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ORIGINAL RESEARCH ARTICLE

A haplotype at the DBH locus, associated with low plasma dopamine β-hydroxylase activity, also associates with cocaine-induced paranoia

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Low levels of dopamine β-hydroxylase (DβH) protein in the plasma or cerebrospinal fluid (CSF) are associated with greater vulnerability to positive psychotic symptoms in several psychiatric disorders. DβH level is a stable, genetically controlled trait. DBH, the locus encoding DβH protein, is the major quantitative trait locus controlling plasma and CSF DβH levels. We therefore hypothesized that DBH variants or haplotypes, associated with low levels of DβH in the plasma, would also associate with greater vulnerability to cocaine-induced paranoia. To test this hypothesis, we first showed that a di-allelic variant, DBH*5′-ins/del, located approximately 3 kb 5′ to the DBH transcriptional start site, significantly associates with plasma DβH activity in European-Americans ($n = 66$). Linkage disequilibrium analysis of that polymorphism and DBH*444g/a, another di-allelic variant associated with DβH levels, demonstrated that alleles of similar association to DβH levels are in positive disequilibrium. We then estimated DBH haplotype frequencies in cocaine-dependent European Americans rated for cocaine-induced paranoia ($n = 45$). As predicted, the low-DβH-associated haplotype, Del-a, was significantly more frequent ($P = 0.0003$) in subjects endorsing cocaine-induced paranoia ($n = 29$) than in those denying it ($n = 16$). Comparison to control haplotype frequencies ($n = 145$ healthy European-Americans) showed that the association predominantly reflected under-representation of Del-a haplotypes in those denying cocaine-induced paranoia. We conclude that: (a) the two DBH polymorphisms we studied are associated with plasma DBH levels; (b) those two polymorphisms are in significant linkage disequilibrium in European Americans, with alleles of similar association to DβH levels in positive disequilibrium; and (c) the haplotype associated with low DBH activity is also associated with cocaine-induced paranoia. *Molecular Psychiatry* (2000) **5,** 56–63.

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More than 60% of experienced cocaine users report paranoia during cocaine intoxication, while the remainder deny even mild cocaine-induced paranoia (CIP), despite reporting similar routes of administration, duration of cocaine dependence, and lifetime and per-binge quantities of cocaine ingested.[1–3] Satel and Edell[4] reported elevated scores on a measure of psychosis proneness in cocaine-dependent subjects who endorsed CIP compared to those who did not. These data suggest the presence of substantial inter-individual variation in vulnerability to CIP.

Evidence for genetic influences on vulnerability to drug-induced paranoia

Several lines of evidence support genetic factors as modulators of individual vulnerability to CIP. Numerous animal studies support the importance of genetic factors in behavioral responses to cocaine.[5] Abstinent human cocaine users who endorse CIP exhibit deficits in P50 sensory gating of auditory stimuli,[6] a heritable phenotype common in schizophrenic patients and their relatives.[7] Moreover, cocaine-abusing schizophrenic patients tend to have their first psychotic breaks at a younger age, and are more likely to be diagnosed as having the paranoid sub-type of schizophrenia.[8,9] Thus, cocaine use may uncover heritable vulnerability to paranoia and other psychotic symptoms in individuals vulnerable to schizophrenia.

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Gelernter and colleagues[10] reported an association between the 9-repeat allele of a variable-number of tandem repeats (VNTR) polymorphism just 3′ to the coding region of SLC6A3 (the gene encoding the dopamine (DA) transporter) and cocaine-induced paranoia. This observation suggests that variation in a gene encoding an important 'receptor' for cocaine may influence vulnerability to cocaine-induced paranoia.

Twin studies have not directly addressed CIP, but have examined related drug-induced behaviors. Nurnberger and colleagues[11] challenged healthy monozygotic (MZ, 11 pairs) and dizygotic (DZ, two pairs) twins with intravenous d-amphetamine. Behavioral excitation was highly correlated in the MZ twins, suggesting genetic control of this psychostimulant-induced behavioral response. Lyons *et al*[12] assessed a factor-analytically derived measure of negative effects of marijuana use, including paranoia and hallucinations, and of positive effects, such as increased creativity and ability to relax, in male twins (352 MZ pairs and 255 DZ pairs). They found a significant inter-twin correlation between scores on the negative factor in MZ twins ($r = 0.29$, $P < 0.001$) but not in DZ twins ($r = 0.06$, NS; MZ *vs* DZ: $P < 0.01$). Model fitting supported effects of additive genetic factors and unique (but not shared) environment on the liability to marijuana-induced negative effects.

