Genetic perspectives on the serotonin transporter

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ABSTRACT: The serotonin transporter (5-HTT) is most well known as the site of action of the serotonin reuptake inhibitors, which were initially developed as antidepressants, but now are the most widely used agents in the treatment of many additional neuropsychiatric and related disorders. The discovery that the gene that expresses the 5-HTT possesses a functional promot- er-region polymorphism, which is associated with tempera- ment and personality traits such as anxiety and negative emo- tionality as well as some behaviors, led to many studies examining this polymorphism in individuals with different neu-ropsychiatric disorders. The subsequent development of mice with a targeted disruption of the 5-HTT in our laboratory has provided an experimental model to examine the many conse- quences of diminished (in +/−, heterozygote mice) or absent (in −/−, homozygote knockout mice) function of the 5-HTT. The 5-HTT-deficient mice were also crossed with other knockout mice, allowing the study of multiple neurobiologic dysfunctions. As multiple genes are probably involved in the expression of complex behaviors such as anxiety, as well as neuropsychiatric disorders, these more genetically complex mice may more closely model disorders with complex etiologies. Thus, the combination of these comparative human and mouse studies may extend the opportunities to examine genetic alterations from a novel “bottom-up” approach [gene knockout or partial gene knockout in a combinational gene × gene (yet unknown) gene approach], which is complementary to the traditional “top-down” genetic approach based upon studies of individuals with diagnosed neuropsychiatric disorders and their family members. © 2001 Elsevier Science Inc.

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INTRODUCTION: “TOP-DOWN” VS. “BOTTOM-UP” GENETIC STRATEGIES

There has been considerable success in identifying a limited num- ber of genes with major contributions to some neuropsychiatric disorders such as Huntington’s disease and, more recently, Alzheimer’s disease [80]. In other disease areas, such as breast cancer, some genes have also been identified as contributing major effects, but only in some subsamples of patients with family-based gene mu- tations. However, in most types of cancer, hypertension, and other common diseases, genes positively linked to the specific disorder have major effects in only a small number (usually <10%) of individuals who develop these disorders.

The majority of individuals developing these common disor- ders are considered as having “disorders of complex genetic/ environmental” etiologies, with multiple genes and environment each making relatively minor additive or interactive contributions to the overall phenotype. This now appears to be the case also for most serious neuropsychiatric disorders, including schizophrenia, bipolar affective disorder, and other affective disorders and anxiety disorders [53,54]. One clinically diagnosed anxiety disorder, obs- sessive-compulsive disorder (OCD), may represent a more homog- enous phenotype and may possibly be associated with the effects of a more limited number of genes [10,13,70]. However, contrary to this hypothesis, co-morbidity between OCD and “OCD spect- rum” disorders such as Tourette’s syndrome (TS) and depression, trichotillomania, attention deficit hyperactivity disorder (ADHD), as well as other complex disorders including schizophrenia, bipo- lar disorder, and eating disorders have been reported [12,78,79,95].

Top-down Strategy

The “top-down” approach most frequently applied in clinical, human genetics has been used to examine affected sib-pairs, trios, or extended family pedigrees with classically identified disorders. DNA from these samples is then subjected to genome-wide or related strategies to locate “hot-spots” in the human genome that fit either typical linkage or more recent and more complex familial and associational analyses [1,6,56,84].

A complementary approach has also been employed to help identify possible candidate genes for these and related disorders. One example of a “candidate gene” approach has been to deliber- ately use physiologically or pharmacologically based data from clinically related clues to select individuals for genotyping to investigate polymorphisms that might represent likely candidate genes. Among many such examples, one pertinent to the serotonin (5-HT) system is 5-HT neurotransmission in obsessive-compulsive disorder (OCD). OCD symptoms are responsive to serotonin re-uptake inhibitors (SRIs) like fluoxetine, citalopram, fluvoxamine, sertraline, and paroxetine. While other anxiety disorders are often effectively treated by these SRIs, these other disorders are equally well treated by other antidepressants (e.g., tricyclics such as desiprime) as well as some anxiolytic agents (e.g., benzodiazepines). In contrast, OCD patients are only effectively treated by SRIs as documented by head-to-head trials of SRIs versus agents such as desipramine which primarily target the norepinephrine system [57]. Thus, the 5-HT system provides a logical source of candidate
Bottom-up Strategy

In contrast to “top-down” approaches, one suggested “bottom-up” approach which we and others have advocated is based on investigations of single gene alterations, specifically those identified from mouse genome data (which is founded upon an estimated 80 million year similarity between mouse and human vertebrate genomes) to predict likely genetic loci that may contribute to similarities between human and mouse phenotypes [59,75].

