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ORIGINAL INVESTIGATION

The fatty acid amide hydrolase 385 A/A (P129T) variant: haplotype analysis of an ancient missense mutation and validation of risk for drug addiction

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Abstract The human fatty acid amide hydrolase (FAAH) missense mutation c.385 C→A, which results in conversion of a conserved proline residue to threonine (P129T), has been associated with street drug use and problem drug abuse. Although a link between the FAAH P129T variant and human drug abuse has been reported, the extent of risk and specific types of substance addiction vulnerability remain to be determined. Here, we investigated the relationship of the FAAH P129T variant to a number of linked single nucleotide polymorphisms to establish a haplotyping system, calculate the estimated age and origin of the *FAAH* 385 C→A mutation and evaluate its association with clinically significant drug addiction in a case control study. The results showed a significant over-representation of the FAAH P129T homozygotes in 249 subjects with documented multiple different drug addictions compared to drug free individuals of the same ethnic backgrounds ($P = 0.05$) using logistic regression analysis controlling for ethnicity. To increase the logistic regression analysis power by increasing the sample size, the data from our previous study (Sipe et al. in Proc Natl Acad Sci USA 99:8394–8399, 2002) were pooled with the present cohort which increased the significance to $P = 0.00003$. Investigation of the *FAAH* chromosomal backgrounds of the P129T variant in both multiple different drug addicted and control subjects revealed a common ancestral haplotype, marked population differences in haplotype genetic diversity and an estimated P129T mutation age of 114,425–177,525 years. Collectively, these results show that the P129T mutation is the only common mutation in the *FAAH* gene and is significantly associated with addictive traits. Moreover, this mutation appears to have arisen early in human evolution and this study validates the previous link between the FAAH P129T variant and vulnerability to addiction of multiple different drugs.

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Introduction

Fatty acid amide hydrolase (FAAH) is a mammalian integral membrane enzyme that terminates the activity of a large class of endogenous nervous system signaling lipids termed the fatty acid amides (endocannabinoids) (Cravatt et al. 1996; Ueda et al. 2000; McKinney and Cravatt 2005). Representative fatty acid amides degraded by FAAH include the endogenous cannabinoid *N*-arachidonoyl ethanolamine (anandamide, AEA), the anti-inflammatory substance *N*-palmitoyl ethanolamine (PEA) and the sleep-inducing lipid 9*Z*-octadecenamide (oleamide, OEA) (McKinney and Cravatt 2005). The central role that FAAH plays in regulating fatty acid amide signaling in vivo has been revealed by animal studies. Most notably, mice with a targeted disruption of the *FAAH* gene ($FAAH^{-/-}$) possess markedly elevated endogenous levels of AEA and related fatty acid amides in the central nervous system and exhibit the typical cannabinoid receptor, type 1 (CB1)-dependent behavioral tetrad consisting of hypomotility, hypothermia, analgesia

and catalepsy (Cravatt et al. 2001). Similarly, administration of FAAH inhibitors to rodents causes a significant increase in brain levels of fatty acid amides that correlates with CB1-mediated anxiolytic and analgesic effects in treated animals (Boger et al. 2000; Lichtman et al. 2004; Jayamanne et al. 2006). These animal studies indicate that FAAH serves as the primary catabolic regulator of fatty acid amide signaling in the nervous system and, therefore, may play an important role in modulating a variety of neurobehavioral processes (Cravatt et al. 2001). Considering that the genetic deletion of *FAAH* in mice results in an adult mammal with greatly increased endogenous brain levels of AEA and related fatty acid amides, polymorphisms in the human *FAAH* gene that produce a functionally altered enzyme also may be expected to shift tonic levels of these CNS signaling lipids (Sipe 2004). Because the brain endogenous cannabinoid system modulates reward and craving pathways, we explored the question of whether naturally occurring human *FAAH* mutations may affect several addictive traits.

We have previously identified a *FAAH* single nucleotide polymorphism (SNP) producing missense mutation (385C→A) that results in the conversion of a proline residue at amino acid position 129 to threonine (P129T) (Sipe et al. 2002). The proline 129 residue is completely conserved among all mammalian and human FAAH enzymes characterized to date (Chiang et al. 2004; Sipe et al. 2002). Subsequent in vivo and in vitro studies in our laboratory demonstrated both reduced cellular expression and activity of the human FAAH P129T variant (Chiang et al. 2004). There is a significant association of the homozygous *FAAH* 385A/385A genotype with an increased frequency of street drug use and problem drug/alcohol use (Sipe et al. 2002), as well as an increased frequency of overweight and obesity in recent case control studies (Sipe et al. 2005).

