Genes for personality traits: implications for psychopathology

Jonathan Benjamin, Richard P. Ebstein and Klaus-Peter Lesch

Abstract
Although 30–60% of the variance in many personality traits is inherited, until recently, little was known about the genes responsible. Preliminary studies of family history in bipolar disorder and of X-linkage of personality traits in colour-blindness suggested a ‘quantitative trait locus’ (QTL) approach to the genetics of normal personality. In methodically similar but independent studies of 124 Israeli and 315 American normal volunteers, an association was found between the dopamine D4 receptor gene (D4DR) and the personality trait of novelty-seeking. In the Israeli sample there was preliminary evidence for an interaction between the D4DR gene and the serotonin 2C receptor gene (5-HT-2C), with a marked effect on the trait of reward dependence. In addition to receptors, monoamine uptake mechanisms, such as the serotonin transporter (5-HTT), are candidate genes for personality traits. 5-HTT gene transcription is modulated by a frequent polymorphism in its promoter region, with resulting effects on 5-HTT expression and 5-HT uptake. In an extended American sample totalling 505 subjects, the 5-HTT polymorphism was associated with anxiety- and depression-related personality traits. The allelic variation in functional expression of the 5-HTT may also be a susceptibility factor for disorders of the affective spectrum. Further investigation of genes for personality traits may provide additional links between normal personality and psychiatric illness.

Introduction
Human personality and behaviour are partly inherited. Twin and family studies have demonstrated that certain behavioural disorders, such as reading disability and male alcoholism, may have larger genetic components than traditional polygenic medical disorders such as hypertension and ischaemic heart disease (Plomin et al., 1994). Normal personality traits, as measured by dimensional scales of personality assessment, present the same pattern, with inheritance typically accounting for between 30 and 60% of the observed variance. One example is provided by the striking similarity of identical twins adopted out and reared apart (the Minnesota Study), some of whom had not known each other prior to the study (Bouchard et al., 1990). On measures of interests, skills and personality traits, these twins had correlations between 34% and 78%. By contrast, fraternal twins studied by the same investigators had correlations between 7% and 59% (Tellegen et al., 1988). Virtually all work in this area supports the idea that there is a substantial heritable component to personality (Henderson, 1982; Loehlin et al., 1988; Plomin, 1990).

Despite the psychometric evidence that personality is influenced by genetic factors, until recently very little was known of the number or nature of the genes responsible. One approach to identifying such genes is linkage analysis, which is based on the principle that if a gene influences a trait, then family members who share a chromosomal region harbouring that gene will be more similar on that trait than relatives who have different versions of that region. The advantages of linkage analysis are that it scans large segments of DNA and does not require prior knowledge of the mode of inheritance or biochemical mechanism of the relevant genes. The disadvantage is that it is relatively insensitive to genes with small effect sizes.
A second approach for identifying genes that are related to personality or other behavioural characteristics is allelic association, which is based on the principle that if a gene influences a trait, then individuals who share a particular allelic variant of the gene should be more similar to one another than are individuals with different alleles. This approach is most powerful when candidate genes that are ‘biologically reasonable’, and that have known functional polymorphisms, are available. The main advantage of allelic association is that it is remarkably sensitive to small effect sizes.

Until recently, there had been no published reports of linkage and association analyses of normal personality traits. In principle, however, such traits should be as amenable to study as physical traits. Quantitative traits (sometimes called ‘dimensional traits’) have been successfully analysed by this method in many non-human species. The idea behind this approach is not to look for a single gene with an ‘all-or-nothing’ effect, as in Huntington’s chorea, but rather for a number of genes, each with a small effect by itself but with large combined effects. Genes with small effects may underlie a dimensional trait, like height or intelligence, and they may also underlie the risk of developing an ‘oligogenic’ disease like pyloric stenosis or perhaps schizophrenia (Benjamin et al., 1996a). If major mental illnesses (and/or personality traits) are due to single genes with large effects (i.e. Mendelian), then it should not be too difficult to find affected relatives in a high proportion of affected probands, provided pedigrees are large enough. Alternatively, a low proportion of probands who have affected relatives suggests an oligo- or polygenic effect; it is unusual for any relative other than an identical twin to share exactly the same combination of genes needed to influence (or even cause) the illness.

Frequency of positive family history in bipolar patients

The Division of Psychiatry of Ben Gurion University of the Negev serves practically all psychiatric patients in its catchment area of 300,000, and its two affective disorder clinics have a record of lithium-treated patients in the area. The files of all bipolar I and schizoaffective patients registered with the clinics in 1991 (n = 236) were reviewed (Alexander et al., 1995). One hundred and seventy-seven probands were personally interviewed about their family histories, as were 50 of their relatives. Since the population prevalence of lithium-treated individuals in Europe is about 1.3 per 1000 (Vestergaard et al., 1989), and over one-third of the population of the Negev is below the age of risk for affective illness, about 250 lithium-treated patients would be expected in a catchment area of 300,000. The present sample of 236 seems to have been fairly comprehensive. About half of the bipolar patients, therefore, have no family history of a condition with an undoubted (partly) genetic etiology. This was also shown about 25 years previously (Winokur, 1973).

We interpreted this as circumstantial evidence that manidepressive disorders are a non-Mendelian disorder.

The case for studying genes for normal personality traits

The difficulties encountered in the search for single major genes for mental illnesses like schizophrenia and manidepressive disorders (Baron et al., 1990; Risch et al., 1996) need to be considered in the light of this possibility, that each of the genes involved contributes only a small part of the variance. And indeed, the new ‘small gene’ approach (Benjamin et al., 1996a) has begun to yield exciting findings for the psychoses, for example, weak but replicated linkages (that is, presumably valid linkages to genes with small effects) to chromosome 18 in manidepressive disorder (Berrettini et al., 1994; Stine et al., 1995) and to chromosome 6 in schizophrenia (Moises et al., 1995; Peltonen, 1995; Schwab et al., 1995; Straub et al., 1995; Wang et al., 1995). A further complication is that it appears increasingly likely that psychiatric diseases are both polygenic and genetically heterogeneous (Belmaker et al., 1994; Cloninger, 1994). If, as has been suggested, the same psychiatric syndrome can result from differing combinations of environmental effects and inherited personality variants (Bleuler, 1952; Goodwin et al., 1990) even in the same family (Cloninger, 1994), then these will be difficult to detect with linkage and association studies, which treat the syndrome as a single phenotype. Instead, further progress may depend on the identification of simpler underlying ‘endophenotypes’ (Gottesman, 1991). A plausible analogy may be drawn from the case of myocardial infarction, where it is cholesterol levels, blood pressure and so on, not the infarct itself, that are inherited.

