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Linkage analysis of plasma dopamine β -hydroxylase activity in families of patients with schizophrenia

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V. K. Lasseter: deceased.

The authors wish to dedicate this manuscript to V. K. Lasseter's memory.

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Abstract

Dopamine β -hydroxylase ($D\beta H$) catalyzes the conversion of dopamine to norepinephrine. $D\beta H$ enters the plasma after vesicular release from sympathetic neurons and the adrenal medulla. Plasma $D\beta H$ activity ($pD\beta H$) varies widely among individuals, and genetic inheritance regulates that variation. Linkage studies suggested strong linkage of $pD\beta H$ to *ABO* on 9q34, and positive evidence for linkage to the complement fixation locus on 19p13.2-13.3. Subsequent association studies strongly supported *DBH*, which maps adjacent to *ABO*, as the locus regulating a large proportion of the heritable variation in $pD\beta H$. Prior studies have suggested that variation in $pD\beta H$, or genetic variants at $D\beta H$, associate with differences in expression of psychotic symptoms in patients with schizophrenia and other idiopathic or drug-induced brain disorders, suggesting that *DBH* might be a genetic modifier of psychotic symptoms. As a first step toward investigating that hypothesis, we performed linkage analysis on $pD\beta H$ in patients with schizophrenia and their relatives. The results strongly confirm linkage of markers at *DBH* to $pD\beta H$ under several models (maximum multipoint LOD score, 6.33), but find no evidence to support linkage anywhere on chromosome 19. Accounting for the contributions to the linkage signal of three SNPs at *DBH*, rs1611115, rs1611122, and rs6271 reduced but did not eliminate the linkage peak, whereas accounting for all SNPs near *DBH* eliminated the signal entirely. Analysis of markers genome-wide uncovered positive evidence for linkage between markers at chromosome 20p12 (multi-point LOD = 3.1 at 27.2 cM). The present results provide the first direct evidence for linkage between *DBH* and $pD\beta H$, suggest that rs1611115, rs1611122, rs6271 and additional unidentified variants at or near *DBH* contribute to the genetic regulation of $pD\beta H$, and suggest that a locus near 20p12 also influences $pD\beta H$.

Introduction

Dopamine β -hydroxylase ($D\beta H$) catalyses the conversion of dopamine to norepinephrine within synaptic vesicles or chromaffin granules of neurons and neurosecretory cells that release norepinephrine and epinephrine. $D\beta H$ protein is released, together with other synaptic contents, during sympathetic neuronal activity (Smith et al. 1970), and also appears to be secreted by a constitutive pathway (Oyarce and Fleming 1991). By virtue of its secretion into the extracellular space, $D\beta H$ enzyme activity can readily be assayed in the plasma or serum. Family and twin studies have demonstrated that plasma $D\beta H$ ($pD\beta H$) activity is a highly heritable trait, with segregation analysis supporting a single major locus responsible for low $pD\beta H$ activity (Ross et al. 1973; Weinshilboum et al. 1975).

A large number of studies (Cubells and Zabetian 2004) have examined $pD\beta H$ for associations with disorders such as schizophrenia (MIM 181500), major depression (MIM 608516) and Parkinson's disease (MIM 168100). Unfortunately, this literature is conflicting and difficult to interpret, in large part, because very few such studies have accounted for the genetic influence on the $pD\beta H$ trait. Despite this shortcoming, one consistent pattern that emerges from studies of $pD\beta H$ is an association between lower $pD\beta H$ and psychotic features in several psychiatric disorders. Perhaps the most consistent observation in this regard is that patients who have unipolar major depression with psychotic features exhibit lower $pD\beta H$ than depressed patients without such symptoms (Cubells et al. 2002; Lykouras et al. 1988; Meltzer et al. 1976; Meyers et al. 1999; Mod et al. 1986; Sapru et al. 1989): five studies