Our previous sequence analysis of the entire *FAAH* coding region failed to find any other mutation that might predispose to addictive traits (Sipe et al. 2002) but there may be other variations linked to the *FAAH* locus that affect FAAH activity. In a further attempt to identify additional polymorphisms that might be present in genes that carry the 385 A/A mutation, this study focused on sequencing the *FAAH* promoter region and establishing a haplotype system for the *FAAH* locus. This has allowed us to compare haplotypes of subjects with multiple different drug addictions to non-drug using control individuals to investigate whether there are haplotypes that are selectively associated with multiple drugs of addiction. Moreover, this *FAAH* haplotyping system has enabled us to gain an insight into the origins and estimated age of the P129T mutation.

Materials and methods

DNA samples from drug addicted individuals and controls

Anonymous DNA samples ($n = 249$) from subjects with documented multiple different drug addiction disorders, the majority (88.4%) of African-American ancestry, were obtained from NIDA-IRP, Baltimore, MD, USA with approval from the NIH Office of Human Subjects Research (OHSR) and Scripps institutional review board (IRB). The drug addiction group included subjects with documented cocaine ($n = 64$), cocaine + alcohol ($n = 56$), ecstasy ($n = 7$), heroin ($n = 11$), methadone ($n = 28$), methamphetamine ($n = 3$), PCP ($n = 1$), alcohol ($n = 5$) and polysubstance ($n = 74$) dependence based on NIDA /IRP structured interviews and examinations. Control anonymous DNA samples from non-drug using, normal weight individuals (Caucasian $n = 579$, Asian $n = 88$, African-American $n = 118$) were obtained with informed consent from the research normal blood donor program and from patients attending a Health Appraisal Clinic in a study approved by the relevant local IRBs. The ethnic origin of each subject was based on self-identification in a comprehensive health assessment questionnaire linked to each anonymous subject's sample. Genomic DNA was extracted from individual whole blood samples using a QIAGEN DNA purification kit and used directly for polymerase chain reaction (PCR) amplifications and genotyping of subjects.

Identification of polymorphic markers

Single nucleotide polymorphism markers flanking the *FAAH* 385 locus were identified by a largely bioinformatics-driven approach. The SNP Consortium Database (http://www.snp.cshl.org/) was searched for SNPs that reside within the 100 kbp interval. This technique identified ten candidate SNPs, of which six were found to be polymorphic (having a minor allele frequency of 5% or greater) by genotyping them in a panel of 20 control DNA samples: rs12075550 [T/C]; rs6658556 [A/C]; −796A > G; rs932816 [G/A]; IVS1 + 22G > A; and rs4660930 [G/A].

PCR amplifications and sequencing

The NCBI sequence AL122001 was used as the reference genomic sequence for the human *FAAH* gene. Oligonucleotide primers were designed for the promoter region and each SNP of the *FAAH* gene using the Primer3 algorithm (http://www.frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The promoter region and each SNP

of interest in the *FAAH* gene were amplified by PCR amplification and purified using spin column purification (Qiagen, CA, USA). Sequencing was then performed by capillary electrophoresis using an ABI PRISM 3100 Genetic Analyzer. Complete *FAAH* promoter sequencing was performed in 20 multiple drug addiction subjects of African-American origin (40 chromosomes). Mutation nomenclature followed standard guidelines (den Dunnen and Antonarakis 2001) using the *FAAH* sequences: cDNA accession number NM_001441; protein ID NP_001432. The nucleotide immediately 5′ to the start ATG codon was designated −1.

Genotype acquisition and haplotype reconstruction

A group of DNA samples from multiple drug addicted ($n = 93$) and control ($n = 171$) subjects were genotyped for the six SNPs and the P129T mutation by standard allele specific oligonucleotide hybridization and restriction endonuclease methods (Sipe et al. 2002, 2005). Haplotypes were reconstructed from genotypic data by means of the Arlequin software ver 2.0 (Schneider et al. 2000). Median-joining (MJ) networks were drawn using the Network program ver 4.X (http://www.fluxus-engineering.com).

