart rate and plasma NE, and decreased plasma DHPG to NE ratio was observed. A457P was the first disease-associated transporter variant in the SLC6 family.

Transient transfection studies revealed A457P to be devoid of NE transport activity and greatly diminished in both the mature, fully glycosylated form of the transporter and its surface expression (**Figure 3**; 6, 44). Importantly, Hahn and coworkers showed that A457P interacts in a complex with wild-type NET and exerts a dominant-negative effect on wild-type NET plasma membrane expression and uptake activity (**Figure 3**; 6). These findings suggest that individuals heterozygous for A457P, or other transporter polymorphisms, may be affected to a greater extent than predicted for harboring one mutant allele. In ongoing studies, we have identified a novel NET variant in an ADHD proband who also exhibits elevated heart rate on standing (M.K. Hahn & R.D. Blakely, unpublished observations). Thus, elevated standing heart rate may be an endophenotype indicative of NET dysfunction that may successfully identify subgroups of subjects with functional NET polymorphisms within populations bearing broader neuropsychiatric diagnoses.

Additional nonsynonymous NET SNPs were identified in studies of populations with cardiovascular phenotypes, both subjects with extreme blood pressure

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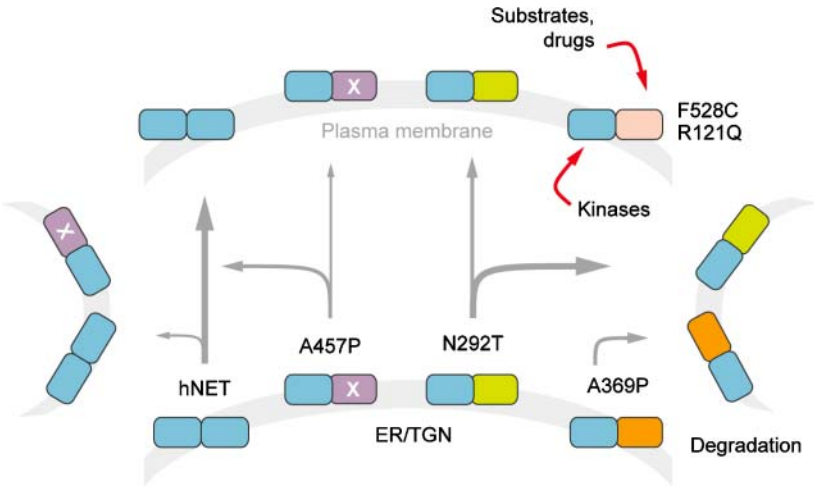

Figure 3

Schematic representation of impact of identified NET variants on biosynthetic progression versus degradation, trafficking, dominant-negative effect on NET wild-type and altered substrate, antidepressant, and protein kinase interactions. ER/TGN, endoplasmic reticulum/trans-Golgi network.

measurements and with long QT syndrome (45, 46). These protein variants express striking effects on transporter protein expression, substrate transport, antagonist interaction, and regulation by kinase-mediated signaling pathways (**Figure 3**; 47). A369P generates a total loss of NE and DA transport, and N292T displays approximately 50% loss of both DA and NE transport. This was at least partly due to a striking disruption in the glycosylation and surface trafficking of these variants. Additionally, A369P and N292T both decreased surface levels of wild-type NET when coexpressed (47). These results support growing evidence that intracellularly retained or mistargeted mutants exert dominant-negative effects on synthesis or trafficking to decrease surface expression and activity of wild-type transporters, facilitated by formation of oligomeric complexes (**Figure 3**; 6, 7).

The variant, R121Q, demonstrates decreased surface levels and decreased transport that is more pronounced for NE versus DA (47). R121 is part of a conserved sequence similar to a motif present in the bacterial Tn10 tetracycline antiporter, and in which the positively charged R is required for transport (48). Substitutions of other amino acids at R121 or other residues of IL1 in NET reduce transport and expression (49). The variant F528C is a more efficient transporter of NE compared with DA and demonstrates an increased K_I for desipramine competition of NE uptake. Remarkably, this variant is resistant to the effects of β-PMA, a phorbol ester that activates protein kinase C (PKC) and downregulates NET activity.

Differences in substrate utilization by R121Q and F528C suggest a potential impact of variants on the balance of catecholamine neurotransmission in areas of the CNS where dual innervation by NE and DA occurs. In the prefrontal cortex (PFC), where both NE and DA fibers are present, released DA is cleared from the extracellular space by NET (**Figure 4**; 50, 51). The therapeutic effect of selective NET blockers in the treatment of ADHD may lie in their ability to elevate both NE and DA (52). Although most of these SNPs appear to be rare in human populations, animal models incorporating NET gene variants such as those with kinase insensitivity or

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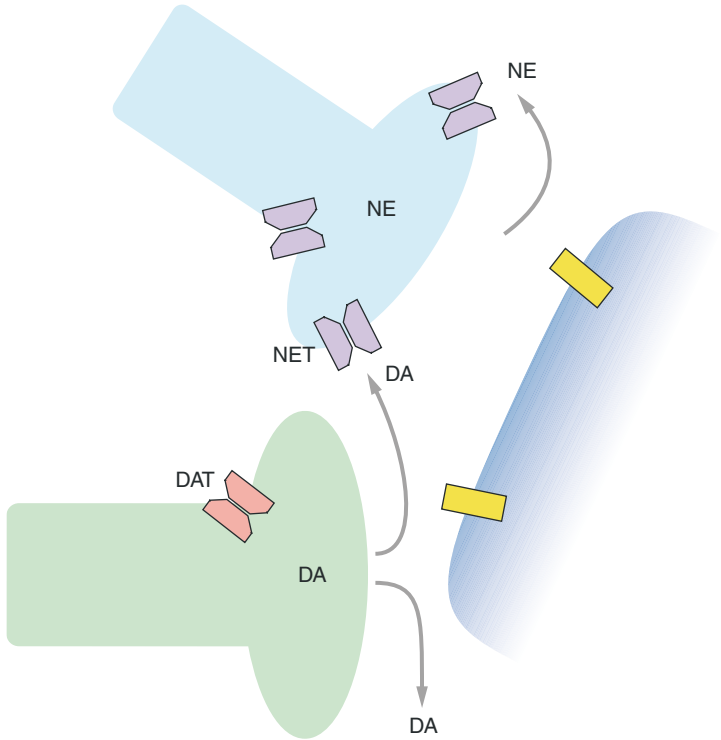

Figure 4

Role of NET in DA clearance in the prefrontal cortex (PFC).

altered substrate efficacies may provide useful tools to understand the requirements for and impact of normal NET function.