have found such a relationship (Cubells et al. 2002; Meltzer et al. 1976; Meyers et al. 1999; Mod et al. 1986), and only one has not (Lykouras et al. 1988). Studies of pD β H in schizophrenia clearly have ruled out a simple association between variation in pD β H and the diagnosis of the disorder, but some studies (Sternberg et al. 1983; van Kammen et al. 1994) have noted differences in symptomatic patterns, premorbid or morbid functioning, or in clinical course, associated with variation in pD β H activity, cerebrospinal fluid D β H activity, or protein levels [which are both highly correlated to pD β H (Castellani et al. 1982; O'Connor et al. 1994)]. The foregoing observations suggest that a detailed understanding of the genetics underlying pD β H might reveal important information about genetic modifiers of psychosis across a variety of psychiatric diagnoses.

Linkage analysis performed with non-DNA markers provided evidence for linkage of pD β H to the *ABO* blood group locus (MIM 110300) (Elston et al. 1979; Goldin et al. 1982; Wilson et al. 1988), which maps to 9q34. This evidence together with the molecular cloning and mapping of the structural gene *DBH* (MIM 609312) to 9q34 (Craig et al. 1988; Gelernter et al. 1991; Lamouroux et al. 1987) strongly suggested *DBH* is a major quantitative trait locus regulating pD β H activity, and this hypothesis was confirmed by reports of associations between molecular markers at *DBH* and variation in pD β H (Cubells et al. 1998; Wei et al. 1997). Zabetian and colleagues (Zabetian et al. 2001) then resequenced DNA samples from individuals with extreme values of pD β H activity, and identified a single nucleotide polymorphism (SNP), consisting of a cytosine to thymidine transition located 970 bp 5' to the transcriptional start site of *DBH* (−970C>T; rs1611115, formerly called −1021C>T) that accounted for 30–50% of the variance in pD β H activity in samples from three human populations of differing geographic ancestry (European Americans, African Americans and Japanese). In all populations examined thus far, the T allele of −970C>T associates in a co-dominant manner with lower pD β H activity (Cubells et al. 2002; Kohnke et al. 2002; Mustapic et al. 2007; Tang et al. 2007). Furthermore, −970C>T accounts for a greater proportion of the variance in pD β H activity than any other SNPs at *DBH* examined to date (Chen et al. 2010; Zabetian et al. 2003). Transient transfection analysis in PC-12 cells of luciferase reporter constructs driven by human *DBH* sequence, together with co-transfection and chromatin immunoprecipitation experiments, recently provided strong evidence that −970C>T is a functional polymorphism influencing transcription of *DBH* (Chen et al. 2010). A non-synonymous SNP located in exon 11 (rs6271) accounts for additional variance in pD β H after allowing for −970C>T statistically (Tang et al. 2006; Zabetian et al. 2001), or by examination of the association of this SNP with pD β H activity in samples chosen for homozygous genotype at −970C>T (Tang et al. 2005).

Some data suggest the presence of at least one additional locus contributing to variation in pD β H. Wilson and colleagues (Wilson et al. 1988), in addition to a strong linkage signal between pD β H activity and *ABO*, also reported a weaker signal at *C3*, the complement fixation locus (MIM 120700), which maps to chromosome 19p13.2-13.3. A re-analysis of those data confirmed those results (Province 2000). No linkage analysis to our knowledge has confirmed the linkage between pD β H and a locus on 9q34, nor evaluated the possible linkage near *C3* on 19p. Indeed, the linkage studies of the pD β H activity phenotype to date have all used phenotypic markers covering only a small proportion of the genome (~5%).

The current study was undertaken for the following purposes: (1) to confirm linkage and association between pD β H and SNP markers at the *DBH* locus; (2) to evaluate possible linkage between pD β H and distal chromosome 19p; (3) to identify evidence that any other loci in the genome show linkage to pD β H activity; and (4) to evaluate the contribution of rs1611115 (−970C>T), rs1611122 and rs6271 to the hypothesized linkage at *DBH*, in preparation for studies of these functional variants as potential modifiers of psychotic symptoms in schizophrenia. We report analysis of linkage between a genome-wide panel of

SNP markers (Holmans et al. 2009) and pD β H activity, assayed in archived plasma samples from participants in family-based studies of schizophrenia.