Normal personality traits, or extreme variants of these, represent prima facie candidates for the role of ‘endophenotypes’ for mental illnesses, and there is some evidence for a continuum between normal personality

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1 If investigators hypothesize a number of genes influencing the trait, with each gene exerting a small effect on its own, the chromosomal regions thought to harbour such genes are sometimes called ‘QTLs’, for ‘quantitative trait loci’.

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traits, personality disorders and psychiatric illness. The normal personality trait of novelty-seeking (see below) is high in ‘cluster B’ type personality disorders like antisocial personality (Svrakic et al., 1993) and alcoholism (Mulder et al., 1994), and the trait of reward dependence (see below) is low in ‘cluster A’ type personality disorders, like schizoid and schizotypal disorders (Svrakic et al., 1993). Consequently, personality disorders may represent extremes of normal personality traits. Mental illnesses, in turn, may be extreme variants of personality disorders, for example, schizoid and schizotypal personality disorders and schizophrenia (Nigg et al., 1994). Basic personality traits like novelty-seeking, harm avoidance and reward dependence may, via a complicated nonlinear path that leads in the first instance to personality disorders, ultimately predispose to psychosis. One possible route could be a maladaptive trait, or traits, which might interact with other biologic and/or environmental risk factors to produce manifest illness. In keeping with this line of thinking, Kendler and colleagues have shown that, in 707 pairs of female twins who completed two scales of the Eysenck Personality Questionnaire and were assessed for the presence of depression, about 70% of the risk of depression was inherited (Kendler et al., 1993a,b). Of this heritability, 45% was direct, while the other 55% was via the effect of the inherited personality trait ‘neuroticism’.

Therefore a series of linkage and association studies of normal (adaptive) personality traits was initiated, with the prediction that positive findings might ultimately elucidate the neurobiology of complex behavioural phenotypes and provide a bridge to the pathophysiology of (maladaptive) personality disorders and serious mental illnesses.

**Linkage between colour-blindness and personality traits**

Because there is substantial evidence of linkage of manic-depressive illness to the colour-blindness locus on the X chromosome (Baron et al., 1987; Souery et al., 1995), it was reasoned that the locus might also play a role in normal personality or variations of normal mood.

Benjamin et al. (1993) adapted the sib-pair method (Suarez et al., 1982) to their QTL approach, and hypothesized that a personality trait or traits would show a significantly higher correlation between pairs of brothers concordant for colour-blindness (both affected) than between pairs of brothers discordant for colour-blindness (only one affected). Colour vision was assessed with Ishihara’s test. To evaluate personality, the Sixteen Personality Factor Questionnaire (16PF) (Cattell et al., 1970) was used. The 16PF questionnaire is based on a factor-analytic model of personality and has been extensively used in four decades of personality research (for example, Loehlin, 1992). It is available in a validated Hebrew version. Seventeen probands participated in this pilot study. A single factor, Q2, correlated significantly between concordant, but not between discordant brothers.

Although the sample was very small and the finding necessarily preliminary, the study was important as a first attempt to map a gene for a personality trait. In the interim there have been reports of personality traits in relation to the debrisoquine metabolism polymorphism (LLerena et al., 1993) and of defence mechanisms in relation to a dopamine D2 receptor polymorphism (Comings et al., 1995). While intriguing, these three studies have not yet been replicated.

**Association studies of the dopamine D4 receptor gene (D4DR)**

There has been renewed interest in the genetics of dopamine receptors, following the discovery of polymorphisms in the dopamine D3 (D3DR) (Rietschel et al., 1993) and D4 receptor genes (D4DR) (Van Tol et al., 1992). Cloninger (1987) had previously described a biological approach to personality assessment, embodied in the Tridimensional Personality Questionnaire (TPQ). On the basis of animal and human studies and statistical analyses, he suggested there were three underlying, inherited dimensions of human temperament, one of which, novelty-seeking, should be mediated by dopamine.

Exploratory behaviour in animals is mediated by dopamine (Kalivas et al., 1988) and so are the effects of cocaine and amphetamines in man (Ritz et al., 1993). Novelty-seeking has been reported to be low in Parkinson’s disease patients, who have dopaminergic deficiencies (Menza et al., 1993).

D4DR seemed a good candidate gene for a behavioural trait: it is expressed in limbic brain regions involved in cognition and emotion (Meador-Woodruff et al., 1994); it was reported to be elevated six-fold in post-mortem brains of schizophrenics (Seeman et al., 1993) and it binds the atypical antipsychotic agent clozapine 14 times more tightly than does the D2 receptor (Van Tol et al., 1992 and references therein). It has recently been reported that mice who have had the D4 receptor ‘knocked out’ behave differently in an open field and after administration of alcohol and cocaine (Rubinstein et al., 1997). The third exon of D4DR contains a highly variable 48 bp repeat sequence, and alleles with different numbers of repeats differ in their ability to bind dopamine (Van Tol et al., 1992) and inhibit adenylyl cyclase (Asghari et al., 1995). Thus the polymorphism might have physiological significance. Despite a lack of evidence for associations
between schizophrenia and D4DR polymorphisms (Nanko et al., 1993; Sommer et al., 1993), a study of personality, and in particular of novelty-seeking, and D4DR in normal individuals seemed warranted.