HUMAN DOPAMINE TRANSPORTER (SLC6A3, DAT)

DA systems are important mediators of motor function, cognition, mood, and reward, particularly the reinforcing properties of drugs of abuse (53, 54). DA systems are implicated in many disorders, including ADHD, bipolar disorder, autism, schizophrenia, drug abuse, Parkinson's disease, and TS (55, 56).

DAT VNTRs

Resequencing of the DAT gene has identified multiple SNPs and VNTRs located throughout the promoter, coding, and 3′ untranslated regions of DAT. Vandenbergh and coworkers identified a VNTR in the 3′ untranslated region of DAT that is composed of repeats of a 40 bp element containing 9 or 10, or more rare, 3, 5, 7, 8, and 11, copies of the repeat unit (57). In the Caucasian population of that study, the 10-repeat allele was present at a frequency of 0.70, followed by the 9-repeat allele at

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0.24. Subsequent studies showed that great heterogeneity exists in allele frequencies across different ethnic groups (58). A VNTR in intron 8 has also been reported that consists of 5 or 6 copies of a 30-bp repetitive element with frequencies of the 6-repeat allele of 0.38 in Caucasians and 0.79 in African Americans (59).

Linkage and Association Studies with the DAT 3′ VNTR

Most linkage and association studies have focused exclusively on the DAT 3′ VNTR, as its location and structure make it a candidate for a functional polymorphism. Mixed or negative results have been found in studies attempting to link or associate the DAT 3′ VNTR with alcohol dependence (60–63). The 9-repeat allele is associated with decreased probability of being a smoker, longer periods of smoking cessation for smokers, and an increased likelihood of quitting (64, 65). However, in a large study of 861 Caucasians, the presence of the 9-repeat allele was not associated with an individual being a current or former smoker or in the nonsmoker group (66). A significant increase in the 9-repeat allele was seen in cocaine-dependent subjects experiencing paranoia (67). 9/9 genotypes are reported to exhibit very little subjective response to amphetamine (67a). Association of the DAT 3′ VNTR with Parkinson's disease has yielded negative results or an association of the rare 11-repeat allele (68, 69). Association studies of DAT polymorphisms and both bipolar disorder and MDD have met with mainly negative results (70, 71). Family-based studies of linkage have yielded intriguing results, whereby the 10-repeat allele of the 3′ VNTR was found to be in LD with bipolar disorder (72). However, other studies failed to replicate this linkage (73, 74). Taken together, these studies paint an inconsistent picture of the role of the 3′ VNTR in disease. All hope is not lost, as researchers have begun to identify and test multiple markers in the DAT gene and such studies have started to yield promising results, particularly in studies of ADHD.

Attention Deficit/Hyperactivity Disorder

Multiple groups have sought to establish linkage and association of the DAT 3′ VNTR and, recently, other markers within DAT with ADHD. In the first study to examine the 3′ VNTR, Cook and coworkers used a haplotype-based haplotype relative risk (HHRR) approach in 56 families to demonstrate that the 10-repeat allele was preferentially transmitted to the affected subject (75). Other within-family designs have demonstrated similar linkage of the 10-repeat allele (76–80). However, other within-family studies, as well as case-control association studies, have failed to replicate these positive findings (81–85). A recent meta-analysis supports the association of DAT with ADHD (86). There is also evidence for preferential transmission of paternal risk alleles, including the DAT 3′ VNTR, to affected ADHD children (87). Such parent-of-origin effects illustrate another level of complexity that, taken into consideration, may further elucidate the relationship between the DAT 3′ VNTR and ADHD.

Imaging studies have examined the level of striatal DAT binding sites in relation to ADHD and the influence of psychostimulant medication response, and attempted to

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establish correlation with the DAT 3′ VNTR genotype. Several groups have reported increases of 15%–70% in DAT binding sites in the striatum in adult attention deficit disorder (88–90). In two of these studies, elevated DAT binding sites were decreased to less than control by methylphenidate treatment (89, 90). In another study, striatal DAT binding sites in ADHD were not elevated compared with controls, but DAT levels in the patient group were positively correlated with hyperactivity severity (91). To the extent that the DAT 3′ VNTR impacts DAT expression (discussed below), this polymorphism may influence the treatment response if, for example, occupancy of a minimum percentage of transporter binding sites is necessary to trigger the neurochemical events that produce favorable drug effects. An equal number of studies have found association of the DAT 3′ VNTR 10-repeat allele with either lack of or better response to methylphenidate (MPH) (92–95). In summary, DAT levels appear altered in ADHD, medication that targets DAT appears to influence these levels, and the 3′ VNTR genotype may be setting a tone for both the disease state and the ability of drugs to rectify the DA uptake system.

Cognitive Processing in ADHD and DAT

Motor impairments are considered central to the symptomatology of ADHD and, indeed, what we know of DAT availability from imaging studies derives from analysis of the nigrostriatal DA pathway. Impairments in executive function, including response inhibition, selective attention, planning, and cognitive flexibility/set shifting, also characterize ADHD and are likely mediated by cortical signaling (96). A study of boys selected from the general population, scoring in either the top or bottom 10% on ADHD assessments, demonstrated a significant excess of DAT 3′ VNTR 10/10 genotypes in the group scoring high for ADHD symptoms (97). Furthermore, subjects with 10/10 genotypes performed worse on measures of selective attention and response inhibition (97). Consistent with these findings, the 10/10 genotype is associated with increased commission errors and impaired impulse control response on the Continuous Performance Task (CPT) (98). Additionally, 10/10 genotypes display increased spatially biased inattention (99). In a study of working memory, an interaction was observed for homozygotes for both the low-activity COMT Met allele and DAT 10-repeat allele to demonstrate a lower blood oxygen level–dependent functional magnetic resonance imaging (BOLD fMRI) signal in the cortex during the task (100). Thus, the effects of DAT genetic variation on phenotypes may be subject to its expression in distinct neuroanatomical pathways that mediate motor or cognitive behaviors, express different levels and patterns of DAT, and encompass a particular repertoire of gene expression. Indeed, striatal DAT is primarily synaptic, whereas PFC DAT is sparse and located away from the synapse, and NET has been shown to clear DAT in PFC (50, 51, 101, 102). Additionally, as COMT appears important in PFC DA clearance through metabolism, epistasis between DAT and COMT may be more likely when examining behaviors mediated primarily in this region. Taken together, these data support the utility of incorporating endophenotypes, assessment of selective brain regions, and multiple gene polymorphisms.