Dopamine β-hydroxylase in serum (or plasma) and in cerebrospinal fluid are biochemical phenotypes that reflect allelic variation at the DBH locus

Dopamine β-hydroxylase (DβH) catalyzes the conversion of DA to norepinephrine (NE). It is localized within neurosecretory vesicles of noradrenergic and adrenergic neurons and neurosecretory cells, where it is present in both soluble and membrane-bound fractions.[13] DβH enzyme activity and levels of DβH immunoreactive protein are measurable in the serum (or plasma) and cerebrospinal fluid (CSF). In either body fluid, enzyme activity and protein level are strongly correlated, and essentially reflect the same biochemical phenotype.[14–16] Thus, we refer here to DβH enzyme activity and DβH protein levels interchangeably as DβH levels.

DβH levels in plasma and CSF are highly heritable traits. Strong evidence shows that: (1) plasma and cerebrospinal fluid DβH levels are genetic traits;[17,18] (2) DBH is the major locus controlling both of these traits,[19–22] accounting for >80% of the genetic variance in plasma DβH levels;[20] and (3) specific alleles of polymorphisms located near the 5′ portion of the DBH gene are associated with lower DβH levels in both plasma and CSF.[22]

DβH phenotypes and psychosis: association of low plasma and CSF DβH levels with vulnerability to positive psychotic symptoms

Previous studies support associations between low plasma or CSF levels of DβH and elevated vulnerability to psychosis. Thus, schizophrenic patients with low CSF levels of DβH exhibit more acute psychotic symptoms that appear to be more responsive to standard antipsychotic medications, compared to patients with higher levels of DβH.[23,24] In unipolar depression, several studies have found significantly lower serum DβH levels in patients with unipolar major depression with psychotic features, compared to those without psychotic features.[25–28]

Current study

We previously showed that a silent single nucleotide polymorphism at the 3′ end of DBH exon 2, DBH* 444g/a, comprised of either guanidine (g) or adenine (a) at cDNA nucleotide position 444[29] associated with plasma and CSF DβH in European-Americans (EA).[22] The a-containing allele, DBH*444a, associates with low DβH levels, while the g-containing allele, DBH* 444g, associates with higher DβH levels. Here, we report a second di-allelic polymorphism at DBH that associates with plasma levels of DβH in EA. This polymorphism, named DBH*5′-ins/del, consists of a 19-bp insertion-deletion approximately 4.7 kb 5′ to the transcriptional start site. The deletion allele (DBH*5′-del) associates with lower levels of plasma DβH, and the insertion allele (DBH*5′-ins) associates with higher plasma DβH. We then demonstrate that in EAs, the low-DβH associated alleles at DBH*5′-ins/del and at DBH*444g/a are in significant positive linkage disequilibrium (LD), as are the respective high-DβH alleles, thus allowing definition of high- and low-DβH associated haplotypes.

Low DβH levels are determined largely by allelic variation at the DBH locus, and low levels of DβH are associated with increased vulnerability to psychotic symptoms in several psychiatric disorders. We therefore hypothesized that DBH alleles or haplotypes associated with lower DβH levels would also associate with CIP. To test this hypothesis, we performed case-control association studies of cocaine dependence or CIP, and DBH haplotypes of known association to plasma DβH levels, in EAs.

Materials and methods

Subjects

Three sets of subjects were examined in this study: Group 1 provided DNA and plasma to characterize relationships between DBH genotype and plasma DβH levels (measured as DβH enzyme activity) in EAs. Group 2 provided data on LD relationships between DBH polymorphisms in EAs and served as the control group for comparison of DBH genotypes and haplotype frequencies to those in the cocaine-dependent subjects. Group 3 was studied to test for associations between DBH haplotypes, and cocaine dependence or CIP. All subjects gave informed consent for participation in genetic studies as approved by the Yale University Human Investigations Committee, the Human Subjects Subcommittee of VA Connecticut Health Care System at West Haven, or the Institutional Review Board of the University of Connecticut Health Center.

