A missense mutation in human fatty acid amide hydrolase associated with problem drug use

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Problem drug use and dependence are neurobehavioral disorders of complex origin. Although environmental factors contribute to drug abuse and addiction, genetic factors also play a significant role estimated at 40–60% of the total risk. Nonetheless, the precise identities of human genes that confer vulnerability to problem drug use remain mostly unknown. Here, we describe a natural single nucleotide polymorphism in the human gene that encodes the principal endocannabinoid-inactivating enzyme, fatty acid amide hydrolase (FAAH), that in homozygous form is strongly associated with both street drug use and problem drug/alcohol use. This single nucleotide polymorphism results in a missense mutation (385C→A) that converts a conserved proline residue to threonine (Pro129→Thr), producing a FAAH variant that displays normal catalytic properties but an enhanced sensitivity to proteolytic degradation. Collectively, these results suggest that genetic mutations in FAAH may constitute important risk factors for problem drug use and support a potential link between functional abnormalities in the endogenous cannabinoid system and drug abuse and dependence.

Drug abuse and dependence are neurobehavioral disorders of complex origin in which both environmental and genetic factors are perceived to contribute to vulnerability (1). Genetic factors have been estimated to account for 40–60% of the risk in susceptible individuals (2). Although the primary molecular sites of action for many drugs of abuse are well characterized, efforts to identify genetic alterations in these neural signaling systems that are associated with problem drug use and addiction have, to date, been mostly unsuccessful (2). One neural signaling pathway generally implicated in drug abuse and addiction is the endogenous cannabinoid system (3). This system includes a G protein-coupled receptor CB1 that binds the principal psychoactive component of marijuana, Δ-9-tetrahydrocannabinol, and the putative endogenous CB1 ligands, anandamide and 2-arachidonoylglycerol (4). Recent evidence suggests that the endogenous cannabinoid system may contribute not only to the development of dependence on marijuana (5) but also other drugs of abuse (6–8). Mice with a targeted disruption in the CB1 receptor exhibit reduced withdrawal responses to morphine (9), suggesting that significant crosstalk exists between endogenous opioid and cannabinoid systems in neural pathways that mediate addiction. Consistent with this notion, withdrawal from cannabinoids is significantly reduced in mice lacking preproenkephalin (10) or the μ-opioid receptor (11).

Recently, a third central component of the endogenous cannabinoid system, the integral membrane enzyme fatty acid amide hydrolase (FAAH), has been identified (12–14). Several lines of evidence suggest that FAAH serves as a primary catabolic regulator of anandamide and related fatty acid amide-signaling molecules *in vivo*. Specifically, mice with a targeted disruption in the *FAAH* gene ($FAAH^{-/-}$) are severely impaired in their ability to degrade anandamide and exhibit exaggerated behavioral responses to this fatty acid amide, including hypomotility, hypothermia, analgesia, and catalepsy (15). Moreover, endogenous brain levels of anandamide and related fatty acid amides are increased over 10-fold in $FAAH^{-/-}$ mice, correlating with enhanced CB1-dependent analgesia in these animals (15). Collectively, these findings suggest that FAAH may control the magnitude and duration of fatty acid amide signaling *in vivo* by setting an endocannabinoid tone in the brain as well as possibly in other parts of the nervous system. Because it is known that exogenous cannabinoids influence the sensitivity or vulnerability to other drugs of abuse in both humans and mammals (6, 8, 16), we interpreted the neurobehavioral and neurochemical abnormalities in $FAAH^{-/-}$ mice as circumstantial evidence that mutations in the human *FAAH* gene may result in dysregulation of endogenous cannabinoid signaling and, thereby produce alterations in brain addiction/reward pathways that could ultimately influence drug use. To explore this hypothesis, we conducted a search for mutations in the human *FAAH* gene in subjects with several neurobehavioral disorders, including street drug use and problem drug or alcohol use.

