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Genetically reduced FAAH activity may be a risk for the development of anxiety and depression in persons with repetitive childhood trauma

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Abstract
Fatty acid amide hydrolase (FAAH) inhibitors are addressed for promising anxiolytics, but human studies on genetically reduced FAAH activity, stress and affective phenotypes are scarce. We investigated the effect of a functional polymorphism of FAAH (*FAAH* C385A or rs324420; low FAAH activity and high anandamide concentration are associated with the A allele) together with childhood adversity on the anxious and depressive phenotypes in 858 subjects from the general population. Phenotypes were measured by the Zung Self-Rating Depression Scale (ZSDS), the depression and anxiety subscales of the Brief Symptom Inventory (BSI-DEP, BSI-ANX) and the State-Trait Anxiety scales (STAI-S, STAI-T). Childhood Adversity Questionnaire (CHA) was used to assess early life traumas. Frequency of the A allele was greater among subjects with high ZSDS scores compared to the CC genotype. Furthermore, *FAAH C385A* and the CHA have shown a robust gene–environment interaction, namely, significantly higher anxiety and depression scores were exhibited by individuals carrying the A allele if they had high CHA scores compared to CC carriers. These data provided preliminary evidence that genetically reduced FAAH activity and repetitive stress in the childhood are associated with increased vulnerability for anxiety and depression in later life. Our results together with earlier experimental data suggest that permanently elevated anandamide level together with early life stress may cause a lifelong damage on stress response

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probably via the downregulation of CB1R during the neurodevelopment in the brain. It may also point to pharmacogenomic consequences, namely ineffectiveness or adverse effects of FAAH inhibitors in this subpopulation.

1. Introduction

The pathological role of stress in the development of affective disorders has been well known for a long time and the underlying molecular mechanism is intensively investigated. Biological evidence supports that childhood trauma leads to marked disturbance of molecular and structural development in the neuroendocrine regulatory system, such as HPA hyper-activity, increased immune activation and reduced hippocampal volume which is also detectable in anxious and depressive disorders (Heim et al., 2008; Frodl and O'Keane, 2013). Further, in epigenetic studies hypermethylation of the DNA in the region of glucocorticoid receptor and BDNF gene promoters have been found in suicide victims who were exposed to childhood trauma (McGowan et al., 2009; Keller et al., 2010).These data suggest that early life trauma causes lifelong changes in the HPA axis, the major regulatory system for the stress response.

The endocannabinoid (eCB) system has a special role in buffering stress reaction due to its unique effect on recovery from stress. The eCB system is composed of receptors (cannabinoid type 1 receptor, CB1R and cannabinoid type 2 receptor, CB2R), endocannabinoids (eCBs, such as 2-arachidonoylglycerol, 2-AG; anandamide, AEA and N-acylethanolamine, NEA), transporter, synthetizing, and catabolic enzymes. The CB1Rs are highly expressed in corticolimbic regions mediating anxiety, including the medial prefrontal cortex, the hippocampus and the basolateral amygdala (Mackie, 2005). CB2Rs are presented mainly in the periphery but are also expressed with less activity in microglia and some neuronal population of the brain (Onaivi et al., 2006). The main substrates of the CB1Rs are the 2-AG and AEA and activated CB1Rs have an inhibiting effect on the release of several neurotransmitters including GABA and glutamate. The fatty acid amide hydrolase (FAAH) enzyme is responsible for the AEA degradation, thus it indirectly regulates the CB1R signaling via the modulation of the availability of AEA.

During the initial phase of acute stress the concentration of AEA is decreased by FAAH hydrolysis in the amygdala and prefrontal cortex which allow HPA activation and optimal fast cortisol response (Hill et al., 2009; McLaughlin et al., 2014). Following this quick stress-response the eCBs, especially 2-AG, plays a significant role in the recovery of the HPA axis to baseline as an effector in the negative feedback mechanism initiated by glucocorticoid receptor (GR) activation in the hippocampus and prefrontal cortex (Wang et al., 2012). In case of chronic stress, data from animal studies suggest that prolonged FAAH activity is resulting in chronic reduction of AEA level which is associated with heightened anxiety. In line with the above observations, CB1R antagonism enhances the basolateral amygdala excitability and

activates the HPA axis inducing anxiety-like behavior (Patel et al., 2005; Newsom et al., 2012; Meye et al., 2013). Consequently, experimental data suggest that increasing AEA level by FAAH inhibition is an effective strategy to reduce HPA axis activation and elicit anxiolytic effect (Haller et al., 2009; Hill et al., 2009), although this effect was dependent on the investigated brain regions and conditions of the experiment (Haller et al., 2009).

Because the AEA level is dependent on the enzymatic activity of the FAAH, the functional genetic variants of the *FAAH* gene may play a crucial role in the development of affective disorders via its regulatory role on the endocannabinoid signal. The *FAAH C385A* polymorphism is a missense functional variant with a significant effect on enzymatic activity. Results from a study of human T-lymphocytes suggested that AA genotype carriers have approximately 50% decreased enzymatic activity and also have higher AEA plasma concentration compared to CC genotype carriers (Chiang et al., 2004). As it is proposed by some reviews the *FAAH C385A* might have significant effect on several mental disorders (Hillard et al., 2012; Gunduz-Cinar et al., 2013a, 2013b), however, only a few human studies addressed this issue in relation to affective disorders so far.