Ebstein et al. (1996) analysed TPQ scores and D4DR exon III alleles in 124 healthy Israeli volunteers; subjects were recruited from the staff of a psychiatric hospital, but did not undergo formal psychiatric screening. As predicted, TPQ novelty-seeking was significantly associated with the D4DR polymorphism \( p = 0.013 \); individuals with the longer, seven-repeat alleles had higher novelty-seeking scores. There was no association with other TPQ trait scores (Table 1a). The results did not appear to represent the false positive association known as ‘population stratification’.

Population stratification refers to chance associations between an allele and a trait, owing to the fact that both are associated with a particular ethnic group. For example, if a particular HLA antigen is common in a certain ethnic group, and so is low stature or high sociability, then a study which includes members of that group and members of other groups will find spurious ‘genetic’ associations between the antigen and the trait in the study population overall. This can be corrected for by ‘transmission tests’, which look for the same association only in families in which subjects have different alleles of the locus in question; individuals, or families whose members all share only one of the alleles being studied, are excluded from analysis.\(^2\) Since members of families are more ethnically similar than members of the population, gene-trait associations within families are presumed to be due to genetic transmission, not artefacts of ethnicity. We found similar allele frequencies and similar allele-TPQ associations in the various ethnic groups in this sample, suggesting that the finding was not a case of population stratification; the absence of families in the study made it impossible to exclude this possibility more conclusively.

Three hundred and fifteen individuals (mostly sib-pairs) were available in an American sample (Benjamin et al., 1996b), who had given blood samples and completed the NEO Personality Inventory – Revised (NEO-PI-R) personality test (Costa et al., 1992, 1996; McCrae et al., 1990) and the 16PF. The NEO is based on the five-factor model of personality, a dominant model in current psychological theories of personality assessment (Digman, 1990). Some of these subjects were from an ongoing X-linkage study of normal personality traits, and others were from an X-linkage study of sexual orientation (Hamer et al., 1993). Subjects were recruited from the general public, and did not undergo formal psychiatric screening. On a self-report screening questionnaire they were asked about individual and family mental problems and substance abuse. Although the NEO-PI-R does not include novelty-seeking as a specific factor, it contains multiple items that are clearly related to questions from the TPQ novelty seeking scale, e.g. ‘I have sometimes done things just for kicks or thrills’, versus ‘I often try new things just for fun or thrills’, and ‘I think things through before coming to a decision’, versus ‘I like to think about things for a long time before I make a decision’. Recent studies of populations administered both the NEO-PI-R and the TPQ have shown correlations of about 70% between novelty-seeking and NEO factors, largely due to positive loading on NEO ‘extraversion’ and negative loading on NEO ‘conscientiousness’. It was therefore predicted that these subjects would show a positive association between long (see below) D4DR exon III alleles and extraversion, a negative association between long D4DR exon III alleles and conscientiousness, and a positive association between long alleles and estimated novelty-seeking, calculated from these data, using appropriately weighted equations derived from the studies using both questionnaires (McCrae et al., 1990, available on request).

Of the 315 subjects there were 291 sibs from 131 families, 7 parents, and 17 unrelated individuals. Ninety-five per cent were males and 92% of the sample were white. As in Ebstein et al. (1996), most of the alleles in this sample included 4 or 7 repeats in the hypervariable exon III. However, because there was a fair number of subjects with 5-, 6- and 8-repeat alleles, and it was thought desirable to use all available information, the length was divided at the mid-point between 4 and 7, that is, 5–6; alleles with 5 or fewer repeats were classified as ‘short’, while those with 6 or more were classified as ‘long’.

(Additional analyses considering only 4- and 7-repeat alleles did not change the results.) As in Ebstein et al. (1996), short-short homozygotes were considered S; the presence of one or two long alleles resulted in a subject’s classification as L. Table 1b shows ANOVA of the 5 major NEO factors and estimated novelty-seeking, with presence or absence of a long allele (L) as the independent variable. As predicted, ‘extraversion’ scores were significantly higher \( p = 0.001 \) and ‘conscientiousness’ scores were significantly lower \( p = 0.03 \) in L subjects. Estimated novelty-seeking was also significantly higher among L subjects \( p = 0.002 \).

To test for true genetic transmission, as opposed to population stratification, all unrelated individuals and all families which included individuals with only short or only long alleles were excluded. This left 60 ‘long-short’ (L-S) pairs, who were subjected to a paired \( t \) test with conservative corrections for non-independence of multiple sib-pairs. Table 2 shows that the same associations were statistically significant as in the sib-pairs. Hence, D4DR exon III polymorphism is associated with personality, and in particular of novelty-seeking, and population stratification.\(^2\) Other corrections also exist.
Table 1. Population associations between D4DR genotype and personality traits

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mean (s.d.) Raw score</th>
<th>F</th>
<th>ΔD4DR</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) TPQ factor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Novelty seeking</td>
<td>15.45 (0.5)</td>
<td>6.34</td>
<td>2.5</td>
<td>0.013</td>
</tr>
<tr>
<td>Reward dependence</td>
<td>18.71 (0.4)</td>
<td>0.47</td>
<td>0.6</td>
<td>ns</td>
</tr>
<tr>
<td>Harm avoidance</td>
<td>12.53 (0.6)</td>
<td>0.25</td>
<td>0.5</td>
<td>ns</td>
</tr>
<tr>
<td>(b) NEO factor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroticism</td>
<td>53.1 (9.1)</td>
<td>0.5</td>
<td>—0.8</td>
<td>ns</td>
</tr>
<tr>
<td>Extraversion</td>
<td>53.4 (9.7)</td>
<td>10.7</td>
<td>3.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Openness</td>
<td>58.5 (9.7)</td>
<td>0.9</td>
<td>1.2</td>
<td>ns</td>
</tr>
<tr>
<td>Agreeableness</td>
<td>46.1 (9.5)</td>
<td>3.6</td>
<td>2.2</td>
<td>ns</td>
</tr>
<tr>
<td>Conscientiousness</td>
<td>45.9 (10.3)</td>
<td>4.7</td>
<td>—2.7</td>
<td>0.03</td>
</tr>
<tr>
<td>TPQ novelty seeking</td>
<td>55.1 (7.9)</td>
<td>10.2</td>
<td>3.0</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* Transformed to a score with a population mean = 50, s.d. = 10.
Modified from (a) Ebstein et al. (1996a) and (b) Benjamin et al. (1996b).