A mouse model of 5-HT transporter (5-HTT) dysfunction [10] helps support the human pharmacology/physiology-based candidate gene approach. As an initial step to pursue the “bottom-up” strategy of mouse-to-human phenotypes, we have begun experiments based upon using the targeted disruption of the 5-HTT gene placed in multiple mice strains (C57BL/6J, CD-1, and 129SJ), to attempt to clarify that this mutation, rather than the background genetics, is well-established as the likely site of action of drugs (e.g., methylphenidate and amphetamine) used in the treatment of attention deficit hyperactivity disorder (ADHD). The co-morbidity of OCD and ADHD suggests that DAT may be a second candidate gene for OCD (Fig. 1).

This is an early example of how a “bottom-up” strategy (using 5-HTT × DAT cross-bred mice) might lead to better candidate gene selection for closely-associated/co-morbid human disorders, as well as substance abuse disorders [66,89,92–94]. We and our colleagues are pursuing other double and, more complex, triple knockout, cross-bred mice (5-HTT × MAO-A; 5-HTT × VMAT; DAT × VMAT; 5-HTT × NET; DAT × NET; 5-HTT × MAO-A × 5-HT1B receptors) [85] to evaluate hypotheses based on epistatic, 1 gene × 1 gene × other gene candidates of complex genetic traits and diseases. It is noteworthy that a strength of these approaches is that heterozygote +/+ mice in some of these studies exhibit significant behavioral and physiological functional alterations compared to +/+ control littermates ([11]; Wichems, et al., submitted). In the case of cocaine reward mechanisms studied in 5-HTT × DAT knockout mice, elimination of only one allele (i.e., +/- mice) of the 5-HTT was needed to obliterate cocaine place preference, whereas knockout of both DAT alleles was required [9]. Genes such as that for the 5-HTT that are able to alter behaviors such as anxiety and reward with only a partial reduction rather than a complete elimination of function are particularly important model candidates.

Thus, the bottom-up strategy is poised to take advantage of the newly-completed human genome “dictionary” of genes, and the soon-to-come mouse genome “dictionary” to help select the most likely candidate genes in which the pleiotropic, complex consequences of one gene may be combined with other genes by knockout and other types of genetic engineering models to identify the candidate genes that may contribute to potential human phenotypes and comorbid conditions. These studies will help close the very large gap that now is present between “top-down” (clinical diagnostic phenotypes) and “bottom-up” (1 gene alone and 1 gene × 1 gene × . . . . . . . . (other genes)) to guide further investigations of individual genes, environment, and additional factors like gender in their multiple contributions to human disorders [5,31,62,71,75] (Fig. 1).
general population samples of mostly sibling pairs were found by multiple regression analysis with corrections for gender, age, and ethnicity [38,58]. Other studies have replicated our original association; however, some studies have reported no association. The lack of association could be due to sample sizes too small to reliably detect this relatively small association, or to population stratification issues (as very large ethnic differences have been found for the prevalence of the s/s or s/l versus the l/l form of the polymorphism [32]), as well as to the use of different measures of personality traits [52,59].

