Association of the Fatty Acid Amide Hydrolase C385A Polymorphism with Alcohol Use Severity and Coping Motives in Heavy-drinking Youth

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Conflict of Interest

RFT has consulted for Quinn Emanuel and Ethismos on unrelated topics. SJK receives funding from Jazz Pharmaceuticals and U.S. NIH NIAAA for studies unrelated to the present investigation. BLF has no relevant COI to declare. Unrelated to the present work: BLF has obtained funding from Pfizer (GRAND Awards, including salary support) for investigator-initiated projects. BLF has some in-kind donation of cannabis product from Aurora and medication donation from Pfizer and Bioprojet and was provided a coil for TMS study from Brainsway. BLF has obtained industry funding from Canopy (through research grants handled by CAMH or University of Toronto), Bioprojet, ACS and Alkermes. BLF has received in kind donations of nabiximols from GW Pharma for past studies funded by CIHR and NIH. All other authors declare no conflict of interest.

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Abstract

Background: Reduced function of fatty acid amide hydrolase, the catabolic enzyme for the endocannabinoid anandamide, can be inherited through a functional genetic polymorphism (*FAAH* rs324420, C385A, P129T). The minor (A) allele has been associated with reduced FAAH enzyme activity and increased risk for substance use disorders in adults. Whether this inherited difference in endocannabinoid metabolism relates to alcohol use disorder etiology and patterns of alcohol use in youth is unknown.

Methods: To examine this question, heavy-drinking youth (n = 302; mean age = 19.74 ± 1.18) were genotyped for *FAAH* C385A. All subjects completed a comprehensive interview assessing alcohol use patterns including the Timeline Follow-back Method, Alcohol Use Disorders Identification Test (AUDIT) and Drinking Motives Questionnaire. ANCOVAs were conducted to assess differences in drinking patterns and drinking motives between genotype groups, and mediation analyses investigated whether drinking motives accounted for indirect associations of genotype with alcohol use severity.

Results: Youth with the *FAAH* minor allele (AC or AA genotype) reported significantly more frequent drinking days (p=0.045), significantly more frequent heavy episodic drinking (p=0.003) and significantly higher consumption patterns (AUDIT p=0.045, AUDIT-C p=0.02). Mediation analyses suggested that the association of *FAAH* C385A with drinking outcomes was mediated by coping motives.

Conclusions: These findings extend previous studies by suggesting that reduced endocannabinoid metabolism may be related to heavier use of alcohol in youth, prior to the onset of chronic drinking problems. Furthermore, differences in negative reinforcement-related drinking might account in part for this association.

KEY WORDS: Alcohol Use Disorders Identification Test (AUDIT), Drinking Motives, Fatty Acid Amide Hydrolase (FAAH), *FAAH* Polymorphism (C385A; rs324420), Youth

Introduction

Alcohol use is common in adolescence and young adulthood. Over 54% of college students in the United States ages 18-22 report alcohol consumption in the past month and 37% indicate at least one occasion of past-month heavy episodic drinking (HED: >4 and 5 drinks per occasion for females and males respectively) (National Institute on Alcohol Abuse and Alcoholism, 2020), a known early risk factor for subsequent development of alcohol use disorder (AUD) (Gowin et al., 2017, Greenfield et al., 2014, Hingson et al., 2017). The progression of occasional drinking to alcohol-related problems including AUD is influenced by multiple factors, including motives to drink (Grigsby et al., 2016), and it has been proposed that young adults are motivated to consume alcohol by the expected positive or negative reinforcement effects (Kuntsche et al., 2005). In particular, drinking to increase pleasure (enhancement motives) is related to higher rates of heavy drinking in youth, while drinking to decrease negative affective states such as anxiety or stress (coping motives) has been more closely linked with AUD and alcohol-related problems (Kuntsche et al., 2005, Neighbors et al., 2007, Hammarberg et al., 2017). Better understanding these motives may assist in investigating how "reward" and/or "relief" drinking might relate to genetic or biological risk factors for AUD.

Recent advancements in understanding AUD have implicated the endocannabinoid system. In particular, fatty acid amide hydrolase (FAAH), the enzyme primarily responsible for the degradation of one of the major endocannabinoid ligands, anandamide, may play a role in the biological vulnerability for AUD (Basavarajappa et al., 2019). In this regard, preclinical studies using FAAH knock-out and knock-in mice models with impaired anandamide degradation, and those using pharmacological inhibition of FAAH, have linked decreased FAAH activity with increased alcohol preference and intake, reduced ethanol sensitivity/intoxication, reduced

alcohol-induced locomotor impairment, and reduced withdrawal effects (Blednov et al., 2007, Zhou et al., 2016, Vinod et al., 2008, Basavarajappa et al., 2006, Zhou et al., 2017). These findings are in line with the observation that rats bred to be alcohol-preferring rats, such as AA Alko and sP Sardinian rats, have lower FAAH protein levels and enzymatic activity prior to alcohol exposure (Vinod et al., 2012, Hansson et al., 2007). Increased alcohol preference and reduced sensitivity to the effects of alcohol are believed to be mediated, at least in part, through the cannabinoid 1 (CB1) receptor, with the majority of preclinical studies suggesting that CB1 stimulation increases alcohol consumption, while CB1 receptor antagonism/inverse agonism decreases alcohol intake (Cippitelli et al., 2005, Linsenbardt and Boehm, 2009, Malinen and Hyytiä, 2008, Hansson et al., 2007, Colombo et al., 2002). It has therefore been proposed that reduced FAAH activity, resulting in increased anandamide, may influence alcohol intake via a CB1 receptor-mediated mechanism (Basavarajappa et al., 2019).