Table 2. Within pedigrees associations between D4DR genotype and personality traits. (n = 60 L-S pairs)

<table>
<thead>
<tr>
<th>Factor</th>
<th>ΔAD4DR*</th>
<th>Paired t†</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraversion</td>
<td>6.8</td>
<td>2.9</td>
<td>0.008</td>
</tr>
<tr>
<td>Conscientiousness</td>
<td>—4.7</td>
<td>2.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Novelty-seeking (estimated)</td>
<td>4.6</td>
<td>2.8</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* ΔAD4DR = mean (L sib’s score – S sib’s score), in each L-S pair.
† Conservatively corrected for nonindependence of sib-pairs from the same family (from Benjamin et al. 1996b).

were found in this family analysis as in the whole study population.

In addition to the strength of the statistical significance, it is important to consider the effect size of the gene. Figure 1 shows that D4DR was responsible for about 5% of the total variance, and affected the scores by about 10%, or 0.4 standard deviations. The effect size in the Israeli study was similar. This is considered a moderate effect (Cohen, 1988). Novelty-seeking is about 40% heritable (Heath et al., 1994), so that D4DR accounts for about an eighth of the genetic effects. Clearly, both other genes and non-genetic influences affect novelty-seeking.

To our knowledge these are the first replicated reports of a gene for a personality trait. They are made more convincing by the fact that the two studies used different ethnic groups and different personality questionnaires,

Figure 1. Distributions of estimated novelty-seeking scores. The x axis shows the estimated TPQ-novelty-seeking scores separated into 8 groups with the indicated median T scores. The y axis shows the distribution, in percent, of subjects with short D4DR exon III alleles (group S, n = 217, hatched bars), and the distribution, in percent, of subjects with long D4DR exon III alleles (group L, n = 98, solid bars).
and yet both studies found essentially the same result. However, two recent Scandinavian studies (Jonsson et al., 1997; Malhotra et al., 1996), three American studies (Gelernter et al., 1997; Pogue-Geile et al., 1998; Vandenbergh et al., 1997), and a New Zealand study (Sullivan et al., 1998), failed to replicate this finding. These studies inevitably differed somewhat in their design and subject populations; in particular, Malhotra et al. recruited alcoholic subjects and relatives, while Gelernter et al. included subjects with substance abuse and normal controls. There have also been two partial replications, one by the present group (Ebstein et al., 1997d) and another in Japan (Ono et al., 1997). In the first of these, the association was in the same direction but was statistically significant with only some of the significance tests employed. In the second study, only the ‘exploratory-excitability’ subscale of the novelty-seeking dimension differed significantly by D4DR allele type. The original reports, heuristic as they have proved, cannot therefore be considered in any way definitive (Baron, 1998; Ebstein et al., 1997a). In the interim, associations have been reported between the long allele and the presence of attention deficit hyperactivity disorder (LaHoste et al., 1997), heroin abuse (Kotler et al., 1997; Li et al., 1997), and gambling (Perez de Castro et al., 1997), all conditions with prima facie resemblance to the impulsive aspect of novelty-seeking.

It has been suggested (Baron, 1998) that the problem of non-replication of association studies could be ameliorated by raising the threshold for significance to correct for multiple-testing, as is customary in linkage studies; a linkage study with 300 markers that found linkage at a particular locus would correct for 300 tests, for example. However, in whole-genome linkage studies, there is no a priori hypothesis concerning any given marker, whereas in association studies there is usually some hypothesis. The initial finding of an association between D4DR and the novelty seeking-scale was predicted, in part, by the personality theory underlying the test employed (Cloninger, 1987). This is even more the case with replication studies. Hopefully, additional research among various ethnic and diagnostic groups will elucidate the relationships between ethnicity, D4DR and novelty-seeking.

**Association study of the dopamine D3 receptor gene (D3DR)**

Like D4 receptors, D3 receptors are expressed in limbic regions and bind antipsychotic drugs. A dopamine D3 receptor polymorphism in a coding region has been described (Rietschel et al., 1993) but its physiological significance is unknown. There have been controversial reports of associations with homozygosity for the polymorphism in schizophrenia (Crocq et al., 1992; Mant et al., 1994), and one report of an association with bipolar disorder (Parsian et al., 1995).

Genotypic D3DR data were available for 124 subjects (Ebstein et al., 1997e). These were subjected to analyses of TPQ factor scores. Table 3 shows that, in contrast to the findings with the D4DR polymorphism, there were no significant associations between various D3DR alleles and the TPQ personality traits (although there was a weak D4DR × D3DR interaction effect on reward dependence; \( p = 0.03 \), not corrected for multiple comparison (Benjamin et al., 1998)).

**Interaction between a 5-HT2C receptor gene polymorphism and D4DR – effect on personality**

It has already been pointed out that two or more genes, each with small individual effects, may exert synergistic effects when acting in concert. These so-called gene–gene interactions may yet provide major breakthroughs in behavioural genetics.

The 5-HT-2C receptor gene is widely expressed in the brain, where it is involved in the regulation of various responses, including the production and secretion of ACTH, oxytocin and prolactin (Lappalainen et al., 1995). The functional state of 5-HT-2C receptors in normal controls and various patient groups has been studied in vivo by administering m-CPP, 5-HT-2C agonist, and measuring hormonal and psychological responses. In alcoholism, panic disorder, seasonal affective disorder and obsessive–compulsive disorder, m-CPP has been shown to induce different hormonal and psychological responses in patients and controls. A 2-allele polymorphism, a cysteine to serine substitution at amino acid 23, with a frequency in the population of 0.13 was recently detected (Lappalainen et al., 1995).

