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L-Histidine Decarboxylase and Tourette's Syndrome

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Summary

Tourette's syndrome is a common developmental neuropsychiatric disorder characterized by chronic motor and vocal tics. Despite a strong genetic contribution, inheritance is complex, and risk alleles have proven difficult to identify. Here, we describe an analysis of linkage in a two-generation pedigree leading to the identification of a rare functional mutation in the *HDC* gene encoding L-histidine decarboxylase, the rate-limiting enzyme in histamine biosynthesis. Our findings, together with previously published data from model systems, point to a role for histaminergic neurotransmission in the mechanism and modulation of Tourette's syndrome and tics.

Tourette's Syndrome is Characterized by Childhood Onset, Waxing and waning symptomatology, and typically, improvement in adulthood. The molecular underpinnings of the disorder remain uncertain, although multiple lines of evidence suggest involvement of dopaminergic neurotransmission and abnormalities involving cortical–striatal–thalamic–cortical circuitry.[1] Current treatment focuses on tic reduction and management of prevalent coexisting conditions such as obsessive–compulsive disorder and attention deficit–hyperactivity disorder. However, therapeutic options have limited efficacy and may carry clinically significant side effects. Consequently, the development of new treatments based on an improved understanding of disease pathophysiology is a high priority.[2]

The large genetic contribution to Tourette's syndrome is well established.[3] However, studies of candidate-gene association and nonparametric linkage have not yet yielded reproducible findings. Rare functional variants in the neuronal transmembrane molecule SLITRK1 (the SLIT and NTRK-like family, member 1 protein) have been associated with Tourette's

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syndrome.[4,5] However, mutations are found in only a small proportion of affected persons, and the protein's normal function and the manner in which it may contribute to Tourette's syndrome are not yet well understood.

In light of the probable genetic heterogeneity underlying Tourette's syndrome, we sought families in which the syndrome is transmitted in a mendelian fashion, which is rare. As has been shown for other complex disorders, gene discovery in such families may help to uncover molecular mechanisms of disease.[6]

Methods

Methods are described briefly here; for complete details, see the Supplementary Appendix, available with the full text of this article at NEJM.org. All studies were approved by the Yale Institutional Review Board, and all participants provided written informed consent.

A nonconsanguineous two-generation pedigree was referred to our laboratory (Fig. 1). The father and all eight offspring met the criteria for Tourette's syndrome in the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition, text revision (Table S1 in the Supplementary Appendix). Two children and the father also have obsessive–compulsive disorder. The mother, her parents, and her extended family are reportedly free from Tourette's syndrome, chronic tics, and obsessive–compulsive disorder.

DNA samples from all family members in the two-generation pedigree were genotyped by means of Affymetrix GeneChip Human Mapping 100K Arrays and short-tandem-repeat markers that had been identified within the lod – 2 linkage interval (the equivalent of a confidence interval around the maximum lod score, consisting of the interval from the maximum lod score to the score minus 2). Parametric analyses were performed with the use of the SimWalk2 program. The coding regions of all 51 known genes within the lod – 2 linkage interval (Table S2 in the Supplementary Appendix) were analyzed by means of Sanger sequencing. Variants were confirmed in all family members. We evaluated messenger RNA from lymphoblastoid cell lines. Detection of copy-number variations was carried out with the use of Illumina 1M-Duo arrays.

The coding, promoter, and untranslated regions of the *HDC* gene, encoding L-histidine decarboxylase, were sequenced in DNA samples from 720 persons with Tourette's syndrome as well as 360 controls not screened for psychiatric disorders. The HDC nonsense mutation, W317X, was genotyped with the use of a custom TaqMan assay in 1500 controls. To ensure an appropriate comparison group, an assessment of the ancestral profile of the pedigree was performed with the use of genotyping data and a multidimensional scaling analysis[7] comparing samples from the index family and 188 HapMap samples (Fig. S1 in the Supplementary Appendix).

Further assessment of control samples for mutations in HDC entailed a review of available databases and an analysis of copy-number variation among DNA samples from 10,571 persons from diverse racial and ethnic backgrounds (Table S6 in the Supplementary Appendix) with the use of three types of gene chips (CNV370-Duo, HumanHap300-v1, and HumanHap550v3, all from Illumina). A stop codon, rs35490626, was identified in the dbSNP database of the National Center for Biotechnology Information. This reported allele, one missense variant identified in a patient, and two missense variants found in controls were further genotyped in 5427 persons who had not been evaluated for Tourette's syndrome or neurologic disorders and in 986 persons who had been screened for, and found not to have, neurologic disease.