In humans, FAAH levels can vary due to a common missense single nucleotide polymorphism (SNP), *FAAH* rs324420 / C385A. This *FAAH* SNP consists of a cytosine-to-adenine substitution (C385A) resulting in the conversion of a conserved proline residue at amino acid position 129 to a threonine residue (P129T). Minor allele (A) homozygotes and heterozygotes (i.e., AA and AC genotypes, respectively), make up approximately 38% of individuals of European descent (Sipe et al., 2002). These individuals have reduced FAAH protein levels and steady-state enzymatic activity compared to CC homozygotes due to a post-translational mechanism (Chiang et al., 2004), which results in a higher concentration of anandamide (Mayo et al., 2018, Dincheva et al., 2015, Sipe et al., 2010, Boileau et al., 2015). Some (Sipe et al., 2002, Flanagan et al., 2006) but not all (Tyndale et al., 2007, Bühler et al., 2014) studies have shown that individuals with the minor (A) allele have increased vulnerability

for alcohol use and addiction. Of note, a recent study in adult humans of Caucasian/European ancestry with AUD reported that individuals with the AC or AA genotype engaged in significantly more drinking days and heavy drinking episodes and had greater self-reported alcohol use severity compared to CC homozygotes (Sloan et al., 2017). Consistent with this finding, low FAAH brain levels, as measured using positron emission tomography (PET), were associated with increased alcohol consumption in a sample of treatment-seeking individuals with AUD (Best et al., 2020). Together, these findings suggest that low FAAH activity may be related to increased alcohol consumption and vulnerability for AUD, though the mechanism by which this might occur requires further investigation.

In addition to alcohol consumption, FAAH is involved in responses to reward (Scherma et al., 2018), anxiety and stress (Gunduz-Cinar et al., 2013). Overexpression of FAAH attenuated stress response and decreased anxiety-like behaviour in one preclinical study (Morena et al., 2019). Other studies have found low FAAH, as indexed by *FAAH AC/AA genotype,* and, specifically, the *FAAH* C385A minor allele, to be related to lower anxiety and stress-relevant traits including enhanced fear extinction and fear extinction recall, as well as increased reward sensitivity, ventral striatal reactivity and impulsivity (Mayo et al., 2018, Hariri et al., 2009, Mayo et al., 2020). Further, some, though not all, studies of those with this variant have reported lower amygdala reactivity to threat during an fMRI task and self-reported anxiety (Dincheva et al., 2015, Hariri et al., 2009). These studies suggest that reduced FAAH activity may increase alcohol drinking and risk for AUD by impacting reward response and/or stress-reactivity, and that this relationship may therefore be mediated by positive or negative reinforcing motives for drinking. In particular, both positive reinforcement (enhancement) and negative reinforcement (coping) motives are associated with alcohol use severity, with coping motives being a particular

risk factor for alcohol-related problems and the development of AUD (Hammarberg et al., 2017, Kuntsche et al., 2005, Galen et al., 2001, Mezquita et al., 2011). Deficient FAAH activity could affect the experience of intoxication, rewarding properties of alcohol, and/or one's response to stress, therefore affecting coping and/or enhancement motives for drinking. However, no research to date has investigated the endocannabinoid system, and more specifically FAAH, in relation drinking motives as risk factors in the development of AUD.

Another unanswered question is whether the association between low FAAH activity (as indexed by AC/AA genotype) and increased alcohol consumption is evident only following the emergence of AUD, or, is observable early in drinking history, potentially influencing the development of alcohol use problems, as suggested by the preclinical literature. Further, there is little known about the mechanisms by which the endocannabinoid system, including FAAH, might relate to motivation to consume alcohol and the risk for developing AUD. As such, the present study sought to investigate whether the FAAH C385A minor (A) allele (i.e., AC or AA genotype) relates to alcohol consumption and drinking motives in youth. Based on the preclinical literature related to the endocannabinoid system and findings in humans with AUD, we hypothesized that youth with the AC or AA genotype (i.e., those presumed to have lower FAAH activity), would exhibit more hazardous patterns of alcohol consumption as compared to CC homozygotes, and that this relationship may be mediated by genotype differences in coping or enhancement drinking motives.

Materials and Methods

Participants and recruitment

Participants were recruited from community and University settings in Toronto, Canada for participation in one of two larger studies investigating the risk for AUD in youth (Hendershot et al., 2017, Best et al., 2020). To be eligible for inclusion in either of the two larger studies, participants were at least 18 years of age, reported at least one occurrence of HED in the previous 30 days, no current medication or medical condition contraindicating alcohol consumption and no current diagnosis or treatment of a psychiatric condition or substance use disorder, as assessed by the Structured Clinical Interview for DSM-IV (SCID-IV) or the Mini International Neuropsychiatric Interview (MINI). Participants reported no current or past attempts to seek treatment for drinking, and no current or past history of significant alcohol related problems, based on telephone screening questions and the brief Michigan Alcohol Screening Test (Pokorny et al., 1972). Age was restricted in one of the two studies to the range of 18-21 years, while the other study included participants aged 19-25. Regular, recreational cannabis use was not an exclusion criterion for one study but was exclusionary for the second. Demographic information by parent study are reported in supplementary material (Supplementary Table 1). The total sample for the current analyses (N=302) comprised all participants with valid genotype data who also had complete data on the Timeline Follow Back (TLFB) and Alcohol Use Disorders Identification Test (AUDIT) questionnaire. Forty additional participants were consented in one of the studies but were not included in the current analyses due to missing genotype data (n = 18) and/or incomplete questionnaires (AUDIT n = 7, TLFB n = 15).

Demographics are reported in Table 1. The sample was comprised of 53% women. 51% identified as Caucasian/of European descent and 78% were currently enrolled as post-secondary students. All participants were 19-25 years of age (mean: 19.88 ± 1.2) and reported at least one

heavy drinking episode in the past month. Alcohol use, AUDIT and Drinking Motive Questionnaire Coping and Enhancement scores are reported in Table 2.

