

Deliberate self-harm is associated with allelic variation in the tryptophan hydroxylase gene (TPH A779C), but not with polymorphisms in five other serotonergic genes

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ABSTRACT

Background. There is a heritable component to suicidal behaviour, encouraging the search for the associated risk alleles. Given the putative role of the 5-HT (5-hydroxytryptamine; serotonin) system in suicidal behaviour, serotonergic genes are leading candidates. In particular, several studies have reported an association with variants in the tryptophan hydroxylase (TPH) gene.

Method. We studied six serotonergic gene polymorphisms in a well-characterized sample of 129 deliberate self-harm subjects and 329 comparison subjects. The polymorphisms were TPH (A779C), 5-HT transporter (5-HTT, LPR S/L), monoamine oxidase A (MAOA G941T), 5-HT1B receptor (HTR1B G861C), 5-HT2A receptor (HTR2A T102C), and 5-HT2C receptor (HTR2C Cys23Ser). Genotyping was done using polymerase chain reaction (PCR)-based assays. The primary analyses compared allele and genotype frequencies between cases and controls. There were a limited number of planned secondary analyses within the deliberate self-harm group.

Results. The TPH A779 allele was more common in deliberate self-harm subjects than in controls (OR 1.38, 95% CI 1.02–1.88; $P=0.03$). None of the other polymorphisms was associated with deliberate self-harm. Within the deliberate self-harm group there were no associations with impulsivity, suicide risk, lifetime history of depression, or family history of deliberate self-harm.

Conclusions. Our data extend the evidence that allelic variation in the TPH gene is a risk factor for deliberate self-harm. No evidence was found to implicate the other polymorphisms.

INTRODUCTION

Deliberate self-harm (DSH) is the best predictor of future suicide (Sakinofsky, 2000). Suicidal behaviour may result from a wide range of factors (Hawton & van Heeringen, 2000). Psychiatric and personality disorders are present in most suicides and DSH patients, and comorbidity of disorders, especially mood and substance abuse disorders, is common (Suominen

et al. 1996; Foster *et al.* 1997; Kessler *et al.* 1999; Haw *et al.* 2001). In addition, impulsivity and aggression are traits of behavioural disinhibition that may underlie a cross-diagnostic propensity for suicidal behaviour (Brodsky *et al.* 1997; Roy, 2001; Van Praag, 2001).

Suicide and DSH show familial clustering (Roy, 1983; Roy *et al.* 2000; Hawton *et al.* 2002). Twin and adoption studies show that this familiarity is due mainly to genetic factors, which differ from the genetic predisposition to associated psychiatric disorders (Roy, 2001; Turecki, 2001). The latter was confirmed by a

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recent case-control study, which found that suicide is independently associated with a family history of suicide, with an odds ratio of 2.58 (95% CI, 1.84–3.61) (Qin *et al.* 2002). An Australian population-based twin study reported that additive genetic effects explained 45% of the variance in suicidal thoughts and DSH (Statham *et al.* 1998), while another twin study reported a 17% genetic contribution to DSH and 36% to suicidal ideation (Fu *et al.* 2002). These findings, in conjunction with the importance of suicide and DSH as public health issues, have focused attention on the search for the susceptibility genes for suicidal behaviour. Investigations to date have been almost entirely case-control association studies in which the frequencies of common polymorphisms in candidate genes have been compared between DSH (or suicide) subjects and a control group from the same population. The most widely studied genes have been those involved in 5-HT (serotonergic) neurotransmission, due to prior evidence of 5-HT dysfunction in suicidal individuals and suicide victims, and in associated factors such as impulsivity, aggression, and low mood (Mann, 1998). For example, cerebrospinal fluid (CSF) levels of the major 5-HT metabolite, 5-HIAA (5-hydroxyindoleacetic acid), are reduced in suicide attempters (Åsberg *et al.* 1976), and suicide victims and those with suicidal ideation exhibit differences in central expression of 5-HT receptors, especially the 5-HT_{2A} receptor (HTR_{2A}), and the 5-HT transporter (5-HTT) (see Mann, 1999).