A significant effect of the *FAAH C385A* has been demonstrated in the emotional-motivational responses in humans by two fMRI studies. Hariri et al. reported that A allele of the *FAAH C385A* was associated with decreased amygdala reactivity during a face allocation task involving fearful and angry faces while an increased ventral striatal reactivity was shown in a gambling test indicating an association with impulsivity and not with trait anxiety (Hariri et al., 2009). In another fMRI study, CC carriers exhibited increased emotional responsiveness towards unpleasant pictures and reduced emotional reactivity in response to pleasant pictures (Conzelmann et al., 2012; Gunduz-Cinar et al., 2013a, 2013b). A allele carriers are better to habituate to fear and show less stress response (negative emotionality). Data on *FAAH C385A* in association with human anxiety or depression are poorly available. Monteleone et al. (2010) reported that A allele was presented in a greater number in patients with bipolar and major depressive disorder than in healthy controls (Monteleone et al., 2010), although an earlier study did not find any association between the FAAH C385A and any psychiatric disorders (Sipe et al., 2002).

Despite the central role of the eCB signaling in stress response, the interacting effect of the *FAAH C385A* and stressful life events has not been investigated on affective disorders so far in human genetic studies.

In this paper we report a significant interaction of the *FAAH C385A* polymorphism and early childhood trauma on anxious and depressive phenotypes. This fact draws attention for a potential pitfall in the recently running clinical trials of FAAH inhibitors.

2.   Experimental procedures

2.1.   Study sample

Nine-hundred and twenty-eight unrelated volunteers (30.2% males and 69.8% females, mean age 31.2 ± 10.5) were recruited in the study from practices of general practitioners, adult students participating in a long-distance learning program and community-based population. The inclusion of subjects was independent of any positive psychiatric anamnesis. After filtering all missing data, a dataset of 858 subjects were analyzed in genetic association tests. All subjects were Hungarian and of Caucasian origin and they have given a written informed consent before entering the study. The study was approved by the Central Ethics Committee.

2.2.   Phenotypic measurements

2.2.1.   Background questionnaire
The Background questionnaire was developed by our team. It collects some information about the subject's medical history, educational, employment and marital status. Lifetime prevalence of the depression was measured based on this instrument. Drug consumption was also questioned in the instrument. Based on these data nobody used any drugs.

2.2.2.   Brief Symptom Inventory
The Brief Symptom Inventory (BSI) is a 26-item self-report measurement developed from its longer version, the SCL-90-R (Derogatis, 1993). With the use of the 26-item brief version questionnaire obsessive-compulsive, interpersonal sensitivity, depression, anxiety and additional symptoms can be evaluated. Each item is scored on a 5-point scale ranging from 0 to 4. In this study we analyzed the anxiety (BSI-ANX) and depression (BSI-DEP) subscales of the BSI in association with *FAAH C385A*. We used BSI-ANX and BSI-DEP as a continuous weighted score similarly to our previously published papers (Juhasz et al., 2014, 2015).

2.2.3.   State-Trait Anxiety Inventory
The State-Trait Anxiety Inventory (STAI) is a 40-item, self-rating instrument developed for measuring two dimensions of anxiety: 'trait' (STAI-T) and 'state' (STAI-S) anxiety (Spielberger, 1970). Both types of anxiety are rated by a 4-point Likert scale scoring from 1 to 4. State anxiety corresponds to the subject's emotional condition during the completion of the questionnaire whereas the trait anxiety is defined as a stable anxious tendency based on daily experiences of the responders.

2.2.4.   Zung Self-Rating Depression Scale
Besides the BSI-DEP subscale, the Zung Self-Rating Depression Scale (ZSDS) was used to assess the depressive phenotype (Zung et al., 1965). ZSDS is a 20-item self-rating instrument (with each item scored on a 4-point Likert scale from 1 to 4) that measures several types of depression symptoms including mood, cognition, impulsivity and suicidal ideation.

The ZSDS is a valid, reliable instrument used in several studies in order to measure depressive symptoms. Higher scores correspond to more frequent symptoms, thus this qualitative scale provided the dependent variable representing the depressive phenotype in the total sample (Biggs et al., 1978; Gabrys and Peters, 1985; Agrell and Dehlin, 1989).

2.2.5.   Childhood Adversity Questionnaire
Childhood adversity (CHA) was evaluated by a shorter version of the Childhood Trauma Questionnaire (Bernstein et al., 1997). All four items related to emotional and physical abuse or neglect are originated from the Childhood Trauma Questionnaire, and scored on a 5-point Likert scale (from 0 to 4). The CHA was completed with an additional question concerning the loss of parents during childhood. The sum of scores was entered into the statistical models in the interaction analyses. All statistical analyses included CHA as a continuous variable. For illustration of the associations between childhood adversity, *FAAH C385A* and phenotypic variant, a categorical variable was created, namely CHA 1 (0 point), CHA 2 (1–3 points) and CHA 3 (more than 3 points).

2.3.   Genotyping procedures

Buccal mucosa samples were obtained from all participants and genomic DNA was isolated according to a protocol described by Freeman et al. (2003).

A functional polymorphism in upstream of the *FAAH* gene (rs324420, also named as C385A) was genotyped using the Sequenom MassARRAY technology (Sequenom, San Diego). The IplexTM assay was performed according to the manufacturer's instructions (http://www.sequenom.com) using 25 ng of DNA. Genotyping was blinded with regard to phenotype. All laboratory work was performed under the ISO 9001:2000 quality management requirements.

2.4.   Statistics

Main effects of the *FAAH C385A* on the phenotypic variables were analyzed with linear regression analyses using PLINK v1.07 software (http://pngu.mgh.harvard.edu/purcell/plink/) under three models (additive, dominant and recessive). To assess the effect of the childhood adversity in interaction with the *FAAH C385A*, linear regression analyses were performed using the PLINK software and for post hoc tests we applied the SPSS 20.0 software. To account for multiple testing we applied the Bonferroni's criteria to correct *p*-values. Since the association tests were performed with five phenotypic instruments under three genetic models *p*-values less than 0.0033 were accepted as significant associations ($0.05/15 = 0.0033$). All analyses were adjusted for age and gender.