To assess the activity of wild-type and W317X mutant HDC proteins, constructs were expressed as N-terminal glutathione *S*-transferase fusion products, isolated under denaturing

conditions, refolded, and subjected to a fluorescence quenching–based assay. Assessment for dominant negative activity (i.e., determination of whether the altered gene product derived from the mutant allele successfully antagonizes the protein product of the wild-type allele) was performed by means of in vitro transcription and translation. We evaluated the effect of mutant proteins on enzymatic activity by using a fixed amount of wild-type DNA template along with increasing amounts of W317X mutant DNA template. The production of histamine from its precursor L-histidine was measured by means of liquid chromatography–mass spectrometry (Table S10 in the Supplementary Appendix).

Results

The occurrence of Tourette's syndrome in two generations of this family and the absence of this and related disorders in the maternal lineage support the conclusion that the syndrome is an autosomal dominant trait in this family. Genomewide analysis, which specified a rate of phenocopy of 1% (i.e., 1% of affected persons may not carry the genetic risk factor), identified complete linkage of a 3.4-centimorgan (cM) segment of chromosome 15 with Tourette's syndrome, with a lod score of 2.05, which approximated the maximum theoretical lod score (2.1) that could be achieved under the model of dominant transmission. No other region had a lod score above 1, and varying the predicted rates of penetrance (i.e., manifestation of the disease in carriers of the genetic risk factor) and phenocopy had no effect on the result (Fig. 2A and 2B). Genotyping of short-tandem-repeat markers defined a lod – 2 interval of 8.13 Mb flanked by the markers D15S126 and GATA153F11 (Fig. 1, and the Supplementary Appendix).

A polymerase-chain-reaction (PCR) assay was used to amplify all coding exons and intron–exon junctions of all genes mapping within the lod – 2 interval that were then sequenced (Table S2 in the Supplementary Appendix). A heterozygous G-to-A transition at nucleotide position 951, resulting in a premature termination codon (W317X), was identified in exon 9 of *HDC*. The mutation was present in the affected father and all affected offspring and absent in the unaffected mother (Fig. 2C). In addition, reverse-transcriptase PCR was performed on messenger RNA samples from transformed lymphoblastoid cell lines. All affected family members had the substitution, providing independent confirmation of the mutation and showing that it may escape nonsense-mediated decay. No other rare nonsense, splice-site, or missense mutations (those with an allele frequency <5%) were identified in the lod – 2 interval. Three previously reported missense substitutions, all with allele frequencies greater than 5%, were found on the linked haplotype (Table S3 in the Supplementary Appendix).

To identify an appropriate control group for evaluating the frequency of W317X, we compared the genomewide genotyping data from the parents of the pedigree with data from 188 subjects of northern and western European (abbreviated as CEU), African, or Asian ancestry in the HapMap database, using a multidimensional scaling analysis.[7] We found that the parents tightly cluster with CEU subjects (Fig. S1 in the Supplementary Appendix). Consequently, we genotyped 3000 control chromosomes from unrelated CEU persons. W317X was not found. Similarly, resequencing of the coding region of *HDC* in 720 patients with Tourette's syndrome and 360 controls not screened for psychiatric disorders revealed no additional nonsense variants and a total of three missense variants, one found in a patient and two in controls (Table S4 in the Supplementary Appendix). All were predicted, by means of standard bioinformatic algorithms (see the Supplementary Appendix), to affect protein function. Review of public databases identified one putative nonsense mutation, rs35490626; however, it did not meet validation criteria established by dbSNP, since it was not found in more than one sequence read and did not undergo PCR confirmation. Nonetheless, we genotyped 12,826 chromosomes from persons from a diversity of ethnic groups (Table S6 in the Supplementary Appendix),

including 1972 chromosomes from neurologically normal controls, and found no further occurrences of any of these variants, suggesting that they are very rare in the general population.