Materials and Methods

***FAAH* Gene Sequencing and PCR Amplification.** Oligonucleotide primers and PCR conditions were designed for each of the 15 *FAAH* exons. Each exon was completely sequenced with patient and control DNA samples for a total of 1,550 sequences. Single nucleotide polymorphisms (SNPs), or microsatellite repeats occurring in more than 1% of samples, were evaluated for predicted amino acid change in the mature protein and for alternative splice sites. SNPs were confirmed by comparison with two SNP databases and numbered based on the predicted ATG start site of the human *FAAH* cDNA (the ATG was considered bases 1–3). The *FAAH* SNP cDNA 385A was identified as potentially significant because this polymorphism predicts the conversion of Pro residue 129, conserved in all mammalian FAAH proteins sequenced to date (human, mouse, rat, pig, and cow), to Thr. Therefore, the cytosine 385 to adenosine (385C→A) missense mutation was selected for further study; because the gene frequency of the C allele is the highest, it is designated throughout as the wild type (wt).

Research Subjects. Patients attending a medical screening clinic were invited to participate with informed consent in DNA screening for genetic disorders. Each subject in this study (Study A) completed a comprehensive health appraisal questionnaire that was linked to the subject's DNA sample. DNA samples and clinical data for this study were from sources not known to the examiner. For Study A subjects, the questionnaire variables that were retained included age, sex, ancestral origin, the presence of medical disorders and neuropsychiatric disorders such as anxiety, depression symptoms, suicidal feelings, history of street drug use, serious problems with drug or alcohol use, nicotine use, and alcohol use. This allowed stratification of variables into groups of patients and matching controls. Patient DNA samples from Study A selected for FAAH 385C→A genotype analysis were all of white ancestral origin because of under-representation of

Abbreviations: SNP, single nucleotide polymorphism; FAAH, fatty acid amide hydrolase; CI, confidence interval.

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Table 1. Subject groups of drug, alcohol, nicotine users, and depressive symptoms

Group	Questionnaire statement or question	Answer
Street drug user	1. Have you used street drugs?	Yes
Problem drug/alcohol user*	2. I am having serious problems with drug or alcohol use.	Yes
Street drug user and problem drug/alcohol user	3. Yes answer on questions 1 and 2.	Yes, Yes
Control I	4. No on questions 1 and 2.	No, No
Alcohol user, no drug use	5. When I drink it is usually 3 or more drinks/day; No on questions 1 and 2.	Yes No, No
Nicotine user, no drug use	6. How many cigarettes do you now smoke per day? How many years have you smoked? No on questions 1 and 2.	20 or more 5 yrs or more No, No
Depressive symptoms, only	7. I often (*a*) feel like crying; (*b*) feel hopeless or down in the dumps; (*c*) have problems with depression. No on questions 1 and 2.	Yes
Suicidal ideation, only	8. I often feel suicidal. And No on questions 1 and 2.	Yes
Control II	9. No on questions 1, 2, 7, and 8.	

The statements or questions represent the exact wording in the questionnaire completed by all patients in the groups shown. When more than one question is listed, an affirmative answer to any one question was regarded as positive in that category.

*Drug/Alcohol problem users included together because there was no separate questionnaire category for each; alcohol users with no drug use were analyzed separately.

other ethnic groups admitting to street drug use and drug or alcohol abuse. The data analysis included subjects and matched controls from Study A stratified by responses to specific questions on the health questionnaire. Table 1 contains the definitions of the subject groups from Study A. In addition, the data analysis included anonymous patient DNA samples obtained from subjects meeting DSM-III-R criteria for the diagnosis of schizophrenia, bipolar disorder, autism, and matched controls. The source of DNA samples and data for schizophrenic and bipolar disorder subjects was the National Institute of Mental Health Center for Genetic Studies, and for autistic subjects, the source was the Coriell Cell Repositories. This protocol was approved by the local Institutional Review Board.