FIG. 1. “Bottom-up” and “top-down” gap between interacting genes, quantitative traits, and behavioral disorders. This figure depicts several major concepts presented in this manuscript: (a) Presently, there is a great gap (-----) between what we understand about the demonstrated genetic association of one gene (specifically, the functional polymorphism in the 5-HTT) with one specific personality trait (‘neuroticism’/negative emotionality) [38,58]. A similar gap (-----) exists for the small top-down literature (two of three studies, including one study of trios), which has found associations between this 5-HTT polymorphism and individuals with OCD [10,13,70]. In another top-down study, neuroticism has been found to be a genetic risk factor contributing to an increased vulnerability to depression in female twin pairs [53]. (b) (Upper part of the figure) There is a well-documented co-morbidity between OCD, depression, Tourette Syndrome/tics, ADHD, eating disorders, and schizophrenia, which suggests that some common genetic contributions of varying magnitudes links these disorders [25,89,97]. (c) (Lower part of the figure) The hypothesis is presented that greater knowledge of the pleiotropic consequences involving altered functions produced by one mutation resulting from disruption of the 5-HTT in several mouse strains [11] combined with, in the present and the future, likely pleiotropic consequences of other, related genetically-engineered single genes—initially alone—but subsequently via cross-breeding of these mice in double (1 gene × 1 gene) [66,92,93], triple [85], and gradually multiple combinations may suggest a bottom-up series of additional hypotheses. These hypotheses can be expected to lead to a multi-candidate, gene-hunting approach for both personality traits with risk factor elements for neuropsychiatric and other disorders. These strategies also provide more data-rich foundations for candidate gene selection for these disorders themselves and also additional likely phenotypes from those currently provided by top-down strategies.

TABLE 1

<table>
<thead>
<tr>
<th>Brain Lymphoblasts [58]</th>
<th>Brain [65]</th>
<th>Platelets [38]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3H-5HT Uptake [11]</td>
<td>−48%**</td>
<td>N/A</td>
</tr>
<tr>
<td>5-HTT mRNA [81]</td>
<td>−35%**</td>
<td>−51%***</td>
</tr>
</tbody>
</table>

5-HTT Promoter region polymorphism s/s + s/l vs. l/l human genotypes
Small genetic associations require large sample sizes and are best studied in sibling pairs to control for population stratification. Three of the four studies of large sample sizes based primarily on studies of sibling pairs found significant differences in personality characteristics in individuals with the 5-HTT polymorphism (summarized in Table 2 [38,58,68]). A large study of a general population sample in Australia that did not use the sibling pair method and did not use the NEO-PI-R but instead used an abbreviated form of the EPQ-R personality inventory, did not find any differences between the two groups [52].

It is not surprising that it has proved difficult to examine individual genes like the 5-HTT, which make only a small contribution to personality trait differences and, perhaps, to heterogenous, multi-ple neuropsychiatric disorders. Several statistically oriented geneticists have recently estimated that any one gene in a complex human disorder may contribute as little as 1–5% to the overall disorder or trait [83,84,98].

**TABLE 2**

<table>
<thead>
<tr>
<th>N (Total and sibs)</th>
<th>Subjects</th>
<th>Significantly different measures</th>
<th>p Values</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>505; 459</td>
<td>General population, USA (93% males)</td>
<td>NEO-PI-R neuroticism 16-PF anxiety TPQ (calculated) harm avoidance</td>
<td>0.03 to 0.004</td>
<td>[58]</td>
</tr>
<tr>
<td>655; 366</td>
<td>Mixed alcoholic violent offenders and controls, Finland</td>
<td>TPQ harm avoidance (HA1, HA2)</td>
<td>0.003</td>
<td>[68]</td>
</tr>
<tr>
<td>759; 0</td>
<td>General population, Australia</td>
<td>EPQ-R (short form)</td>
<td>NS</td>
<td>[52]</td>
</tr>
<tr>
<td>397; 340</td>
<td>General population, USA (87% females)</td>
<td>NEO-PI-R neuroticism, agreeableness (–)</td>
<td>0.02 to 0.001</td>
<td>[38]</td>
</tr>
</tbody>
</table>

**PERTINENT DATA ON THE GENETICS OF THE SEROTONIN TRANSPORTER FROM GENETIC ENGINEERING STUDIES IN MICE**

In homozygote (5-HTT −/−, knockout) mouse strains developed by our laboratory, 5-HTT binding sites were totally absent in different brain regions (brain stem, frontal cortex, hippocampus, and striatum) [11]. As expected, these binding sites were reduced by approximately 50% in the heterozygote (5-HTT +/−) mice compared with their control (5-HTT +/+ littermates [11]. Marked reductions in tissue concentrations of serotonin were seen in the same brain tissue regions. Although the magnitude differed, they were generally reduced by 40% to 60% in the 5-HTT −/− mice.