Statistical analysis

Statistical analyses included comparison of each ethnic group's drug addicted subjects with ethnically matched non-drug using, normal weight controls for the frequency of the FAAH 385 A/A (P129T/P129T) genotype versus heterozygotes plus wild type based on our previously published work (Sipe et al. 2002). Logistic regression analysis controlling for ethnicity was performed to determine the contribution of the FAAH P129T homozygous mutant carrier status to drug addiction in the entire drug addicted population in this study because of the small numbers of Caucasians and Asians. For all analyses, *P*-values of ≤ 0.05 were considered statistically significant and odds ratios greater than 1 were considered statistically significant. After the first logistic regression analysis, the FAAH P129T data in the control and drug abusing population from our previous study (Sipe et al. 2002) were combined with the data in this study to provide the second logistic regression analysis of a much larger multi-ethnic data set.

Results

The promoter region (−1,000 to −1 bp) of the *FAAH* gene was analyzed by sequencing. Complete sequencing of the *FAAH* promoter in 20 multiple drug addicted subjects of African-American ancestry (40 chromosomes) revealed no significant new mutations within the promoter region. The FAAH 385 C→A (P129T) missense mutation was examined by comparing the FAAH 385 genotypes of multiple different drug addicted subjects ($n = 249$) to individuals of Caucasian ($n = 579$), Asian ($n = 88$) or African-American ($n = 118$) origin with no history of drug use or addiction. We focused on the 385 locus because our previous sequencing of the entire *FAAH* coding region indicated that the P129T is the only frequent mutation in the *FAAH* gene. The drug addicted group, consisting of 88.4% African-Americans, was genotyped for the P129T mutation. We performed logistic regression analysis on the entire multiple drug addicted population, controlling for ethnicity to compare the P129T/P129T genotype frequencies versus the heterozygote plus wild type homozygote frequencies separating statistically the ethnic groups of Caucasians, Asians, and African-Americans. Logistic regression analysis combining all three groups and controlling for ethnicity was employed to elucidate the contribution of the FAAH P129T mutant homozygous carrier status to drug addiction in the multi-ethnic cohort. In agreement with our previous studies in a Caucasian population (Sipe et al. 2002), the overall contribution of the P129T mutation was higher ($P = 0.05$) in the multiple drug addicted group than in matched non-drug using controls (For P129T carrier status, controlling for race [odds ratio = 2.25 (0.98–5.13, 95% CI) (Table 1)]. When the data from our previous study (Sipe et al. 2002) were combined with the current study's data and logistic regression analysis was performed on this much larger sample set, the overall significance of the contribution of the P129T carrier status to drug addiction was $P = 0.00003$ [odds ratio = 3.20 (1.86–5.51, 95% CI)]. This is in agreement with our previous studies in a single ethnic Caucasian population. All populations were found to be in Hardy-Weinberg equilibrium for the FAAH P129T mutation.

To compare the *FAAH* chromosomal backgrounds of drug addicted and control individuals, a haplotyping system was established for the *FAAH* locus. Six polymorphic SNPs at the *FAAH* locus: rs12075550 [T/C]; rs6658556 [A/C]; −796A > G; rs932816 [G/A]; IVS1 + 22G > A; and rs4660930 [G/A] were combined with previously characterized P129T (385C > A) mutation to form the basis of the haplotype framework (Fig. 1). The compound haplotype flanks the 20 kbp *FAAH* locus on chromosome 1 (nt. 46,572,012–46,591,529), covering an approximately 100 kbp interval (nt. 46,532,012–46,631,529). Analysis of the interval using

Table 1 Population frequencies of the P129T genotypes

Population, abbreviated name (number of subjects)	Caucasian controls, C (n = 579)	Caucasian drug add, CD (n = 27)	Asian controls, AC (n = 88)	Asian drug add, AD (n = 2)	African-American controls, AFC (n = 118)	African-American drug add, AFD (n = 220)
Individual genotypes						
P129T/P129T	12 (2.1%)	1 (3.7%)[a]	5 (5.7%)	1 (50%)[b]	8 (6.8%)	25 (11.4%)[c]
P129T/Wt	179 (30.9%)	6 (22.2%)	30 (34.1%)		47 (39.8%)	106 (48.2%)
Wt/Wt	388 (67.0%)	20 (74.1%)	53 (60.2%)	1 (50%)	63 (53.4%)	89 (40.4%)
Overall allele frequency of P129T	17.8%	14.8%	22.7%	50%	**26.7%**	**35.5%**