We next screened for copy-number variations at the *HDC* locus in the general population, investigating 21,142 chromosomes using three detection algorithms (Table S6 in the Supplementary Appendix). No deletions or amplifications disrupting the transcript were identified. However, the probe density in this region limited the detection of copy-number variations to a minimum size of approximately 15,000 base pairs. Review of the Database of Genomic Variants revealed no *HDC* deletions and a duplication, identified by means of a bacterial-artificial-chromosome array, in two reportedly phenotypically normal individuals.[8]

Taken together, the data from genotyping, resequencing, and assessment of copy-number variations establish that the W317X mutation is extremely rare and that loss-of-function alleles at this locus are likely to be very uncommon.

The index family is striking because of not only the number of affected individuals but also the large number of miscarriages (Fig. 1). Consequently, we exhaustively evaluated the sequencing data for the linkage interval in the mother and father and performed high-resolution analysis of copy-number variations in both, in search of compound heterozygote mutations that could provide an alternative explanation for the cosegregation of one paternal haplotype among all live births. Apart from W317X in the father, we found neither a rare, putatively functional sequence variation nor any identifiable structural variation in the linkage interval in either parent.

The W317X nonsense mutation is predicted to result in a truncated protein of 316 amino acids (35 kD) missing key segments of the active domain (Fig. 3). Full-length HDC, the activity of which depends on the coenzyme pyridoxal 5′-phosphate, is initially produced as a 74-kD product. This is processed to produce multiple isoforms, including a stable isoform of approximately 54 kD, which then forms active homodimers of about 110 kD each.[9] Evidence has strongly suggested that the truncating mutation would result in haploinsufficiency[9] and lead to decreased histamine in the central nervous system.[10] However, given the possibility of incomplete or absent nonsense-mediated decay in the pedigree, and given that the major active form of the HDC enzyme is a homodimer, we speculated that the retained mutant might act through a dominant negative mechanism.

To test this hypothesis, we evaluated the enzymatic activity of wild-type HDC, the 35-kD mutant, and the combination of the two. A fluorescence-quenching–based approach confirmed that the mutant form lacked enzymatic function (Fig. 3C). We subsequently used liquid chromatography–mass spectrometry to determine the dose–response characteristics of histamine production when various ratios of wild-type and mutant HDC were combined,[11] and we found evidence for a dominant negative effect of the mutant protein (Fig. 3E and 3F).

Discussion

We have characterized a highly unusual two-generation pedigree in which Tourette's syndrome is segregated in an autosomal dominant fashion. Given that Tourette's syndrome has a population prevalence of 1% and a rate of recurrence of 10 to 15% among first-degree relatives of an affected person, it is extremely unlikely that all eight offspring of an affected parent would have Tourette's syndrome by chance, suggesting that this kindred indeed carries a mendelian form of the syndrome. The finding of a single segment of the genome that is shared among all affected members with a clearly deleterious mutation within this interval is also highly unlikely to occur by chance alone. Though the accepted standard for statistical significance on the basis of parametric linkage of a mendelian trait is a lod score of 3.0, these findings strongly point to a causal relationship between the identified mutation and Tourette's syndrome in this family.

Previous research on the role of HDC within the central nervous system lends additional support to our genetic data. Although HDC expression is highly constrained within the posterior hypothalamus, axons from histamine-containing neurons project throughout the brain. Histaminergic signaling is mediated by four known G protein–coupled receptors — H1 through H4 — that are located both presynaptically (H3 and H4) and postsynaptically (H1, H2, and H3) and are known to modulate circadian rhythms, appetite, memory, and behavior.[12] Moreover, H2 and H3 receptors are highly enriched in the striatum and cortex, regions widely implicated in the pathophysiology of Tourette's syndrome.[12]

$Hdc^{-/-}$ mice[10] are viable but have altered mast-cell morphology and subtle abnormalities in locomotion, arousal, learning, and memory.[13-15] In addition, they have several traits relevant to features of Tourette's syndrome. The mice have exaggerated locomotor responses to methamphetamine and enhanced behavioral sensitization: the progressive and persistent augmentation of locomotor and stereotypic behaviors resulting from repeated administration of dopamine agonists.[14] Such stimulant-induced stereotypic movements, including rearing, sniffing, and biting, have previously been proposed as a model of human tics.[16] In addition, multiple studies have confirmed that $Hdc^{-/-}$ mice display behavior more consistent with anxiety than littermate controls,[15,17] similar to the phenotype recently observed in mice deficient in Slitrk1.[5]

The links between histaminergic neurotransmission and tics, suggested by our genetic findings, are further supported by previous studies of pharmacologic agents that target the presynaptic autoreceptor H3R. Highly selective antagonists and inverse agonists increase histaminergic neurotransmission and decrease both the locomotor effects of stimulants and the behavioral sensitization seen in rodents.[18-20] Several such compounds have been developed and are under investigation in humans. The convergence of our findings with data from mouse models and the presence of therapeutic agents that would be expected to moderate deficits in histaminergic neurotransmission suggest an approach to treatment that may prove relevant not just to our rare kindred but also to others with Tourette's syndrome.