This polymorphism was typed in the 124 Israeli subjects described above (Ebstein et al., 1997e). The traits of reward dependence, which describes sentimentality and sensitivity to others, and persistence, which includes perseverance and industriousness, were reduced about 10% by the substitution (main effect of 5-HT-2C on three-way ANOVA \( F(2,116) = 10.7, p < 0.001 \). This effect was markedly accentuated by the simultaneous presence of the rarer 5-HT-2C and the rarer D4DR alleles; for example, reward dependence was 14.1 (S.E. 0.7) in 30 subjects with the common 5-HT-2C allele and long D4DR alleles, and 8.0 (S.E. 2.0) in the 3 subjects with long D4DR alleles, who were also homozygous for the rare 5-HT-2C allele (Figure 2). This provocative effect size amounts to about 10 times the pooled mean standard error of the study population. However, in the light of the rarity of
Table 3. Population associations between D3DR genotype and personality traits

<table>
<thead>
<tr>
<th>Traits</th>
<th>D3 1,1 (n = 60)</th>
<th>D3 1,2 (n = 57)</th>
<th>D3 2,2 (n = 7)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novelty seeking</td>
<td>16.2 (4.9)</td>
<td>16.1 (5.3)</td>
<td>16.3 (4.3)</td>
<td>0.99</td>
</tr>
<tr>
<td>Harm avoidance</td>
<td>11.6 (5.8)</td>
<td>13.3 (5.9)</td>
<td>14.9 (5.3)</td>
<td>0.17</td>
</tr>
<tr>
<td>Persistence</td>
<td>5.4 (1.5)</td>
<td>4.6 (2.3)</td>
<td>5.4 (2.9)</td>
<td>0.09</td>
</tr>
<tr>
<td>Reward dependence</td>
<td>13.8 (3.3)</td>
<td>14.1 (3.5)</td>
<td>12.4 (3.2)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

MANOVA: Wilk’s lambda = 0.92, df = 8, 236, overall p value = 0.27.

Modified from Ebstein et al. (1997e).

Figure 2. The effect of the 5-HT2C serotonin receptor polymorphism on reward dependence (RD 1–4 and RD123) and persistence (RD2) in subjects with the long D4DR exon III repeat allele.

Allelic variation in functional 5-HT transporter expression, anxiety-related personality traits and susceptibility to affective disorders

The 5-HT transporter (5-HTT) is another interesting piece in the mosaic-like texture of personality. Recent advances have resulted from the functional and molecular characterization of the 5-HTT, from pharmacologic studies relating the actions of psychoactive drugs (for example, tricyclics, selective 5-HT reuptake inhibitors (SSRIs), psychostimulants), to discrete effects on 5-HT uptake and release, and from the molecular dissection of the complex changes in functional expression as a consequence of altered gene transcription. Elucidation of 5-HTT structure has provided the means for studying 5-HT uptake processes in humans at the molecular level (Lesch et al., 1993). Progress has also been made in the dissection of the 5-HTT’s genomic organization including 5’-flanking transcriptional regulatory sequences, as well as in modelling 5-HTT-related brain dysfunction in mice by targeted disruption of the 5-HTT gene. The influence of genetic and environmental factors on 5-HTT function and its impact on event-related synaptic plasticity in disease and therapeutic intervention is of particular interest. Highest densities of 5-HTT are found on serotonergic cell bodies in the midbrain raphe complex and in its projection areas including cortical areas, entorhinal cortex, CA3 region of the hippocampus, amygdala, substantia nigra, caudate putamen, and hypothalamus. 5-HTT-dependent functions are likely to play a meaningful role in developmental neuroplasticity, thus determining the expression of complex traits and their associated behaviour throughout adult life (Lesch et al., 1996b). In this regard, anxiety-related traits like ‘neuroticism’ on the NEO questionnaire, ‘tension’ on the 16PF and ‘harm avoidance’ on the TPQ are excellent examples of fundamental, enduring, and continuously distributed dimensions of normal personality. Although twin studies have indicated that individual variation in measures of anxiety-related traits are 40–60% heritable, none of the relevant genes has yet been identified. Moreover, it is becoming increasingly evident that allelic variation of 5-HTT activity, together with inadequate adaptive responses to environmental stressors, are likely to contribute significantly to the aetiopathogenesis of affective disorders, such as depression and manic-depressive illness.

5-HTT function appears to be dysregulated in a variety of complex behavioural traits and disorders such as depression, bipolar, anxiety, obsessive-compulsive, schizophrenic, and neurodegenerative disorders, substance abuse and eating disorders (for review, see Ellis et al., 1994; Owen et al., 1994). Moreover, 5-HT uptake capacity has been proposed to be a trait variable for affective disorder, since it remains decreased after recovery. Twin studies suggest that 5-HT uptake is genetically controlled (Meltzer et al., 1988), which is supported by the fact that 5-HTT function is lower in first-degree relatives of patients with depression who th
selves display decreased 5-HT uptake. The hypothesis that genetic control of and disease-related alteration in 5-HTT function is more likely to be related to differential regulation of 5-HTT expression than to amino acid substitutions, is further supported by the fact that mutation-screening of the gene's translated exons in several samples of patients with affective spectrum and obsessive-compulsive disorders (OCD) detected only rare coding variants (Altemus et al., 1996; Di Bella et al., 1996; Lesch et al., 1995). This led the present group to the search for regulatory sequence variations that might modify the expression rather than the structure of the transporter protein. The human 5-HTT gene is composed of 14 exons spanning ~35 kb (Lesch et al., 1994) and comparison of the human and murine 5-HTT genes revealed a striking conservation of both the exon/intron organization and the 5'-flanking regulatory sequences (Bengel et al., 1997).

A common insertion/deletion polymorphism is located in the transcriptional control region upstream of the 5-HTT coding sequence (Heils et al., 1996).

**Characteristics of the 5-HTT-linked polymorphic region (5-HTTLPR)**

The 5-HTTLPR is located approximately 1 kb upstream of the 5-HTT gene transcription initiation site in a GC-rich region and is composed of 14–16 repeat elements with the consensus sequence CCCCCCCCTGCACCCCCCAGCAT. The polymorphism, which consists of the presence or absence of a 44 bp segment involving repeat element 6–8, is highly prevalent. PCR-based genotype analysis of 505 American volunteers revealed allele frequencies of 57% for the long (l) allele and 43% for the short (s) allele. The 5-HTTLPR genotypes were distributed according to Hardy–Weinberg equilibrium: 32% l/l, 49% l/s, and 19% s/s. Although other variations, such as rare single nucleotide substitutions, have been detected within 5'-flanking promoter sequences (Bengel et al., 1997; G. Stober and K.-P. Lesch, manuscript in preparation), it remains to be clarified whether these sequence variants affect promoter activity.