Minor allele frequencies and Hardy-Weinberg equilibrium were calculated using the HaploView 4.2 software.

To estimate the power of the analyses we used the Quanto 1.2 software.

3.   Results

3.1.   Descriptive statistics

FAAH C385A allele frequencies were 0.79 for C and 0.21 for A. The genotype distribution (CC=532, CA=287 and AA=42) was in accordance with the Hardy-Weinberg equilibrium ($p=0.382$) and corresponded to available genotype data from other European populations (HapMap CEU release #28).

Lifetime prevalence of depression and anxiety disorders reported by the participants were 20.9% and 19.7% in the

study sample which is not deviated significantly from the national average (Szadoczky et al., 1998). The proportion of subjects who scored more than 48 points on the ZSDS was 5.8% which is an estimated prevalence of current episode of depression and it is also in accordance with the Hungarian statistics. The mean of Childhood adversity scores was 1.39 ± 0.68 and the most frequent answer was zero. Mean scores and standard deviations on depression and anxiety scales are shown in Table 1.

Both depressive and anxious phenotypic scores were higher in women than in men. However, CHA did not significantly differ between the genders.

3.2. Genetic association tests

Main effect of *FAAH C385A* (rs324420) was not significant on any of the phenotypic variables in any genotypic model (Table 2). However, carrying A allele meant a two-fold higher risk to score more than 48 points on ZSDS compared to CC genotype with a nominal significance (OR=2.053, p=0.016).

Interaction analyses with childhood adversity and *FAAH C385A* yielded significant associations with anxious and depressive phenotypes under a dominant model. In regression analyses A allele carriers showed significantly higher BSI-ANX and STAI-T scores than individuals with homozygous CC genotype of *FAAH C385A* if they had higher CHA scores ($p_{interact}$=0.00002; $p_{interact}$=0.0023, respectively; Table 3). Depressive phenotypes were also associated with *FAAH C385A* and childhood adversity in a similar manner. Significantly higher BSI-DEP and ZSDS scores were observed in *FAAH C385A A* carriers when they reported childhood adversities compared to CC carrier population ($p_{interact}$=0.0005; $p_{interact}$=0.004, respectively; Table 3). In case of STAI-S and ZSDS scales the associations did not remain significant after correction for multiple testing.

Figure 1 shows the mean scores of phenotypic scales in different genotypes and CHA subgroups (Figure 1).

The estimated power to detect interaction on BSI-DEP was 93.3% (β_G=−0.068, β_E=0.053, β_{GE}=0.054) and on BSI-ANX was 98.1% (β_G=−0.176, β_E=0.0367, β_{GE}=0.069).

4. Discussion

This is the first report on a significant interaction effect of *FAAH C385A* polymorphism with childhood adversity on anxiety or depressive phenotypes. We found that those A allele carriers who experienced high childhood adversity scored higher on BSI-ANX, STAI-T, BSI-DEP and ZSDS scales compared to CC genotype carriers with a massive significance. In addition, our results on higher frequency of A allele among subjects with more than 48 points on the ZSDS compared to others are consistent with findings of Monteleone et al. (2010). They reported that A allele of *FAAH C385A* was presented with a higher number in patients with bipolar and major depressive disorder (MDD) than in healthy control population (Monteleone et al., 2010). Nevertheless, significance of this association failed to hold after correction for multiple testing both in our study and in the study of Monteleone et al. (2010) suggesting that this result needs to be replicated and clarified in further studies. Interesting to note that the *FAAH C385A* A allele was the risk variant for MDD in the Psychiatric Genomics Consortium latest mega-analysis, although it only showed a trend (p=0.065) (Major Depressive Disorder Working Group of the Psychiatric GC, 2013).

There are four important aspects to discuss of our results on the significant interaction between childhood adversity and *FAAH C385A* on anxiety or depressive phenotypes. First, the molecular consequence of the genetic variant of FAAH. Second, the effect of the repetitive stress on the eCB signaling during neurodevelopment. Third, the role of gender in the association between eCB and affective phenotype. The fourth is the potential pharmacogenomic consequences of these results.

4.1. The molecular consequence of the genetic variant of FAAH

Previous data suggested that A allele of the FAAH C385A is associated with reduced enzymatic activity and as a consequence with higher AEA level. Several experimental studies demonstrated that acute inhibition of FAAH through the increased AEA level was correlated with anxiolytic effect. However, human data are controversial. Significantly increased AEA level has been found in patients with MDD (Hill et al., 2008; Ho et al., 2012) and elevated levels of eCB have been observed in post mortem brain tissues of alcoholic suicide victims (Vinod et al., 2005).

The anxiolytic effect of AEA is mediated by the CB1Rs which has inhibitory properties on glutamate release which is responsible for the activation of HPA axis in stress. As it was described earlier, this mechanism depends on the amount of AEA availability and sensitivity and number of CB1R. Permanently elevated AEA level inhibits CB1R expression and lower density of the CB1Rs are associated with impaired inhibitory function of the endocannabinoid system resulting in sustained HPA hyperactivity and increased vulnerability to anxiety. Accordingly, accumulating evidence suggest that repeated treatment with URB597, a selective FAAH antagonist (corresponding similar condition to genetically reduced enzymatic activity), resulted in a decreased CB1R expression accompanied by impaired neurogenic cell proliferation and cell survival in hippocampus and hypothalamus of the rat brain (Rivera et al., 2015). These data together suggest that decreased expression of CB1R lead to definitive changes in brain structure during neurodevelopment determining later life behavior. Thus we can assume that in case of A allele carriers, similarly to chronic FAAH inhibition (Pacher and

Table 1 Mean scores on the depression and anxiety scales in the total sample.