Procedures

All procedures were approved by the Centre for Addiction and Mental Health Research Ethics Board. All participants completed an initial phone screening questionnaire; those who met age and alcohol consumption criteria (at least one occasion of HED in the past month) without any current, serious pre-existing medical, psychiatric or substance use conditions were invited for a baseline session. Data were collected during the baseline session prior to the completion of any other laboratory and/or neuroimaging sessions that the participant may have qualified for. Questionnaires were administered to assess recent alcohol use (described below). In addition, recent cannabis use was assessed and the Barratt Impulsivity Scale (BIS) was used to evaluate impulsivity. Urine samples were collected to test for recent use of other drugs of abuse.

Measures

Alcohol Consumption. A Timeline Follow Back (TLFB) interview (Sobell and Sobell, 1992) was used to assess alcohol use over the past 90 days. The TLFB is a calendar-based assessment that uses cues to facilitate accurate recall of recent drinking behavior. Participants reported the number of standard drinks they consumed on each day over the past 90 days, and data were used to derive indices of past 90-day drinking frequency, average number of drinks consumed per drinking occasion, and frequency of heavy episodic drinking (defined as 4/5 or more drinks per occasion for women/men). Participants completed the 10-item AUDIT (Saunders et al., 1993), which contains items assessing alcohol consumption, alcohol-related

harms, and alcohol dependence symptoms. The AUDIT-C (comprising the AUDIT consumption items, items 1-3, range: 0-12) provides an index of consumption, whereas the AUDIT total score (range: 0-40) provides a continuous index of hazardous drinking severity.

Drinking Motives. Two 5-item subscales of the Drinking Motives Questionnaire-Revised (Cooper, 1994) were used to assess internal reinforcement motives for positive reinforcement and negative reinforcement drinking (i.e., enhancement and coping motives). The enhancement motives scale is comprised of the sum of 5 items assessing the frequency of drinking for the enjoyment or pleasure derived from alcohol consumption (e.g. "Because it gives you a pleasant feeling"). The coping motives scale is, likewise, comprised of the sum of 5 items assessing the frequency of drinking to reduce negative mood and to help put worries out of one's mind (e.g., "To forget about your problems"). Participants rated each item on a scale from 1 = *almost never/never* to 5 = *almost always/always.* Other drinking motives domains (e.g., social and conformity motives) were not examined because our hypotheses concerned *FAAH* genotype differences in internal (rather than external/social) aspects of alcohol reward or reinforcement.

Cannabis and Nicotine Use. The NIDA-Modified Alcohol Smoking and Substance Involvement Screening Test (NM ASSIST) was used to assess recent, self-reported cannabis use frequency over the last 90 days. Participants responded on a 5-point scale ranging from *Never* to *Daily or Almost Daily* Participants recruited through the smaller of the two parent studies reported their recent use of cannabis using the 90 day TLFB, if applicable. From this, the number of days per week on which cannabis was used was determined and this data was adapted to the NM ASSIST. For example, participants reporting no occasions of cannabis use in the past ninety days were scored *"Never"* on the NM ASSIST, whereas participants reporting one or two occasions of cannabis use on the 90-day TLFB were scored as *"once or twice"*. Those reporting

weekly cannabis use less than once per week, but at least once per month were scored on the NM ASSIST as "*monthly"*. Finally, one participant reported using cannabis once per week, and was scored *"weekly"* on the NM ASSIST. It should be noted that positive cannabis urine toxicology was an exclusion criteria for this smaller study, and, as such, these participants reported minimal cannabis use (see Supplementary Table 1 for more details). Nicotine use was self-reported, and nicotine dependence was assessed using the Fagerström test of Nicotine Dependence (Heatherton et al., 1991).

Genotyping. DNA was extracted from either venous blood samples (n = 23) or saliva samples (n = 279); a 260/280 ratio of above 1.8 was the minimal quality for DNA used. *FAAH* genotyping (rs324420) was performed as previously described (Boileau et al., 2015). Briefly, the *FAAH* genotype (rs324420) was determined using the Taqman SNP genotyping assay set performed on a ViiA7 thermal cycler (Life Technologies, Burlington, Ontario, Canada) with appropriate controls. Five µl of 2x GTXpress Master mix (cat#4401892, Life Technologies) was mixed with 10 ng of DNA and the 40 × probe (cat#C_1897306_10, Life Technologies) in a final volume of 10 µl and run for 50 cycles of 95°C for 1 second and 60°C for 20 seconds. Positive controls include DNA samples of all three genotypes and negative controls include samples with DNA replaced by water; a minimal ViiA7 quality score of 98% was required.

Data Analysis

Prior to analyses, distributions of all variables were screened for extreme outliers (defined as greater than 3.29 standard deviations from the mean and being notably disconnected from the other scores (Tabachnick et al., 2007). Extreme outliers were observed on the drinking frequency (n=1) and heavy drinking frequency (n=2) variables; these values were Winsorized

(i.e., recoded to one unit greater than the next most extreme value) to reduce their influence in the models. We then examined descriptive statistics comparing *FAAH* genotype groups on demographic variables. Given that only n=14 participants were homozygous for the A allele, we had low power to examine these participants separately in the main analyses. Thus, we combined participants that were AA homozygous with those AC heterozygous into an AC/AA, genotype group *(AC/AA group)* (n=116) and compared them to participants homozygous for the C allele (*CC group*) in all analyses (Sloan et al., 2017, Dincheva et al., 2015). Differences in demographic characteristics, including cannabis use and BIS impulsivity scores, were assessed using independent samples t-tests or chi-squared tests, where applicable.

