Individual differences in frontolimbic circuitry and anxiety emerge with adolescent changes in endocannabinoid signaling across species


Anxiety disorders typically emerge during adolescence, when the incidence of mental illness peaks (1, 2). The developmental phase of adolescence is characterized by dynamic changes in gene expression, frontolimbic circuitry (3, 4), and overall tone of the endocannabinoid system, which are implicated in anxiety (5–7). The corticolimbic endocannabinoid system undergoes dynamic changes across development. The onset of adolescence is marked by the highest expression of type 1 cannabinoid receptor (CB1) in both cortical and subcortical brain regions, with CB1 expression declining to adult levels throughout adolescence (8, 9). Across the amygdala and prefrontal cortex, fatty acid amide hydrolase (FAAH) expression shows a transient increase from postnatal day 35 (P35) to P45 during adolescence in mice (10). Consistent with the regulatory role of FAAH, anandamide (AEA) levels show an inverse pattern of a peak at P35 and subsequent decrease during adolescence (10, 11). AEA is an endogenous ligand for the CB1 receptor, suggesting that the concurrent changes in AEA and CB1 expression reflect decreasing endocannabinoid signaling during adolescence (12), which may be associated with increasing risk for anxiety (Fig. 1).

A common human polymorphism in the gene encoding FAAH (C385A; rs324420), the primary catabolic enzyme of the prototypical endocannabinoid AEA, regulates FAAH activity. The variant FAAH A385 allele destabilizes the FAAH protein, causing lower levels of FAAH enzymatic activity and/or increased levels of AEA in T lymphocytes and brain (13, 14). Phenotypic expression of common polymorphisms may vary as a function of developmental changes in AEA signaling, gene expression, and neural circuit maturation. Defining the precise interaction of developmental and genetic variation in AEA signaling can advance understanding of the molecular basis of anxiety disorders and facilitate developing FAAH (C385A) as a biomarker for precise targeting of treatments to the biological state of the developing brain.

To enhance the reliability of human genetic studies of FAAH C385A, we have developed a knock-in mouse that biologically recapitulates the human FAAH A385 allele, displaying reduced FAAH protein levels and enzymatic activity and increased AEA levels in the brain relative to wild-type (WT) mice, which express the ancestral FAAH C385 allele (14). We have shown that the variant FAAH A385 allele is associated with selective enhancement of frontolimbic connectivity implicated in anxiety (15–18) and greater fear extinction in both adult humans and mice (14). In light of the quantitative differences in AEA levels across development and because of the genotype at FAAH C385A, we hypothesized that there would be a gene by development interaction, in which the effects of FAAH C385A on frontolimbic connectivity and anxiety emerge during adolescence.

In this study, we implemented parallel studies in mice and humans across development (19) to determine the developmental...
course of FAAH C385A effects on frontolimbic circuitry and anxiety-related behavior. Diffusion tensor imaging data from 1,050 human participants ages 3–21 y old (518 females) from the Pediatric Imaging, Neurocognition, and Genetics (PING) Study (20) and tract tracing in FAAH knock-in and WT mice at P23–P75 were used to measure the interaction of FAAH C385A genotype and age on frontolimbic circuitry relative to control tracts. We specifically hypothesized that FAAH A385 allele effects on enhanced frontolimbic circuitry and anxiety would emerge during adolescence, when AEA levels begin to decrease (10, 11), and therefore, may be less sensitive to the additional quantitative effects of the FAAH A385 allele.

Results

**FAAH C385A-Associated (Phenotypic) Differences in Frontolimbic Circuitry Emerge in Adolescence in Humans and Mice.** Species-specific analyses were performed to assess the effects of FAAH C385A genotype on frontolimbic connectivity across age. In humans, we examined connectivity within the uncinate fasciculus (UF), a white matter tract in the human brain that connects limbic structures in the anterior temporal lobe with ventral and medial prefrontal cortices (21–24) implicated in anxiety (15). This fiber bundle shows a protracted developmental trajectory, with continuing development well into young adulthood (25, 26). We measured fractional anisotropy (FA), an index of connectivity derived from diffusion tensor imaging, and controlled for sex, ancestry, and site (i.e., imaging facility at which the scan occurred). A significant linear effect of age [F(1,1,031) = 305.985; P = 2.2 × 10^-10] on FA was observed in the UF (Fig. S1). Consistent with our earlier results of enhanced adult frontoamygdala connectivity as a function of the FAAH A385 allele (14), there was a main effect of the FAAH genotype on UF FA [F(1,1,031) = 17.858; P = 2.59 × 10^-5].