Several suggestive associations between serotonergic genes and either suicide or DSH have been reported, in particular with allelic variation in tryptophan hydroxylase (TPH) (Mann *et al.* 1997), HTR_{2A} (Du *et al.* 2000a), 5-HTT (Du *et al.* 1999) and 5-HT_{1D} β receptor (HTR_{1B}) (New *et al.* 2001). However, as reviewed by Turecki (2001) and Mann *et al.* (2001), although the first two associations have been replicated at least once, other studies have failed to do so, while still others have reported associations with different serotonergic genes (see Discussion). To extend this body of work, we have examined these four polymorphisms, together with two other 5-HT-related candidate genes (5-HT_{2C} receptor (HTR_{2C}) and monoamine oxidase A (MAOA)), in a well-characterized sample of DSH subjects and a relatively large

comparison group drawn from the same population.

METHOD

Sample selection and recruitment

The subjects were patients aged 15 years and over who presented to the general hospital in Oxford following an episode of DSH. The definition of DSH was that given for 'parasuicide' in the WHO/EURO Multicentre Study of Suicidal Behaviour (Platt *et al.* 1992). From the 217 individuals approached, 150 took part in the original interview study, and from these, 129 (52 male, 77 female; mean age 38 years (s.d. = 14; range 20–72)) provided DNA for the present study. The demographic, psychiatric and personality characteristics of the 150 DSH patients have been documented in detail (Haw *et al.* 2001); the 129 patients studied here were demographically indistinguishable from the full sample. Subjects were assessed using a structured interview (Haw *et al.* 2001), generating diagnoses according to ICD-10 research criteria (World Health Organization, 1993). Subjects were also rated using the Beck Suicide Intent Scale (Beck *et al.* 1974), the Brown–Goodwin Aggression Scale (Brown *et al.* 1979), and the Plutchik Impulsivity Scale (Plutchik *et al.* 1989).

The salient clinical characteristics of the sample were as follows. The majority (96%) of the DSH acts were self-poisoning. Two-thirds had previously self-harmed at least once. Most (91%) patients had an Axis I psychiatric disorder at index. The commonest diagnoses were depressive episode (72%), alcohol harmful use or dependence (29%), and neurotic/stress-related disorders (16%). Seventy-eight subjects (64% from a total of 122) had a lifetime history of depressive disorder of at least moderate severity. A self-reported family history of suicide and/or DSH was present in 40% of subjects (Hawton *et al.* 2002).

The control sample comprised 329 individuals (138 male, 191 female), mean age 38 years (s.d. = 13, range 19–71), recruited from blood donor clinics within Oxfordshire. It excluded individuals who reported any history of psychiatric disorder or current psychotropic medication in a self-report questionnaire. All DSH and control subjects were Caucasian and born

in the UK, as were their parents and all four grandparents.

DNA extraction and genotyping

Participants provided two buccal swabs from which DNA was extracted using standard techniques (DNAce Clinipure Kit, Bioline, UK). Genotypes for the polymorphisms in the HTR2A, HTR2C, TPH, HTR1B and MAOA genes were determined using a previously described sequence-specific PCR assay (Marshall *et al.* 1999). Briefly, the assay comprises two reactions – each consisting of the relevant allele-specific primer, the consensus primer, and two control primers (to determine amplification success). Allele-specific primers differ in the 3'-terminal nucleotide to allow the different genotypes to be discriminated (details on request). In reactions where the specific primer binds appropriately, two bands are observed – one for the allele specific PCR product and one for the control primer. Each reaction was carried out in a 13 µl volume consisting of 67 mM Tris base, pH 8.8; 2 mM magnesium chloride; 0.01% v/v Tween 20; 16.6 mM ammonium sulphate; 200 µM of each dNTP; between 100–300 ng of DNA template; and 0.2 U BioTaq DNA polymerase (Bioline). All primers were designed to operate within the same range of melting temperatures; the final primer concentrations were 192 pg/µl, with the exception of the HTR2C primers (96 pg/µl) and the control primers (38 pg/µl). Thermal cycling parameters consisted of an initial denaturation at 96 °C for 60 s followed by five cycles of 96 °C for 20 s, 67 °C for 45 s and 72 °C for 25 s. Two subsequent stages followed – first, 26 cycles of 96 °C for 25 s, 60 °C for 50 s and 72 °C for 30 s, then four cycles of 96 °C for 30 s, 55 °C for 60 s, and 72 °C for 90 s. Samples were cooled to 4 °C, centrifuged, and visualized on 1.5% agarose gels containing 0.5 µg/ml ethidium bromide.