In order to test the hypothesis that the AC/AA group would drink more heavily and hazardously relative to the CC group, we conducted a series of ANCOVAs comparing frequency of drinking, average drinks per occasion, frequency of heavy episodic drinking, and total AUDIT score between the AC/AA group and CC group. We also ran an ANCOVA with enhancement and coping motives as outcomes to explore whether there were genotype differences in coping-related drinking motivations. ANCOVAs included participant sex and race (white vs. non-white) and age as covariates. We also examined models including Genotype*Sex and Genotype*Race interactions to explore whether sex and race moderated any effects of genotype on drinking outcomes. Although the original studies were not powered based on this research aim, the sample size allowed for detection of effect sizes as small as partial $\eta^2 = 0.026$ for the covariate adjusted mean differences between *FAAH* genotype groups with 80% power and alpha of 0.05. For comparison, the current study was powered to detect standardized differences in unadjusted means as small as $d = 0.33$, which is on the lower end of the range reported in the largest study on *FAAH* and alcohol to date, which found genotype differences in AUDIT scores with effect

sizes ranging from $d = 0.26$ (African American sample, n = 144) to $d = 0.74$ (Caucasian sample, n = 63).

Next, we tested the hypothesis that coping or enhancement drinking motives might mediate the relationship between genotype and drinking outcomes. To do so, for any motive subscale that was significantly different across genotype groups (based on the ANCOVAs described above), its role as a mediator was examined using the SPSS PROCESS macro, which uses bootstrapping to derive bias corrected confidence intervals (CI) of the mediated effect (Hayes, 2017). In each model, we specified sequential indirect effect pathways from genotype to coping motives to alcohol consumption to AUDIT. Specifically, genotype group (CC=0, AC or AA=1) was specified as a predictor of coping or enhancement motives (z-score transformed), which in turn was specified as a predictor of each alcohol consumption index (drinking frequency, average drinks per occasion, heavy episodic drinking frequency, each examined in a separate model). Given that heavier alcohol consumption is thought to underlie hazardous/harmful drinking outcomes, each model then included hazardous drinking severity assessed with the AUDIT as the final outcome. For all mediation models, sex, race and age were included as covariates in all estimated paths. Support for significant mediation was observed when the 95% bias corrected confidence interval for the indirect effect did not contain zero. SPSS Statistics software was used for all analyses, and level of significance was set at $p < 0.05$.

Results

Table 1 shows participant demographics by genotype group. The groups did not differ significantly with respect to sex, ethnicity and proportion of currently enrolled students. Of the 302 participants, 61.6% were CC homozygotes, 33.8% AC heterozygotes and 4.6% AA

homozygotes. The minor (A) allele frequency in this sample (.215) was consistent with other reports involving predominantly Caucasian participants (e.g., Sloan et al., 2017), and genotype distributions did not diverge from Hardy-Weinberg equilibrium. The AC/AA group had a significantly higher mean age than the CC genotype group, though all enrolled participants were between 18 and 25 years of age. Scores on the Barratt Impulsivity Scale did not significantly differ between genotype groups. Further, we found no significant difference in frequency of smokers, level of tobacco dependence or cannabis use between groups.

Table 2 shows the covariate-adjusted means, standard deviations, *F* tests, and effect size estimates (partial η^2) from the ANCOVAs comparing the AC/AA and CC groups on alcohol-related outcomes and enhancement and coping drinking motives (controlling for sex, race, and age). Supplementary Table 2 contains the unadjusted means, standard deviations and standardized mean differences (*d*) between the genotype groups on each of the outcomes. As shown in Table 2, the AC/AA group reported significantly greater frequency of alcohol consumption and significantly greater heavy episodic drinking frequency, without a significant difference in the average number of drinks per occasion (Figure 1a). The AC/AA group also had a significantly higher total AUDIT scores (Figure 1a) than the CC group (Table 2). AUDIT-C (consumption) scores were also significantly elevated in AC/AA group (Figure 1a) compared to the CC group. Finally, the AC/AA group had significantly greater endorsement of coping motives for drinking (Figure 1b), but no difference in enhancement drinking motives (Figure 1b). Effect sizes for all of the significant group differences were small (partial η^2 ranged from .013 to .029), suggesting that that genotype group uniquely accounted for 1%-3% of the variance in these outcomes. Of note, none of the interactions between genotype and sex or genotype and race were significant in any of these models (all $p>.05$), suggesting that sex and race did not moderate

the effects of genotype. Further, when including sample source (i.e., which of the two studies the participants were drawn from) as an additional covariate in the ANCOVAs, none of the main effects for genotype changed, and there were no main effects of sample source on any of the outcomes (all $p > 0.21$).

Significant main effects of sex were observed for the AUDIT, AUDIT-C, and drinks per occasion (all $p < .05$), with men having higher means than women. Significant main effects of race were observed for AUDIT-C, drinking frequency, and heavy episodic drinking frequency (all $p < .05$), with white participants having higher means than non-white participants. Age was also a significant covariate in several models, with older participants reporting more frequent drinking, lower average drinks per occasion, lower AUDIT total scores, and lower coping and enhancement motives (all $p < .05$).

Next, coping motives were investigated as a potential mediator of the relationship between genotype and each alcohol consumption outcome (frequency, quantity, heavy drinking episode), which in turn were specified as mediators of the association between coping motives and AUDIT score. As enhancement motives did not differ between genotype groups, only coping motives were tested in these mediation analyses. The results of these analyses are depicted in Figure 2. In all models, genotype was a significant predictor of coping motives, and coping motives, in turn, significantly predicted the alcohol outcomes. The alcohol outcomes, in turn, predicted AUDIT scores in all three models. In all models, the indirect association from genotype to coping motives to alcohol consumption (frequency, quantity, or heavy episodic drinking) to AUDIT was statistically significant as indicated by 95% CIs that did not contain zero. In addition, in the models that included drinking frequency and average drinks per occasion, the direct paths from genotype to the drinking variable was not significant when

controlling for the mediated pathway through coping motives (see Figure 2a and 2b), suggesting that coping motives fully mediated the pathway from genotype to alcohol use and AUDIT in these models. Further, in all models, the direct path from coping motives to AUDIT was statistically significant, suggesting that alcohol consumption variables only partially mediated the link between coping motives and AUDIT in these models.