Comparison of the 5-HTT gene promoter in different species revealed that the 5-HTTLPR is ‘foreign’ DNA, possibly of viral origin and unique to humans and non-human primates, including rhesus monkeys (Bengel et al., 1997; A. Heils and K.-P. Lesch, unpublished observations). The 5-HTTLPR displays a complex G string-dependent tetrastrand-like structure, silences transcriptional activity in non-serotonergic cells, and contains positive response elements (Heils et al., 1995). In addition, the 5-HTTLPR confers allele-dependent differential transcriptional activity on 5-HTT gene promoter activity when fused to a luciferase reporter gene and transfected into human placental choriocarcinoma (JAR) cells (Heils et al., 1996). Cyclic AMP- and protein kinase C (PKC)-dependent mechanisms induced transcriptional activity in both variants of the 5-HTT promoter, but the dose-dependent increase following induction remained proportionally smaller in the allele with the deletion.

**Allelic variation of functional 5-HTT expression**

Lesch et al. (1996a) determined the effects of 5-HTTLPR variability on functional gene expression by studying the relationship between 5-HTTLPR genotype, 5-HTT gene transcription, and 5-HT uptake activity in human lymphoblastoid cell lines. Because appropriate cell models for human serotonergic neurons do not exist and the JAR cells are homozygotic for the 5-HTTLPR, lymphoblastoid cells represent a feasible compromise and cell lines with the complete range of different 5HTTLPR genotypes can readily be obtained. The effect of the 5-HTTLPR on transcriptional activity was first assessed by transfecting lymphoblastoid cells with constructs in which a luciferase reporter gene was fused to ~1.4 kb of 5'-flanking sequences (subsequently referred to as the 5-HTT promoter) containing the l or s form of the 5-HTTLPR. Comparison of the allelic variants revealed that the basal activity of the l variant was significantly higher than the s form of the 5-HTT promoter, thus confirming our findings in JAR cells. Activation of adenyl cyclase with forskolin or stimulation of PKC by a phorbol ester-induced transcriptional activity in both the l and s variants, but the dose-dependent increase following induction of cAMP- and PKC-dependent mechanisms remained proportionally smaller in the s promoter variant.

Although transfection experiments with reporter gene constructs are useful in assaying the transcriptional competence of cloned promoter DNA sequences, they could lead to false results due to the absence of more distant control elements. Therefore, the present group next studied the expression of the native 5-HTT gene in lymphoblastoid cell lines with different 5-HTTLPR genotypes. Cells homozygous for the l form of the 5-HTT promoter showed significantly higher 5-HTT expression and 5-HT uptake activity than did cells containing one or two copies of the s variant. The genotype-dependent differences in functional 5-HTT expression persisted proportionally when 5-HTT gene transcription was induced with forskolin or phorbol ester.

**Association between the 5-HTTLPR and anxiety-related traits**

To determine the contribution of 5-HTTLPR variability to individual phenotypic differences in personality traits, a combined population and family genetic study in an
Table 4. Population association between 5-HTT genotype and anxiety- and depression-related personality facets; NEO-PI-R
T-scores (mean ± s.d.) (n = 505)

<table>
<thead>
<tr>
<th>Group</th>
<th>L (n = 163)</th>
<th>S (n = 342)</th>
<th>F ratio</th>
<th>S-L</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEO domains</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N Neuroticism</td>
<td>53.1 ± 11.5</td>
<td>56.4 ± 11.8</td>
<td>9.3</td>
<td>3.3</td>
<td>0.002</td>
</tr>
<tr>
<td>E Extraversion</td>
<td>54.2 ± 10.6</td>
<td>53.0 ± 11.1</td>
<td>1.4</td>
<td>−1.2</td>
<td>ns</td>
</tr>
<tr>
<td>O Openness</td>
<td>59.4 ± 11.6</td>
<td>58.1 ± 11.3</td>
<td>1.3</td>
<td>−1.3</td>
<td>ns</td>
</tr>
<tr>
<td>A Agreeableness</td>
<td>48.2 ± 10.7</td>
<td>46.1 ± 11.6</td>
<td>4.0</td>
<td>−2.1</td>
<td>0.045</td>
</tr>
<tr>
<td>C Conscientiousness</td>
<td>45.9 ± 10.9</td>
<td>44.8 ± 12.3</td>
<td>1.0</td>
<td>−1.1</td>
<td>ns</td>
</tr>
<tr>
<td>Neuroticism facets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1 Anxiety</td>
<td>52.5 ± 11.6</td>
<td>54.9 ± 11.6</td>
<td>4.9</td>
<td>2.4</td>
<td>0.027</td>
</tr>
<tr>
<td>N2 Angry hostility</td>
<td>50.5 ± 10.8</td>
<td>53.9 ± 11.6</td>
<td>9.9</td>
<td>3.4</td>
<td>0.002</td>
</tr>
<tr>
<td>N3 Depression</td>
<td>52.3 ± 11.0</td>
<td>55.3 ± 11.8</td>
<td>7.3</td>
<td>3.0</td>
<td>0.007</td>
</tr>
<tr>
<td>N4 Self-consciousness</td>
<td>53.0 ± 11.1</td>
<td>54.3 ± 11.9</td>
<td>1.4</td>
<td>1.3</td>
<td>ns</td>
</tr>
<tr>
<td>N5 Impulsiveness</td>
<td>54.1 ± 11.0</td>
<td>56.9 ± 10.9</td>
<td>7.0</td>
<td>2.8</td>
<td>0.008</td>
</tr>
<tr>
<td>N6 Vulnerability</td>
<td>51.6 ± 10.9</td>
<td>52.8 ± 11.3</td>
<td>1.3</td>
<td>1.2</td>
<td>ns</td>
</tr>
</tbody>
</table>

F, F value for one-way ANOVA comparing S and L genotype groups.
S-L, Mean score for S genotypes (s/s and s/l) minus mean score for L genotypes (l/l).
ns, not significant (p > 0.05).