Moreover, consistent with our hypothesis, there was an age by genotype interaction [age × genotype interaction: F(1,1,031) = 5.893; P = 0.0154] (Fig. 2A), with the genotype effect apparent after 12 y of age [F(1,491) = 14.02; P = 0.0002; trend toward dose dependence association] (Fig. S2) but not before [F(1,523) = 0.513; P = 0.474]. Specificity of the FAAH genotypic effects on frontolimbic circuitry was shown using the corticospinal tract as a control, which showed a similar main effect of age [F(1,1,031) = 531.616; P = 2.0 × 10^-18] but no effect of genotype [F(1,1,031) = 2.699; P = 0.101] or age by genotype interaction [F(1,1,031) = 0.009; P = 0.927] (Fig. S3). The age by genotype interaction on UF FA was significant, even when controlling for corticospinal FA [F(1,1,030) = 6.532; P = 0.011].

We have proposed that knock-in mice that recapitulate common human polymorphisms facilitate controlled experiments on an inbred genetic background and provide a valuable source of validation of human candidate gene associations (19). Therefore, we performed connectivity analyses in FAAH A385 allele knock-in mice (FAAH/AA) and WT littermate controls (FAAH/CC). Given previous findings of FAAH C385A effects on descending infralimbic (IL) to basolateral amygdala (BLA) projections but not ascending BLA to IL projections (14), we focused on IL to BLA projections. The anterograde tracer phaseolus vulgaris leucoagglutinin (PHA-L) was injected into the IL prefrontal cortex of mice to label axonal projections from IL to BLA as a measure of frontoamygdala connectivity (27). Connectivity analyses were performed as a function of age in FAAH CC and FAAH AA mice after PHA-L injections at P23, P45, and P75 corresponding to preadolescence, adolescence, and adulthood, respectively (n = 7 per genotype per age), by quantifying fiber density in the BLA. Consistent with the human findings, the trac tracing experiment revealed a significant age by genotype interaction [F(2,36) = 58.72; P = 0.0001] on fiber density in the BLA (Fig. 2B).

Posthoc Student’s t tests between genotypes within each age group showed enhanced projections from IL to BLA in adolescent [t(12) = 13.81; P < 0.0001] and adult [t(12) = 12.49; P < 0.0001] FAAH AA mice relative to FAAH AA mice but no differences in fiber density in preadolescent mice [t(12) = 0.56; P = 0.583] as a function of FAAH C385A genotype.

**FAAH A385 Allele Is Associated with Lower Anxiety in Adolescence but Not in Childhood Across Humans and Mice.** Prior studies have shown that anxiety disorders peak during adolescence (1, 2) when there are dynamic changes in AEA levels (10, 11) that impact anxiety-related frontolimbic circuitry (28–31). Moreover, we have shown that the FAAH A385 allele is associated with increased frontolimbic connectivity and lower anxiety in both mice and humans in adulthood (14). Based on this culmination of findings and our imaging results showing phenotypic differences in frontolimbic circuitry that emerge during adolescence, we hypothesized that there would be a similar age by genotype interaction with FAAH A385 allele carriers showing less anxiety during adolescence than noncarriers and no phenotypic differences during childhood. We tested this hypothesis in a subset of the PING Study participants with self-reported anxiety symptoms (n = 214) using our imaging results and participant mean age of 12 y old to determine a priori age group boundaries for childhood (less than 12 y of age) and adolescence (12 y of age and older). We showed an age group by genotype interaction for anxiety controlling for sex, ancestry, and site [F(1,198) = 0.23; P = 0.5134] (Fig. 3A). Posthoc analyses controlling for age, sex, ancestry, and site within each age group showed that adolescent FAAH A385 allele carriers had lower self-reported anxiety than noncarriers [F(1,102) = 8.96; P = 0.003], a phenotypic pattern that did not reach significance in children [F(1,83) = 3.82; P = 0.054].

To constrain our interpretation of our human findings and control for genetic and environmental background differences, we tested anxiety-like behavior in FAAH/CC and FAAH AA mice using the elevated plus maze (EPM) at P23, P45, and P65–P75. Consistent with the human findings, the EPM test showed a significant age group by genotype interaction on anxiety-like behavior [F(1,45) = 4.92; P = 0.0316] as measured by percentage of time in the open arms (Fig. 3B).