Measurement	Mean ± S.D.
BSI-ANX	0.69 ± 0.70
BSI-DEP	0.55 ± 0.68
STAI-S	37.62 ± 11.26
STAI-T	39.66 ± 10.59
ZSDS	38.60 ± 5.69

BSI-ANX, Anxious subscale of the Brief Symptom Inventory; BSI-DEP, Depressive subscale of the Brief Symptom Inventory; STAI-S, State anxiety subscale of the State-Trait Anxiety Inventory; STAI-T, Trait subscale of the State-Trait Anxiety Inventory; ZSDS, Zung Self-Rating Depression Scale.

Table 2 Main effects of *FAAH C385A* on the depression and anxiety scales.

	n	ZSDS	*n*	BSI-DEP	*n*	BSI-ANX	*n*	STAI-S	*n*	STAI-T
ADD										
CC	529	38.5 ± 5.5	532	0.54 ± 0.67	532	0.69 ± 0.7	527	37.1 ± 10.9	528	39.5 ± 10.3
CA	284	39.3 ± 5.9	284	0.65 ± 0.77	284	0.73 ± 0.78	282	38.6 ± 11.8	283	41.3 ± 11.4
AA	41	37.4 ± 8.3	42	0.52 ± 0.70	42	0.65 ± 0.63	41	38.2 ± 12.3	41	37.4 ± 9.9
DOM										
CC	529	38.5 ± 5.5	532	0.54 ± 0.67	532	0.69 ± 0.69	527	37.1 ± 10.9	528	39.5 ± 10.3
CA+AA	325	39.0 ± 6.3	326	0.63 ± 0.76	326	0.72 ± 0.77	323	38.5 ± 11.9	324	40.8 ± 11.3
REC										
AA	41	37.4 ± 8.3	42	0.52 ± 0.65	42	0.65 ± 0.63	41	38.2 ± 12.3	41	37.4 ± 9.9
CA+CC	813	38.8 ± 5.8	816	0.58 ± 0.70	816	0.70 ± 0.72	809	37.6 ± 11.2	811	40.1 ± 10.7

BSI-ANX, Anxious subscale of the Brief Symptom Inventory; BSI-DEP, Depressive subscale of the Brief Symptom Inventory; STAI-S, State anxiety subscale of the State-Trait Anxiety Inventory; STAI-T, Trait subscale of the State-Trait Anxiety Inventory; ZSDS, Zung Self-Rating Depression Scale, ADD, additive genetic model; DOM, dominant genetic model; REC, recessive genetic model.

Table 3 Interaction effects of *FAAH C385A* and childhood adversity on phenotypic variables in linear regression analyses.

	Beta	S.E.	95% C.I.	STAT	*p*
BSI-DEP					
FAAH C385A (DOM)	-0.07	0.06	-0.19-0.06	-1.08	ns.
CHA	0.05	0.01	0.03-0.07	5.48	$5.64E-08$
FAAH C385A (DOM) × CHA	0.05	0.02	0.02-0.08	3.50	0.0005
BSI-ANX					
FAAH C385A (DOM)	-0.18	0.07	-0.30-0.05	-2.71	0.007
CHA	0.04	0.01	0.02-0.06	3.71	0.0002
FAAH C385A (DOM) × CHA	0.07	0.02	0.04-0.10	4.32	$1.72E-05$
ZSDS					
FAAH C385A (DOM)	-0.58	0.53	-1.61-0.45	-1.10	ns.
CHA	0.37	0.08	0.22-0.53	4.65	$3.78E-06$
FAAH C385A (DOM) × CHA	0.37	0.13	0.12-0.62	2.88	0.004
STAI-S					
FAAH C385A (DOM)	-0.73	1.06	-2.81-1.35	-0.69	ns.
CHA	0.59	0.16	0.27-0.90	3.64	0.0003
FAAH C385A (DOM) × CHA	0.60	0.26	0.09-1.10	2.29	0.0219
STAI-T					
FAAH C385A (DOM)	-0.97	0.97	-2.88-0.93	-1.00	ns.
CHA	0.69	0.15	0.40-0.98	4.69	$3.11E-06$
FAAH C385A (DOM) × CHA	0.72	0.24	0.26-1.19	3.05	0.0023

BSI-ANX, Anxious subscale of the Brief Symptom Inventory; BSI-DEP, Depressive subscale of the Brief Symptom Inventory; STAI-S, State anxiety subscale of the State-Trait Anxiety Inventory; STAI-T, Trait subscale of the State-Trait Anxiety Inventory; ZSDS, Zung Self-Rating Depression Scale; CHA, Childhood Adversity; DOM, dominant genetic model.

Kunos, 2013) downregulation of CB1R occur and serves as a possible mechanism underlying the anxiogenic and depressogenic effect of this polymorphism via sustained HPA hyperactivity and decreased cell proliferation during the neurodevelopment (Figure 2).

Animal studies repeatedly demonstrated that chronic stress eventually leads to blunted eCB signaling in the brain through also downregulation of CB1Rs (Wamsteeker et al., 2010; McLaughlin et al., 2014; Hill et al., 2005). It has been proposed that during chronic stress the reduced CB1R density is due to the inhibitory effect of high corticosteroid level on CB1R expression. As it has been mentioned above, lower CB1R density exerts weaker inhibition on glutamate release which sustains HPA activation. Thus our findings may reflect

the CB1R downregulating effect of the repetitive stress. However, our findings underpinned that for phenotypic manifestation of the affective vulnerability, a combined effect of the A allele of *FAAH C385A* with the repetitive early life stress are needed on CB1R expression (Figure 2).