The number of individuals (n) included in the logistic regression analysis for each group is given in brackets. Using Logistic Regression analysis of the entire drug addicted population, controlling for ethnicity, the results including all groups comparing the P129T mutant carrier status [a,b,c] with ethnically matched controls resulted in an overall P value = 0.05. When a larger sample size was similarly compared by addition of subjects and controls from our previous study (Sipe et al. 2002) the contribution of the mutant P129T carrier status to drug addiction was more highly significant (P = 0.00003) [odds ratio = 3.20 (1.86–5.51, 95% CI)]

the HapMap data [http://www.hapmap.org; January 2006 release] indicates that there is a high degree of linkage in the approximate 46,532,012–46,631,529 interval on chromosome 1.

To examine the chromosomal background of the P129T mutation and wild-type chromosomes, a sample set of P129T homozygotes (Caucasian n = 34, African-American n = 35 and Asian n = 15) and wild-type individuals (Caucasian n = 30, African-American n = 27 and Asian n = 30) were obtained. These individuals (n = 171) had no history of drug use or addiction. A multiple different drug addicted group (n = 93 individuals; 89 P129T alleles; 97 non P129T alleles) was also analyzed. This group consisted of 45 polysubstance, 24 methadone, 10 heroin, 11 cocaine and 3 ecstasy dependant subjects of African-American ancestry. Each DNA sample was genotyped and haplotypes were assigned according to Arlequin haplotype reconstruction. The haplotypes are defined in the marker order rs12075550 [T/C]; rs6658556 [A/C]; −796A > G; rs932816 [G/A]; IVS1 + 22G > A and rs4660930 [G/A] (Fig. 2a).

Analysis of the P129T alleles showed that the mutation was associated with five compound haplotypes of which H6 [T A A G G G] was the most common, occurring at an overall frequency of 80.6% (Table 2). The MJ network of the P129T compound haplotypes (Fig. 2a) showed a minimally extended network centered on this majority, presumably ancestral, H6 haplotype. In contrast, the MJ network of wild-type *FAAH* chromosomes (Fig. 2b) was more extended and contained nine haplotypes (Table 3). The most common haplotype in the wild type populations was also H6 [T A A G G G] occurring at a frequency of 28.3, 35.0 and 42.6% in the Caucasian, Asian and African-American populations, respectively. When non-P129T chromosomes were analyzed to compare multiple different drug addicted subjects versus control individuals, there were no significant differences in *FAAH* wild-type haplotype frequencies.

Estimates of genetic diversity (h) revealed substantial differences in the level of diversity associated with P129T haplotypes between the populations sampled (Table 2). The Caucasian (0.140 ± 0.056) and Asian (0.186 ± 0.088) P129T populations had lower haplotype diversity compared to the African-American (0.457 ± 0.063) P129T population. Haplotype genetic diversity data for wild-type chromosomes showed similar levels of diversity across all populations samples with an overall $h = 0.781 \pm 0.015$ (Table 3).

To investigate the age of the P129T mutation, the δ parameter of Risch et al. (1995) was used to estimate the degree of linkage disequilibrium (LD) across the