Materials and methods

The data were generated from plasma and DNA samples donated by a subset of 123 European American families, multiplex for schizophrenia, comprising 921 individuals (Holmans et al. 2009). A total of 284 individuals from 70 pedigrees had samples of plasma available for assay of pD β H activity. Of the 70 pedigrees, 59 had two generations, 10 had 3 generations, and 1 pedigree had 4 generations.

Samples of acid-citrate anti-coagulated plasma, collected at the time of participant ascertainment, were stored frozen at -80°C until D β H-activity assay. pD β H activity was determined in duplicate 5- μL aliquots of plasma as the rate of conversion of tyramine to octopamine using a method modified from that of Nagatsu et al. (Cubells et al. 1998; Nagatsu and Udenfriend 1972). Octopamine was measured by a column-switching, reverse phase high performance liquid chromatography (HPLC) system, using coulometric electrochemical detection, and synephrine as internal standard. D β H activity is reported as μmol of octopamine formed per minute from a solution of tyramine (0.2 mol L^{-1}) by 1 L of plasma at 37°C ($\mu\text{mol min}^{-1} \text{ L}^{-1}$). The average ($\pm\text{SD}$) duration of storage was 10.5 ± 2.0 years.

Genotypes were obtained on 210 SNP markers on chromosome 9 with mean spacing 0.76 cM, and 153 markers on chromosome 19 with mean spacing 0.73 cM. These genotypes were all from the Illumina Version 4 Linkage Panel, except genotypes for rs1611115 and rs6271, which were determined using the Taqman 5' exonuclease method. The genome-wide analyses employed a total of 5584 markers on 22 autosomal chromosomes passing quality control checks. X-linked markers were not analyzed because familial correlations, calculated by the S.A.G.E. program FCOR, gave no suggestion of X-linkage (supplementary Table 1).

In order to perform model-based linkage analysis, the trait model parameters (the trait genotypic means, a common variance at the trait locus, and the trait locus allele frequencies) were estimated using a diallelic model with the SEGREG program of S.A.G.E. 6.01. In a model-based linkage analysis, it is important to have an appropriate model. Therefore, we explored various modes of inheritance and power transformations of the data values to determine appropriate distributional assumptions for the model used. The Box-Cox transformation is useful in this regard because it comprises a family of power transformations that includes as a special case the logarithmic and square root transformations. Under the assumption of Mendelian transmission, low-value dominant, low-value recessive and additive models were fitted for each of the following four transformations of pD β H activities: (1) no transformation, (2) square root transformation, (3) logarithmic transformation, and (4) Box-Cox transformation with estimated power λ_1 . Therefore, a total of 12 trait models were produced for the pD β H activity trait. A trait-locus type probability file, which provides individual-specific penetrance functions for linkage analysis, was produced by SEGREG for each of the trait models.

We planned a priori to examine linkage first on chromosome 9, followed by linkage on chromosome 19, because of the prior evidence for linkage on those chromosomes. For the chromosome-specific analyses, two-point and multipoint model-based linkage analyses were, respectively, performed under the trait models produced in SEGREG. Using diallelic trait models produced by SEGREG, two-point linkage was conducted with the LODLINK program, and multipoint linkage was performed with MLOD, these being programs in

S.A.G.E. 6.01. After confirming linkage on chromosome 9 but not on chromosome 19, genome-wide linkage analysis was performed.

In addition, model-free linkage was conducted with SIBPAL, with the sibship mean estimated by the best linear unbiased predictor [BLUP; (Wang and Elston 2004)], and the regression method W4 was selected to set the dependent variable as a weighted combination of the squared trait difference and the squared mean-corrected trait sum (Shete et al. 2003). For this analysis, exact multipoint IBD sharing distributions for full siblings were estimated at 2 cM intervals by GENIBD. Both SIBPAL and GENIBD are part of the S.A.G.E. package.