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DAT 3′ VNTR and DAT Expression

The 3′ untranslated region of genes may serve a variety of functions, including sorting of mRNA to distinct compartments in the cell, regulating rate of translation and signaling stabilization, or destabilization of mRNA to influence turnover rate (103–105). Several studies have examined the relationship of the DAT 3′ VNTR genotype to DAT binding sites in the brain. A study of abstinent alcoholics and controls demonstrated a 22% increase in [^{123}I]β-CIT binding in the putamen of subjects with a 10/10 genotype (with no genotype difference between controls and alcoholics) (106). These above findings are consistent with the association of the 10-repeat allele with ADHD and an increase in striatal DAT binding sites in ADHD that has been reported. However, other studies also utilizing radiolabeled β-CIT as the ligand have found either a decrease or no change in binding sites associated with the 10-repeat (107–109). Finally, alcoholics admitted to detoxification treatment demonstrate decreased striatal [^{125}I]β-CIT binding compared with controls that increased to control levels following four weeks of abstinence, highlighting the potential of a disease state to confound results (110). Results in transfected cells have been equally discordant (111–113). In summary, it is not apparent, or probable, based on current studies that the 3′ VNTR exerts autonomous control over DAT expression in the brain but, rather, it interacts with other sites within the DAT gene as well as the environment.

Contribution of Other DAT Risk Alleles and Haplotypes

Recent studies have begun to examine the full expanse of the DAT gene in earnest, identifying multiple markers, defining the haplotype structure of the gene, and examining the contribution of haplotypes, rather than simply the 3′ VNTR, to both expression and disease. LD analyses revealed the presence of almost complete disequilibrium between seven SNPs in the 5′ region of DAT (promoter through intron 6), as well as a high degree of LD between seven SNPs in the 3′ region (exon 9 through exon 15), indicating that there are two major blocks of the DAT gene with limited chromosomal recombination (114, 115). Analysis of a sample of 50 parent-proband triads for bipolar disorder revealed a highly significant association of haplotypes comprising five SNPs in the 3′ region (114). An analysis of the 3′ VNTR, an exon 9 SNP (rs6347), and an intron 9 SNP (rs8179029) found that haplotypes containing the 3′ VNTR 10-repeat allele and the G allele of the intron 9 polymorphism but differing in the presence of the A allele of exon 9 polymorphism were preferentially transmitted to ADHD-affected children (116). Expanding this initial study of 102 families (116) to 178 ADHD families, Feng et al. found biased transmission of the haplotype containing the G allele of an Msp1 SNP (rs27072) located 480 bp 5′ of the 3′ VNTR, the 10-repeat allele of the 3′ VNTR, the exon 9 and intron 9 SNPs (117). Examining two separate populations of ADHD, one English and one Taiwanese, Brookes and coworkers found association and linkage of the 3′ VNTR and association of the intron 8 VNTR (118). Furthermore, this study revealed that a haplotype of the 3′ VNTR 10-repeat and the intron 8 VNTR 6-repeat was

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preferentially transmitted in both populations, with an increased risk if mothers drank alcohol during pregnancy. This interaction could reflect an environmental influence through the effects of alcohol on fetal development or alcohol-related behavioral patterns that influence the child. It may also signify that transmitted genes associated with promoting alcohol use also have a bearing on ADHD. A recent study has associated the 6-repeat of the intron 8 VNTR with cocaine abuse in a Brazilian population (119). These researchers also explored the functional influence of the intron 8 VNTR through expression in SN4741 cells, derived from substantia nigra and thus providing a native environment. The 5-repeat exhibited 50% more activity than the 6-repeat, cocaine selectively decreased expression from the 6-repeat, and depolarization and forskolin treatments increased expression of the 6-repeat (119). These data provide exciting evidence that the intron 8 VNTR is associated with ADHD and cocaine abuse and may be a functional variant modulating expression to influence a phenotype, although the mechanism mediating the influence of the intron 8 VNTR on expression remains to be elucidated.

The DAT promoter region also appears to contain determinants of both expression and disease association. Expression of two constructs of common promoter hoplotypes in SN4741 cells reveals differences in levels of reporter expression and evidence for repressor elements (120). Another study of the promoter region spanning 5 kb 5′ of the transcriptional start site through the start of exon 2 identified common haplotypes that direct as much as a twofold difference in expression in transfected PC12 cells (121). Variation in the 5′ region may also contribute to disease susceptibility. One group has shown that an increase in the T allele of a DAT promoter SNP, −67 A/T, associates with bipolar disorder (122). One study shows that the 3′ VNTR 10 repeat is preferentially transmitted to ADHD children, and that a haplotype of the 3′ VNTR plus a 5′ microsatellite marker was even more strongly associated (123). In summary, there are polymorphic regions located throughout the DAT gene that may participate in conjunction with or independently of the 3′ VNTR to determine expression levels and impact phenotypes, and further elucidation of the ability of these polymorphisms to act as regulators of transcription, splicing, or mRNA processing will suggest a set of highly informative markers.