Genotyping of the 5-HTT LPR short/long promoter variant was determined separately, using primers directed to the adjacent upstream and downstream regions of the insertion/deletion site. A 20 µl reaction volume consisted of 1.5 mM magnesium chloride; 200 µM of each dNTP; 5% dimethylsulphoxide; approximately 200 ng of DNA template; and 1 U BioTaq DNA polymerase together with the appropriate

volume of 10X BioTaq reaction buffer. The final concentration of the forward (5'-GCG TTG CCG CTC TGA ATG C-3') and reverse (5'-GGA CTG AGC TGG ACA ACC AC-3') primers was 625 pg/µl. Cycling parameters were: 95 °C for 120 s, two cycles at 95 °C for 30 s, 63 °C for 30 s, and 72 °C for 60 s, prior to two cycles at 95 °C for 30 s, 62 °C for 30 s and 72 °C for 60 s, followed by 35 cycles at 95 °C for 30 s, 61 °C for 30 s and 72 °C for 60 s, with a final elongation step for 8 min at 72 °C. PCR fragments were resolved on a 3% agarose gel and visualized with 0.5 µg/ml ethidium bromide.

Every polymorphism was genotyped twice for each individual, and alleles were scored independently by two people. For the few samples where there was an inconclusive result or discrepancy between duplicates, genotyping was repeated until an unequivocal genotype was obtained.

Statistical analysis

The primary analysis was the comparison of genotype and allele frequencies between cases and controls using χ^2 tests. For the HTR2C and MAOA polymorphisms, men and women were analysed separately since males are hemizygous for X-linked genes. In addition, four planned secondary analyses were performed within the DSH sample, limited to those polymorphisms that showed a positive overall association with DSH in the present study, or where a prior association had been reported. The secondary analyses were: (1) the presence or absence of a lifetime history of major depression; (2) the presence or absence of a family history of suicidal behaviour; (3) the relationship with impulsivity, assessed using the Plutchik scale, for which scores ranged from 21–53, with a mean (s.d.) of 36.0 (6.4), available in 121 subjects; (4) finally, we produced a composite score for risk of suicide ('suicide risk score'), which was the sum of the quartile scores obtained from the Brown–Goodwin Aggression Scale, the Beck Suicide Intent Scale, and the number of lifetime DSH episodes divided into quartiles. This gave a range of possible scores from 3 to 12, and was available for 120 of the DSH patients. In males, the suicide risk score ranged from 4 to 12, with a mean of 7.8, and in females ranged from 3 to 12, with a mean of 6.9.

Table 1. *Allele and genotype frequencies in DSH and control groups, for the autosomal genes*