4.2. The effect of repetitive childhood adversity on eCB signaling during the neurodevelopment

Although the gene encoding the CB1R has been investigated in interaction with stressful life events (Juhasz et al., 2009; Agrawal et al., 2012), the *FAAH* gene together with stress, especially with childhood traumas were not investigated in
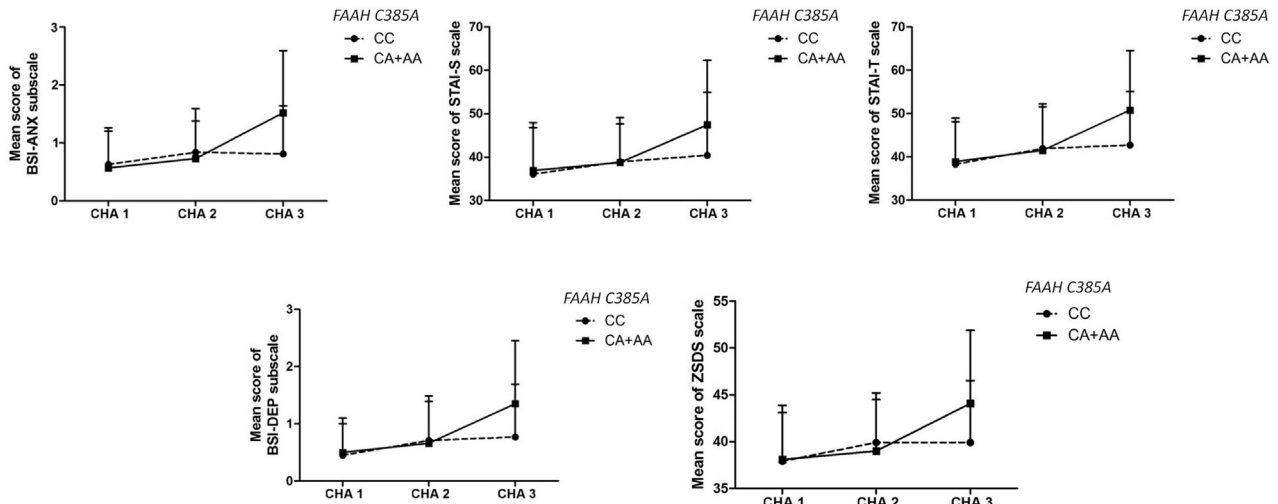
Figure 1 Interactions between FAAH C385A and childhood adversity on anxious and depressive phenotypes.

glutamate

glutamate receptor

FAAH C385

FAAH 385A

CB1R

AEA

Glucocorticoid receptor
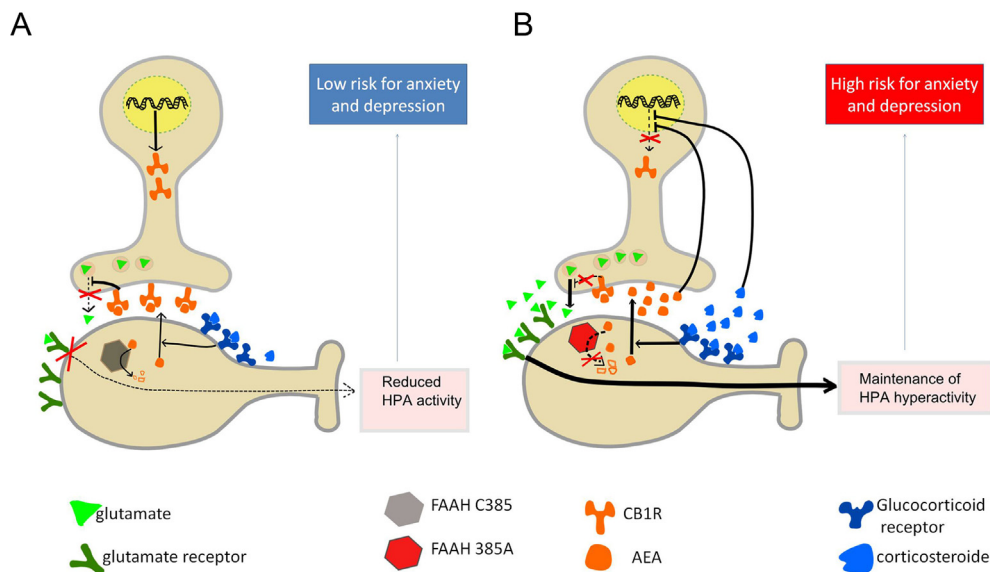
corticosteroide

Figure 2 Different regulatory mechanisms of HPA activations in the hippocampus due to genetic, epigenetic effects in association with anxiety and depression. *Part A*: the HPA axis is regulated by a negative feedback mechanism in the hypothalamus involving eCBs as retrograde messengers. In case of stress response elevated concentration of corticosteroids activates the glucocorticoid receptors (GR) and they increase the AEA release to the synaptic cleft. The AEA binds to the presynaptically expressed CB1R which mediates an inhibiting effect on the glutamate release (Wang et al., 2012). With the attenuated level of glutamate the HPA activation declines to the baseline. Thus, in healthy individuals with a balanced neuroendocrine function, the excitatory effect of the glutamatergic signal is inhibited by endocannabinoids in association with a low risk for anxiety. *Part B*: in A allele carriers of FAAH C385A the inhibitory effects of endocannabinoids on glutamatergic signal is impaired due to a strong reduction of CB1R expression. Reduced activity of the FAAH resulted in continuously high AEA level causing a downregulation of the CB1R as it was demonstrated by repeated treatment with FAAH inhibitor (Rivera et al., 2015). Besides, in chronic stress the highly increased corticosteroids block the CB1R expression, too. This double inhibiting effect on CB1R expression results in a low density of CB1R presynaptically and it leads to a suppressed inhibition on the glutamate release (Wamsteeker et al., 2010). The higher level of glutamate maintains the activation of the HPA axis with a high risk for anxiety.