Posthoc t tests revealed that FAAH AA mice spent more time in the open arms than FAAH/CC mice during adolescence [t(15) = 2.49; P = 0.0261] and adulthood [t(15) = 2.45; P = 0.0326] but not during preadolescence [t(15) = 0.932; P = 0.3687]. There was no FAAH C385A genotype by age group interaction effect on total distance traveled in the EPM, a nonanxiety-related behavior (Fig. S4).
Discussion

This developmentally informed translational approach with parallel studies in mice and humans provides converging evidence that effects of the FAAH C385A polymorphism on frontolimbic circuitry emerge during adolescence. FAAH<sup>A/A</sup> knock-in mice and human FAAH A385 allele carriers show stronger frontolimbic connectivity beginning in adolescence and extending into young adulthood but not in childhood. Consistent with stronger structural connectivity, adolescents but not younger mice or humans with the FAAH A385 allele showed lower anxiety, further suggesting developmental specificity in the phenotypic effects of the FAAH C385A polymorphism. This previously unidentified gene by development interaction may elucidate mechanisms underlying risk for anxiety disorders and suggest an important step toward precision medicine approaches that tailor treatment based on genetic variation and developmental stage (e.g., adolescence).

The adolescent emergence of FAAH C385A effects on frontolimbic circuitry in mice and humans suggests that normative developmental processes moderate the effects of the FAAH C385A polymorphism on frontolimbic development. CB1 expression, FAAH activity, and AEA levels are relatively stable during childhood but fluctuate dynamically across adolescence (10, 11, 32, 33). During this period of fluctuation, the developing system may be especially sensitive to differential expression of FAAH and consequently, AEA levels associated with the variant allele, especially as AEA levels wane. Specifically, as AEA levels decrease during midadolescence, additional quantitative effects of the FAAH A385 allele may increase. The moderation of phenotypic differences by developmental stage was not evident for a control tract, suggesting some specificity of the phenotypic effects to frontolimbic circuit development.

Consistent with the developmental emergence of FAAH C385A effects on frontolimbic connectivity, the effects of the FAAH A385 allele on anxiety also emerged during adolescence. Developmental alterations in the activity of FAAH and consequently, the levels of AEA in the system, may influence the development of neural circuitry and the emergence of anxiety (4). The endocannabinoid system has been associated with anxiety (34, 35), and we have shown that the FAAH A385 variant allele strengthens frontolimbic circuitry, enhances fear extinction, and decreases anxiety-like behaviors in mice and humans during adulthood (14).

Effects of the FAAH C385A polymorphism on frontolimbic circuitry implicated in anxiety (15–18) are a likely mechanism underlying phenotypic differences in anxiety associated with the A385 allele. Specifically, functional effects of the A385 allele may enhance prefrontal regulation of the amygdala during fear (6, 7) through stronger frontoamygdala connectivity (14). In addition to revealing their developmental trajectory, here we extend these findings to structural connectivity in humans. Our prior work suggests that phenotypic differences resulting from the FAAH C385A polymorphism are specific to top-down connections from the IL to BLA, which were not apparent for the BLA to IL, or the connections between the BLA and prelimbic cortex (14). Thus, greater regulation by top-down control of the amygdala may mediate the gain of function that FAAH A385 allele carriers exhibit in the domain of anxiety. Given the smaller sample of participants with anxiety scores in this study and the substantial effects of age on frontolimbic connectivity over this age range, it was not possible to uncouple these effects in a mediation model.

Despite the need for identification of differential genetic effects across development to inform risk and treatment of psychiatric disorders, gene by development interactions have remained largely elusive. Moreover, the challenges of mapping psychiatric disorders onto neurobiology, including nonreplication of past candidate gene studies, has hindered progress in clinical practice. Conducting parallel studies across species allows for...
greater control of environmental and genetic background (e.g., in rodents) while enabling rapid translational discovery by leveraging conserved elements of neural and behavioral systems to identify genotype-phenotype relationships (19, 36–38). By integrating a developmentally informed approach with parallel rodent and human studies (Fig. 4), we have established convergence and cross-species validation of the developmental emergence of FAAH C385A effects on frontolimbic circuitry. Ultimately, such translational studies can facilitate scientific consensus that will permit the implementation of developmental biomarkers guiding clinical practice.