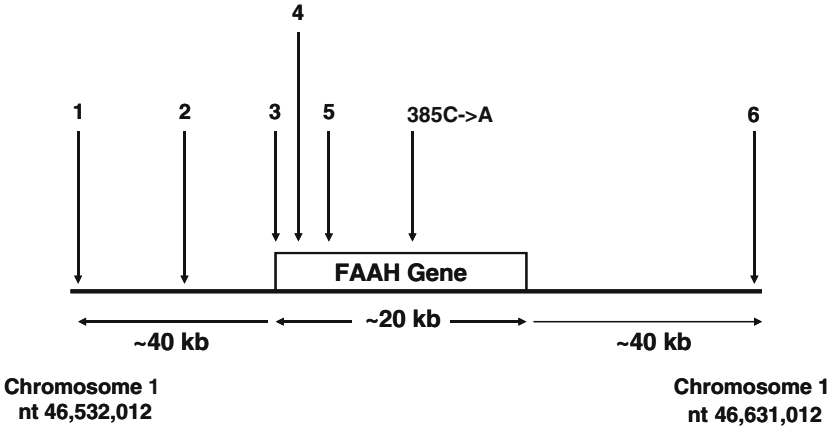

Fig. 1 Haplotyping system for the FAAH 385 locus. The location of the 385C > A (P129T) mutation is shown. The haplotype is defined in the marker order: *1*, rs12075550 [T/C]; *2*, rs6658556 [A/C]; *3*, −796A > G; *4*, rs932816 [G/A]; *5*, IVS1 + 22G > A; and *6*, rs4660930 [G/A]. The entire haplotype spans approximately 100 kbp on chromosome 1p

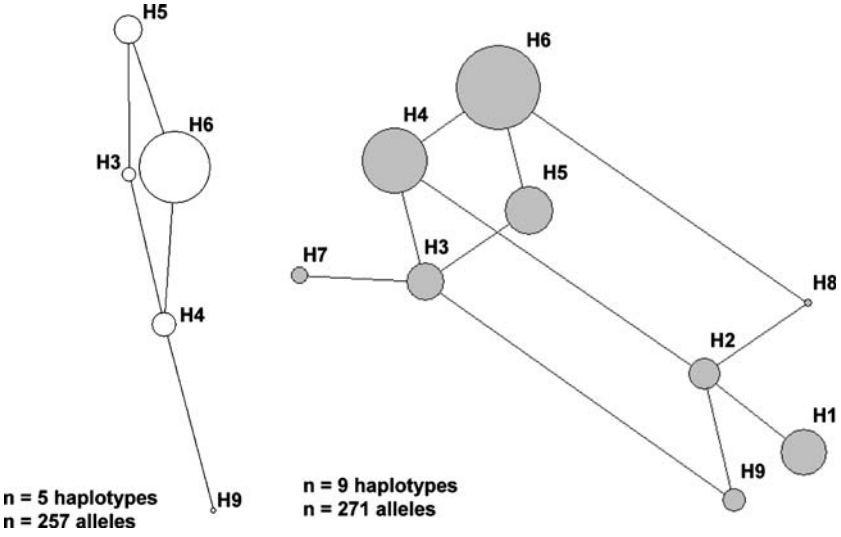

Fig. 2 **a** Median-joining (MJ) network of P129T haplotypes. **b** MJ network of non-P129T haplotypes. For both networks, the size of each node is proportional to the frequency of the haplotype of that node. The haplotype name is also given for each node

Table 2 Haplotypes of the P129T mutation

P129T haplotype	All alleles (n = 257)	Caucasian (n = 68)	Asian (n = 30)	African-American (n = 70)	Multiple drug addicted (n = 89)
H3 C A A G G A	2.3%	–	–	4.2%	3.4%
H4 C A A G G G	7.0%	–	10.0%	5.8%	12.4%
H5 T A A G G A	9.7%	5.9%	–	18.7%	9.0%
H6 T A A G G G	80.6%	92.6%	90%	71.3%	75.2%
H9 C A G A A G	0.4%	1.5%	–	–	–
Genetic diversity, h	0.349 (±0.069)	0.140 (±0.056)	0.186 (±0.088)	0.457 (±0.063)	0.413 (±0.060)

The haplotypes are defined in the marker order rs12075550 [T/C]; rs6658556 [A/C]; −796A > G; rs932816 [G/A]; IVS1 + 22G > A; and rs4660930 [G/A]. Values of haplotype genetic diversity (h) are given as mean ± standard deviation. The number of chromosomes (n) included in the analysis for each population is given in brackets

interval spanned by the *FAAH* compound haplotype (Table 4). The highest values of δ were observed in the markers located closest to the *FAAH* gene, with a major decline of δ values at the 3′ end of the haplotype. To perform the Risch age estimate, the closest SNP marker (rs12075550) to the P129T mutation demonstrating a significant decrease in δ from the ancestral H6 haplotype was used (P_D = 0.903; P_N = 0.498; δ = 0.807). Analysis of the chromosome 1 *FAAH* region showed that the recombination rates are between 0.84 M = 1 Mb and 1 M = 1 Mb for the *FAAH* locus (Payseur and Nachman 2000). This gave a recombination rate of 0.0042 M–0.005 M between the rs12075550 SNP and the P129T mutation (~50 kbp). Age estimation based upon this information yielded an age for the P129T mutation between 114,425 and 177,525 years, using the method of Risch (Table 5).