From Lesch et al. (1996b).

extended American sample totalling 505 subjects was conducted (Lesch et al., 1996a); this sample was recruited in the same manner as the sample described in Benjamin et al. (1996b). Anxiety-related and other personality traits were assessed by the NEO-PI-R. There was a significant association between 5-HTTLPR genotype and NEO ‘neuroticism’ (Table 4). Individuals with either one or two copies of the s form of the 5-HTT promoter (subsequently referred to together as group S) had higher ‘neuroticism’ scores than individuals homozygous for the l variant of the 5-HTTLPR (referred to as group L). The scores for the l/s and s/s genotypes were not significantly different, indicating that the polymorphism has more of a dominant/recessive than of a codominant/additive effect on neuroticism, just as it does on 5-HTT gene expression. The distributions of ‘neuroticism’ scores in the S and L groups were overlapping but had means that were separated by 3.4 T score units, a difference of 0.29 s.d. units (Table 4 and Figure 3). The effect of the 5-HTTLPR genotype on personality was specific for neuroticism. Scores on three of the other five major NEO personality factors, ‘extraversion’, ‘openness’ and ‘conscientiousness’, were not significantly associated with the genetic variant.

The 16PF questionnaire was used as a secondary psychometric instrument. A significant and specific association between 5-HTTLPR genotype and the 16PF ‘anxiety’ factor was found; this was primarily due to associations with the two ‘anxiety’-related 16PF traits of ‘tension’ and ‘suspiciousness’. The final method of personality assessment was based on Cloninger’s biosocial model. Although our subjects did not complete the TPQ, it was possible to estimate TPQ scores from the NEO-PI-R data using weighted regression equations. The 5-HTTLPR genotype was found to be associated with estimated scores for ‘harm-avoidance’ but not the other three TPQ traits. Analysis of the subscales for ‘harm-avoidance’ showed significant associations with the scales for ‘worry and pessimism’, ‘fear of uncertainty’ and ‘fatigability’, but not to ‘shyness’. These results indicate that the 5-HTTLPR influences a pattern of traits related to anxiety. Across the three personality measures, the 5-HTT polymorphism contributes a moderate 7–9% of the genetic variance, based on estimates from twin studies using these and related measures.

The association between 5-HTTLPR genotype and personality traits was then analysed in 78 sib-pairs that were discordant for the 5-HTTLPR. Despite the reduction in sample size, the difference between the L and S siblings was statistically significant even after conservatively correcting for the non-independence of sib-pairs from the same family. These family studies show that the observed associations between 5-HTTLPR genotype and anxiety-related traits are due to genetic transmission rather than population stratification. Overall, however, the associations reported here represent only a small portion of the genetic contribution to anxiety-related traits observed in this non-random population sample.
Table 5. Genotype and allele frequency of the 5-HT transporter-linked polymorphic region (5-HTTLPR) in affective disorders

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 570)</th>
<th>Combined affective disorder (n = 454)</th>
<th>Bipolar disorder (n = 304)</th>
<th>Unipolar depression (n = 150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l/l</td>
<td>191 (33%)</td>
<td>128 (29%)</td>
<td>82 (27%)</td>
<td>46 (31%)</td>
</tr>
<tr>
<td>l/s</td>
<td>278 (49%)</td>
<td>211 (46%)</td>
<td>148 (49%)</td>
<td>63 (42%)</td>
</tr>
<tr>
<td>s/s</td>
<td>101 (18%)</td>
<td>115 (25%)</td>
<td>74 (24%)</td>
<td>41 (27%)</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>9.5</td>
<td>6.4</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.009</td>
<td>0.04</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>(b) Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l</td>
<td>660 (58%)</td>
<td>467 (51%)</td>
<td>312 (51%)</td>
<td>155 (52%)</td>
</tr>
<tr>
<td>s</td>
<td>480 (42%)</td>
<td>441 (49%)</td>
<td>296 (49%)</td>
<td>145 (48%)</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>8.5</td>
<td>7.0</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.004</td>
<td>0.008</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

From Collier et al. (1996b).

Figure 3. Distributions of neuroticism (NEO-PI-R factor N) scores. The x axis shows the neuroticism scores separated into 8 groups with the indicated median T scores. The y axis shows the distribution, in percent, of subjects with short 5-HTT alleles (group S, n = 342, empty bars), and the distribution, in percent, of subjects with long 5-HTT alleles (group L, n = 163, solid bars).

There is considerable evidence indicating that increased serotonergic neurotransmission (which would be an evident consequence of the reduced 5-HT uptake capacity found in individuals with short 5-HTTLPR alleles) is anxiogenic in animal models as well as in humans. At the clinical level, reduced 5-HT uptake or reduced inhibitor binding to the 5-HTT has been one of the most consistent psychobiological findings in individuals with depression and several anxiety disorders. The associations reported here represent only a small portion of the genetic contribution to anxiety-related personality traits. If other genes were hypothesized to contribute similar gene-dosage effects to anxiety, approximately 10–15 genes might be predicted to be involved.

Since the original observation, a number of similar studies have continued to explore the influence of the 5-HTTLPR (Ball et al., 1997; Ebstein et al., 1997b; Nakamura et al., 1997; Ricketts et al., 1997) and other polymorphisms (Evans et al., 1997) at the 5-HTT gene locus on anxiety-related traits. Ricketts et al. confirmed the association, while the other three studies of the 5-HTTLPR did not. Thus this association, like the D4DR – novelty-seeking association, cannot be considered definitive.