The finding that FAAH C385A effects on frontolimbic circuitry and anxiety emerge during adolescence may prove valuable in enhancing personalized medicine for anxiety disorders, which are the most common psychiatric disorders during adolescence. Despite dynamic changes across brain development, many treatments are based on principles studied in adults; delineating the biological state of the developing brain is critical for optimizing treatment for children and adolescents (3). Tailoring clinical practice based on developmental stage and individual genotypic differences may indicate which treatments will be most effective for which patients. Although any single common polymorphism is unlikely to be sufficient for personalizing treatments, this work may contribute to efforts to target treatments that rely on endocannabinoid signaling (e.g., FAAH inhibitors). Most recently, the role of endocannabinoids in treatment with selective serotonin reuptake inhibitors has been elucidated, suggesting additional implications for use of selective serotonin reuptake inhibitors or their combination with exposure-based therapies. Specifically, BLA-specific CB1 receptors and fluoxetine-induced increases in AEA mediated the effects of fluoxetine (but not citalopram) on fear extinction (39).

Given the high incidence of anxiety disorders during adolescence (1) and prior evidence of diminished fear extinction during adolescence across species (40–42), these findings in the context of recent work on treatment mechanisms related to the endocannabinoid system have significant potential to optimize treatment for anxiety during this sensitive window of development.

### Conclusions

A developmentally informed approach integrating multiple levels of analysis across parallel studies in humans and mice engineered to recapitulate common human polymorphisms can identify reliable and precise genotype-phenotype relationships. In this report, we have shown that the effects of FAAH C385A genotype on frontolimbic connectivity vary as a function of developmental stage, emerging in early adolescence when endocannabinoid signaling is decreasing. This developmental trajectory coincides with the onset of anxiety disorders in many individuals and may contribute to interindividual variation in anxiety disorders and their treatment during adolescence and beyond. Our results underscore the importance of considering normative developmental neurobiology in characterizing the phenotypic effects of common human polymorphisms and their association with atypical development. Moreover, these findings highlight how phenotypic expression of common polymorphisms varies as a function of developmental changes in gene expression and neural circuit maturation and have broad implications for the development of common polymorphisms as potential biomarkers guiding psychiatric care.

### Methods

**Human Participants.** Data used for this study were obtained from the PING Study database (pingstudy.ucsd.edu) (SI Methods) (20). Participants included an initial sample of 1,221 typically developing individuals ranging in age from 3 to 21 y old who had diffusion scans collected in the multisite PING Study. Details on the recruitment, ascertainment, behavioral, genetic, and neuroimaging methods and acquisition in the PING Study are summarized below (details are in refs. 43–46). All participants provided informed consent (age 18 y old and older) or parental informed consent with child assent (ages 7–17 y old) in accordance with policies of the Institutional Review Boards of the sites of participation. Compensation was provided as agreed on during the consent process. In total, 1,050 individuals (mean age = 12.11 y old; SD = 4.80; range = 3.0–21.0; 518 females) from the PING Study database were included in this work after 171 participants from the initial
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2.8; range n The FAAH genotype was acquired by the PING Genomics
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resulting in an average UF FA value for each participant. To test the
were inspected visually to ensure a trajectory consistent with the known shape
resulting probabilistic UF masks (both hemispheres) for each participant
with a single threshold (4,000) held constant for all participants (Fig. 2
any tracts traveling posteriorly from the seed. The FAAH C385A polymorphism on brain biochemistry,
neurocircuitry, behavior, and symptoms (i.e., vertical integration across multiple
levels of analysis) during development and identified a previously unidentified gene by development interaction.

diffusion imaging data preprocessing and FA analysis. Diffusion MRI data were
obtained from the PING Study database. Acquisition protocols for a high-resolu-
tion, T1-weighted scan and diffusion-weighted scans were standardized across
sites dependent on the make of scanner, with most parameters constant across all
locations (SI Method). Image preprocessing and analysis were performed with a
customized processing pipeline using Analysis of Functional Neuroimages (47)
and FMRIB Software Library software packages (48). Images were corrected for
both susceptibility-induced and eddy current distortions (49). Participant head
movement during image acquisition was calculated, and realignment was per-
formed across gradient direction slices. Gradient vector directions were rotated
based on the motion correction. Gradient slices that were found to have head
displacement greater than one-half of a voxel (1.25 mm) were excluded from the
diffusion directions used for additional analyses, because data for that
gradient direction were likely inaccurate because of movement. If more than
one-half of the gradient slices in a participant’s data exceeded the movement
threshold of 1.25 mm, the participant was excluded from additional analysis.