association with affective phenotypes in human genetic studies so far. Negative life events in the childhood have more profound effects on the phenotype than those in the adulthood since they affect the neurodevelopment of the brain. Several studies suggest that stressful experiences in adolescents have prolonged effects to the adulthood yielding increased anxiety behavior (Avital and Richter-Levin, 2005;

Wamsteeker et al., 2010). Thus it is important to note that the effect of genetic variants in the *FAAH* gene persists throughout the neurodevelopment in contrast to a pharmacologic inhibition of FAAH in adulthood. Strong evidence supports that eCB system is involved in the neural development, including neurogenesis, glia formation and axonal elongation. Further, a progressive increase of CB1 receptor

concentration was demonstrated in numerous brain areas between fetal period and adulthood and it was particularly higher in the limbic area suggesting a significant role of CB1R in the emotional regulation (Fride, 2008). That is why a genetically reduced FAAH activity can have an altering effect on the development of neuronal circuits responsible for stress response. However, genetic variant of FAAH alone does not yield affective disturbances in our study. It may suggest that A allele carriers with altered FAAH enzymatic activity dispose a vulnerable HPA regulation associating with less psychological resilience and in case of repetitive trauma, only a maladaptive responsiveness can be produced. Since we investigated the effect of childhood trauma on anxiety and depression related phenotypes in adults it can be assumed that these stressful factors in the early age together with the *FAAH A385* may cause irreversible transformation in the stress response by exerting molecular and neuroplastic changes that affect these phenotypes lifelong (Fride, 2008).

4.3. The role of gender in the association between eCBs and affective phenotypes

There are some data showing that endocannabinoid genes are differently expressed in males and females in multiple brain areas, although these data are not consistent in details. In animal studies lower CB1 receptor expression and increased CB1 receptor function have been reported in the hippocampus and the prefrontal cortex of females compared to males (Mateos et al., 2011; Llorente-Berzal et al., 2013). Further, in an animal study Marco et al. found that early life stress was associated with increased endocannabinoid-related gene expression in the hippocampus in females whereas in males higher expression of the eCB genes were found in the frontal cortex (Marco et al., 2014).

However, our results did not show any difference between men and women. Despite the fact that females were overrepresented in our sample, it seems that our significant results are not due to genders. We analyzed the association of FAAH C385A and CHA on the affective phenotypes separately in men and women and the interactions were nominally significant on all phenotypic measurements in both gender subgroups except on ZSDS. This suggests that the presented $G \times E$ interaction may be independent from the gonadal regulation. On the other hand, behind this genotype–phenotype association a genetically reduced enzymatic activity is assumed which affects all brain areas during the neurodevelopment.

4.4. Potential pharmacogenomic consequences

As the selective FAAH inhibitors are considered as potential anxiolytics and they are under pharmacological development (www.clinicaltrials.gov/ct2/show/NCT01665573), our results suggest that evaluating the treatment outcome in human trials may require the complex analysis of genetic risk factors and environmental adversities, even in lights of the recently announced fatal outcome in a clinical trial of FAAH inhibitors (http://www.nature.com/news/scientists-in-the-dark-after-french-clinical-trial-proves-fatal-1.19189). Although prompt effect of FAAH inhibitors can be anxiolytic, experimental data on repeated treatment of FAAH inhibitor and chronic stress

together with our results suggest that individuals with different genetic variants of the *FAAH* gene and with or without early life trauma may exhibit divergent reaction (or even resistance) to an extrinsic FAAH inhibitor because of molecular alterations in the stress response neurocircuits during neuro development. Thus, as we previously proposed, pharmacogenomic testing of relevant biological pathways and measures of stressors in different time windows of life are useful and in some cases necessary tools to predict antidepressant/anxiolytic or depressogenic/anxiogenic effect of an agent (Lazary et al., 2009, 2011; Kirilly et al., 2012, 2013).

In conclusion our data revealed a significant interaction between *FAAH C385A* polymorphism and childhood trauma on affective phenotypes. In contrast with the acute FAAH inhibition during acute stress, our results confirmed that genetically reduced FAAH activity together with chronic early life stress are anxiogenic. This difference can be explained by a reduced CB1R receptor expression during neurodevelopment in the human brain, but further studies are needed to support this hypothesis. Besides, better understanding of the pathological role of distinctive stressors in the relationship between eCB system and affective vulnerability may have great impact on clinical guidelines related to the recently developing FAAH inhibitors as promising anxiolytic agents.

Limitations

Smoking habits which can be a confounding factor in the genetic associations on FAAH, CHA and affective phenotypes were not investigated in our study.

Our sample was not balanced regarding gender ratios; females were overrepresented in our sample. However, statistical analyses were adjusted for gender and we replicated the interaction analyses in both genders.

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Contributors

Judit Lazary – sample collecting, statistical analysis, MS preparing; Nora Eszlari – database building, statistical analysis; Gabriella Juhasz – study design, MS preparing; Gyorgy Bagdy – study design, MS preparing.

Conflict of interest

Authors declare no conflict of interest.