Discussion

The present study reports that youth with the A allele (AC or AA genotype) of the *FAAH* C385A polymorphism (i.e., those with presumed reductions in endogenous FAAH) engage in more hazardous drinking patterns through more frequent HED and reporting higher AUDIT scores. This group also reported significantly higher endorsement of coping motives for drinking. Further, our mediation models suggest that there is a significant indirect effect of genotype with risk for more hazardous drinking, such that the AC/AA group has a greater tendency to drink for coping-related reasons, which in turn relates to more frequent and heavier alcohol consumption, and more hazardous drinking outcomes (AUDIT).

Our findings support and extend the current literature in which lower FAAH, through genotypic differences or pharmacological manipulation, has been associated with AUD (Sloan et al., 2017, Best et al., 2020), increased severity of alcohol consumption (Basavarajappa et al., 2019). The present study reports complementary data to that which has incorporated neuroimaging and blood phenotypes to investigate the endocannabinoid system in relation to AUD and risk for addiction (Best et al., 2020, Boileau et al., 2016, Mansouri et al., 2020). As previous studies have focused on preclinical and/or adult populations, the present study is the second (and largest to date) to investigate this relationship in a youth population. Consistent with our findings, Sloan *et al*. reported that older Caucasian adults (mean age of 39 years) with the

AC/AA genotype with AUD had a significantly higher frequency of heavy drinking episodes and higher AUDIT scores compared to CC homozygotes (Sloan et al., 2017). As expected, based on the difference in populations investigated, Sloan *et al.,* reported higher rates of alcohol use compared to the present study. Effect sizes for genotype differences in drinking outcomes were in the small range in the present study (Table 2), which is consistent with the lower end of the range of effect sizes reported in Sloan *et al*. (see also Supplementary Table 2 for comparison), and would be expected in genetic association studies. As such, any clinical significance of these findings remains unclear. Nonetheless, our finding of a mean difference in AUDIT scores of 1.33 could imply clinical significance in some contexts, given that a 1-point difference in AUDIT score could reflect changes in consumption of 1-2 drinks per day or up to 30 drinks per week, depending on the item. Together, these two studies represent the largest samples investigated to date and suggest an association between AC/AA genotypes for the *FAAH* polymorphism and increased vulnerability for more hazardous alcohol consumption that persists across age and diagnostic groups.

By focusing on a youth sample, the current study begins to translate to humans the preclinical literature in which alcohol-naïve alcohol-preferring rats were found to have low FAAH (Vinod et al., 2012, Hansson et al., 2007) and genetically engineered mice homozygous for the A-allele of the *FAAH* C385A SNP showed increased "binge" alcohol consumption (Zhou et al., 2016). Our finding is, however, inconsistent with another smaller study in youth which used a different method of measuring 'risky drinking'(Bühler et al., 2014), as compared to the 90 day TLFB used in the present and other related study (Sloan et al., 2017). By studying both alcohol consumption and drinking motives in a large sample of young drinkers, the present study is the first to investigate in humans whether the relationship of low FAAH activity (due to the

410 *FAAH* C385A minor allele) and increased hazardous alcohol consumption is relevant to AUD
411 etiology, and specifically, whether the association between *FAAH* genotype and drinking
412 motives reflects a possible mechanism.
413 Here we report the association of inherited low FAAH activity with increased hazardous
414 alcohol consumption and drinking motives, specifically negative reinforcement/coping motives
415 (i.e., drinking to reduce negative affect); we found no association with positive
416 reinforcement/enhancement motives (i.e., drinking to enhance positive affect) or impulsivity.
417 There are multiple ways that the presence of this *FAAH* C385A polymorphism, which has been
418 shown to decrease FAAH activity in brain and increase anandamide across species (Dincheva et
419 al., 2015, Mayo et al., 2018, Boileau et al., 2015, Spagnolo et al., 2016, Sipe et al., 2010), might
420 increase alcohol consumption. One possibility is that impaired anandamide degradation may
421 increase the rewarding properties of alcohol through the established, yet complex interaction
422 between endocannabinoids and the dopaminergic system. In relation to the *FAAH*
423 polymorphism, heterozygous AC mice have enhanced mesolimbic dopamine circuitry (Burgdorf
424 et al., 2020), while humans and mice with the *FAAH* C385A polymorphism have significantly
425 elevated D3 receptor levels in the brain (Mansouri et al., 2020), and increased striatal reward
426 reactivity (Hariri et al., 2009). This suggests that dysregulation of the endocannabinoid system
427 might impact the dopaminergic response to reward, in turn driving drug-seeking behavior.
428 In addition, alcohol's rewarding or negative reinforcement properties may also be
429 enhanced through functional interactions of the endocannabinoid and opioid systems (Haller et
430 al., 2008), or through modulation of alcohol-induced GABAergic neurotransmission. As
431 indicated by studies in FAAH KO mice, low FAAH increased ethanol-reward behavior,
432 decreased sensitivity to ethanol-induced effects and reduced mu-opioid activation as compared to

433 wild-type animals (Vinod et al., 2008). It has also been suggested, based on studies in mouse
434 models, that decreased FAAH activity results in reduced sensitivity to alcohol's effects, including
435 intoxication and sedation, and that differences in GABAergic neurotransmission may contribute
436 to these effects (Pavon et al., 2019, Zhou et al., 2016). In humans, this could translate to
437 differential sensitivity to acute alcohol effects, which in turn might influence alcohol
438 consumption. In particular, our finding that inherited low FAAH activity relates specifically to
439 coping, but not enhancement, motives, raises the possibility that individuals with lower FAAH
440 may show differences in the magnitude of sedative or anxiolytic effects experienced under acute
441 alcohol. An alternate possibility is that sedative or anxiolytic effects of alcohol do not differ in
442 magnitude but are nonetheless experienced as more rewarding for these individuals. Either
443 possibility could promote greater use of alcohol for negative reinforcement reasons (i.e. drug
444 taking that alleviates a negative emotional state (Koob, 2014)).