D4DR and 5-HTTLPR in neonates

Neonatal infants are at a phase of development when environmental influences are minimal and least likely to confound associations between temperament and genes. The possible influence of D4DR and/or 5-HTTLPR on temperament in 2-wk-old human infants was evaluated using the Brazelton neonatal assessment scale (Ebstein et al., 1997c). D4DR was associated most significantly with the cluster of items termed ‘orientation’, that is, attention to novel stimuli. The homozygous short 5-HTTLPR genotype lowered orientation scores in infants who also lacked a long D4DR allele. We may speculate that ‘orientation’ is a prima facie neonatal equivalent of novelty-seeking, and that, in the absence of its enhancement by a long D4DR allele, it is opposed by a genotype which acts to increase the neonatal equivalent of anxiety, neuroticism or harm avoidance.
5-HTT expression and susceptibility to affective disorders

Genetic susceptibility factors contribute to the aetiology of affective illness, including bipolar affective disorder and unipolar depression, and heritability is estimated to be up to 86% for bipolar disorder (McGuffin et al., 1994) and 30 to 37% for depression (Kendler et al., 1994). Although both disorders may share factors which determine depressive symptomatology with an associated high risk of suicide (Winokur et al., 1995), genetic heterogeneity and a substantial but varying environmental component complicates identification of predisposing genes.

To test the influence of 5-HTTLPR-dependent variability in functional 5-HTT expression on susceptibility to affective illness, a genetic case-control study was performed on independently ascertained samples from 3 different European centres (Collier et al., 1996b). English (n = 277) and German (n = 86) subjects satisfied DSM-IV criteria for bipolar I disorder or major depression; Italian subjects (n = 97) satisfied DSM-III-R criteria for the same disorders. Table 5 shows the allele and genotype frequencies in the affected subjects (n = 570) versus the controls (n = 454). When the data for each centre were analysed separately, no significant differences were found in the allele or genotype frequencies of the 5-HTTLPR between cases and controls for any diagnosis, although there was an excess of the low-activity allele (short 5-HTTLPR) over the high activity allele (long 5-HTTLPR) for affected subjects from all countries (Table 5). When all samples were combined by diagnosis, there was a significant excess of the low activity allele in both bipolar affective disorder (n = 304; p = 0.008) and unipolar depression (n = 150; p = 0.05) in comparison to controls (n = 570). Significant differences were also found in the genotype frequencies of the 5-HTTLPR between cases and controls for both diagnoses. Differences in genotype and allele frequencies were most significant for the combined affective disorders category (n = 454; p = 0.009 and p = 0.004, respectively). There was no statistical evidence for heterogeneity of samples between centres for either patients or controls. The odds ratio (OR) and relative risk (RR) for the association of the low-activity allele with the combined affective disorders were similar and both indicated a relative risk of 1.7-fold for homozygotes for the short 5-HTTLPR variant, whereas the effect in heterozygotes did not reach significance (RR, 1.14). The population-attributable risk (AR) resulting from the low-activity allele was calculated to be 9% (5% confidence interval, 2.7–16.3%) for the combined affective disorders category.

Linkage between the 5-HTT locus on human chromosome 17q12 and bipolar affective disorder has not been detected, although individual families with lod scores of approximately 1 have been observed (Kelsoe et al., 1996). This is not necessarily unexpected, since linkage analysis does not have the sensitivity to detect quantitative trait or vulnerability loci with modest effects; nevertheless, linkage would bolster confidence in the association finding. Previous analysis of the 5-HTT gene for polymorphic variants revealed, in addition to some rare polymorphisms which were not associated with affective disorders (Altemus et al., 1996; Di Bella et al., 1996; Lesch et al., 1995), a VNTR located in intron 2 with two common (with 10 or 12 repeats) and one rare allele (with 9 repeats) (Lesch et al., 1994). This rare allele was reported to be associated with unipolar but not bipolar affective disorder (Ogilvie et al., 1996). While this finding has not been replicated (Collier et al., 1996a; Stober et al., 1996), analysis of a larger sample of subjects with affective disorders indicated a strong association between allele 12
Our findings indicate the low transporter-mediated 5-HT uptake function resulting from reduced activity of the 5-HTT gene’s transcriptional apparatus increases susceptibility to both bipolar affective disorder and unipolar depression. Although the increase in risk for homozygotes for the low 5-HT uptake activity allele is relatively modest, the observation of a similar effect of this allele in both unipolar depression and bipolar disorder may indicate that affective spectrum disorders share a general genetic susceptibility locus for anxious and depressive symptoms (Figure 4). To further validate the concept of the 5-HTT gene as a susceptibility locus for anxiety- and depression-related symptomatology this group is currently pursuing a transgenic strategy (Bengel et al., 1996). This approach addresses the question to what extent targeted disruption of the 5-HTT gene affects biochemistry, electrophysiology and pharmacology of the 5-HT system and modulates neural development and synaptic plasticity. It also may provide a system to dissect successive events that lead to disease states related to anxiety and depression as well as to test novel therapeutic concepts.

Conclusion

The difficulties of finding genes directly responsible for psychiatric disorders have been discussed and evidence presented for the possibility that normal personality traits, extreme deviations of normal personality traits (personality disorders), and mental illnesses may represent differing positions on a genetic continuum. Following preliminary studies which indicated the importance of investigating genes for normal personality, a direct and replicated genetic association was reported between a candidate gene for a receptor of undoubted relevance to psychiatry, the dopamine D4 receptor, and the normal personality trait of novelty-seeking. A possible marked effect of an interaction between two genes on the trait of reward dependence was also reported. Moreover, the 5-HTT gene, thought to be a site of action of 5-HT reuptake blocking antidepressants, was cloned and characterized; a functional polymorphism in the promoter region of the gene was described, as was an association between this polymorphism and anxiety-related personality traits. Evidence for possibly equivalent effects was also seen in neonates. Finally, there is evidence that allelic variation in functional 5-HTT expression may be a susceptibility factor for disorders of the affective spectrum.

We believe that both normal personality traits and psychiatric disorders are amenable to genetic study, and that the genetic component of the former may contribute to the latter. The task ahead is to demonstrate the links between these and other genetic findings in personality disorders and psychiatric illnesses. The work described so far elevates this objective from the status of a scientific hypothesis to that of an ongoing research programme.

Acknowledgements


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Genes for personality traits


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