SNP Genotype Screening Using Allele-Specific Oligonucleotide Hybridization (ASOH). An ASOH protocol (17) was used to determine the SNP genotype at nucleotide position 385 in DNA samples whose sources were not known to the examiner. Oligonucleotide 17mer primers were designed for the *FAAH* 385 wild-type C and mutant A alleles, and individual genotypes were determined by using radiolabeled primers and digital imaging from the Cyclone PhosphoImager. Statistical calculations included Fisher's exact test, χ^2 with Yates correction, relative risk [with 95% confidence interval (CI)] and odds ratio (with 95% CI) on 2×2 and 2×3 contingency tables. Significant P values were corrected for 10 group comparisons by the Bonferroni method.

Expression and Purification of Rat and Human FAAH Variants. The 385C→A point mutation was introduced into both the human and rat *FAAH* cDNA sequences by using Stratagene QuickChange procedures and was validated by sequencing. Wild-type and Pro-129→Thr (P129T) mutant rat FAAH enzymes were expressed as N-terminal His-6-fusion proteins in *Escherichia coli* lacking the N-terminal transmembrane domain of the wild-type protein (ΔTM-FAAH), as described (18). Pure FAAH protein (≈1.0 mg) was isolated per liter of culture volume for both the wild-type and ΔTM-FAAH variants. Wild-type and P129T human FAAH variants were expressed as full-length proteins by transient transfection in COS-7 cells, as described (13).

FAAH Catalytic Activity Assays. The rates of FAAH-catalyzed hydrolysis of oleamide and anandamide were determined by using ^{14}C-labeled substrates and purified rat ΔTM-FAAH variants, as described (18). K_m and k_{cat} values were determined from Lineweaver-Burk plots of six substrate concentrations performed in triplicate. Heat-inactivation assays were conducted with human FAAH variants expressed in COS-7 cells. COS-7 cells transiently transfected with either FAAH variant exhibited over 100-fold higher FAAH activity than cells transfected with empty vector, with the wild-type protein expressing to approximately two-fold higher levels than the P129T variant (as judged by relative oleamide hydrolysis activities and Western blotting with anti-FAAH antibodies). Samples of transfected COS-7 membrane extracts were incubated for 30 min at one of seven different temperatures ranging from 37 to 55°C, after which the samples were cooled to room temperature; FAAH activity was then measured in triplicate (60 min reaction at 37°C with 100 μM [^{14}C]oleamide; reaction buffer, 50 mM Tris/125 mM NaCl, pH 8.0).

Urea Denaturation Curves of Wild-Type and P129T FAAH Variants. The stability of purified wild-type and P129T rat ΔTM-FAAH variants was measured as a function of urea concentration in 50 mM phosphate buffer at pH 7.5. Each protein (0.15 mg/ml) was incubated for 24 h with freshly prepared solutions of urea, with final urea concentrations ranging from 0 to 9.75 M. The tryptophan fluorescence spectra of each FAAH variant was recorded over the range of 310–410 nm by using an ATF 105 Aviv spectrofluorometer equipped with a thermostated cell holder employing excitation at 295 nm. Native FAAH proteins exhibited a fluorescence maximum at 335–340 nm, whereas unfolded proteins showed maximum emissions between 350 and 355 nm. The 350/340 nm emission intensity ratio was used to follow the denaturation of each FAAH variant as a function of urea concentration.

Far UV-Circular Dichroism Spectrometry of Wild-Type and P129T FAAH Variants. Purified wild-type and P129T rat ΔTM-FAAH samples were diluted to a concentration of 0.4 mg/ml and their respective circular dichroism (CD) spectra obtained with an Aviv stopped-flow CD spectrometer, as described (19).