	DSH group (<i>N</i> = 129)	Control group (<i>N</i> = 329)	χ^2	<i>P</i>
TPH A779C				
Genotype				
A779/A779	20 (0.16)	44 (0.13)	6.55	0.04
A779/C779	67 (0.52)	135 (0.41)		
C779/C779	42 (0.34)	150 (0.46)		
Allele				
A779	107 (0.41)	223 (0.34)	4.62	0.03
C779	151 (0.59)	435 (0.66)		
HTR1B G861C				
Genotype				
G861/G861	77 (0.6)	194 (0.59)	0.17	0.91
G861/C861	44 (0.34)	111 (0.34)		
C861/C861	8 (0.06)	24 (0.07)		
Allele				
G861	198 (0.77)	499 (0.76)	0.08	0.77
C861	60 (0.23)	159 (0.24)		
HTR2A T102C				
Genotype				
T102/T102	17 (0.13)	43 (0.13)	1.01	0.60
T102/C102	70 (0.54)	163 (0.50)		
C102/C102	42 (0.33)	123 (0.37)		
Allele				
T102	104 (0.40)	249 (0.38)	0.48	0.49
C102	154 (0.60)	409 (0.62)		
5-HTT LPR				
Genotype				
S/S	25 (0.19)	65 (0.20)	0.14	0.93
S/L	66 (0.51)	160 (0.49)		
L/L	38 (0.30)	100 (0.31)		
Allele				
S	116 (0.45)	290 (0.44)	0.01	0.92
L	142 (0.55)	360 (0.56)		

Numbers in parentheses indicate allele or genotype frequency.

RESULTS

Allele and genotype data for the six polymorphisms in the DSH and control groups are given for the autosomal genes in Table 1, and the X-linked genes in Table 2. All results were in Hardy–Weinberg equilibrium and were very similar to previously reported allele frequencies within Caucasian populations (Gelernter *et al.* 1997; Marshall *et al.* 1999). The main positive finding is that the TPH A779 allele was more common in the DSH sample than in the controls (Table 1: OR = 1.38, 95% CI 1.02–1.88; $P = 0.03$). There were no significant differences or trends between cases and controls for any of the other polymorphisms (all $P > 0.25$).

Planned secondary analyses within the DSH group

Table 3 summarizes the secondary analyses. No significant differences in allele or genotype

frequencies were found related to a family history of DSH, lifetime history of depression, or with suicide risk scale or impulsivity score, for any of the four genes for which these planned comparisons were carried out. In addition, because of the finding of Evans *et al.* (2000), we examined whether the HTR2C Ser23 variant was associated with impulsivity score, but found no such relationship (Cys, 36.7 ± 7.2 , $N = 44$; Ser, 38.8 ± 4.8 ; $N = 5$).

DISCUSSION

Allelic variation in 5-HT-related genes and DSH

We found a significant association between DSH and the A779 polymorphism of the tryptophan hydroxylase gene (Table 1). (The A779 and C779 alleles are alternatively referred to as 'U' and 'L' respectively.) This replicates the original finding of Mann and colleagues (1997) in a group of subjects with major depression. The A779C polymorphism is in almost complete linkage disequilibrium with another TPH polymorphism, A218C. That is, virtually all individuals either have A alleles at both positions, or C alleles at both positions. Hence, it is notable that four studies have shown an association of suicidal behaviour with the A218 allele, since these are effectively also replications of the A779 finding (Persson, 1999; Tsai *et al.* 1999a; Abbar *et al.* 2001; Courtet *et al.* 2002). These data together make a relatively convincing case that the A779/A218 variant of the TPH gene is associated with DSH. However, other results have been negative (Furlong *et al.* 1998; Zalsman *et al.* 2001) or equivocal (Geijer *et al.* 2000), and no association has been found with completed suicide (Bennett *et al.* 2000; Du *et al.* 2000b; Ono *et al.* 2000; Turecki *et al.* 2001). Moreover, the opposite association (i.e. with the C779 allele) has also been reported (Nielsen *et al.* 1994, 1998), albeit in a sample of alcoholic, violent offenders and arsonists, who represent a markedly different clinical population from that of typical DSH subjects.