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References

Agrawal, A., Nelson, E.C., Littlefield, A.K., Bucholz, K.K., et al., 2012. Cannabinoid receptor genotype moderation of the effects of childhood physical abuse on anhedonia and depression. Arch. Gen. Psychiatry 697, 732-740.

Agrell, B., Dehlin, O., 1989. Comparison of six depression rating scales in geriatric stroke patients. Stroke 209, 1190-1194.

Avital, A., Richter-Levin, G., 2005. Exposure to juvenile stress exacerbates the behavioural consequences of exposure to stress in the adult rat. Int. J. Neuropsychopharmacol. 82, 163-173.

Bernstein, D.P., Ahluvalia, T., Pogge, D., Handelsman, L., 1997. Validity of the childhood trauma questionnaire in an adolescent psychiatric population. J. Am. Acad. Child Adolesc. Psychiatry 363, 340-348.

Biggs, J.T., Wylie, L.T., Ziegler, V.E., 1978. Validity of the Zung self-rating depression scale. Br. J. Psychiatr. 132, 381-385.

Spielberger, C.D., 1970. Manual for the State-Trait Anxiety Inventory. Consulting Psychologist Press.

Chiang, K.P., Gerber, A.L., Sipe, J.C., Cravatt, B.F., 2004. Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. Hum. Mol. Genet. 1318, 2113-2119.

Conzelmann, A., Reif, A., Jacob, C., Weyers, P., et al., 2012. A polymorphism in the gene of the endocannabinoid-degrading enzyme FAAH (FAAH C385A) is associated with emotional-motivational reactivity. Psychopharmacology (Berlin) 2244, 573-579.

Derogatis, L.R., 1993. BSI Brief Symptom Inventory: Administration, Scoring and Procedure Manual, fourth ed. National Computer Systems Pearson Inc., Minneapolis.

Freeman, B., Smith, N., Curtis, C., Huckett, L., Mill, J., Craig, I.W., 2003. DNA from buccal swabs recruited by mail: evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. Behav. Genet. 33, 67-72.

Fride, E., 2008. Multiple roles for the endocannabinoid system during the earliest stages of life: pre- and postnatal development. J. Neuroendocrinol. 20 (Suppl. 1), S75-S81.

Frodl, T., O'Keane, V., 2013. How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. Neurobiol. Dis. 52, 24-37.

Gabrys, J.B., Peters, K., 1985. Reliability, discriminant and predictive validity of the Zung self-rating depression scale. Psychol. Rep. 573 (Pt. 2), 1091-1096.

Gunduz-Cinar, O., Hill, M.N., McEwen, B.S., Holmes, A., 2013a. Amygdala FAAH and anandamide: mediating protection and recovery from stress. Trends Pharmacol. Sci. 3411, 637-644.

Gunduz-Cinar, O., MacPherson, K.P., Cinar, R., Gamble-George, J., et al., 2013b. Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. Mol. Psychiatry 187, 813-823.

Haller, J., Barna, I., Barsvari, B., Gyimesi Pelczer, K., et al., 2009. Interactions between environmental aversiveness and the anxiolytic effects of enhanced cannabinoid signaling by FAAH inhibition in rats. Psychopharmacology (Berlin) 2044, 607-616.

Hariri, A.R., Gorka, A., Hyde, L.W., Kimak, M., et al., 2009. Divergent effects of genetic variation in endocannabinoid signaling on human threat- and reward-related brain function. Biol. Psychiatry 661, 9-16.

Heim, C., Mletzko, T., Purselle, D., Musselman, D.L., et al., 2008. The dexamethasone/corticotropin-releasing factor test in men with major depression: role of childhood trauma. Biol. Psychiatry 634, 398-405.

Hill, M.N., McLaughlin, R.J., Morrish, A.C., Viau, V., et al., 2009. Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic-pituitary-adrenal axis. Neuropsychopharmacology 3413, 2733-2745.

Hill, M.N., Miller, G.E., Ho, W.S., Gorzalka, B.B., et al., 2008. Serum endocannabinoid content is altered in females with depressive disorders: a preliminary report. Pharmacopsychiatry 412, 48-53.

Hill, M.N., Patel, S., Carrier, E.J., Rademacher, D.J., et al., 2005. Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. Neuropsychopharmacology 303, 508-515.

Hillard, C.J., Weinlander, K.M., Stuhr, K.L., 2012. Contributions of endocannabinoid signaling to psychiatric disorders in humans: genetic and biochemical evidence. Neuroscience 204, 207-229.

Ho, W.S., Hill, M.N., Miller, G.E., Gorzalka, B.B., et al., 2012. Serum contents of endocannabinoids are correlated with blood pressure in depressed women. Lipids Health Dis. 11, 32.

Juhasz, G., Chase, D., Pegg, E., Downey, D., et al., 2009. CNR1 gene is associated with high neuroticism and low agreeableness and interacts with recent negative life events to predict current depressive symptoms. Neuropsychopharmacology 348, 2019-2027.

Juhasz, G., Gonda, X., Hullam, G., Eszlari, N., et al., 2015. Variability in the effect of 5-HTTLPR on depression in a large European population: the role of age, symptom profile, type and intensity of life stressors. PLoS One 103, e0116316.

Juhasz, G., Hullam, G., Eszlari, N., Gonda, X., et al., 2014. Brain galanin system genes interact with life stresses in depression-related phenotypes. Proc. Natl. Acad. Sci. USA 11116, E1666-E1673.

Keller, S., Sarchiapone, M., Zarrilli, F., Videtic, A., et al., 2010. Increased BDNF promoter methylation in the Wernicke area of suicide subjects. Arch. Gen. Psychiatry 673, 258-267.