In order to adjust for the linkage signal on chromosome 9 prior to testing for linkage on chromosome 19 and elsewhere, the square root of $pD\beta H$ activities were regressed on (1) the SNPs rs1611115, rs1611122 and rs6271 in the *DBH* gene, coded by 14 covariates to represent their additive, dominant and interaction effects, and (2) the 25 SNPs in the 40 cM linkage region on chromosome 9, coded by 40 additive and/or dominant covariates (including the 14 covariates for the 3 SNPs in *DBH*). The square root of $pD\beta H$ activities was used for this purpose both because it gave the strongest linkage signal and because our previous study (Zabetian et al. 2001) showed that, for rs1611115 genotypes, a plot of the genotype-specific variance of $pD\beta H$ activity against mean $pD\beta H$ activity produced approximately straight lines passing through the origin for three ethnically different populations (see supplementary Fig. 1), suggesting that a square root transformation would be the appropriate transformation to produce a stable variance for the three genotypes (Healy 1968). From the total of 45 SNPs in the 40 cM region, the 25 SNPs individually showing the most significant associations (p value for each covariate less than 0.2 by a likelihood ratio test) were selected for this purpose. The regressions were performed using the ASSOC program in S.A.G.E. 6.01, which allows for familial correlations in the trait. The residuals of the two regression models, which were the differences between the square root of $pD\beta H$ and the sum of weighted SNP covariate values, were first adjusted to be on a scale comparable to that of the original $pD\beta H$ activities by adding a constant and then squaring (Wilson et al. 1990); they were then used as new traits. Best-fitting segregation models were fitted to the new traits, to produce a new set of low-value dominant, low-value recessive and additive models under no transformation, a square root transformation, and a logarithmic transformation. Then linkage analyses with these new trait models were performed on chromosome 19 and elsewhere as described above.

Results

Segregation analysis strongly rejected environmental transmission of the trait $pD\beta H$ under all models (data not shown). Mendelian dominant, recessive and additive transmission models under no transformation, square root transformation, logarithmic transformation, and Box-Cox transformation with the power λ_1 estimated were used in both two-point and multipoint linkage analyses. The model producing the best genomewide linkage signal was the low-value dominant model under the square root transformation (Table 1). In addition, there was no significant effect of schizophrenia on $pD\beta H$ activity when schizophrenia status was included as a covariate of penetrance in the segregation analysis.

The strongest linkage signal was on chromosome 9q (Fig. 1). The highest multipoint linkage LOD score was 6.33 at the position 2.8 cM proximal to the *DBH* locus. At the three SNPs (rs1611115, rs1611122, rs6271) in the *DBH* gene, the multipoint LOD score was 6.0. Two-point linkage under this model produced LOD scores at rs1611115, rs1611122 and rs6271 that were, respectively, 2.32, 3.6 and 0.29, each with a recombination fraction of 0. Association analysis of the square root of $pD\beta H$, performed using ASSOC within S.A.G.E.,

showed that SNP rs1611115 associated with $\text{pD}\beta\text{H}$ in a co-dominant manner, with means 4.20, 2.90 and 1.06 ($\mu\text{mol min}^{-1} \text{L}^{-1}$)^{0.5}, i.e. 17.64, 8.41 and 1.12 $\mu\text{mol min}^{-1} \text{L}^{-1}$, respectively, for the CC, CT and TT genotypes ($p = 1.14 \times 10^{-18}$; Table 2).