Microdialysis studies using conscious, freely moving 5-HTT mutant mice conducted by Dr. Anne Andrews’ group demonstrated that there was a ~fivefold increase in extracellular serotonin concentrations in the striatum of the 5-HTT −/− mice compared to their 5-HTT +/+ littermate controls [4,67]. Anticipated similar increases in other brain regions are currently being studied. There were no differences in striatal dopamine concentrations at baseline, and equal dopamine release and reuptake were found with potassium chloride substitution for sodium chloride in the dialysis medium in the control, 5-HTT +/−, and 5-HTT −/− mice, indicating that disruption of the 5-HTT transporter had negligible effects on dopamine transport [4]. Turnover/synthesis studies using a classic decarboxylase inhibition technique showed negligible changes in norepinephrine and dopamine synthesis and turnover in the 5-HTT −/− mice. As expected, there were marked changes in serotonin synthesis and turnover in the 5-HTT −/− mice. (Murphy et al., in preparation, 2001).

5-HT-mediated functions and brain concentrations are influenced by many factors including anxiety/affect, aggression/im-pulse control, appetite/satiety, cardiovascular function, circadian rhythms/sleep, endocrine regulation, motor activity, gastrointestinal function, pain, sensory function, and sex drive (e.g., [74]). Originally, we hypothesized that a 5-HTT knockout mouse might be a lethal mutant because 5-HT is important in many physiological functions and behaviors as well as in neurodevelopment [73, 85,90]. In contrast, our initial findings showed that the 5-HTT knockout mice behaved like their control littermates, with no obvious differences in development or mortality rate and no overt behavioral defects [11]. Initial weight gain and size were also normal [11]. Therefore we searched for more subtle phenotypes of these mice, an enterprise that provided a wealth of results.

Two conflict/exploration models [29,86,88] were examined in studies of two different background strains of mice with this disrupted 5-HTT, the C57BL/6J strain and the CD-1 strain, and yielded similar results, indicating increased fear/"anxiety" behaviors in both strains [96], (Wichems et al., submitted). This is important because mouse strains vary considerably in such behav-iorial tests. When we examined measures such as time in the open maze areas, latency in entering the open areas, head dips, and other associated phenomena, we obtained similar genotype-dependent results. Similar results were found in male mice, although behav-ioral differences were somewhat less marked in several of the ancillary measures such as head dips in male mice [96], (Wichems et al., submitted). As with the zero maze data, there were marked genotype-related differences in the light/dark box, with 5-HTT −/− and 5-HTT +/+ of both background strains showing behaviors that were consistent with an increased level of fearfulness and "anxiety" compared to the control littermates. These models sug-gest that a major behavioral phenotype of the 5-HTT mutant mouse is characterized by increased "anxiety" and fearfulness. These observations have proved replicable in a third background strain, and are currently under investigation [51] in additional "anxiety," aggression, and other behavioral models, (e.g., [24,50]).

In experiments of stress responsiveness, marked differences were observed in hormonal responses in 5-HTT −/− mice. Marked increases in the stress hormone adrenocorticotropic (ACTH) were found in plasma of 5-HTT −/− and +/+ mice compared to their control littermates [63]. In contrast, simply decapitating the mice and measuring baseline ACTH levels re-
5-HTT mutant mice (data reformatted from [63]). Nucleus revealed a marked reduction in spontaneous increased stress-responsive phenotype [21,35,63].

Vealed lower ACTH concentrations and no genotype-related differences (Fig. 2). These data suggest that these mice exhibit an

FIG. 2. No differences in plasma ACTH were found across genotypes of 5-HTT mutant mice when the mice were removed from their cages and quickly decapitated (right portion of figure). In contrast, when the mild stress of an intraperitoneal injection of saline followed by 15 min of time delay with the mice returned to their cages, a small increase of plasma ACTH was found in the +/+ littermate control mice, while very large (> threefold) increases in ACTH were observed in both the +/− and −/− 5-HTT mutant mice (data reformatted from [63]).

vealed lower ACTH concentrations and no genotype-related differences (Fig. 2). These data suggest that these mice exhibit an increased stress-responsive phenotype [21,35,63].