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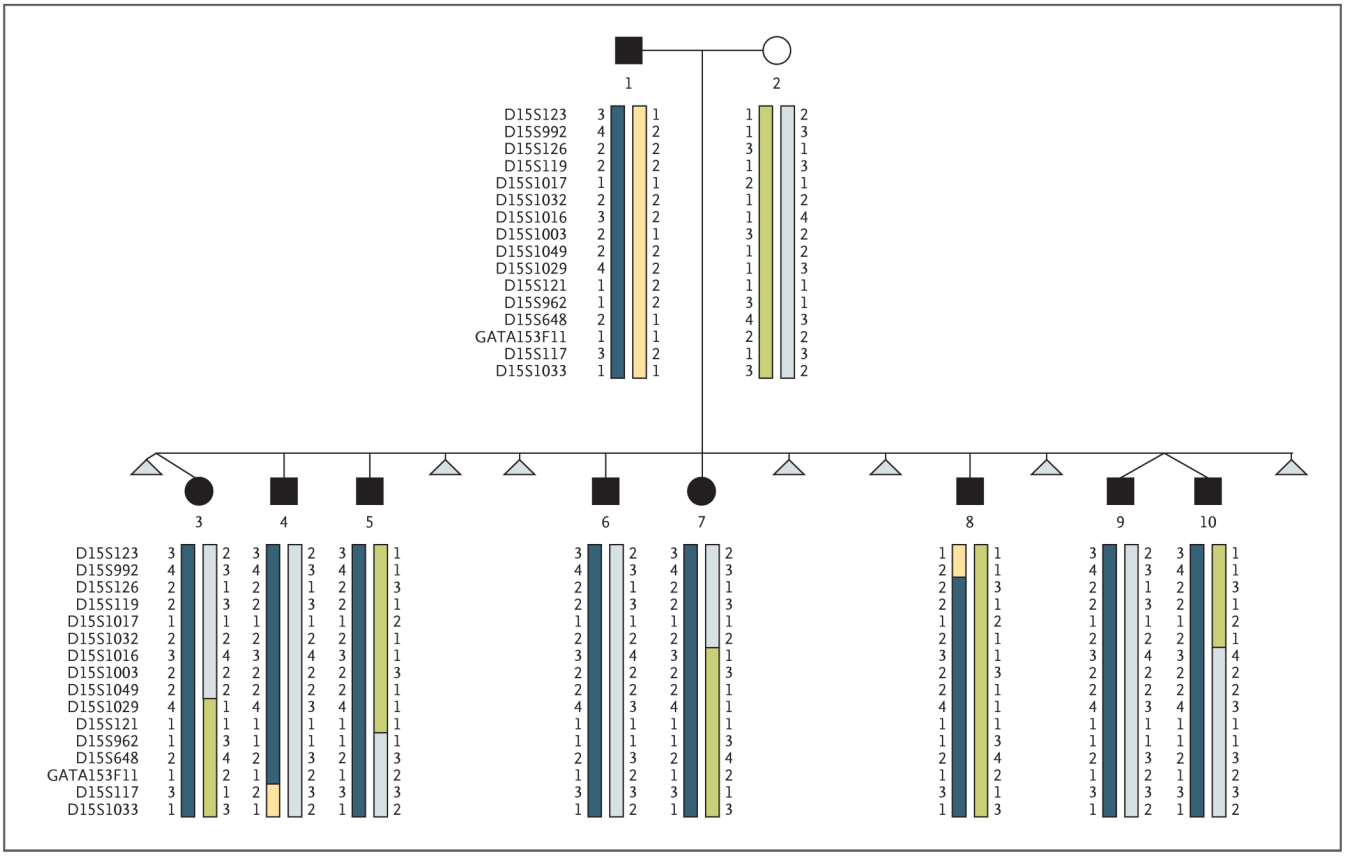