Linkage analysis was repeated on the new traits formed after adjusting for the linkage signal on chromosome 9, using dominant, recessive, and additive segregation models fitted for the new traits under no transformation, square root, and logarithmic transformations. Using a new dominant model without transformation for the new trait, having adjusted for rs1611115, rs1611122, and rs6271 (Table 1), the best linkage signal was again detected on chromosome 9, but decreased by 47%. The highest multipoint LOD score was 3.37 on chromosome 9q34, 9.5 cM from the *DBH* gene, with the one-LOD support interval encompassing the *DBH* gene (Fig. 1). For the new trait obtained on adjusting for the 25 SNPs in the linkage region on chromosome 9, no suggestive linkage was identified at the *DBH* locus (Fig. 1: the best linkage signal on chromosome 9 was identified under a new additive model using the logarithmic transformation—multipoint LOD score of 1.39 at 6.6 cM from the *DBH* gene), which suggests that the original linkage signal at *DBH* was now adjusted out.

Using the new trait models fitted after adjusting for the 25 SNPs, no linkage was found around the *C3* locus (19p13) on chromosome 19. Among all these models, a Mendelian dominant model under logarithmic transformation produced the best, but quite weak, multipoint linkage signal on chromosome 19q13 (multipoint LOD = 1.0). The position of that peak was very far from the *C3* locus (Supplementary Fig. 2). To confirm the absence of a significant linkage signal on chromosome 19, and to account for the possibility that gametic disequilibrium between *DBH* and a locus on chromosome 19 explains the lack of a signal after accounting for *DBH*, we also examined linkage on chromosome 19 without adjusting out *DBH*. That analysis failed to show any significant linkage across the entire chromosome, the best linkage signals being identified by a Mendelian additive model under logarithm transformation, at positions 0 and 7.7 cM on 19p, with multipoint LOD scores of 1.32 and 1.15, respectively. The positions are 21.6 and 13.9 cM away from the *C3* locus (Supplementary Fig. 2).

Model free linkage analysis indicated that the most significant linkage peak for the square root of $\text{pD}\beta\text{H}$ activity was at 9q34, 9.4 cM from the *DBH* gene, with p value = 7.6×10^{-12} . After adjusting for the 25 SNPs in the linkage region, the model free linkage signal of the new trait on chromosome 9 decreased to $p = 0.01$ at 9.4 cM from the *DBH* gene, and to $p = 0.13$ at the gene itself (Fig. 2). There was no significant linkage for the new trait on chromosome 19 (Supplementary Fig. 3).

The proportion of the square root $\text{pD}\beta\text{H}$ heritability accounted for by the linkage on chromosome 9 was estimated in two ways. First, using the 266 individuals for whom the 25 selected SNPs were available, the estimated heritability of square root $\text{pD}\beta\text{H}$ activity was 0.62 ± 0.14 . After adjusting for the 25 SNPs in the *DBH* region, the heritability of the square root of the new trait (comparable to the square root of $\text{pD}\beta\text{H}$ in scale) decreased to 0.37 ± 0.18 , indicating that the markers at the *DBH* region accounted for 40% of the genetic component of the square root $\text{pD}\beta\text{H}$ activity. Second, the genetic variance of the square root of $\text{pD}\beta\text{H}$ due to the *DBH* gene is equal to the regression coefficient of the proportion of alleles shared IBD at the gene, estimated to be 1.91 for square root $\text{pD}\beta\text{H}$. After adjusting out the linkage signal on chromosome 9, the genetic variance at the *DBH* gene was estimated to be 0.69 for square root $\text{pD}\beta\text{H}$. Thus the decrease in the genetic variance at the *DBH* locus is estimated to be 64%. Knowing that the total variance of square root $\text{pD}\beta\text{H}$ (based on all 284 individuals for whom $\text{pD}\beta\text{H}$ is available) is 3.09, the heritability by this

method is estimated to decrease by 39% (consistent with the above method accounting for 40% of the genetic component of pD β H activity).

To provide preliminary data on linkage between loci on other chromosomes and plasma D β H activity, we examined linkage results for the new trait formed by adjusting for the 25 SNPs with markers from the entire genome. The most significant model-based linkage result for the new trait was obtained under the new additive model with a square root transformation (Table 1), and revealed a positive significant peak on chromosome 20p12 (multi-point LOD = 3.1 at 27.2 cM; Fig. 3). No other substantial peaks were observed (Supplementary Fig. 4). The most significant linkage for pD β H other than the one on chromosome 9 was also obtained on chromosome 20p12 (Fig. 3, multipoint LOD = 2.4 at 43.2 cM), under the same segregation model that produced the best linkage signal on the *DBH* locus (Table 1, dominant model under the square root transformation). Thus adjusting for the effects of the markers on chromosome 9 made the linkage signal on chromosome 20 become somewhat stronger, but its position moved.