Table 3 Haplotypes of the non-P129T *FAAH* alleles

Non-P129T Haplotype	All alleles (n = 271)	Caucasian (n = 60)	Asian (n = 60)	African-American (n = 54)	Multiple drug addicted (n = 97)
H1 C G G A A G	11.4%	30.0%	5.0%	3.7%	8.2%
H2 C A G A A G	5.5%	5.0%	–	5.6%	9.3%
H3 C A A G G A	7.4%	3.3%	3.3%	16.7%	7.2%
H4 C A A G G G	21.8%	20.0%	20.0%	25.9%	21.6%
H5 T A A G G A	12.5%	6.7%	30.0%	5.6%	9.3%
H6 T A A G G G	36.9%	28.3%	35.0%	42.6%	40.2%
H7 C G A G G A	1.5%	5.0%	–	–	11.0%
H8 T A G A A G	0.4%	1.7%	–	–	–
H9 C A G A A A	2.6%	–	6.7%	–	3.1%
Genetic diversity, h	0.781 (±0.015)	0.792 (±0.027)	0.752 (±0.028)	0.730 (±0.039)	0.769 (±0.030)

The haplotypes are defined in the marker order rs12075550 [T/C]; rs6658556 [A/C]; −796A > G; rs932816 [G/A]; IVS1 + 22G > A; and rs4660930 [G/A]. Values of haplotype genetic diversity (h) are given as mean ± standard deviation. The number of chromosomes (n) included in the analysis for each population is given in brackets

Table 4 Calculation of the linkage disequilibrium parameter (δ) of the *FAAH* haplotype markers to P129T

Haplotype marker	Main allele with P129T	Distance from 385C > A (kbp)	All P129T pops. (P_D)	All non-P129T pops (P_N)	δ (LD)	African-Americans non-P129T (P_N)	δ (LD)
rs12075550	T	~50	0.903	0.498	0.807	0.482	0.813
rs6658556	A	~25	1.000	0.871	1.000	0.964	1.000
−796A > G	A	~11	0.996	0.801	0.980	0.908	0.957
rs932816	G	~11	0.996	0.801	0.980	0.908	0.957
IVS1 + 22G > A	G	~10	0.996	0.801	0.980	0.908	0.957
rs4660930	G	~60	0.880	0.760	0.500	0.778	0.481

Table 5 P129T allele ages assessed by the linkage disequilibrium method of Risch et al. (1995)

Haplotype marker	Distance from 385C > A	θ (1 M = 1 Mb)		θ (1 M = 1.19 Mb)	
rs12075550	~50 kbp	0.005 M		0.0042 M	
		Age (generations)	Age (years)[a]	Age (generations)	Age (years)[a]
P129T all populations		5005	125,125	7101	177,525
P129T African-Americans		4577	114,425	6495	162,375

The allele ages were estimated using two recombination values (θ) and using either all populations combined or the African-American control non-P129T populations as the ancestral population

[a] Generation time of 25 years

Discussion

FAAH enzyme expression and activity in vivo play a central role in regulating fatty acid amide neuromodulatory signaling lipids (Bisogno et al. 2002; McKinney and Cravatt 2005). From previous studies, it has been shown that a *FAAH* P129T mutation causes decreased FAAH enzyme activity and protein expression (Chiang et al. 2004). This reduction in FAAH activity may result in altered endocannabinoid signaling in reward and craving pathways, which consequently may affect several addictive traits. To now ensure that there were no further mutations at the *FAAH* locus, we have screened the *FAAH* promoter and coding region in a cohort of multiple different drug addiction and control individuals. There were no other mutations found in the *FAAH* region, indicating that the P129T mutation is the only common missense mutation in the human *FAAH* gene. In this study, the NIDA multiple drug different addicted individuals of mostly African-American ancestry were found to have a higher frequency of the P129T mutant allele (35.5%) compared to control non-drug use individuals (frequencies of 17.8, 22.7 and 26.7% in the Caucasian, Asian and African-American populations, respectively). An appropriate method to analyze the contribution of the FAAH P129T mutant carrier status to drug addiction in the entire multi-ethnic sample is to employ logistic regression analysis