Group 1 consisted of 66 EA patients (38 female, 28

male) with a variety of mood and anxiety disorders, who donated blood for DNA and plasma analysis. The demographic and clinical characteristics of these subjects, as well as a discussion of the generalizability of DBH results from these subjects to other EA populations, are presented elsewhere.[22] Group 2 consisted of 145 unrelated EA subjects. All were screened for the absence of DSM-IV Axis-I diagnoses, using screening procedures described previously.[30,31] Group 3 consisted of 45 unrelated EAs who met DSM-IIIR criteria for cocaine dependence ($n = 45$), who were described in detail previously[10] To test for associations between DBH alleles and haplotypes and cocaine-induced paranoia in European-Americans, we divided Group 3 subjects into those who endorsed CIP (P(+) subjects) and those who did not endorse CIP (P(–) subjects) according to the Cocaine Experience Questionnaire (CEQ).[1,10]

Genotyping

Genotypes at DBH*444g/a were determined by PCR amplification of DBH exon 2 and surrounding intronic sequence, followed by digestion with EcoN1 and agarose gel electrophoresis, as described previously.[22]

DBH*5′-ins/del was originally described as part of a haplotype containing a nearby simple tandem repeat (STR) polymorphism.[32] We isolated it from the STR using PCR amplification with the following primers (cf Genbank Accession No. X63418), to generate PCR products of 144 bp (DBH*5′-del) or 163 bp (DBH*5′-ins):

sense: 5′-GCA AAA GTC AGG CAC ATG CAC C-3′;
antisense: 5′-CAA TAA TTT GGC CTC AAT CTT GG-3′.

PCR reactions (15 μl final volume) contained 25 ng of genomic DNA, 10 nmoles of each primer, 0.03 units of Klen Taq polymerase (AB Peptides, St Louis, MO, USA), and 1× strength of the PC-2 buffer supplied by the manufacturer. Temperature conditions were: 95°C for 5 min, followed by 30 s each of 98°C, 68°C, and 72°C for 30 cycles. The resulting fragments were resolved on 2% agarose gels, stained with ethidium bromide, trans-illuminated with UV light, and digitally imaged for genotyping.

Determination of plasma DβH activity

DβH activity was measured in plasma samples from Group 1. Separation and detection of the enzyme product, octopamine, in the presence of a large excess of the substrate, tyramine, was accomplished using a high performance liquid chromatographic-fluorometric system, as described previously.[22]

LD analysis, estimation of haplotype frequencies, and statistical analysis

LD between alleles at DBH*5′-ins/del and DBH*444g/a was evaluated in Groups 2 and 3, using the expectation-maximum (EM) method and hypothesis testing strategy of Long and colleagues,[33] and the program 3locus.[33] The test statistic, G, reflects the likelihood ratio of the restricted model (null hypothesis: no disequilibrium) and the specified model (in this case, linkage disequilibrium between DBH*5′-ins/del and DBH*444g/a). To determine whether LD was statistically significant, G was calculated in Monte-Carlo simulations in which genotypes were generated randomly from the observed allele frequencies. The significance level was then estimated as the proportion of times the simulated G reached or exceeded the G calculated from the actual data.[33] Values of D′ (describing LD as a proportion of the maximum possible value of the LD coefficient D, given the observed allele frequencies) were calculated as described by Lewontin.[34]

To compare haplotype frequencies, we estimated numbers of the respective haplotypes between the paranoia(+) and paranoia(–) groups by multiplying the haplotype frequencies estimated by 3locus[33] by the number of chromosomes examined, and rounded to the nearest integer value. To evaluate whether the P(+) and P(–) groups exhibited heterogeneity in haplotype frequencies, we used a 2×4 contingency table and a χ^2 test of heterogeneity. Given significant overall heterogeneity, each haplotype was then examined in 2×2 comparisons. To avoid inaccurate P values arising from low values in individual cells, we employed the T1 algorithm of Sham and Curtis,[35] as implemented in their CLUMP program, to estimate significance levels of the χ^2 values.