Discussion

The present study, to our knowledge, is the first linkage study of pD β H activity to employ genotypes inferred directly from DNA markers as opposed to protein phenotypes. The results confirm evidence from linkage studies that used protein and blood group markers some 30 years ago, indicating that a major quantitative trait locus regulating pD β H activity resides on distal 9q34 near *ABO* (Goldin et al. 1982; Province 2000; Wilson et al. 1988), and shows with finer resolution that the linkage peak encompasses the structural gene *DBH*. Those results are consistent with strong association evidence implicating *DBH* as a QTL regulating pD β H activity (Chen et al. 2010; Zabetian et al. 2001). However, the present analysis fails to support prior findings suggesting a second QTL on chromosome 19 near *C3*, which had been reported in one of the earlier linkage studies (Province 2000; Wilson et al. 1988). The prior linkage studies, based on non-DNA markers, covered only ~5% of the genome, and only included one marker, adenosine deaminase, on chromosome 20q. The current analysis, using a genome-wide SNP marker set, revealed an additional positive (but not significant) linkage peak on chromosome 20p12. On the basis of the model-free regression coefficient at the peak signal, the locus-specific variance here is estimated to be 99.6, corresponding to 49.6% of the variance of the original pD β H activity. This estimate, however, is necessarily an overestimate on account of being determined from the point at which the linkage signal is most significant.

The association analysis performed on the markers spanning *DBH* confirms the well-replicated finding that rs1611115 is strongly associated with pD β H in a co-dominant pattern, in which the T allele associates with lower activity than the C allele. However, the best linkage results were found under a low-value dominant model. The most likely reason for this discrepancy is that the distribution of values of pD β H activity assayed in the present study appears to be somewhat compressed toward the lower end of the expected range based on prior studies (Kohnke et al. 2002; Zabetian et al. 2001). There are two likely explanations for that compression of values. First, the samples assayed for pD β H activity had been stored at -80° C for more than a decade, and it is possible that some loss of activity occurred during storage. Second, the plasma samples assayed in this study were anticoagulated in acid citrate buffer, whereas prior studies have examined either heparin-anti-coagulated plasma (Zabetian et al. 2001) or serum (Weinshilboum 1978). Unpublished experiments performed by R.W.B and J.F.C show that acid citrate diminishes activity by approximately 50% (presumably due to copper chelation by acid citrate). The compression of values due to those, and possibly other factors, is likely to affect higher values more substantially than lower values owing to a “floor effect.” Such asymmetric compression of the distribution of

values would, therefore, be expected to exaggerate the influence of the low-activity-associated alleles, leading an additive system to appear low-value dominant. The results were not affected by including the duration of sample storage as a covariate but, since almost all of the samples were stored for more than 10 years, that observation cannot exclude the possibility of a sample-storage effect. Samples of serum or heparin-anticoagulated plasma were not available, so we were unable to evaluate the influence of anti-coagulant directly in this sample set. Regardless of the possible effects of factors such as duration of sample storage or anticoagulants, the strong replication of the 9q34 linkage, in addition to replication of the strong association of rs161115 with pD β H activity, indicate that the data from this study are useful and reasonably comparable to prior data.