DAT Protein Variants

Five nonsynonymous polymorphisms, V55A, R237Q, V382A, A559V, and E602G, have been identified in DAT (63, 124, 125). The A559V and E602G mutations were identified in bipolar disorder and A559V was also identified in an ADHD cohort (125, 126). Of these, V382A, although not identified in an affected population, demonstrates functional effects. V382A is decreased on the cell surface and has diminished transport capacity in several heterologous cell lines (127, 128). In addition, V382A shows a downregulation in activity in response to PMA treatment but internalization is impeded (128). Normally, internalization of transporters in response to PMA is synchronous with decreases in activity, and is typically presumed to be the mechanism of downregulation. These and other recent data suggest that transporter inactivation and trafficking can be uncoupled (14).

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SEROTONIN TRANSPORTER (SLC6A4, HTT, 5-HTT, SERT)

5-HT plays a role in mood, aggression, response to alcohol, appetite, sleep, cognition, and sexual and motor activity, and it likely contributes to multiple mental illnesses related to these biological processes, including depression, suicide, anxiety, autism, obsessive-compulsive disorder (OCD), eating disorders, schizophrenia, and alcohol abuse (129–131). 5-HTT is a target for SSRIs, which block 5-HTT, and are the most efficacious and commonly employed pharmaceutical tools in use. 5-HTT binding sites are reportedly reduced in both platelets of depressed patients and brains of depressed individuals who committed suicide (130, 132). 5-HT and 5-HTT are also implicated in diseases of peripheral systems, particularly irritable bowel syndrome and primary pulmonary hypertension (132a, 132b).

5-HTT Polymorphisms

Two VNTR polymorphisms in 5-HTT have been studied most extensively. One polymorphism, termed the 5-HTTLPR, was initially identified as comprising two alleles, long (l) and short (s), with frequencies in Caucasians of 0.39 and 0.61, respectively (133). There is large variation in allele frequencies of the 5-HTTLPR among different ethnic populations, as well as rare, longer alleles (134–136, 136a, 162). Novel alleles of s and l that differ in sequence composition of the individual repeat units have also been found (137). Expression of promoter constructs containing the l or s allele in cell culture demonstrated that the l allele confers threefold greater basal transcriptional activity (133, 137, 138). Lymphoblast cell lines generated from individuals demonstrated that the ll genotypes had greater 5-HTT mRNA levels, [^{125}I]RTI-55 membrane binding and 5-HT uptake (138, 139). Enhanced 5-HT uptake associated with the 5-HTTLPR ll genotype has also been observed in platelets and smooth muscle cells (140) but other studies have failed to replicate this finding in peripheral cell types (141, 142).

A VNTR has been identified in intron 3[2] of 5-HTT that is composed of 17-bp repeats, the most prevalent alleles being the 10- and 12-repeat (143). As demonstrated for other polymorphic sites, there is a wide range of frequencies of the intron 3 VNTR among ethnic groups (135, 136). Expression in transgenic embryos and embryonic stem cells suggests that the intron 3 VNTR polymorphism acts as a regulator of transcription (144, 145).

5-HTT Polymorphisms: Linkage and Association Studies

In addition to identifying the 5-HTTLPR, Lesch and coworkers also demonstrated the first association of this polymorphism with a phenotype. S-containing genotypes were associated with higher scores on the neuroticism scale in the NEO personality inventory (NEO-PI-R), which is based on anxiety and depressive symptoms (139). Several studies employing a variety of personality inventories have failed to associate

[2] Intron 3 was formerly designated intron 2 prior to the identification of a novel upstream exon in 5-HTT.

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neuroticism with the 5-HTTLPR (146–151). Others have confirmed the association between the 5-HTTLPR s allele and personality assessments of anxiety (152). Lesch and colleagues returned to this issue and replicated their findings in a new population, both alone and as a combined group with the population from the initial study (153). Furthermore, a meta-analysis of all of these studies determined that there is an association of the 5-HTTLPR with neuroticism on the NEO personality inventories (154).

Family-based linkage studies and case-control association studies have provided some evidence for risk associated with the s allele of the 5-HTTLPR with MDD, bipolar depression, and seasonal affective disorder (SAD) (155–159). A number of reports have also failed to find linkage or association with depressive disorders (70, 160–164). Efforts to identify an association of the 5-HTT intron 3 polymorphism with affective disorder have yielded mixed results (157, 162, 165–170).

More convincing evidence supports that the 5-HTTLPR genotype has predictive power for SSRI treatment response, with association of the l allele with better antidepressant response. MDD patients treated with SSRIs did not respond as well, as measured by scores on the Hamilton Depression Rating Scale (HDRS), if they were of the ss genotype (171–173). Furthermore, this difference in response was eliminated when antidepressant treatment was supplemented with pindolol (171, 172). Interestingly, this effect does not appear to extend to non-SSRI compounds, as there is no difference in the response by genotype to nortriptyline, an uptake inhibitor that acts with greater selectivity at NET (174). There is also highly significant association of the s allele with manic episodes experienced by bipolar patients and believed to be provoked by their antidepressant treatment (175). The ability of the s allele to predict poorer or adverse response to SSRIs presents an opportunity for a pharmacogenomic approach to the treatment of affective disorder whereby selection of therapeutics can be matched to genetic background.

5-HT systems are hypothesized to mediate aggressive, impulsive behavior that may be present across a broad spectrum of psychiatric syndromes (129). There have been efforts to establish an association of 5-HTT gene polymorphisms with clinical conditions that involve elements of impulsivity, such as alcohol dependence and suicide. Examining the link of the 5-HTTLPR to alcoholism alone has generated mixed results (136, 176, 177). However, utilizing endophenotypes that include impulsivity as a trait have generated interesting results. The Type 2 subgroup of alcoholics are defined by early onset, with dissocial and impulsive-aggressive behavior and also display alterations in aspects of serotonergic system function and high novelty seeking and low harm avoidance scores on the TPQ personality assessment (178). One study reported a trend for an excess of ss genotypes in dissocial alcoholics (179). The increased frequency of the s allele was replicated in impulsive, violent, early-onset alcoholics compared with late-onset alcoholics (180). However, this finding was not replicated in German alcohol-dependent patients, divided into high and low-impulsivity groups, compared with controls (181).