Figure 1. The Two-Generation Pedigree and Results of Haplotype Analysis

Squares indicate male family members; circles, female family members; solid symbols, persons with Tourette's syndrome; the open circle, the unaffected mother; and gray triangles, miscarriages. The haplotypes illustrated below the pedigree symbols correspond to the 8.13-Mb segment that cosegregates with Tourette's syndrome at 15q21.1–15q21.3. The short-tandem-repeat markers used to confirm linkage are listed to the left, with the corresponding genotypes at these markers represented as numerals 1 through 4 for each chromosome of each proband. The haplotype that segregates with Tourette's syndrome is indicated by the dark blue bar and is bounded by markers D15S126 and GATA153F11. Detailed clinical information is presented in the Supplementary Appendix.

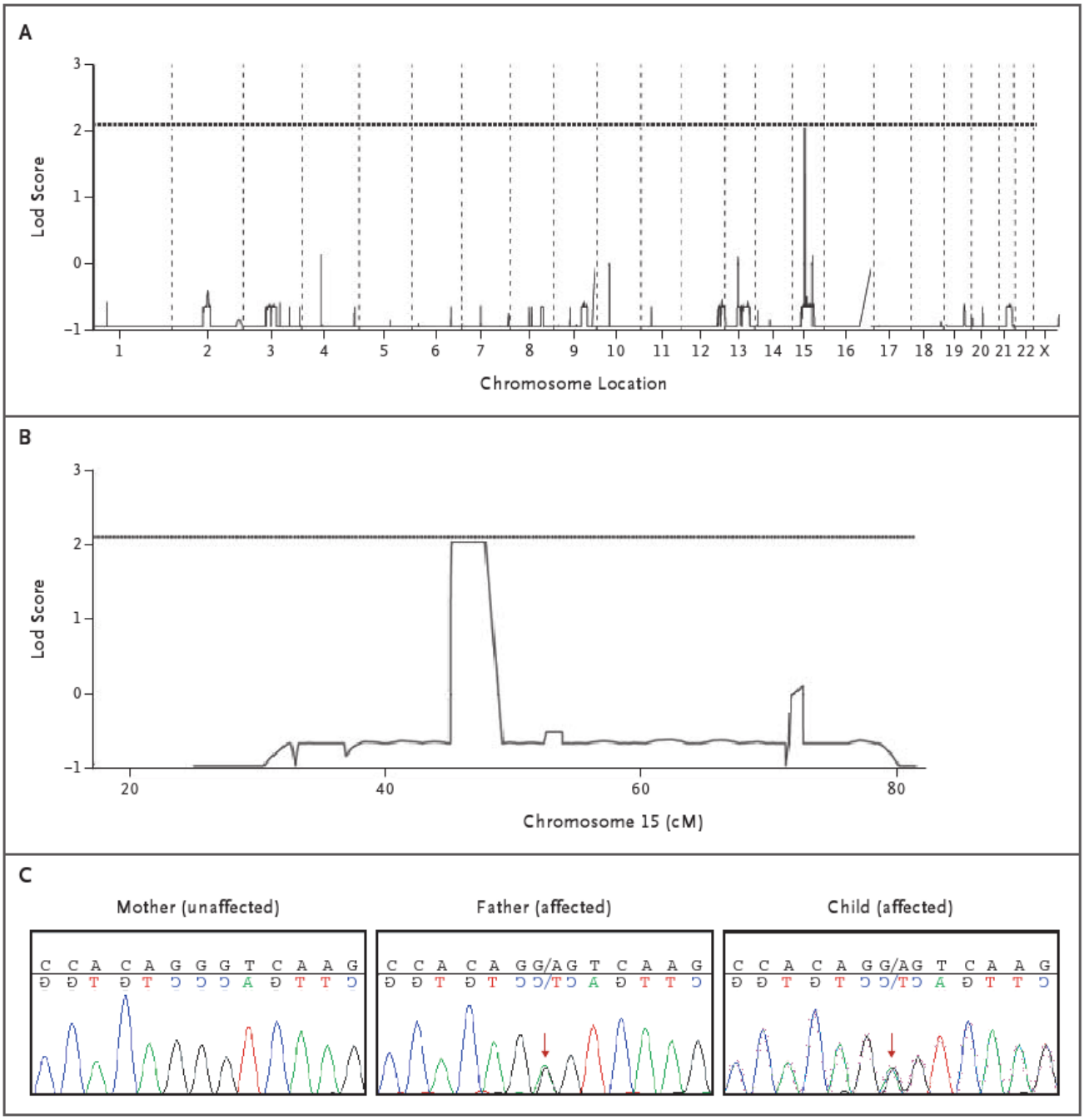

Figure 2. Mutation in *HDC* within the Lod – 2 Linkage Interval

Panel A shows the results of the genomewide parametric linkage analysis, expressed as lod scores. The maximum theoretical lod score for this family is 2.1, as indicated by the horizontal dashed line. The vertical dashed lines show the boundaries of each chromosome. Genotyping was performed on the GeneChip Human Mapping 100K Array (Affymetrix). The analysis modeled dominant inheritance of a rare allele (allele frequency, 0.001); a rate of phenocopy of 1% (i.e., 1% of affected persons may not carry the genetic risk factor), which is equal to the population prevalence of Tourette's syndrome; and 99% penetrance. Panel B shows the lod scores for chromosome 15q; the horizontal dashed line indicates the maximum theoretical lod score for this family (2.1). Panel C shows the sequence chromatograms at the nucleotide

position 951 of the *HDC* transcript. The affected father and an affected child have a G-to-A substitution (indicated by red arrows), which is predicted to result in a change from the wild-type tryptophan to a stop codon at amino acid 317 (W317X). The sequence of the mother, who is unaffected, is the wild-type sequence. Resequencing of both DNA strands, as well as a reverse-transcriptase–polymerase-chain-reaction assay of messenger RNA, revealed this variation in all the affected family members.