It is important to note that accounting for the SNPs rs161115, rs161122 and rs6271 substantially diminished, but did not completely eliminate, the linkage signal at 9q34, resulting in a residual multi-point lod score of 3.37 under a low-value dominant model. However, accounting for all SNPs in the 9q34 linkage region completely eliminated the signal, strongly suggesting that additional variants at or near *DBH* contribute to regulation of pD β H activity. One evidence-supported candidate for such a variant is rs1989787. Chen and colleagues (Chen et al. 2010) recently showed that SNP to associate with pD β H activity after statistically accounting for rs161115. In addition, analysis of reporter-gene transcription indicated that rs1989787 appears to alter transcription driven by the *DBH* promoter (Chen et al. 2010). Prior association studies from our laboratory have also suggested the presence of at least one additional functional variant, in linkage disequilibrium with a SNP within intron 5 of *DBH* but not with rs161115 or rs6271 (Tang et al. 2006).

The finding of positive (but not genome-wide significant) evidence for linkage between markers on chromosome 20 and variation in pD β H is an entirely new observation. Although the fact that the chromosome-20 model-based linkage peak became stronger after correcting for the linkage at *DBH* is encouraging, only replication in independent studies will distinguish a true linkage from a chance finding. We note here that the limited sample size in the current study substantially reduced our power to detect linkage in a genome-wide scan, and this is an important limitation of the study. If further research verifies a chromosome-20 linkage to pD β H, it will be interesting to examine how much of the unaccounted-for genetic variance such a linkage explains. It should be noted in passing that the segregation model that best fits the trait data alone is not always the segregation model that, when genetic markers are included for a model-based linkage analysis, gives the strongest linkage signal. We cannot exclude the interesting possibility that the positive linkage signal on chromosome 20 (or the lack of such a signal on chromosome 19) is due to the fact that the current study examines pedigrees ascertained for schizophrenia. However, without additional evidence to support such a hypothesis, we believe it unlikely.

Prior studies of pD β H in psychiatric illness reveal a pattern of association of lower pD β H or CSF levels of D β H with psychosis-related phenotypes, including psychotic features in major depression (Cubells et al. 2002; Lykouras et al. 1988; Meltzer et al. 1976; Meyers et al. 1999; Mod et al. 1986), more florid symptoms of schizophrenia described in the original publications as “reactive” as opposed to “process” schizophrenia (Sternberg et al. 1983; van Kammen et al. 1994), and higher risk for psychosis following treatment with disulfiram (which inhibits D β H) in alcoholics (Major et al. 1979). Furthermore, genotypes and haplotypes at *DBH* that associate with lower pD β H have also been reported to associate with cocaine-induced paranoia in chronic users of cocaine (Cubells et al. 2000; Kalayasiri et al. 2007), and poor outcome in patients with schizophrenia (Yamamoto et al. 2003). Those findings, taken together, suggest that *DBH* might influence the phenotypic expression of psychosis in a variety of drug-induced and idiopathic psychotic disorders. While the precise relationship between pD β H and central regulation of D β H activity remains unclear, a single

gene encodes the central and peripheral protein, suggesting that molecular mechanisms regulating central and circulating $D\beta H$ could at least partially overlap. The associations just cited, between variation in $pD\beta H$ and differences in psychiatric phenomena almost certainly originating in the brain might reflect such common mechanisms. The present results set the stage for more detailed molecular-genetic investigation of that hypothesis in families segregating schizophrenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Model-based linkage analysis of $pD\beta H$ activity on chromosome 9. Model-based linkage analysis of $pD\beta H$ activity was performed using MLOD within S.A.G.E., as described in the text. The *red solid-line profile* shows the multi-point linkage for $pD\beta H$ activity under a square root transformation, low-value dominant model. The *green dash-dot line* shows the linkage for the new trait formed after adjusting for three SNPs in the *DBH* gene, under the new untransformed, dominant model. The *blue dotted-line profile* is the multi-point linkage for the new trait formed after adjusting for the 25 SNPs in the linkage region on chromosome 9, under the new additive, logarithmic transformation model

Fig. 2.

Model-free linkage analysis of pDBH activity on chromosome 9. Model-free linkage analysis was performed in SIBPAL, as described in the text. The *red solid line* shows linkage of square root pDBH activity. The *green dash-dot line* shows the linkage of the new trait formed by adjusting for three SNPs in the DBH gene. The *dotted-line profile* is for the new trait created by additionally adjusting for the 25 SNPs in this linkage region

Fig. 3.