Trypsin Sensitivity of Wild-Type and P129T FAAH Variants. Wild-type and P129T human FAAH-transfected COS-7 cell membranes normalized for equal FAAH levels were treated with trypsin (5 μM final concentration; Sigma) and incubated at 30°C (reaction

Table 2. Summary of FAAH 385 C→A data analysis by subject groups

Group	*n*	385A/385A, %	385A/wt, %	wt/wt, %	*P* (385A/385A vs. Control)	Odds ratio (95% CI)
Control I	1737	3.7	28.3	68		
Problem drug or alcohol user	80	**15**	21	64	0.0001 0.001*	4.54 (2.17–8.39)
Street drug user and problem drug or alcohol user	69	**16**	16	68	0.0001 0.001*	4.88 (2.45–9.73)
Street drug user	233	7.7	27.5	64.8	0.0083 0.08*	2.15 (1.25–3.7)
Alcohol user, no drug use	68	2.9	30.9	66.2	NS	0.79 (0.19–3.3)
Nicotine user, no drug use	34	0	12	88	NS	
Control II	1144	3.75	30	66.25		
Depressive symptoms (only)	222	3.6	25	71.4	NS	0.96 (0.44–2.01)
Suicidal ideation (only)	98	4	33	63	NS	1.09 (0.38–3.10)
Schizophrenic (NIMH sample)	48	2	48	50	NS	0.55 (0.07–4.0)
Bipolar disorder (NIMH sample)	20	10	55	35	NS	2.8 (0.65–12.8)
Autistic (Coriell sample)	18	0	83	17	NS	

NS, not significant.

**P* values corrected for 10 group comparisons by the method of Bonferroni; results with significant corrected *P* values shown in bold.

buffer, 50 mM Tris/125 mM NaCl, pH 8.0). At six time points, ranging from 5 to 60 min, reactions were terminated by the addition of soybean trypsin inhibitor (15 μM final concentration; Sigma). Samples then were divided into two fractions for the analysis of FAAH protein levels. One set of fractions was analyzed by Western blotting by using anti-FAAH polyclonal antibodies, as described (13). The second set of fractions was treated with a rhodamine-tagged active site-directed probe, FP-rhodamine, for 30 min at room temperature, following published methods (20, 21). FP-rhodamine labeling reactions were quenched with one volume of 2× SDS/PAGE loading buffer (reducing), separated by SDS/PAGE, and the levels of FAAH were measured with a flatbed fluorescence scanner (Hitachi FMBio II, MiraiBio). For each time point, fluorescent band intensities were calculated from three separate trials; the average of these trials was reported as a percentage of control values obtained from FP-rhodamine reactions with FAAH samples lacking trypsin. The relative trypsin sensitivities of wild-type and P129T mutant FAAH proteins were compared statistically by using a Student's unpaired *t* test, where a value of $P < 0.05$ was considered significant.

Results

***FAAH* 385A/385A Homozygous State Is Associated with Street Drug Use and Drug/Alcohol Abuse.** In the first data analysis, the frequencies of the mutant 385A allele in the white problem drug or alcohol user group ($n = 80$) and white control group I composed of subjects denying street drug use and problem drug or alcohol use ($n = 1737$) were 0.256 and 0.1796, respectively. These allele frequencies differed significantly (Fisher's exact test, $P = 0.0190$). The allele frequency of the same white problem drug or alcohol user group ($n = 80$) also differed significantly from control group II composed of white neuropsychologically normal subjects denying street drug use and problem drug or alcohol use ($n = 1144$; Fisher's exact test, $P = 0.0408$).

A more detailed analysis of the occurrence of the *FAAH* cDNA 385A SNP revealed that 385A/wt heterozygotes were not overrepresented in the street drug user or problem drug/alcohol user groups relative to the distribution of the wt/wt FAAH genotype (Table 2). In contrast, comparisons of 385A/385A individuals with wt/wt individuals or pooled groups containing the 385A/wt and wt/wt genotypes revealed a strong and selective association of the 385A/385A genotype with street drug use and problem drug/alcohol use (Table 2 and Fig. 1; $P = 0.0001$). The odds ratio for risk of problem drug or alcohol use in individuals with the 385A/385A homozygous genotype was 4.5, with a 95% CI between 2.2 and 8.4 (Table 2 and Fig. 2). Similarly, the odds ratio for risk of street drug use in 385A/385A individuals was 2.2 (95% CI, 1.3–3.7; Table 2 and Fig. 2). Finally, the odds ratio for risk of combined street drug use and problem drug/alcohol use in 385A/385A individuals was 4.9 (95% CI, 2.5–9.7; Table 2 and Fig. 2).