Genotype and haplotype nomenclature

The following abbreviations for haplotypes will be used: DBH*5′-ins/DBH*444a: Ins-a; DBH*5′-ins-DBH*444g: Ins-g; DBH*5′-del/DBH*444a: Del-a; DBH*5′-del/DBH*444g: Del-g.

Results

*DBH*5′-ins/del associates with plasma DβH activity in EA subjects*

Figure 1 shows that EA subjects (Group 1) with different genotypes at DBH*5′-ins/del differ significantly in mean plasma DβH activity (one-way ANOVA: $F(2, 62) = 5.2$; $P < 0.01$). Thus, DBH*5′-insdel is a di-allelic polymorphism in which one allele (DBH*5′-ins)

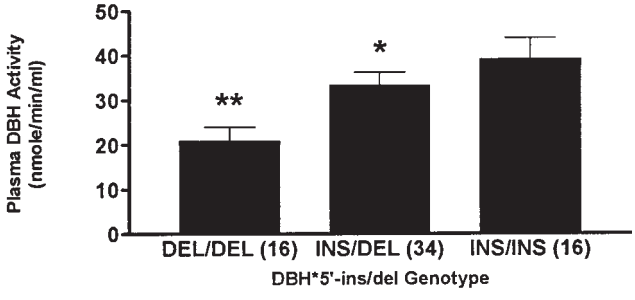

Figure 1 Relationship between genotype at DBH5′-ins/del and plasma DβH. Plasma DβH activity and DBH*5′-ins/del genotypes were determined in European-American patients with mood or anxiety disorders (n per group shown in parentheses) as described in Methods. One-way ANOVA: $F_{2,62} = 5.2$; $P < 0.01$. $^{**}P < 0.01$, $^{*}P < 0.05$, compared to the ins/ins group, Newman-Keuls post-hoc tests.

associates with higher levels of plasma DβH, and the other (DBH*5′-del) with lower levels of plasma DβH. Genotype frequencies are in Hardy–Weinberg equilibrium.

*DBH*5′-ins/del and DBH*444g/a: alleles of like association to DβH activity are in positive LD*

Genotypes were determined at DBH*5′-ins/del and DBH*444g/a in Group 2, and the resulting data subjected to LD analysis (Table 1). We observed significant LD ($P < 0.0001$, 10 000 simulations) between the two polymorphic loci, such that alleles associated with lower plasma DβH levels (DBH*5′-del and DBH*444a) were in positive disequilibrium, as were alleles associated with higher DβH activity (DBH*5′-ins and DBH*444g). These result demonstrate that Del-a is a low-DβH associated haplotype, and Ins-g is a high DβH associated haplotype. This conclusion is also supported by comparison of plasma DβH levels among Group 1 subjects homozygous for each haplotype [mean ± sem: Del-a/Del-a: $n = 5$; 9.3 ± 3.6 nmoles min^{-1} ml^{-1}; Del-g/Del-g: $n = 3$; 32.7 ± 3.8; Ins-a/Ins-a: $n = 5$; 31.4 ± 5.4; Ins-g/Ins-g: $n = 4$; 41.3 ± 12.2 ($P = 0.038$, Kruskal–Wallis ANOVA by ranks)].

Neither DBH haplotype frequencies nor patterns of DBH allelic LD differ between cocaine-dependent and healthy EAs

There were no significant differences between allele, genotype, or haplotype frequencies at either DBH*444g/a or DBH*5′-ins/del in the healthy EA group as compared with the cocaine-dependent group (haplotype frequencies: Table 1; χ^2, 3 df = 1.42, NS). The pattern and strength of LD among alleles at DBH*444g/a and DBH*5′-ins/del were virtually identical in the two groups (Table 1). The low value of G, and resulting marginal significance of LD in the cocaine-dependent group ($P = 0.053$), presumably resulted from the small number of cocaine-dependent subjects examined.