The molecular mechanism by which the A779C and A218C polymorphisms might mediate their effects on DSH remains unclear. Both are intronic and therefore do not alter the amino-acid sequence. Two alternative possibilities remain: they may affect the processing of the TPH gene transcript (e.g. alter RNA

Table 2. Allele and genotype frequencies for the X-linked genes in the DSH and control groups

	DSH males (N=52)	Control males (N=138)	χ^2	P	DSH females (N=77)	Control females (N=191)	χ^2	P
HTR2C Cys23Ser								
Genotype								
CysCys					51 (0.66)	136 (0.71)	2.03	0.36
CysSer					22 (0.29)	51 (0.27)		
SerSer					4 (0.05)	4 (0.03)		
Allele								
Cys	45 (0.87)	112 (0.81)	0.76	0.38	124 (0.81)	323 (0.85)	1.29	0.26
Ser	7 (0.13)	26 (0.19)			30 (0.19)	59 (0.15)		
MAOA G941T								
Genotype								
GG					5 (0.07)	22 (0.12)	1.54	0.46
GT					34 (0.44)	81 (0.42)		
TT					38 (0.49)	88 (0.46)		
Allele								
G	17 (0.33)	37 (0.27)	0.64	0.42	44 (0.29)	125 (0.33)	0.88	0.35
T	35 (0.67)	101 (0.73)			110 (0.71)	257 (0.67)		

Numbers in parentheses indicate allele or genotype frequency.

splicing), or else they are merely markers of another, functional, variant which is in linkage disequilibrium with them both. The first possibility is unlikely since neither variant is associated with the production of aberrant TPH splice products (Nielsen *et al.* 1997). Recent evidence does lend some support to the second proposal. There are four polymorphisms within the TPH promoter sequence, which are in varying degrees of linkage disequilibrium with the A779C and A218C polymorphisms. Certain haplotypes (combinations of these polymorphisms) are more frequent in suicide attempters (Rotondo *et al.* 1999) and completers (Turecki *et al.* 2001). Promoter polymorphisms have functional consequences by virtue of quantitative effects on expression of the gene. Although this has not been shown directly for TPH, preliminary evidence is consistent with the possibility (Rotondo *et al.* 1999). Whatever the molecular mechanism, the polymorphism is functional, at least in men, with the A779 variant being associated with lower CSF 5-HIAA levels (Jonsson *et al.* 1997) and an attenuated prolactin response to fenfluramine (Manuck *et al.* 1999).

Previous studies of the 5-HTT LPR polymorphism and suicidal behaviour have produced conflicting results. The S (short) allele, which is associated with a lower basal transcriptional activity of the 5-HTT promoter, has been found to be more common in female suicide attempters (Baca-García *et al.* 2002) and in alcoholics who attempt suicide, especially

where the method is violent (Bondy *et al.* 2000). Conversely, the long allele was reported to be more frequent in depressed suicide victims (Du *et al.* 1999), and the LL genotype was more frequent in a subgroup of suicidal in-patients who scored highly for hopelessness and suicidal intent (Russ *et al.* 2000). Several other reports indicate no association with either allele (Mann *et al.* 2000; Geijer *et al.* 2000), and our finding (Table 1) adds to the tally of negative studies.

The HTR2A C102 allele has been associated with suicidal ideation (Du *et al.* 2000a) and a history of suicidal behaviour (Arias *et al.* 2001) among patients with major depression. However, in line with three other studies of suicide and suicidal behaviour (Crawford *et al.* 1999; Tsai *et al.* 1999b; Turecki *et al.* 1999) we did not find any association of this polymorphism with DSH (Table 1), or in the subgroup comparisons within the DSH sample (Table 3). Moreover, Zhang *et al.* (1997) reported a weak association between DSH and the T102 allele. Overall therefore, as with the 5-HTT LPR polymorphism, there is no consistent evidence regarding a relationship between HTR2A gene variation and DSH.