We examined structural connectivity for an a priori tract of interest, the UF,
based on its identification as a major white matter tract connecting the pre-
frontal cortex and amygdala (22) and our prior evidence of phenotypic differ-
cences across mice and humans in frontoamygdala connectivity (14).
Probabilistic tractography was used to determine UF masks on a participant
by participant basis. Diffusion data were modeled using a crossing fiber tech-
nique described previously (Bayesian estimation of diffusion parameters
obtained using sampling techniques) (50). A two-fiber model was created to
perform probabilistic tractography and FA analysis. To determine the location
of the UF in each participant, a mask in standard Montreal Neurological In-
istute (MINI) space was created to use as a seed region for tractography. The
seed was placed at the site where the UF is known to ascend from the tem-
poral lobe and loop anteriorly into the frontal lobe (Fig. 2A). An exclusionary
mask was also placed posteriorly to the seed region to prevent detection of
any tracts traveling posteriorly from the seed.

Participant FA maps were transformed into MNI space using a standard FA
map, and probabilistic tractography was performed using the seed described.
This technique resulted in individualized probabilistic maps, where the highly
connected voxels showed a clear UF-shaped pattern that was easily isolated
with a single threshold (4,000) held constant for all participants (Fig. 2A).
The resulting probabilistic UF masks (both hemispheres) for each participant
were inspected visually to ensure a trajectory consistent with the known shape
of this major white matter tract (51). Each participant’s FA map was then
masked using individualized UF masks. The FA values of included voxels
were averaged, resulting in an average UF FA value for each participant. To test the
specificity for results of analyses of the UF, analyses were also performed on the
corticospinal tract, which served as a control tract (SI Methods and Fig. 53).

Human genotyping. The FAAH genotype was acquired by the PING Genomics
Core by genome-wide genotyping on extracted DNA from saliva samples using the Illumina Human 660W-Quad Beadchip with markers passing quality control
filters (sample cell rate >98%; SNP call rate >95%; minor allele frequency >5%). To assess ancestry and admixture proportions, a supervised clustering approach
was implemented in the ADMIXTURE software (52), which clustered participant
data into six clusters corresponding to six major continental populations: Afri-
can, Central Asian, East Asian, European, Native American, and Oceanic.

Anxiety self-report. A subset of child and adolescent participants in the
PING Study sample provided self-report on the Screen for Child Anxiety-
Related Emotional Disorders (53). All child and adolescent participants
who had genotype and self-reported anxiety (n = 217; mean ± 12.69 ±
SD = 2.8; range = 7.25–17.52; 100 females) were examined in the
analyses of anxiety; three of these participants were excluded because of
extreme scores on the anxiety measures (outliers <1.5x or >1.5x inter-
quartile range), resulting in a total sample of 214 (Table S2).

Mouse model and anterograde tract tracing analyses. We have previously validated
a knock-in mouse model of FAAH C385A; generation of FAAH C385A mice is de-
tailed in our recent work (14). WT mice expressing the human ancestral C385T
replacement with the variant A385 allele resulted in knock-in mice having lower
levels of FAAH protein and higher levels of AEA than WT mice. The FAAH knock-in
mice were backcrossed >10 generations onto C57Bl6 mice (Charles River Labora-
tories). Only male mice were used for all experiments. All animals were kept on a
12:12-h light-dark cycle at 18 °C to 22 °C with food and water available ad libitum.

Five mice were housed together per cage. All procedures were in accordance with
the NIH Guide for the Care and Use of Laboratory Animals (54) and approved by
the Institutional Animal Care and Use Committee of Well Cornell Medical College.

Based on our prior hypothesis about genotypic effects on the development
of frontoamygdala circuitry, we used anterograde tracer in our FAAH knock-
in mouse model to examine connectivity with the IL. Mice were microinjected with
20 μL of PHA-L (2.5% solution (wt/vol) in 0.05 M Tris-buffered saline (TBS) at pH
7.4; catalog no. A5-2300; Vector Laboratories) into the IL at P23, P45, and P75 to
anterograde injections to BI (Fig. 2B and Table S3). Bilateral injections of PHA-L
resulted in all areas analyzed. After PHA-L injections, animals were deeply anesthetized with Euthasol (0.1 mL/10 g
body weight) and perfused through the heart with 30 mL 0.9% saline
followed by 120 mL 4% (wt/vol) paraformaldehyde in a 0.1 M phosphate buffer.

Brains were removed and postfixed with 4% (wt/vol) paraformaldehyde in a
0.1 M phosphate buffer at 4 °C overnight and then, transferred to a sucrose
solution (30% sucrose in 0.1 M phosphate buffer) at 4 °C at 24 °C with food and water available ad libitum.