The Del-a haplotype associates with CIP in EA cocaine-dependent subjects

Table 2 presents the results of LD analyses of DBH haplotypes in P(+) compared to P(–) cocaine-dependent subjects. The low Del-a haplotype frequency in the P(–) cocaine-dependent subjects appears to alter the pattern of LD in this group. The sign of pairwise LD is reversed for Del-a and Ins-g, while the frequencies of Del-g and Ins-a are elevated in the P(–) group compared to the P(+) (Table 2). Thus, dividing the cocaine-dependent group according to the presence or absence of CIP appears to identify subgroups of cocaine users in which DBH haplotype frequencies and LD relationships differ, while there are no such differences when comparing cocaine-dependent with healthy EA subjects (Table 1).

Figure 2 compares estimated haplotype frequencies in control EAs, P(+), and P(–) cocaine-dependent EAs. There is a significantly lower frequency of the low-DβH-associated Del-a haplotype in cocaine users who did not endorse cocaine-induced paranoia compared to the frequency in those who did ($\chi^2 = 11.92$, $P = 0.0003$, in 10 000 simulations). This result predominantly reflects a dearth of Del-a haplotypes in the P(–) subjects, rather than a surplus in the P(+) subjects: The frequency of Del-a haplotypes was significantly lower in P(–) cocaine-dependent subjects than in control European-Americans ($\chi^2 = 10.54$, $P = 0.0010$ in 10 000 simulations), while the frequencies in P(+) cocaine-dependent subjects did not differ from those in the controls ($\chi^2 = 0.86$, NS).

Discussion

The present study yields three main findings. First, alleles at DBH*5′-ins/del are associated with variation in plasma DβH levels in EA subjects. The observation adds another DBH polymorphism to a growing list associated with differences in plasma, serum or CSF DβH levels.[21,22] Second, analysis of LD between DBH*

Table 1 Linkage disequilibrium (LD) analysis of DBH*5′-ins/del and DBH*444g/a in healthy European Americans (EA) and in cocaine-dependent EA

Haplotype	*Healthy EA (n = 145)*		*Cocaine-dependent EA (n = 45)*	
	Estimated haplotype freq.	*D, D′*	*Estimated haplotype freq.*	*D, D′*
Del-a	0.36	0.091, 0.37	0.33	0.080, 0.35
Del-g	0.16	–0.091, –0.37	0.15	–0.080, –0.035
Ins-a	0.15	–0.091, –0.37	0.19	–0.080, –0.035
Ins-g	0.32	0.091, 0.37	0.33	0.080, 0.035
G:	22.1		3.7	
Significance	$P < 0.0001$		$P = 0.053$	

Haplotype frequencies and corresponding values of G were estimated in each group as described by Long and colleagues.[33] D′ was calculated by the method of Lewontin.[35] DBH alleles that associate with lower values of DβH activity (Del and a, respectively), and with higher levels of DβH (Ins and g, respectively), are in positive LD (for relationship of alleles at DBH*444 g/a to plasma DβH activity, see Sternberg *et al*.[23] The distributions of estimated haplotype counts (calculated by multiplying haplotype frequencies by 2N, and rounding to the nearest integer), did not differ significantly across groups (χ^2, 3 df = 0.81; NS).

Table 2 DBH haplotype frequencies and linkage disequilibrium (LD) in cocaine-dependent subjects who endorse or deny cocaine-induced paranoia

Haplotype	*Paranoia(+) (n = 29)*		*Paranoia(–) (n = 16)*	
	Estimated haplotype freq.	*D, D′*	*Estimated haplotype freq.*	*D, D′*
Del-a	0.42	0.099, 0.431	0.07	–0.075, –0.534
Del-g	0.13	–0.099, –0.431	0.28	0.075, 0.534
Ins-a	0.16	–0.099, –0.431	0.34	0.075, 0.534
Ins-g	0.28	0.099, 0.431	0.32	–0.075, –0.534
G:	4.28		0.82	
Significance	0.042		NS	

Haplotype frequencies, D,D′, and the significance of LD were evaluated as described in Methods. 2×4 comparison of estimated haplotype frequency distributions: $\chi^2 = 13.74$, $P = 0.003$ in 1000 simulations. *2×2 comparison of Del-a haplotypes to all others in P(+) *vs* P(–): $\chi^2 = 12.39$, $P = 0.0005$ in 10 000 simulations.