New *et al.* (2001) reported that the intronic G861 variant of the HTR1B gene was associated with a history of suicide attempt in personality disorder subjects. We did not find any association of this polymorphism with DSH (Table 1) or with any of the DSH subgroups (Table 3). Our negative result is in line with the report by

Table 3. *Allele and genotype frequencies within subgroups of the DSH sample*

	Family history of suicidal behaviour		Lifetime history of depression		Suicide risk score (<i>N</i> = 120)	Impulsivity score (<i>N</i> = 121)
	Present (<i>N</i> = 52)	Absent (<i>N</i> = 77)	Present (<i>N</i> = 78)	Absent (<i>N</i> = 44)		
TPH A779C						
Genotype						
AA	8 (0.15)	12 (0.15)	11 (0.14)	7 (0.16)	7.5 ± 2.3	36.2 ± 7.6
AC	24 (0.39)	43 (0.56)	42 (0.54)	21 (0.48)	7.3 ± 2.3	35.9 ± 5.6
CC	20 (0.46)	22 (0.29)	25 (0.32)	16 (0.36)	7.1 ± 2.0	36.7 ± 6.9
Allele						
A	40 (0.38)	67 (0.44)	64 (0.41)	35 (0.40)		
C	64 (0.62)	87 (0.56)	92 (0.59)	53 (0.60)		
HTR1B G861C						
Genotype						
GG	31 (0.60)	46 (0.60)	45 (0.58)	26 (0.59)	7.3 ± 2.1	36.1 ± 6.3
GC	19 (0.36)	25 (0.32)	31 (0.40)	13 (0.30)	7.5 ± 2.4	35.6 ± 5.9
CC	2 (0.04)	6 (0.08)	2 (0.03)	5 (0.11)	5.7 ± 2.0	37.7 ± 10.0
Allele						
G	81 (0.78)	117 (0.76)	121 (0.78)	65 (0.74)		
C	23 (0.22)	37 (0.24)	35 (0.22)	23 (0.26)		
HTR2A T102C						
Genotype						
TT	5 (0.10)	12 (0.16)	9 (0.12)	7 (0.16)	7.4 ± 2.6	37.4 ± 7.3
TC	30 (0.58)	40 (0.52)	43 (0.55)	24 (0.55)	6.9 ± 2.2	35.9 ± 6.5
CC	17 (0.33)	25 (0.32)	26 (0.33)	13 (0.30)	7.7 ± 1.9	35.7 ± 5.9
Allele						
T	40 (0.38)	64 (0.42)	61 (0.39)	38 (0.43)		
C	64 (0.62)	90 (0.58)	95 (0.61)	50 (0.57)		
5-HTT LPR						
Genotype						
SS	13 (0.25)	12 (0.16)	13 (0.17)	12 (0.25)	7.3 ± 2.6	36.3 ± 6.1
SL	25 (0.48)	41 (0.53)	41 (0.53)	25 (0.45)	7.1 ± 2.2	36.0 ± 7.0
LL	14 (0.27)	24 (0.31)	24 (0.31)	14 (0.29)	7.5 ± 2.0	35.9 ± 5.7
Allele						
S	51 (0.49)	65 (0.42)	67 (0.43)	49 (0.48)		
L	53 (0.51)	89 (0.54)	89 (0.59)	53 (0.52)		

Numbers in parentheses indicate allele or genotype frequency. Scores are mean ± s.d.

Huang *et al.* (1999) in suicide victims. We also found no association between DSH and the silent G941T polymorphism in the MAOA gene, in either men or women (Table 2), in agreement with Ho *et al.* (2000).

Frequencies for the HTR2C Cys23Ser polymorphism did not differ in this study between DSH and control men or women (Table 2). There have been no prior investigations of this association with DSH, although the Ser23 variant has been associated with higher impulsivity scores in men, but not women, presenting after DSH (Evans *et al.* 2000). We did not find a relationship between the Ser23 allele and impulsivity, rated with the Plutchik scale. A possible reason for the differing results is that Evans and colleagues (2000) used a different measure of impulsivity. They also had a larger sample, making our study relatively under powered to replicate the finding.