Generation and Initial Characterization of Wild-Type and P129T Mutant FAAH Proteins. To study the effects of the P129T mutation on the catalytic and structural properties of FAAH, this mutation was introduced into both the rat and human FAAH proteins. Proline129 resides in a region of primary structure that is highly conserved between the rat and human FAAH proteins (complete sequence identity from amino acids 120–145), indicating that both species variants should represent acceptable models for investigating the biochemical effects of the mutation of this residue. However, rat FAAH variants offer the advantage of superior recombinant expression in *E. coli* (data not shown); therefore, wild-type and P129T rat FAAH proteins were used for biochemical and biophysical studies that required large quantities of pure protein. In contrast, for experiments that were compatible with unpurified protein preparations, we examined

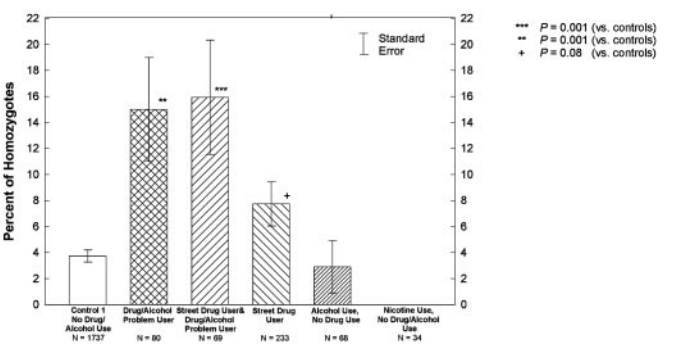

Fig. 1. Percentage of FAAH 385A/385A homozygous subjects in groups of patients reporting street drug use and/or problem drug or alcohol use, matched controls with no drug/alcohol use, alcohol use but no drug use, and nicotine use but no drug/alcohol use. Corrected *P* values are shown; Bonferroni corrections are also shown in Table 2. Error bars represent standard errors calculated for a binomial distribution of the indicated homozygote frequencies.

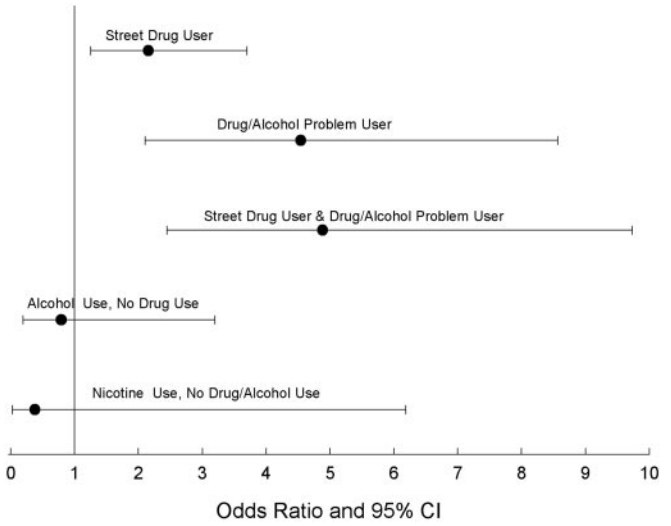

Fig. 2. Distribution of odds ratios and 95% CIs for data analyses summarized in Table 3. An odds ratio of 1 indicates that the probability of an individual with the homozygous mutation having the specific user trait indicated is no different from the probability of the genotype appearing in the control population.

the human wild-type and P129T FAAH variants expressed by transient transfection into COS-7 cells.