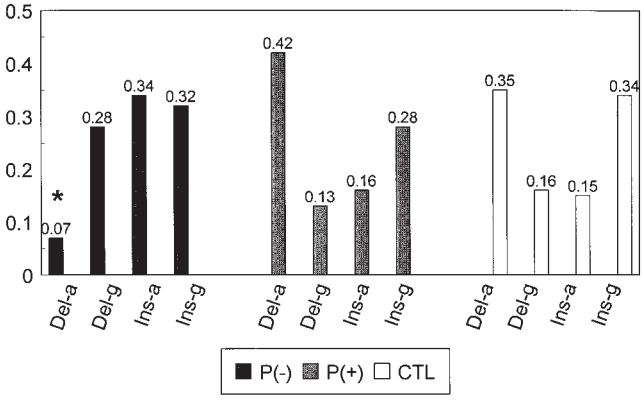

Figure 2 DBH haplotype frequencies in control EAs, P(+) cocaine-dependent EAs, and P(–) cocaine-dependent EAs. Subjects were cocaine-dependent EAs assessed for cocaine-induced paranoia as described in Methods. *2×2 comparison of Del-a haplotypes to all others in P(+) *vs* P(–): $\chi^2 = 12.39$, $P = 0.0005$ in 10 000 simulations.

5′-ins/del and another plasma DβH-associated di-allelic variant, DBH*444g/a,[22] shows that alleles of similar association to plasma DβH levels are in positive LD, suggesting they identify a haplotype containing a common functional variant(s) that lowers plasma DβH levels. Third, the low-DβH associated haplotype, Del-a, associates with vulnerability to cocaine-induced paranoia in EA cocaine-dependent individuals. The observation that Del-a is significantly less common in P(–) subjects is consistent with our initial hypothesis, that DBH variants associated with low DβH also associate with greater vulnerability to cocaine-induced paranoia. This finding suggests that heritable variation in DBH alters vulnerability to CIP.

Allelic and haplotypic association of DBH to plasma DβH

The haplotype defined by DBH*5′-ins/del and DBH*444g/a spans approximately 8.4 kb (cf Genbank accession Nos. AC001227 and AC000404). Given this proximity, and our previous observation that a DBH STR, located 118 bp 3′ to DBH*5′-ins/del,[32] is in LD with DBH*444g/a,[22] the presence of LD is not surprising. The *pattern* of LD is remarkable because alleles of like association to plasma DβH levels are in positive LD. These data suggest that a functional variant(s) of DBH, which lowers plasma DβH levels, is in LD with the Del-a haplotype in EAs. The results strongly support the hypothesis suggested by ABO linkage data[19,20] and DBH mapping data,[36–38] that DBH is the major quantitative trait locus controlling plasma DβH levels.

A DBH haplotypic association to cocaine-induced paranoia

The cocaine-dependent EA subjects studied here, taken as a group, did not exhibit significant differences in DBH haplotype frequencies when compared to control EAs. However, when the cocaine-dependent group was divided into those endorsing or denying CIP, significant differences in DBH haplotype frequencies emerged. Thus, as predicted, the low-DβH haplotype, Del-a, was significantly more common in P(+) cocaine users than in P(–) cocaine users. The frequencies of the high-DβH haplotype, Ins-g, and both haplotypes of mixed association to DβH levels (Del-g and Ins-a) did not differ between P(+) and P(–) groups. These results suggest that a functional DBH variant(s), in LD with Del-a and associated with lower plasma DβH levels, also modifies vulnerability to paranoia in habitual cocaine users.

Neither DBH*5′-del or DBH*444a alone significantly associated with cocaine-induced paranoia, presumably because some of the alleles at each individual polymorphism occurred on different haplotype backgrounds, and were therefore in weaker LD with low-DβH functional variants. This conclusion is supported by the pattern of LD in P(–) subjects. In this group, the 'mixed' haplotypes Del-g and Ins-a accounted for greater respective proportions of the DBH*5′-del and DBH*444a genotypes than in the P(+) subjects or the healthy controls.