Methodological issues

Our findings are potentially affected by various limitations of case-control association studies (Malhotra & Goldman, 1999; Cardon & Bell, 2001). The first, hidden population stratification, is relatively unlikely given our careful matching of groups for ethnicity. In any event, recent empirical data suggest that it is less of a concern than was thought (Wacholder *et al.* 2000; Ardlie *et al.* 2002). Secondly, like most studies in the field, our *P* values are uncorrected for multiple testing, and their interpretation must take into account the low prior probability that a given polymorphism, even within a convincing candidate gene, is truly associated with a trait. However, our positive finding with the TPH A779C allele was a replication, and so may be considered less vulnerable to this criticism; moreover, we have not studied

any polymorphisms other than those presented here, partially reducing the problem of multiple testing. We also note that there is no accepted statistical solution to the issue, and Bonferroni corrections may be inappropriate (Cardon & Bell, 2001). Regarding the converse problem – false negative results due to lack of power – our sample size was larger than most prior studies, for which the average has been approximately 90 DSH subjects and 100 controls (Roy, 2001; Turecki, 2001). Furthermore, none of the polymorphisms showed a non-significant trend towards an association with DSH, which might be suggestive of a type II error (Tables 1 and 2). Nevertheless, because of the risks of false positive and false negative results, additional and larger studies are needed to confirm the present findings, as well as to ascertain the role of rarer allelic variants in serotonergic genes.

A strength of the present study is that the DSH sample was particularly well characterized (Haw *et al.* 2001; Hawton *et al.* 2002), and representative of DSH patients attending hospital in the UK (with the exclusion of repetitive self-cutting). A weakness, as in most studies of behavioural phenotypes, is that we lacked full information about the relevant behaviour in the comparison group. Epidemiological studies indicate that between 1.1 and 4.6% of adults report a lifetime history of DSH (Moscicki, 1997; Kessler *et al.* 1999) and we assume that these data apply to blood donors. However, it seems highly unlikely that this could have confounded either the positive TPH A779 result, or the negative findings regarding the other polymorphisms.

A final question concerns the actual trait with which the TPH A779C polymorphism is associated. Genetic factors may be more closely related to intermediate phenotypes such as impulsivity, aggression, or severity of suicidal intent, rather than to DSH itself (e.g. Nielsen *et al.* 1998; Manuck *et al.* 1999; Evans *et al.* 2000). For these reasons, we analysed the impulsivity score, and created the composite suicide risk score which included a measure of aggression. We found no evidence that either of these indices was related to the polymorphisms (Table 3), suggesting that these factors do not account for the association between DSH and the TPH A779 allele. However, it may instead be that the various scales do not adequately capture the

behaviour of interest, or reflect the lack of power of these subgroup analyses. Also, there are many other variables about which we had no information and which might correlate with DSH; for example, the TPH A779 variant has been associated with smoking behaviours (Lerman *et al.* 2001; Sullivan *et al.* 2001). These considerations indicate that future genetic studies will benefit from even more extensive characterization of subjects in both DSH and control groups.

Summary

The heritability of DSH, like that of other complex behavioural phenotypes, presumably reflects the influence of many genes and their interactions, both with each other, and with environmental factors. Association studies of the kind reported here provide initial clues as to the alleles and genes involved. However, they suffer from intrinsic limitations, as mentioned above. Therefore, while well-replicated positive results, such as the association between the TPH gene and DSH are noteworthy and merit further investigation, as well as meta-analyses when sufficient comparable data are available, significant progress in understanding the genetic contributions to suicidal behaviour will also require family-based association studies, genome-wide scans and haplotype analyses.

This work was funded by the American Foundation for Suicide Prevention, and the Anglia and Oxford Research and Development Committee. Edward C. Pooley held a Medical Research Council studentship. Keith Hawton is funded by the Oxfordshire Mental Healthcare Trust. Technical assistance was provided by Khatija Parekh and Louise Hutchinson; Ms Parekh is funded by a grant to the Medical Research Council 'Neurobiology of Mood Disorders' Co-Operative Group. We are very grateful to Drs S. Marshall and K. Welsh for their help establishing the allele-specific PCR, Drs P. Burnet and M. Sodhi for technical advice and Drs E. Townsend and C. Haw for assistance in the collection and processing of the clinical data.

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