Association of the 5-HTTLPR s and l allele and no association with suicide have all been reported (182–184). [^{3}H]cyanoimipramine binding to 5-HTT is decreased in the prefrontal cortex of depressed patients and suicide victims, but the 5-HTTLPR allele

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status has no bearing on this (185). However, an association has been made between the 5-HTTLPR and suicidal behavior with elements of violence. A compelling study reported that 91% of suicides, mostly committed by violent means, but only 67% of controls, had an s-containing genotype (186). Another group replicated this finding (187). Taken together, the data suggest an association of the s allele of the 5-HTTLPR with impulsive, violent behavior that is present across a broad spectrum of psychiatric disorders.

Cook and coworkers were the first to identify linkage of the s allele of the 5-HTTLPR gene polymorphism in autism (188). However, other family-based TDT studies, including subgroup analyses, support preferential transmission of the l allele (189–191). Two of these reports agree in finding no preferential transmission of the intron 3 VNTR (188, 189). Case-control studies have found a lack of association of the 5-HTTLPR with autism (192). A genome-wide linkage analysis of 152 sib pairs revealed significant LOD scores on chromosome 17 using a marker in intron 3 of 5-HTT and demonstrating increased paternal transmission (193). Evidence of linkage on chromosome 17 was also found by Sutcliffe and colleagues in a sample of 341 families, with particularly striking linkage to 17q11.2 in a subgroup of families containing only affected males (194). Furthermore, association of rigid compulsive behaviors was revealed when multiple 5-HTT polymorphisms identified in the families, including functional protein variants, were analyzed in aggregate (194). Taken together, these data point to exciting advances that can be made in studies of autism, and likely other disorders, when experimental design and analyses consider parent-of-origin effects, allelic heterogeneity, and trait clusters.

Analyses of the 5-HTTLPR and OCD have yielded evidence of both linkage of the l allele and association of the ll genotype with OCD (195, 196). However, other studies have failed to find a relationship (197, 198). Using an A/G SNP within the l allele of the 5-HTTLPR as an additional marker (see further discussion below), Du and coworkers found association and preferential transmission of the l-A allele in OCD. That OCD is uniquely treated by 5-HTT-preferring antidepressants suggests pharmacogenomic approaches to treatment of this disorder may prove useful and advocates further examination of 5-HTT gene variation in OCD.

5-HTTLPR and 5-HTT Expression in Brain

In vivo SPECT and PET imaging and postmortem studies of 5-HTT radioligand binding have yielded mixed results. Three imaging studies, two using [^{123}I]β-CIT as the SPECT ligand, report no effect of 5-HTTLPR on 5-HTT binding in several diencephalon and mesencephalon brain regions of healthy subjects (107, 199, 200). Another study reported higher 5-HTT binding sites in ss genotypes in a healthy, primarily Caucasian population (201). Using a novel approach, Lim et al. tested for allelic imbalance in the amount of RNA expressed from the l versus s allele in individuals that were heterozygous for the 5-HTTLPR (202). The authors found no imbalance between alleles of heterozygous sl individuals in either rostral pons or lymphoblastoid cell lines and no differences in absolute mRNA levels among ll, ls, and ss genotypes.

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Two reports examining the 5-HTTLPR and 5-HTT binding sites in the brain in alcohol-dependent subjects and cocaine users reveal an intriguing genotype-disease interaction that highlights the difficulties in understanding on a simple level the relationship of the 5-HTTLPR to expression. In one report, sl genotypes were accompanied by less postmortem 5-HTT [^{125}I]β-CIT binding and mRNA in the dorsal raphe in a group combining cocaine users and controls compared with ll genotypes (203). However, [^{125}I]β-CIT binding was increased in alcoholics in the same study with s-containing genotypes compared with cocaine user and control groups. In the second study, examining controls only, those with the ll genotype demonstrated twofold greater [^{123}I]β-CIT binding, measured by in vivo SPECT in the dorsal brainstem, than did ss-carriers (204). However, alcohol-dependent subjects with the ll genotype demonstrated less [^{123}I]β-CIT binding than ll controls. This apparent interaction of genotype with alcoholism on 5-HTT binding sites could be due to a different array of genetic contributions in alcoholics or due to the effects of alcohol and many other environmental factors associated with chronic alcohol use. This emphasizes that experience may interact with genotype to affect 5-HTT expression.

An A to G SNP, identified by Nakamura and coworkers, exists solely in the l allele of the 5-HTTLPR, contained within the repeat region unique to this allele (137). The presence of the l-G allele contributed to decreased 5-HTT mRNA expression in lymphoblastoid cell lines and decreased reporter construct expression in RN46A cells, similar to levels generated by s (205). The alleles were codominant, suggesting that rather than being considered as a triallelic SNP, l-G and s alleles might be functionally grouped. Gel shift assays confirmed that an AP-2 site created by l-G binds AP-2 to repress transcription. Case-control and TDT linkage demonstrate association and preferential transmission, respectively, of the l-A allele in OCD. However, applying this partition of alleles, Parsey et al. were unable to find an influence of 5-HTTLPR genotype on 5-HTT binding potential in multiple brain regions in medication-free patients with MDD or in healthy volunteers (206). Characterization of the l-G allele exemplifies how a complete understanding of the polymorphic sites in 5-HTT that dictate expression level will likely help eliminate some of the hurdles that impede linking 5-HTT genetic variation to disease.

5-HTTLPR and Vulnerability to Stressors

During the early postnatal period, 5-HT plays a pivotal role in the development of the CNS (207). Multiple environmental influences, including environment richness, stressful life events, and exposure to drugs, are likely to impinge on 5-HTT expression via transcriptional and posttranscriptional mechanisms, as well as influence development of the 5-HT system and receptor expression. Thus, the level of 5-HTT driven by s and l alleles could interact with all of these environmental factors to influence 5-HT system structure and function. Studies in which 5-HTTLPR genotypes were stratified by occurrence of traumatic life events reveal a striking interaction between extreme stress and ss genotype on the number of depressive symptoms and diagnoses suffered (208–211).