Injection sites were confirmed by referring to the Allen Mouse Brain Map
(Allen Co.). Sections then were cut on a freezing microtome (40 μm). Free-floating serial sections (take
one out of every three) were washed (three times for 10 min each) in TBS and
incubated for 24 h at 4 °C overnight. Sections were washed (three times for 10 min each) in TBS and
incubated 30 min in a blocking solution containing 4% normal donkey serum
and 1% BSA in TBS with 0.2% Triton X-100. Sections were
washed (three times for 10 min each) in TBS and
incubated for 2 h with the antigen-specific primary antibody (i.e., Fluor-labeled donkey anti-rabbit Alexa Fluor
555 (1:500; Invitrogen). Sections were mounted, coverslipped, and sealed with nail polish.

Injection sites were confirmed by referring to the Allen Mouse Brain Map
(Allen Co.). Sections were observed under a Nikon 80i Fluorescent Microscope.
Digital photography was performed using a MicroFire camera and FireFrame
software (Optronics), and stereological estimation of cell density was per-
formed using StereoInvestigator software (MicroBrightfield). Contours of the IL
and BLA were drawn, and random sampling was applied to contours. Total
volume of BLA was estimated using the Cavalieri method. Total cell numbers in
IL were estimated using a fractionator, with counting frame size of 25 × 25 ×
40 μm and sampling grid size of 100 × 100 μm. Detection of fiber density in
BLA was performed using a stereological method. Briefly, sections were traced
under a 4x lens, and then, perimetrics probe analysis was done under a 40x
lens. Counting frame was set to 25 × 25 μm, and radius of the Merz coherent
test system was set to 5 μm. Total length of all sampling sites was automati-
cally calculated. For each animal, fiber density is obtained from the sum of the
lengths divided by the sum of the areas for all sections. There were no dif-
fences in injections across all genotypes and age groups (Fig. 55).

EPM to measure anxiety-like behavior in mice. The EPM task was conducted as
described previously (SI Methods) (56). All mice studies were blinded to the in-
vestigator with regard to genotype for both the tract tracing and behavior studies.

Statistical analyses. Statistical analyses were performed within the R statistical
software package, version 2.15.1 (R Core Team). For human analyses, ANOVA

<table>
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<th>Gene</th>
<th>FAAH C385A</th>
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<td>Molecular Phenotype</td>
<td>Decreased FAAH activity, increased AEA levels</td>
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<tr>
<td>Neural Phenotype</td>
<td>Increased connectivity of frontolimbic circuitry</td>
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<tr>
<td>Behavioral Phenotype</td>
<td>Decreased anxiety</td>
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<tr>
<td>Clinical relevance</td>
<td>Potential use of FAAH inhibitors for anxiety</td>
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was conducted to examine the effects of age group (under 12 y old versus 12 y old and older) and FAAH genotype [homozygous CC vs. a allele carriers (AA, AC) on FA in mice]. The hypothesis was tested within each age group using a GLM controlling for age (continuous given the wide age range), sex, ancestry, and site. Given a significant age group by genotype interaction was detected, posthoc general linear model (GLM) analyses examined the effect of genotype within each age group in children (under 12 y old) as well as adolescents and young adults (12 y old and older) controlling for age (continuous given the wide age range), sex, ancestry, and site. For mice analyses, ANOVA tested for an age group (P23, P45, or P75) by genotype (WT vs. knock in) on fiber density and the percentage of time spent in the open arms during the EPM. For a significant age group by genotype effect, posthoc Student’s t tests tested the effect of genotype within each age range. For humans, second exploratory analyses used a GLM to test for a dose-dependent effect of genotype on FA (AA > AC > CC) (Fig. 5A) and asymmetry (AA < AC < CC) (Fig. 5B). Statistical tests were two-sided; values of P < 0.05 were considered statistically significant. Data are presented as means ± SEMs.

ACKNOWLEDGMENTS. This work was supported, in part, by the Samuel W. Perry III, MD Distinguished Award in Psychiatric Medicine (to D.G.G.); NIH Grants T32MD017397 (to the Weill Cornell/Rockefeller/Soan Kettering Tri-Institutional MD/PhD Program, R.N.F., A.L., and A.T.D.), RZCD029475 (to A.M.D., T.L.J., and B.J.C.), and U01DA041174 (to B.J.C.); a gift from the Mortimer D. D. D. Foundation; a National Science Foundation Graduate Research Fellowship (to A.D.C.); and the New York-Presbyterian Younger Anxiety Center (F.S.L.).