Most genetic association studies rely on relatively nonspecific 'two-tailed' or 'multi-tailed' hypotheses of the general form, 'one allele or another should associate with one phenotype compared to another.' In the present study, strong association between DBH alleles and plasma DβH levels (references 21, 22, and present data), anchored in prior linkage data,[19,20] allowed us to use plasma DβH phenotypes to guide construction of a specific 'one-tailed' hypothesis. Thus, prior associations of low DβH levels to psychotic symptoms[23–28] strongly suggested that a specific low-DβH associated haplotype, Del-a, would associate with greater vulnerability to cocaine-induced paranoia. Consistent with this hypothesis, Del-a was significantly more frequent in P(+) cocaine-dependent subjects as compared to P(–) cocaine-dependent subjects. The association arose from significant under-representation of Del-a haplotypes in the P(–) cocaine-dependent group, as compared either to the P(+) cocaine-dependent group, or the healthy control group. These observations suggest the Del-a haplotype is in LD with a functional polymorphism(s) at the DBH locus that lowers both plasma DβH and the threshold for a paranoid reaction to cocaine relative to that associated with higher DβH and 'not-Del-a' haplotypes.

DBH is expressed specifically within noradrenergic and adrenergic neurons in the adult nervous system,[13] and is present in a single copy per haploid genome.[29] Thus, variation at DBH that causes lower plasma DβH levels will also be expressed within noradrenergic and adrenergic neurons. The present results, combined with previous observations at the biochemical level,[23–28] therefore suggest that variation at the DBH locus can modify individual vulnerability to drug-induced and idiopathic psychotic symptoms by modifying noradrenergic and/or adrenergic neuronal phenotypes.

Limitations

The results of this study must be viewed with caution, pending replication. As in any case-control association study, the results may reflect population stratification.[31,39–41] Inspection of the patterns of LD in the P(+) and P(–) cocaine-dependent groups suggests different haplotype frequencies and LD relationships in the two groups. Population stratification could account for such a result. However, the patterns of LD in the combined cocaine-dependent group were identical to those observed in a large group of healthy EAs. Thus, a DBH association between the cocaine-dependent group and other EAs was not detected, despite the larger sample sizes and greater statistical power of these comparisons.

Another observation mitigating against population stratification as an explanation for the present results is that prior studies of psychotic symptoms and DβH levels have consistently reported associations between greater vulnerability to psychosis in unipolar depression, and lower DβH levels.[25–28] Two of those studies were performed in predominantly EA populations,[25,28] one in an Eastern European population,[26] and one in a subcontinental Asian population.[27] Regardless of almost certain differences in allele and haplotype frequencies among at least some of the populations in those studies, a phenotypic association was consistently observed between psychotic symptoms and lower DβH levels. Biologically meaningful associations between DBH genotypes and psychosis therefore appear to involve functional polymorphisms at DBH that diminish plasma DβH levels, but that may occur on different haplotype backgrounds in different populations. Thus, in addition to replication of the present findings in case-control and family-based association studies (currently underway in this laboratory), delineation of the functional polymorphism(s) at DBH that cause differences in DβH biochemical phenotype will be necessary to evaluate the relationship between DBH variants and vulnerability to psychosis. Another important area of research necessary to clarify whether and how DBH variants might influence biochemical and clinical phenotypes is delineation of DBH genotype/DβH phenotype relationships in non-European populations. To our knowledge, no such studies have been published.

A second limitation of the present study is the small numbers of cocaine-dependent subjects. Replication in a larger, independently ascertained group of subjects is necessary to confirm the present results. Finally, the P(+) and P(–) phenotypes in this study were ascertained using a single, retrospective method (the CEQ).[1,10] We are currently refining multifaceted methods for ascertainment of subjects vulnerable to CIP, and other cocaine-induced psychotic symptoms such as hallucinations.

Clinical implications

Recent evidence suggests that disulfiram (a potent inhibitor of DβH activity) may be a useful treatment for cocaine dependence.[42,43] In addition, some cocaine-dependent subjects experience intense dysphoria during nasal cocaine self-administration after pretreatment with disulfiram.[44–46] CSF levels of DβH predict disulfiram-induced paranoia and dysphoria in alcoholic subjects.[47] Paranoid reactions to cocaine are consistently described by cocaine users as highly unpleasant, and often motivate users to seek treatment for cocaine dependence.[1] These observations, together with the present results, suggest that understanding DBH genotype-DβH phenotype relationships may facilitate identification of cocaine-dependent patients most likely to benefit from treatment with disulfiram or other inhibitors of DβH.

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