Wild-type and P129T rat FAAH proteins were expressed in *E. coli* as His-6-tagged fusion proteins lacking the predicted N-terminal FAAH transmembrane domain (ΔTM-FAAH proteins) and purified as described in *Materials and Methods*. Deletion of the N-terminal transmembrane domain of FAAH has been shown to assist in the expression and purification of the enzyme without affecting its general catalytic properties (18). Purified wild-type and P129T FAAH proteins were found to display equivalent gel filtration elution profiles and UV CD spectra (data not shown), indicating that the quaternary and tertiary structures of FAAH were not dramatically altered by the P129T mutation. Both wild-type and P129T FAAH proteins also exhibited similar K_m and k_{cat} values for the hydrolysis of two representative endogenous fatty acid amide substrates, oleamide and anandamide (Table 3), indicating that the P129T mutation did not significantly impact the catalytic properties of the enzyme.

Relative Structural Stabilities of Wild-Type and P129T Mutant FAAH Proteins. The global structural stabilities of wild-type and P129T rat FAAH proteins were compared by determining their respective urea denaturation curves over a concentration of urea ranging from 0 to 9.5 M. Wild-type and P129T FAAH proteins exhibited similar urea denaturation curves (data not shown) and midpoint of denaturation values (7.4 M urea), suggesting that global protein stability was not altered by the P129T mutation. Consistent with this notion, wild-type and P129T variants of human FAAH expressed in COS-7 cells were found to exhibit similar heat-inactivation profiles (Fig. 3*A*).

Table 3. Comparison of the kinetic properties of wild type and P129T FAAH (means ± SD; $n = 3$)

	Oleamide		Anandamide	
FAAH	K_m (μM)	k_{cat} (s^{-1})	K_m (μM)	k_{cat} (s^{-1})
Wild type	33.3 ± 4.4	10.3 ± 0.9	16.9 ± 2.4	5.8 ± 1.0
P129T	38.7 ± 8.6	9.6 ± 0.6	12.6 ± 3.8	4.3 ± 0.3

To evaluate the effect of the P129T mutation on the local structure of FAAH, the relative protease sensitivities of the wild-type and P129T human FAAH variants were compared by using a combination of Western blotting with anti-FAAH antibodies and chemical labeling with a fluorescent active site-directed probe (FP-rhodamine; ref. 21). By either method, the P129T FAAH protein was found to exhibit a significantly greater sensitivity to degradation by the protease trypsin (Fig. 3*B*). A quantitative comparison of the fluorescence intensities of FP-rhodamine-labeled wild-type and P129T FAAH proteins revealed that nearly 80% of the latter protein was proteolyzed after a 10-min incubation with trypsin, whereas only 40% of wild-type FAAH had been degraded at this time point (Fig. 3*B*). Likewise, significant levels of wild-type FAAH protein, but not the P129T mutant, could be detected by Western blotting and FP-rhodamine-labeling after a 40–60 min incubation with trypsin (Fig. 3*B Inset*).

Discussion

The role of genetic factors in vulnerability to drug use and addiction is currently a topic of major interest. In particular, familial, twin, and adoption studies support a significant genetic contribution to addiction (1, 2). The identification of genetic abnormalities that contribute to the individual risk of drug use and addiction may provide new molecular targets for screening, prevention and treatment of these disorders.

Here, we report a naturally occurring single nucleotide polymorphism in the human *FAAH* gene, 385A, that is strongly associated with street drug use and problem drug/alcohol use. This association seems especially relevant to illegal drug use, because neither alcohol nor nicotine abuse alone showed any significant relationship to the 385C→A missense mutation (Table 2). Moreover, we did not observe an association of the 385A SNP with any of the other neurobehavioral or neuropsychiatric disorders examined by our questionnaire, including depressive symptoms, suicidal ideation, schizophrenia, bipolar disorder, and autism.