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Work in primates supports an interaction between the 5-HTT gene and environment to promote pathology. In rhesus macaques, there is a polymorphic repeat region homologous to the human 5-HTTLPR (212), and in transfected JAR cells, the s allele also directs less transcriptional activity (213). The primate 5-HTTLPR has thus been a valuable tool to examine the interaction of the 5-HTTLPR and early life events that can be manipulated and controlled in monkey colonies to a far greater extent than in human populations. Differences in temperament, including emotional reactivity, are observed by one month of age in sl versus ll rhesus monkeys (214). This is reminiscent of the observation that the 5-HTTLPR can predict differences in temperament in human infants (215). Monkeys reared in stressful conditions by peers (PR), but not maternally reared monkeys (MR), demonstrate abnormal social behavior, a preference for and altered behavioral response to ethanol, and lower levels of CSF 5-HIAA if they harbor s alleles or sl genotype (213, 214, 216–218). In response to a social isolation stress, PR monkeys demonstrated an enhanced ACTH response if they had the sl genotype (219). Taken together, these data support the conclusion that the s allele acts as a predisposing factor with which environmental stressors interact to reset neurotransmitter and hormonal signaling and produce long-lasting changes in mood, behavioral response to stress, and substance-taking behavior.

5-HTTLPR and Networks of Affective Responding

fMRI studies have revealed a relationship between the 5-HTTLPR and brain activity in response to stimuli evoking affective responding. Hariri et al. demonstrated that individuals carrying the s allele viewing fearful and angry faces exhibit a fivefold greater activation of the amygdala (220). Furthermore, fMRI also revealed functional connectivity between anterior cingulate cortex and amygdala in fearful responding, which was disrupted in the s allele carriers (221). Heinz and colleagues demonstrated a BOLD response in the amygdala, which was increased to a greater extent in ss carriers, in response to viewing aversive images and was positively coupled to ventromedial (vmPFC) response (222). These latter data indicate that the emotional saliency of aversive stimuli or events may be enhanced in s allele carriers. That these papers came to dissimilar conclusions regarding coupling between cortical-subcortical regions may be due to differences in amygdala interactions with anterior cingulate versus vmPFC, different 5-HT signaling in these regions, and/or differential effects of s allele-driven expression on neurotransmission in different target regions. It is noteworthy that Heinz et al. described increased coupling between amygdala and vmPFC in s carriers relative to ll, as evidence supports a role for the vmPFC in reward processing (223). Another group has examined the effect of acute tryptophan depletion (ATD) to deplete brain 5-HT in reward responding in the context of 5-HTTLPR genotype (224). There were interactions between genotype, ATD, and reward responding, indicating that l and s alleles influence brain reward systems differentially, both at baseline and if 5-HT levels are manipulated (224). Taken together, these data indicate that the 5-HTTLPR influences activity in brain networks mediating affect and reward, important components of the dysfunction in mood disorders.

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5-HTTLPR s and l Alleles and 5-HT Signaling in Brain

Most studies consider the influence of the s and l alleles on 5-HTT expression, but ongoing studies of network properties and developmental influences make it clear that more needs to be learned about effects of this polymorphism on the development of 5-HT systems and 5-HT tone in the brain under both basal and activated circumstances. The influence of s and l alleles may be the result of adaptations of the anatomical connections of 5-HT pathways, expression of pre- and postsynaptic receptors, and excitability of 5-HT neurons. Recent studies show that the s allele correlates with decreased 5-HT1A receptor binding in humans, leading to a hypothesis that a higher level of extracellular 5-HT may cause compensatory downregulation of receptors (225). 5-HTT knockout mice display altered 5-HT neuron density, firing rate, and receptor sensitivity, possibly due to the long-term developmental effects of reduced 5-HT reuptake (226, 227). The proposed reduction in transcriptional activation of the s compared with l allele in response to signaling cascades may also play a role in the influence of the 5-HTTLPR on expression and disease susceptibility. A diminished responsiveness of the s allele to signal transduction pathways may not meet the demands of 5-HT neurotransmission and lead to vulnerability to the influence of environmental factors that could be buffered by more dynamic changes in 5-HTT expression from the l allele. Thus, the more dynamically regulated l allele would be beneficial in the response to antidepressants or under conditions requiring transporter mobilization. Under certain conditions the l allele could also play a role in a disadvantageous sensitization to life stressors. What is needed is to expand our thinking beyond the designation of one allele as "healthy" and one allele as "disease-promoting," to incorporate recent data suggesting that expression is under the control of a complex interplay of gene and environment, and to realize that the influence of alleles may vary with the nature and intensity of the stimulus and previous experience.

5-HTT Protein Variants

Several studies have identified nonsynonymous 5-HTT SNPs, a substantial number of which have effects on transport function and appear to be enriched in select diagnostic populations and in families with high incidence of psychiatric disorders (124, 228). I425V was found in two families with multigenerational incidence of psychiatric diagnoses, including OCD and Asperger's syndrome (229). I425V exhibits enhanced transport in transfected cells (230). In a study of 341 autism families, G56A, F465L, L550V, and I425L were identified in affected probands, the latter two variants representing novel SNPs (194). When all the coding variants were combined, there was a significant association with rigid compulsive behaviors in the families with linkage at 17q11.2, suggesting that these variants may indeed contribute to a subgroup of autistic subjects (194). The G56A variant is also notable in that although its frequency in the general population is estimated at 0.5%–1.0%, in the autistic families selected on strength of contribution to the linkage peak at 17q11.2, the frequency is increased to 2.3%, including the presence of three minor allele homozygotes (194, 228). Lymphoblastoid cell lines homozygous for A56 displayed enhanced

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5-HT uptake compared with G56 homozygotes (194). Functional characterization of other 5-HTT SNPs has revealed that T4A, G56A, S293P, L362M, and I425V display enhanced basal 5-HT transport activity, and one variant, P339L, has markedly reduced uptake activity (231).