Model-based linkage analysis of pDBH activity on chromosome 20. The *red solid line* shows the multi-point linkage of pDBH activity under the square root transformation, low-value dominant model. The *blue dotted-line* shows the multi-point linkage analysis of the new trait created by adjusting for the 25 SNPs in the chromosome 9 linkage region, under the new additive model, with square root transformation

Table 1
Parameter estimates of the segregation models for pD β H activity and the models for the new trait that produced significant linkage signals

Trait	Transformations	Model	q_A^a	μ_{AA}^b	μ_{AB}^c	μ_{BB}^d	σ	λ_1	λ_2	$\ln(L)^e$	Akaike's AIC
pD β H activity	No transformation	Dominant ^f	0.59	10.63	10.63	42.47	8.46			-1,100.28	2,210.55
		Recessive ^f	0.59	10.63	10.63	42.47	8.46			-1,100.28	2,210.55
		Additive	0.89	10.11	39.41	68.71	8.26			-1,105.41	2,220.82
	Square root	Dominant ^g	0.52	8.07	8.07	34.46	7.58	0.5	0	-1,052.17	2,114.33
		Recessive	0.84	7.35	31.53	31.53	7.33	0.5	0	-1,050.63	2,111.26
		Additive	0.87	8.02	33.54	59.06	7.54	0.5	0	-1,052.6	2,115.20
	logarithmic	Dominant ^f	0.47	1.65	15.04	15.04	7.10	0	0	-1,037.13	2,084.25
		Recessive ^f	0.47	1.65	15.04	15.04	7.10	0	0	-1,037.13	2,084.25
		Additive	0.45	1.46	11.66	21.86	6.76	0	0	-1,041.67	2,093.34
	Estimated power λ_1	Dominant	0.24	4.01	4.01	19.39	7.31	0.11	1	-1,049.64	2,111.27
		Recessive	0.51	2.06	15.69	15.69	7.02	-0.12	1	-1,043.22	2,098.44
		Additive	0.48	1.79	12.27	22.74	6.71	-0.15	1	-1,047.88	2,107.76
New trait (adjusted for 3 SNPs)		Dominant	0.62	11.69	11.69	27.05	27.93			-906.7	1,823.40
New trait (adjusted for 25 SNPs)	Square root	Additive	0.69	9.76	17.72	25.67	29.55	0.5	0	-899.17	1,808.34

^aThe trait locus allele frequency

^{b, c, d}Median unbiased estimates of means of the trait genotypic distributions for AA, AB and BB, respectively, on the untransformed pD β H scale

^e L is the Jacobian transformed likelihood

^fNote that because of the change in gene frequency the dominant and recessive models are identical

^gThe model that produce the best genome-wide linkage signal for pD β H activity

Table 2
The association p values of square root of pDBH to individual SNPs in the linkage regions on chromosome 9 and chromosome 20, determined by likelihood ratio tests with ASSOC (polygenic, marital, and sibling variance components were allowed, no transformation was used)

SNP	Chromosome	MAF	Association p value	
			Additive	Dominant
rs1611115	9	0.24	1.14×10^{-18}	8.59×10^{-4}
rs1611122	9	0.48	4.38×10^{-7}	0.69
rs6271	9	0.07	2.27×10^{-3}	NA *
rs705670	9	0.30	1.80×10^{-3}	0.87
rs12009	9	0.48	0.61	2.06×10^{-2}
rs877954	9	0.35	2.90×10^{-2}	0.46
rs1572306	9	0.20	3.89×10^{-2}	0.43
rs908831	9	0.37	4.41×10^{-2}	0.91
rs727472	20	0.44	4.64×10^{-3}	0.18
rs362987	20	0.47	2.24×10^{-2}	0.22

* Not available because only CC and CT genotypes appeared in the data, TT is missing