FAAH is the primary catabolic regulator of a family of neural-signaling molecules, the fatty acid amides, that includes the endogenous cannabinoid, anandamide (14). Previous efforts to identify genetic relationships between the endogenous cannabinoid system and drug and alcohol abuse have focused on the analysis of the *CB1* receptor gene and have produced mixed results. A frequent silent mutation in *CB1* has been identified (1359G→A; ref. 22), but its functional significance and relevance to drug and alcohol abuse remains controversial (23, 24). In contrast, pharmacological studies have provided substantial evidence that activation of the cannabinoid system leads to key molecular and cellular changes associated with addiction. For example, cannabinoids have been shown to enhance mesolimbic dopaminergic transmission in a manner similar to heroin (25). Likewise, long-term cannabinoid administration alters corticotropin-releasing factor activity, a neuroadaptive process generally related to drug dependence (26).

Considering that the genetic deletion of *FAAH* in mice results in an adult mammal with greatly increased endogenous brain levels of anandamide and related fatty acid amides (15), polymorphisms in the human *FAAH* gene that produce a functionally altered enzyme also might be expected to shift tonic levels of these signaling lipids. The *FAAH* SNP identified in this study produces a missense mutation (385C→A) that results in the conversion of a Pro residue to Thr (P129T). Interestingly, this Pro residue is completely conserved among all mammalian FAAH enzymes characterized to date, including the human, mouse, rat, pig, and bovine FAAH orthologues. Although the P129T mutation was not found to alter the general catalytic properties or structural stability of FAAH, it did produce a FAAH variant with significantly increased sensitivity to proteolytic degradation. Whether this enhanced protease sensitivity