445 The current study is the first to report coping motives as a mediator of the previously
446 reported finding that individuals with the *FAAH* C385A minor allele report increased frequency
447 and more hazardous patterns of alcohol consumption (Sloan et al., 2017). While this suggests
448 that reduced FAAH activity may influence increased alcohol consumption in part through
449 negative reinforcement mechanisms, the association between FAAH and coping motives is
450 complex and there is limited literature exploring the endocannabinoid system, coping and
451 drinking motives, specifically. In line with our findings, it has been established that youth who
452 endorse drinking to cope report increased alcohol consumption and have increased risk for
453 negative outcomes related to alcohol (Corbin et al., 2013, Grigsby et al., 2016). However, the
454 preclinical and clinical literature generally suggests that lower FAAH is associated with reduced
455 anxiety during alcohol withdrawal (Cippitelli et al., 2008), lower trait anxiety, reduced amygdala

reactivity to threats and lower stress reactivity (Dincheva et al., 2015, Mayo et al., 2018, Hariri et al., 2009, Patel et al., 2017). Acute and chronic stress has been shown to upregulate FAAH protein expression, such that individuals with the AC or AA genotype (Gray et al., 2015, Gray et al., 2016), those with less functional FAAH protein, would catalyze less anandamide than CC homozygotes under conditions of stress. Furthermore, endocannabinoid system activation via pharmacological FAAH inhibition has been shown to attenuate stress-induced hypothalamic-pituitary-adrenal axis activity (Gray et al., 2015).

Given evidence that a genetic predisposition to lower FAAH activity generally relates to lower anxiety/stress reactivity, the current evidence for an association with *greater* motivation for negative reinforcement drinking may at first appear contradictory. However, the anxiolytic effects of decreased FAAH activity are notably more prominent under conditions of high environmental aversiveness (Patel et al., 2017) and FAAH inhibition has also been shown to attenuate stress-induced reductions in salience and reward-seeking behavior (Rademacher et al., 2008). Further, FAAH and endocannabinoid tone may influence coping behavior such that FAAH inhibition and increased anandamide promote more focused problem solving in light of anxiety-inducing environmental stimuli (Aliczki et al., 2016). Finally, alcohol-related stress-response has been shown to be context-dependent, whereby acute alcohol intoxication dampens aversive stress-reactivity to uncertain or variable threat (Gorka et al., 2020, Bradford et al., 2013). Together with the decreased experience of alcohol intoxicating effects seen in preclinical investigations, individuals with the AC or AA genotype may therefore be inclined to consume alcohol for the alleviation of negative affect in a context-dependent manner. Though negative reinforcement is traditionally associated with later stages of AUD development, the present relationship with coping motives presents a potential mechanism by which those with the *FAAH*

minor allele may be at greater risk for developing AUD, but future research is necessary to further elucidate these relationships.

Our study is not without limitations. While other studies investigating the *FAAH* C385A polymorphism and addiction or alcohol use have found ethnic-specific effects, our limited sample size did not allow for investigation of the impact of *FAAH* genotype on alcohol consumption patterns within an ethnic group. One previous study found an association of the AA homozygous genotype with alcohol abuse and addiction in a predominantly African American group (Flanagan et al., 2006), while another only found an effect of the A-allele in Caucasian individuals with AUD (Sloan et al., 2017). Though ethnicity was taken into consideration as a covariate, it is difficult to predict how this may have impacted our results. Another limitation of the present and other clinical studies investigating FAAH and AUD is that alcohol consumption data have been based on self-report using measures such as the AUDIT or TLFB. To more thoroughly investigate this relationship and support the translation of findings from alcohol-administration procedures in preclinical studies, laboratory-based alcohol challenge studies are warranted in humans.

Other limitations of the current study include the cross-sectional design and the inability to examine individuals with the AA homozygote genotype independently, as we may not be able to account for differential effects in this specific genotype, as some studies have reported. We acknowledge that there has been limited assessment of other relevant phenotypes, including anxiety, and as such, cannot rule out the potential impact of this on our results. Additionally, comprehensive diagnostic information was not collected on all participants included in the present analyses, therefore it cannot be ruled out that some participants may have reported early symptoms of AUD. Finally, the present study did not directly measure FAAH or FAAH substrate

concentrations (e.g. anandamide, oleoylethanolamide or palmitoylethanolamide). It cannot be ruled out that the presently reported association between the *FAAH* polymorphism and increased alcohol consumption may also be influenced by increased FAAH substrates other than anandamide (e.g. oleoylethanolamide and palmitoylethanolamide) and future studies would benefit from these measurements in addition to genotype. Nonetheless, the present study provides a developmental context for the investigation of the impact of FAAH on AUD by suggesting that innate differences in the endocannabinoid system relate to differences in alcohol consumption patterns in youth, potentially via differences in the negative reinforcement value of alcohol consumption. The association of low FAAH and increased risk for the development of AUD may indeed predate the onset of alcohol problems, and more research is warranted to further understand the mediators of this relationship.