Examination of the response of 5-HTT variants to cytokines, neurotransmitters, and second messengers has revealed further striking impact of these protein variants. Acute treatment with IL-1β, TNFα, and other neurotransmitters that signal through protein kinase G (PKG) and p38 MAP kinase (p38MAPK) stimulates 5-HTT activity, altering both K_M and V_{MAX} for transport (232). I425V, in addition to elevated transport, was reported to be unresponsive to nitric oxide stimulation of guanylyl cyclase (230). Remarkably, T4A, G56A, G215K, K605N, and P621S (but not I425V) are completely insensitive to PKG and p38MAPK activation (231). Additionally, G56A was also shown to be hyperphosphorylated in the basal state and phosphorylation was not enhanced by use of 8BrcGMP to stimulate PKG (231). In transformed lymphocytes, A56 homozygotes are also insensitive to regulation by PKG and p38MAPK, recapitulating the observations in heterologous cell lines (194).

A fascinating insight from these studies is that individuals harboring SNPs that affect 5-HTT protein regulation by PKG/p38MAPK may also be subject to the concomitant influence of other polymorphic sites that mediate transcriptional regulation by these signaling pathways. Long-term exposure to IL-1β increases 5-HT uptake, attributable to enhanced production or stability of 5-HTT mRNA (233). Furthermore, the antiinflammatory cytokine, IL-4, was found to decrease 5-HT uptake into lymphoblast cell lines only of 5-HTTLPR ll genotype (234). Elevated proinflammatory cytokine levels have been found in a number of psychiatric conditions, including depression (235). Taken together, these data support the idea that the ongoing, dynamic influence of kinases on both 5-HTT transcription and protein trafficking provides another level of complexity for association of polymorphic state with measures of 5-HTT mRNA and binding sites. Similar regulation exists for PKC, which downregulates 5-HTT protein at the plasma membrane and influences transcription rate, with a greater effect on the l versus s allele of the 5-HTTLPR (139, 236). Future studies that consider previous drug exposure and environmental influences that activate kinase pathways, and even assess kinase tone at the time of sample or data collection, may reveal the influence of l and s on expression as a function of interaction with these factors.

CREATINE TRANSPORTER (SLC6A8, CT1)

Creatine plays a key role in energy transmission and storage in cells and tissues with high energy demands (**Figure 5**; 237). The dephosphorylation of phosphocreatine yields energy in the form of ATP. Creatine is acquired by dietary intake and synthesized primarily in the liver and also in kidney and pancreas. Most other tissues, including heart, brain, and skeletal muscle, rely on the creatine transporter (CT1) for uptake of creatine (238). Brain function appears to be linked in a number of ways with creatine metabolism, although the relationships are not yet fully understood. There is coupling of creatine kinase with neurotransmitter release, growth cone migration and restoration of ion gradients following depolarization (239, 240).

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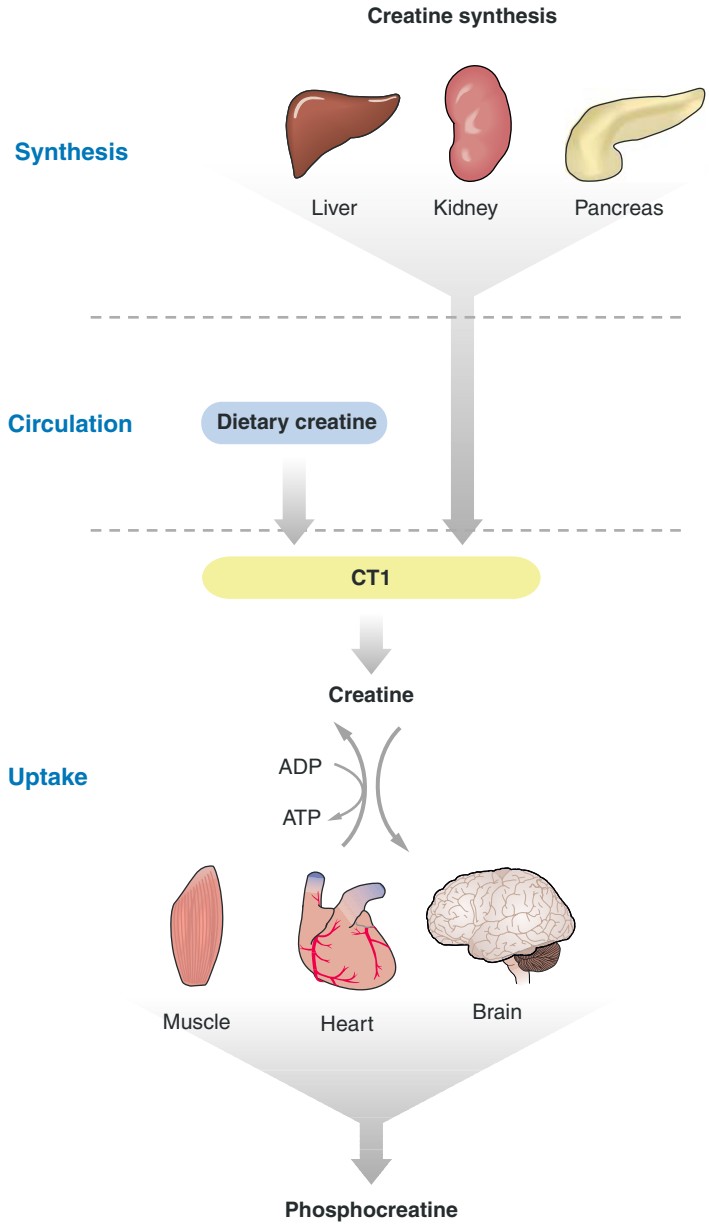
Figure 5

Creatine acquisition, synthesis, and uptake. Creatine can be acquired through diet or synthesized in liver, kidney, and pancreas. Creatine in the circulation is transported by CT1 into tissues that lack creatine synthesis, including muscle, brain, and heart, where it is converted to phosphocreatine, an energy store.