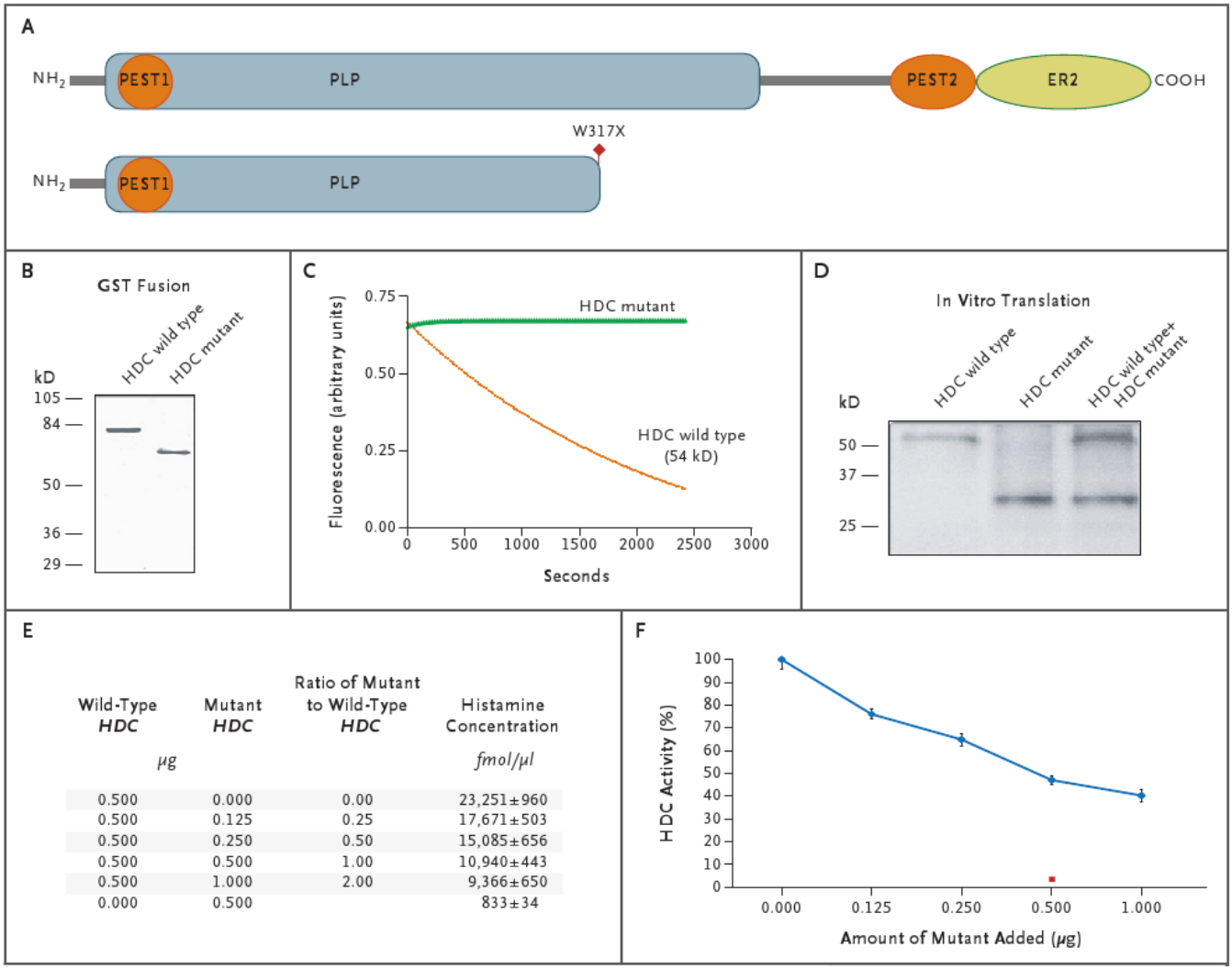

Figure 3. Results of Functional Analysis of Mutant HDC (L-Histidine Decarboxylase) Activity

The wild-type HDC protein (Panel A, top), 74 kD (662 amino acids) in size, contains a pyridoxal 5′-phosphate (PLP) domain at the N-terminal end (NH_2), two PEST domains (PEST1 and PEST2), and a C-terminal (COOH) intracellular targeting domain (ER2). The mutant HDC (Panel A, bottom) is predicted to be truncated at the site of the W317X mutation (red diamond), resulting in a 35-kD product (316 amino acids) lacking a portion of the PLP domain, one of two PEST domains, and the entire ER2 domain. The wild-type 54-kD isoform (representing the active, processed form of HDC) and the truncated mutant HDC (35-kD) were expressed as glutathione *S*-transferase (GST) fusion proteins in *Escherichia coli*. The resulting proteins were largely insoluble, primarily found in inclusion bodies, and therefore were isolated under denaturing conditions and refolded. These isolated proteins were analyzed with the use of sodium dodecyl sulfate–polyacrylamide-gel electrophoresis (SDS-PAGE) and Coomassie staining. Both proteins were the expected size (after accounting for the size of the glutathione *S*-transferase tag) (Panel B). A fluorescence-quenching–based assay was then performed to assess HDC activity. The turnover of histidine to histamine was measured by means of displacement of the fluorescent dye (2-aminoethyl)sulfonamide (Dapoxyl), which serves as a histamine substitute. A steady decline in fluorescence was seen with the wild-type HDC, reflecting enzymatic activity, whereas no activity was observed in the W317X mutant HDC