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Table 1: Baseline demographics of participants

Characteristics of the Study Sample	CC Genotype (n = 186)	AC/AA Genotype (n = 116)	p value	χ2 / t value
Sex (Females/Males), n (%)	100 (54%)/86 (46%)	59 (51%)/57 (49%)	.623	0.24
Age, Years†	19.74 (1.18)	20.10 (1.3)	.012*	-2.53
Ethnicity (Caucasian/Non-caucasian), n (%)	98 (53%)/88 (47%)	55 (47%)/61 (53%)	.373	0.80
Currently Enrolled as a Student, n (%)	151 (81%)	85 (73%)	.328	2.23
Current Cigarette Smoker, n (%)	41 (22%)	29 (25%)	.560	0.34
Fagerstrom Test of Nicotine Dependence†[a]	1.12 (1.35)	1.33 (1.73)	.574	-0.56
Self-reported Current Frequency of Cannabis Use, past 90 days				
"Never", n (%)	62 (33%)	38 (33%)	.069	8.70
"Once or twice", n (%)	49 (26%)	23 (20%)		
"Monthly", n (%)	14 (8%)	12 (10%)		
"Weekly", n (%)	33 (18%)	13 (11%)		
"Daily or almost daily", n (%)	26 (14%)	29 (25%)		
Barratt Impulsivity Scale - Total†	64.6 (10.67)[b]	63.60 (10.80)	.411	0.82

Table 2: Results of the the ANCOVA analyses of covariate-adjusted mean differences between the genotype groups on the alcohol variables

	FAAH CC Genotype (n=186)		*FAAH* AC/AA Genotype (n=116)				
	Adjusted Mean†	**SE**	**Adjusted Mean†**	**SE**	***F***	***p***	**partial η^2**
TLFB 90 Days							
Number of drinking days	18.25	0.86	21.06	1.09	4.064	0.045*	0.013
Average Number of Drinks per Drinking Occasion	4.82	0.15	5.28	0.19	3.37	0.068	0.011
Number of Heavy Drinking Episodes	9.77	0.73	13.29	0.93	8.79	0.003**	0.029
AUDIT							
Total Score	9.84	0.41	11.17	0.52	4.05	0.045*	0.013
AUDIT-C	5.71	0.13	6.22	0.17	5.48	0.02*	0.018
Drinking Motives Questionnaire							
Coping Motives	10.59	0.33	12.12	0.42	8.13	0.005**	0.027
Enhancement Motives	16.14	0.36	17.16	0.46	3.07	0.081	0.01

Table Legends

Table 1 - †Values represented as mean (SD); *p < 0.05 for independent t-tests or Pearson's chi square tests conducted between genotype groups of the same parent study. [a]based on a subset of participants who reported regular nicotine use and completed the Fagerström Test of Nicotine Dependence (n = 68). [b]based on a subset of participants who completed the Barratt Impulsivity Scale (n = 278).

Table 2 - Covariate-adjusted means, standard deviations, *F* tests, and effect size estimates (partial η^2) from the ANCOVAs comparing the AC/AA and C/C groups on outcomes from the 90-day Timeline Followback (TLFB), the Alcohol Use Disorders Identification Test (AUDIT) and the Drinking Motives Questionnaire. †means adjusted for covariates sex, race and age; *p < 0.05; **p < 0.01.

Figure 1.

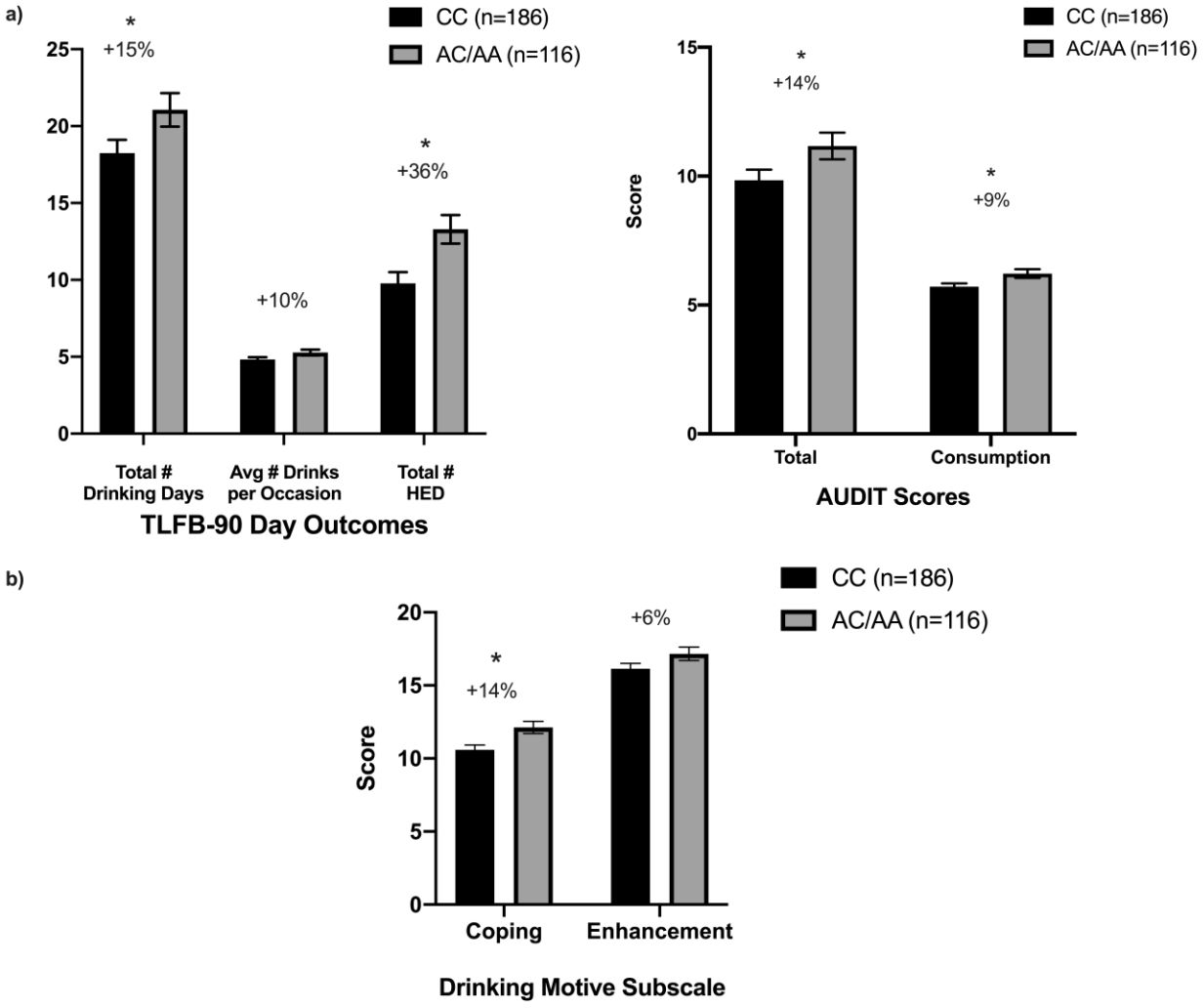
a)
CC (n=186)
AC/AA (n=116)
*
+15%
+10%
*
+36%
25
20
15
10
5
0
Total # Drinking Days
Avg # Drinks per Occasion
Total # HED
TLFB-90 Day Outcomes
*
+14%
*
+9%
Score
Total
Consumption
AUDIT Scores
b)
+6%
*
+14%
Coping
Enhancement
Drinking Motive Subscale