CT1 Mutations and X-linked Mental Retardation

Mental retardation (MR) affects 2%–3% of the population in Western nations, with males comprising 70% of the cases (241). X-linked mental retardation (XLMR) is estimated to account for 5%–12% of all cases of MR, with a relatively large contribution of fragile-X syndrome (fraX) (242). In 2001, Salomons et al. provided the

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first discovery of a mutation in SLC6A8 in a family with XLMR (243). In a young boy diagnosed with mild MR and delayed language development, proton magnetic-resonance spectroscopy revealed an almost complete absence of creatine in the brain. He was found to harbor a CT1 SNP that causes the conversion of R514 to a stop codon. Indeed, creatine uptake into fibroblasts was negligible in the patient but normal in the female carriers in the family. The female carriers demonstrated equal mRNA expression from both the normal and mutant allele. The lack of symptoms in the female carriers, coupled with normal creatine transport, indicates that one functional allele is sufficient to sustain creatine homeostasis. However, the mother and grandmother reported learning disabilities. Thus, despite some measures of normal creatine function, female carriers are most likely affected in more subtle ways. Indeed, a female carrier in this family was subsequently found to have a 63% decline in brain creatine levels in the basal ganglia, suggesting that the brain is less tolerant of this allele loss (244). Skewed X-inactivation leading to between-cell mosaicism of wild-type and mutant CT1 expression may also explain the difference in clinical phenotype of female carriers, even within a single family. Subsequent to identification, the R514X index patient was placed on a creatine supplementation diet for 3 months without any clinical improvement or spectroscopic evidence of creatine restoration in the brain (245). However, this treatment produced normal CSF and muscle creatine, indicating a transport mechanism in the choroid plexus and muscle not present or sufficient in brain.

New families with SLC6A8 mutations have been identified; these include truncations caused by incorrect splice site usage and single amino acid deletions (246–248). Most of the affected males are recognized owing to delays in expressive speech and language; the phenotype also includes severe MR, behavioral abnormalities, epilepsy in 50% of cases, and autism (248). The patients exhibit elevated creatine in the urine, reduced or absent creatine in brain as measured by magnetic resonance spectroscopy, nondetectable creatine in CSF, and impaired creatine uptake in fibroblasts (248). Although some families show disturbed motor tone in upper limbs, dystonic gait, and choreoathetoid movements of the face and upper limbs, some patients display no evidence of myopathies (246, 248), which may be somewhat surprising because muscle normally contains the highest concentration of creatine and creatine depletion in animals causes severe muscle weakness.

A large-scale study of 288 families from the European XLMR panel identified almost 20 CT1 variations: insertions, deletions, missense mutations, intron SNPs, and 3′ UTR SNPs (247). Remarkably, no variants were found in 276 controls examined simultaneously, and the intron SNPs all appeared in more than one family while being absent in controls. If these nonsynonymous and noncoding SNPs are found to be functional, they would account for approximately 6% of the patients in this XLMR sample. While not nearly as prevalent as FMR 1 mutations of FraX syndrome, this is a higher rate of disease-causing mutations than exists for other members of the SLC6 family, likely due to the critical role of creatine in energy homoeostasis and the single allele status of CT1 in males. These polymorphisms await characterization in expression systems, such as fibroblasts, of possible deficits in transcriptional activation, splicing mechanisms, protein expression, trafficking, and substrate utilization.

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Although by birth many developmental processes are already dramatically disrupted by creatine deficiency, further degeneration can occur throughout the lifetime of affected individuals. A study of a patient with delF107 revealed the characteristic near-absence of creatine and phosphocreatine in brain, and, additionally, there was atrophy of white matter, apparent with MRI analysis, which progressed over the course of several years (249). This continued deterioration argues for the development of ameliorative interventions and their use at an early stage in development. The determination of the mechanisms of CT1 dysfunction may inform treatment options for progression of XLMR. For example, one family found to have a splice error that causes skipping of exon 10 had 5%–15% of normal brain creatine levels, suggesting some use of the correct splice site (250). Such patients might benefit from creatine dietary supplementation, perhaps by producing a slowing of the neurodegeneration described. It is thus critical to assess function of each mutation identified and determine if creatine levels or transporter function can be positively manipulated and treatment matched to individual families.

CONCLUDING REMARKS

Advances have been made to demonstrate that transporter SNPs, both rare and common, help explain individual variation in behavior and disease. Regulatory region SNPs, by nature of their frequency, will have consequence in more individuals than protein variants. These regulatory region polymorphisms with functional consequences must be identified and examined in concert with other markers in the same and other genes. Examination of individuals and families carrying protein variants also provides a unique opportunity to understand the impact of dramatic changes in transporter function on behavior. Furthermore, to the extent that certain protein polymorphisms are represented in a substantial proportion (1%) of the population, their contribution to phenotypes should be examined in the context of other common SNPs to understand the influence of multiple assaults to the same protein at the levels of transcription and protein regulation. Furthermore, the regulation of expression at the levels of transcription; translation; trafficking; and regulation by signaling pathways, antidepressants, and drugs of abuse by similar mechanisms, such as kinase activation, emphasizes that the complexity of multiple genes should not be overlooked and indeed should guide future use of multiple markers in genes linked by functional pathways, such as kinases, phosphatases, and enzymes of biosynthesis and metabolism.

The influence of polymorphisms may only become apparent when we "divide and conquer," looking at endophenotypes, environmental influences, early life events, history of drug abuse, response on a task that tests a specific behavioral repertoire, response to treatment, and network properties rather than by assessing static measures of brain activity. For example, greater understanding of the neuroanatomical circuitry mediating an endophenotype, behavior, or task under consideration will guide the regions of interest in imaging studies. This will help establish meaningful relationships between genotype, behavior or performance, and function of the gene product and neuronal activity in the engaged area of the brain. These efforts must

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also keep in mind the early life and continual influence of the environment to modulate behavior. The future success will depend on continued efforts by researchers to apply all of the tools available to establish a relationship between transport genes and behavior.

SUMMARY POINTS

1. Genetic studies need to utilize haplotypes and markers in multiple genes, especially in functional pathways.
2. Identification of functional SNPs through evaluation in relevant model systems is necessary.
3. Endophenotypes or selective traits can reveal relationship between genotype and phenotype.
4. One must examine environmental interactions with genes that influence disease.

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First identification of a 5-HTT protein variant tracked within two families with multiple instances of psychiatric disorder.

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NOTE ADDED IN PROOF

After submission of this review, the first human variants in GLYT2 were found in neurological startle disease (see Ref. 251).

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