(Panel C). Expression of HDC proteins generated by in vitro translation (Panel D) showed that the wild-type HDC and W317X mutant HDC were translated separately as well as cotranslated. Biotinylated transfer RNA was added to the reaction to allow for visualization of the resulting proteins by means of SDS-PAGE and streptavidin–horseradish peroxidase. The wild type and the W317X mutant were the expected 54-kD and 35-kD sizes, respectively. Evaluation of dominant negative activity by means of liquid chromatography–mass spectrometry was performed on the cotranslated protein products (Panel E). The amount of wild-type DNA template used in the in vitro translation is listed in the first column and the amount of W317X mutant DNA template added to the cotranslation is listed in the second. The resulting ratio of the amounts of W317X mutant DNA to wild-type DNA is noted in the third column. After the reactions were incubated with L-histidine, the resulting mean (±SD) amount of histamine produced was measured by means of liquid chromatography–mass spectrometry. The dose–response curve for HDC activity (Panel F) shows the percent activity, as calculated by setting to 100% the amount of histamine produced with wild-type HDC DNA template only (23,251 fmol per microliter). As more W317X mutant DNA template was added, a decrease in overall activity was noted, according to the amount of W317X mutant added to a 0.5-g sample of wild-type HDC. The red square indicates a sample of W317X mutant only. The data are the means of three technical replicates of a single cotranslation experiment (see the Supplementary Appendix). The I bars indicate standard deviations.