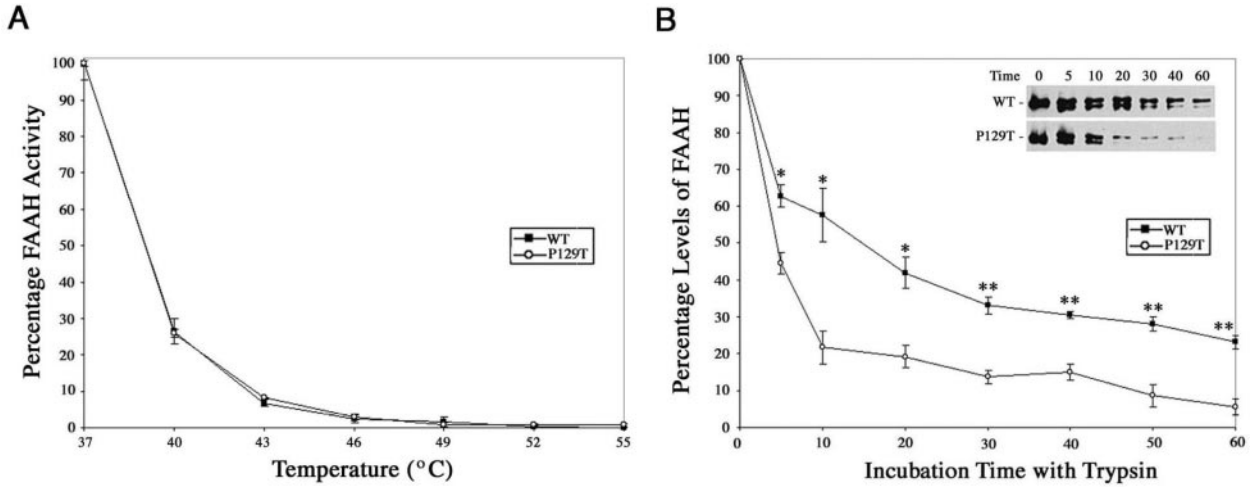

Fig. 3. Relative structural stability of wild-type and Pro129→Thr human FAAH proteins. (*A*) Heat-inactivation profiles for wild-type and Pro129→Thr FAAH variants. Membranes from COS-7 cells transfected with human FAAH proteins were preincubated for 30 min at the indicated temperatures and then assayed for oleamide hydrolysis activity. Activities are presented as a percentage of the activity observed for control reactions preincubated at 37°C ($n = 3$ for each temperature). Control activities for oleamide hydrolysis were 2.36 nmol × mg^{-1} × min^{-1} and 1.59 nmol × mg^{-1} × min^{-1} for wild-type and Pro129→Thr FAAH variants, respectively. (*B*) Protease sensitivity profiles of wild-type and Pro129→Thr FAAH variants. Membranes from COS-7 cells transfected with human FAAH proteins were preincubated with trypsin for the time points indicated, after which the levels of remaining FAAH protein were measured by covalent labeling with an active site-directed fluorescent probe, FP-rhodamine. Levels of FAAH protein are presented as a percentage of the levels observed for control samples run in the absence of trypsin ($n = 3$ for each time point). *, $P < 0.025$; **, P values < 0.01, Student's t test. (*Inset*) Levels of FAAH estimated by Western blot analysis after incubation of the enzymes with trypsin for the indicated time points.

results in changes in the steady-state levels and/or half-life of FAAH *in vivo* remains unknown. However, it is interesting to note that only the homozygous *FAAH* 385A/385A genotype was significantly associated with an increased frequency of street drug use and problem drug/alcohol use. In virtually every lipid metabolic enzyme deficiency disorder described to date, including Gaucher disease, Tay-Sachs disease, Niemann-Pick disease, Krabbe disease, metachromatic leukodystrophy, and many others (27), only the homozygotes exhibit the clinical phenotype. In these cases, the normal phenotype in heterozygous individuals is presumably due to the presence of a single copy of the wild-type gene, which affords an intermediate level of enzyme activity sufficient to prevent full expression of the disease phenotype. The lack of association of the *FAAH* 385A/wt genotype with drug-related neurobehavioral disorders fits with this autosomal recessive model of enzyme deficiency in which the amount of FAAH activity manifested in heterozygotes would be predicted to maintain essentially normal endocannabinoid balance and prevent vulnerability to street drug use and problem drug/alcohol use. Consistent with this notion, $FAAH^{+/-}$ mice exhibit behavioral phenotypes much closer to those of wild-type animals than those of $FAAH^{-/-}$ mice (15).

Although these findings are provocative in that they offer a potential link between functional alterations in the endogenous cannabinoid system and a predisposition to illegal drug use, alternative interpretations of the data are also possible. One confounding possibility is that of linkage disequilibrium, in which the *FAAH* 385C→A mutation would be linked to a nearby risk-determining gene mutation. Data interpretation also could be complicated by stratification bias inherent in the clinical grouping by questionnaire responses. However, clinical studies have demonstrated that a high rate of comorbidity exists between alcoholism and polydrug abuse (28), indicating that these diseases may share a common molecular and cellular foundation. Finally, one must consider that addiction is a complex trait (1), and the *FAAH* cDNA 385C→A mutation is undoubtedly only one of many genetic and environmental factors that may influence the expression of the pathological phenotype. Nonetheless, the *FAAH* 385A/385A genotype was sufficiently prevalent in street drug users and problem drug or alcohol users to detect above the background of many other genetic factors in the populations examined, and, therefore, may prove to be a useful diagnostic predictor of individuals at risk for drug-related disorders. More generally, the association of a naturally occurring human *FAAH* mutation with problem drug use provides further support that the endogenous cannabinoid system may play an important role in neural circuits that underlie drug abuse and dependence.

Data and biomaterials were collected in three projects that participated in the National Institute of Mental Health Schizophrenia Genetics Initiative. Data and biomaterials were collected in four projects that participated in the National Institute of Mental Health Bipolar Disorder Genetics Initiative. Data and biomaterials from autistic subjects were obtained from the Coriell Cell Repositories. We thank Songpon Deechongkit and Jeffery Kelly for assistance with CD and fluorescence measurements as well as helpful suggestions with the manuscript. This work was supported by the Skaggs Scholars in Clinical Science Program from The Scripps Research Institute and by National Institutes of Health Grants DK53505–02, RR00833, DA13173, and DA15197, and by the Stein Endowment Fund. This manuscript is number 14704-MEM from The Scripps Research Institute.

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