controlling for all ethnic groups. When the multiple drug-addicted population was compared to ethnically matched controls using logistic regression analysis to control for ethnicity, the result found a significant ($P = 0.05$) contribution of the P129T homozygous mutant carrier state to multiple drug addiction. Although the present drug addicted and control groups are relatively small, when we performed a second logistic regression analysis for the P129T mutation again controlling for ethnicity by addition of the control and drug use data from our previous study (Sipe et al. 2002) to the data of this study, the result was highly significant ($P = 0.00003$). There is still the possibility of a Type 1 (false positive) error due to population stratification in these data. Specifically, the African-American population contains variable degrees of European admixture. Since the gene frequency of the P129T mutation is higher among Africans, the association between the mutation and drug addiction could be due to a higher proportion of African genes in drug-addicted individuals. It may be possible, and the future, to examine this possibility by studying other markers that have a higher frequency in the African than in the European genome and by determining the degree of population stratification.

To further examine the impact of the P129T mutation and to assess the origins of the P129T mutation, a haplotyping system was established. Haplotypes are a combination of alleles at different markers along the same chromosome that are inherited as a unit. Mutations with a single origin tend to be found predominantly on the same haplotype background, though there may be some decay of the haplotype over time due to recombination. Recurrent mutations will normally be found on several different haplotype backgrounds. Analysis of the haplotype backgrounds of a specific mutation can therefore determine whether the mutation is likely to have had a single or multiple origins (Kan and Dozy 1980). Examination of the P129T alleles showed that the mutation was associated with five compound haplotypes. However, there was no detectable association of a particular haplotype with specific drugs of addiction or risk of becoming addicted.

There was a predominant P129T haplotype (H6), occurring at an overall frequency of 80.6% (Table 2). The H6 haplotype was the majority haplotype in each P129T population analyzed, suggesting that the P129T mutation had a single origin. The minimally extended MJ network of the P129T haplotypes was also consistent with a single origin of the P129T mutation on an H6 chromosomal background. This mutation has then undergone a founder effect to allow spread of the mutation and there has been decay of the H6 haplotype since. When genetic diversity (h) of the haplotypes was estimated, there was a substantial difference in the level of diversity associated with P129T haplotypes between the populations sampled (Table 2). The African-American P129T populations, either drug addiction or non-drug addicted groups, had the highest degree of haplotype diversity. In contrast, wild-type chromosomes showed similar levels of diversity across all populations. As more haplotype divergence is expected where the mutation has resided longest, the P129T mutation would appear to have its origins in an African or African-American population. Interestingly, the P129T variant has its highest frequency in these populations and in this study; the wild-type H6 haplotype had its highest frequency in the African-American population.

To investigate the age of the P129T mutation, the method of Risch et al. (1995) was used to calculate an estimate based upon recombination frequency. This method estimated an age of origin between 114,425 and 177,525 years ago (Table 5), depending on the population and recombination values used to calculate age of the P129T mutation. Extensive archeological and genetic data indicate that modern humans originated within the past 200,000 years in Africa (Mountain et al. 1992; Mountain and Cavalli-Sforza 1997; Tishkoff and Verrelli 2003) There was a suspected migration of modern humans out of Africa within the past 50–100,000 years with subsequent rapid expansion throughout a broad geographic region. These expansions led to the Paleolithic colonization event of Europe (~40,000 years ago) and to the divergence of Caucasian and Asian peoples (~100,000 years ago) (Ammerman and Cavalli-Sforza 1984; Nei and Roychoudhury 1993). From the age estimates of the P129T mutation, the mutation would be suspected to have occurred early in human history and has probably had a single origin in an African population. Subsequent evolutionary forces of genetic drift, human migration, and/or selection would then account for the worldwide incidence of the P129T mutation.

Thus, haplotype analysis of the *FAAH* locus indicates that the P129T mutation is the sole common mutation of the *FAAH* gene and this mutation is significantly associated with addictive traits. Our analysis demonstrates that this mutation has arisen early in human evolution and, in agreement with our previous study (Sipe et al. 2002), it is also over-represented in a population of predominantly African-Americans with addiction to several different drugs.

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