Investigating of firing rates of 5-HT neurons in the dorsal raphe nucleus revealed a marked reduction in spontaneous firing rates [33]. Neuroanatomic studies are beginning to reveal anomalies in thalamocortical association fields in 5-HTT −/− mice [77,85]. Somatosensory barrel fields fail to develop normally in 5-HTT −/− mice, an effect which persists into adulthood. There was also a diminution in the density of barrel fields in the 5-HTT +/− mice. This effect in 5-HTT mutant mice was reversed by administration of the 5-HTT synthesis inhibitor, parachlorophenylalanine, on postnatal days 1 and 2, suggesting that the absence of barrel fields in 5-HTT −/− mice is a result of excess 5-HT present during early postnatal development. Mice with a targeted disruption of the MAO-A gene also have markedly higher brain 5-HT concentrations and fail to develop barrel fields [17,85].

The 5-HTT is a site of action of SRIs used to treat patients with neuropsychiatric, depressive/anxiety and related disorders. The 5-HTT is also a site of action of drugs of abuse such as MDMA ("ecstasy"), cocaine, LSD [11,16,94], as well as 2′-amino MPTP, an analogue of MPTP with selective 5-HT neurotoxic effects [2,3].

As the SRIs are usually given to neuropsychiatric patients for relatively short periods, this use of SRIs is quite different from the situation of the 5-HTT −/− or +/− mice, where the altered 5-HTT function is present from early development throughout adulthood. In contrast to SRI use, humans with the "s" allele form of the 5-HTT promoter region polymorphism do manifest this genetic difference from the beginning of development, and the magnitude of the functional change in 5-HTT function is similar to that found in 5-HTT +/− mice [39,47–49,58,65] (Table 1).

In investigations of human infants whose mothers were treated with SRIs during pregnancy; most studies clearly showed no major teratogenic effects of SRIs given during the first trimester of pregnancy, however, a few studies have noted shorter gestational periods and increased prenatal complications when SRIs were given during the third trimester [19,30,34,72]. With due caution regarding species differences, it may be prudent to consider whether subtle behavioral or physiological consequences may result from altered 5-HT availability during early development due to SRI use [46,73,90] or the presence of the short form of the 5-HTT promoter polymorphism. Conversely, the potential that enhanced 5-HTT function during development, as in individuals with the I/I form of the 5-HTT polymorphism, may also have developmental or other functional consequences needs further evaluation. 5-HTT appears to have many sites of regulation [5,14,60,82] and continued study of this regulation, as well as of the genetic consequences of life-long 5-HTT alterations, would thus seem of high importance.

CONCLUSIONS

This overview of recent studies of the 5-HTT transporter in mice and humans attempts to open new areas for discussion and define concepts not yet comprehensively reviewed or reconciled in the literature on serotonin function. These studies highlight the multiple roles of the serotonin transporter in genetic disorders, neurodevelopment, and as a target for drugs widely used to treat neuropsychiatric and other disorders, as well as a target for drugs of abuse and neurotoxins. This paper does not attempt to address the complexity of the 14+ 5-HT receptors and their splice variants, or RNA-edited and other variants [7,15,20]. The roles of the 5-HTT and 5-HT receptors in the gut, lungs, heart, adrenal glands, and in the many peripheral sites where it is expressed [22,45,46,61] are also not discussed. A more extensive and detailed review might have covered the mixed human genetics literature on 5-HTT polymorphisms/mutations, with many interesting exploratory investigations of the potential role of these 5-HTT variants in alcohol abuse, suicide, Alzheimer’s disease, autism, eating disorders and therapeutic responses to antidepressant drugs (e.g., [9,26,36,55,76,91,99]).

Rather, this review is meant to emphasize that insights gained from in-depth studies of reduced and absent function of the 5-HTT in mice may suggest potential phenotypes in humans that “top-down” genetics might not reveal. Equally importantly, we suggest that follow-up studies of epistatic 1 gene × 1 gene interactions are worthy of study. Hypotheses may be tested in studies of 1 gene × 1 gene × multi-gene manipulations that, despite their complexity, provide complementary information to traditional “top-down” genetics in yielding insights into complex human disorders.

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REFERENCES