Figure 2.

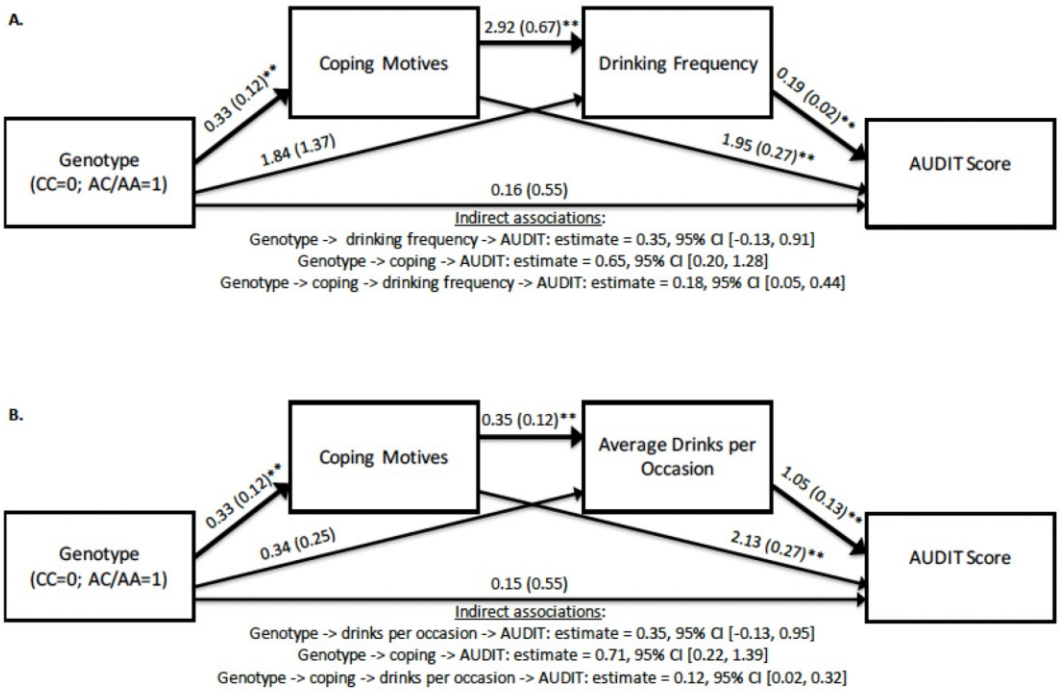
A.
Genotype
(CC=0; AC/AA=1)
Coping Motives
Drinking Frequency
AUDIT Score
0.33 (0.12)**
2.92 (0.67)**
0.19 (0.02)**
1.84 (1.37)
1.95 (0.27)**
0.16 (0.55)
Indirect associations:
Genotype -> drinking frequency -> AUDIT: estimate = 0.35, 95% CI [-0.13, 0.91]
Genotype -> coping -> AUDIT: estimate = 0.65, 95% CI [0.20, 1.28]
Genotype -> coping -> drinking frequency -> AUDIT: estimate = 0.18, 95% CI [0.05, 0.44]
B.
Genotype
(CC=0; AC/AA=1)
Coping Motives
Average Drinks per Occasion
AUDIT Score
0.33 (0.12)**
0.35 (0.12)**
1.05 (0.13)**
0.34 (0.25)
2.13 (0.27)**
0.15 (0.55)
Indirect associations:
Genotype -> drinks per occasion -> AUDIT: estimate = 0.35, 95% CI [-0.13, 0.95]
Genotype -> coping -> AUDIT: estimate = 0.71, 95% CI [0.22, 1.39]
Genotype -> coping -> drinks per occasion -> AUDIT: estimate = 0.12, 95% CI [0.02, 0.32]

Figure Legends

Figure 1 – Comparison of covariate adjusted means from alcohol-related outcomes between FAAH C385A genotype groups (CC genotype vs AC/AA genotypes) using ANCOVAs with age, sex, and race as covariates. Alcohol consumption outcomes include the (a) Timeline Followback Method assessment of total number of drinking days (average number of drinks per drinking occasion and total number of heavy episodic drinking occasions in the past 90 days); (b) Alcohol Use Disorders Identification Test (AUDIT) total and consumption scores; and (c) Drinking Motives Subscale scores for coping and enhancement motives. * indicates a statistically significant difference ($p<0.05$).

Figure 2 – Mediation models of indirect associations from genotype to coping motives to alcohol consumption (A. drinking frequency; B. average drinks per occasion; C. heavy episodic drinking frequency) to AUDIT score. Sex, race (white vs. non-white) and age are controlled in each model by regressing each outcome variable (coping motives, alcohol consumption and AUDIT) on each of these covariates. Estimates of the indirect associations contained within each model are shown below each path diagram with 95% bias-corrected confidence intervals (CI). *$p<0.05$, **$p<0.01$.