Kirilly, E., Gonda, X., Bagdy, G., 2012. CB1 receptor antagonists: new discoveries leading to new perspectives. Acta Physiol. (Oxf.) 2051, 41-60.

Kirilly, E., Hunyady, L., Bagdy, G., 2013. Opposing local effects of endocannabinoids on the activity of noradrenergic neurons and release of noradrenaline: relevance for their role in depression and in the actions of CB(1) receptor antagonists. J. Neural Transm. 1201, 177-186.

Lazary, J., Juhasz, G., Hunyady, L., Bagdy, G., 2011. Personalized medicine can pave the way for the safe use of CB(1) receptor antagonists. Trends Pharmacol. Sci. 325, 270-280.

Lazary, J., Lazary, A., Gonda, X., Benko, A., et al., 2009. Promoter variants of the cannabinoid receptor 1 gene (CNR1) in interaction with 5-HTTLPR affect the anxious phenotype. Am. J. Med. Genet. B Neuropsychiatr. Genet. 150B8, 1118-1127.

Llorente-Berzal, A., Assis, M.A., Rubino, T., Zamberletti, E., et al., 2013. Sex-dependent changes in brain CB1R expression and functionality and immune CB2R expression as a consequence of maternal deprivation and adolescent cocaine exposure. Pharmacol. Res. 74, 23-33.

Mackie, K., 2005. Distribution of cannabinoid receptors in the central and peripheral nervous system. Handb. Exp. Pharmacol. 168, 299-325.

Major Depressive Disorder Working Group of the Psychiatric GC, 2013. A mega-analysis of genome-wide association studies for major depressive disorder. Mol. Psychiatry 184, 497-511.

Marco, E.M., Echeverry-Alzate, V., Lopez-Moreno, J.A., Gine, E., et al., 2014. Consequences of early life stress on the expression of endocannabinoid-related genes in the rat brain. Behav. Pharmacol. 255-256, 547-556.

Mateos, B., Borcel, E., Loriga, R., Luesu, W., et al., 2011. Adolescent exposure to nicotine and/or the cannabinoid agonist CP 55,940 induces gender-dependent long-lasting memory impairments and changes in brain nicotinic and CB(1) cannabinoid receptors. J. Psychopharmacol. 2512, 1676-1690.

McGowan, P.O., Sasaki, A., D'Alessio, A.C., Dymov, S., et al., 2009. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat. Neurosci. 123, 342-348.

McLaughlin, R.J., Hill, M.N., Gorzalka, B.B., 2014. A critical role for prefrontocortical endocannabinoid signaling in the regulation of stress and emotional behavior. Neurosci. Biobehav. Rev. 42, 116-131.

Meye, F.J., Trezza, V., Vanderschuren, L.J., Ramakers, G.M., et al., 2013. Neutral antagonism at the cannabinoid 1 receptor: a safer treatment for obesity. Mol. Psychiatry 1812, 1294-1301.

Monteleone, P., Bifulco, M., Maina, G., Tortorella, A., et al., 2010. Investigation of CNR1 and FAAH endocannabinoid gene polymorphisms in bipolar disorder and major depression. Pharmacol. Res. 615, 400-404.

Newsom, R.J., Osterlund, C., Masini, C.V., Day, H.E., et al., 2012. Cannabinoid receptor type 1 antagonism significantly modulates basal and loud noise induced neural and hypothalamic-pituitary-adrenal axis responses in male Sprague-Dawley rats. Neuroscience 204, 64-73.

Onaivi, E.S., Ishiguro, H., Gong, J.P., Patel, S., et al., 2006. Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. Ann. N.Y. Acad. Sci. 1074, 514-536.

Pacher, P., Kunos, G., 2013. Modulating the endocannabinoid system in human health and disease – successes and failures. FEBS J. 2809, 1918-1943.

Patel, S., Cravatt, B.F., Hillard, C.J., 2005. Synergistic interactions between cannabinoids and environmental stress in the activation of the central amygdala. Neuropsychopharmacology 303, 497-507.

Rivera, P., Bindila, L., Pastor, A., Perez-Martin, M., et al., 2015. Pharmacological blockade of the fatty acid amide hydrolase (FAAH) alters neural proliferation, apoptosis and gliosis in the rat hippocampus, hypothalamus and striatum in a negative energy context. Front. Cell. Neurosci. 9, 98.

Sipe, J.C., Chiang, K., Gerber, A.L., Beutler, E., et al., 2002. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc. Natl. Acad. Sci. USA 9912, 8394-8399.

Szadoczky, E., Papp, Z., Vitrai, J., Rihmer, Z., et al., 1998. The prevalence of major depressive and bipolar disorders in Hungary. Results from a national epidemiologic survey. J. Affect. Disord. 502-503, 153-162.

Vinod, K.Y., Arango, V., Xie, S., Kassir, S.A., et al., 2005. Elevated levels of endocannabinoids and CB1 receptor-mediated G-protein signaling in the prefrontal cortex of alcoholic suicide victims. Biol. Psychiatr. 575, 480-486.

Wamsteeker, J.I., Kuzmiski, J.B., Bains, J.S., 2010. Repeated stress impairs endocannabinoid signaling in the paraventricular nucleus of the hypothalamus. J. Neurosci. 3033, 11188-11196.

Wang, M., Hill, M.N., Zhang, L., Gorzalka, B.B., et al., 2012. Acute restraint stress enhances hippocampal endocannabinoid function via glucocorticoid receptor activation. J. Psychopharmacol. 261, 56-70.

Zung, W.W., Richards, C.B., Short, M.J., 1965. Self-rating depression scale in an outpatient clinic. Further validation of the SDS. Arch. Gen. Psychiatr. 136, 508-515.