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Insulin-Like-Growth Factor 1 Moderates the Influence of the BDNF P.Val66Met Variant on Depression Severity in Adolescent Depression

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Abstract

Background: A genetic influence for adolescent depression has been described in numerous studies. The product of the brain-derived neurotrophic factor gene (*BDNF*; rs6265 c.196G>A; p.Val66Met) has been identified playing an important role in genesis and course of depression. It interacts with serological BDNF and other growth factors such as the Insulin-like-Growth Factor 1 (IGF-1), influencing memory-associated brain regions (i.e. hippocampus, parahippocampal gyrus and entorhinal cortex) as well as reward-related brain circuits (cf. nucleus accumbens, amygdala and frontal areas).

Methods: 40 patients with adolescent depression aged 13 to 17 years, were included. Assuming a significant influence of the *BDNF* p.Val66Met variant on depression severity, we performed moderation analyses to further examine the interplay between *BDNF* p.Val66Met variant, serological IGF-1, regional brain volume and depression severity.

Results: IGF-1 was found to moderate significantly the influence of *BDNF* p.Val66Met variant on depression severity. Brain volumes of (para) hippocampal, and amygdala as well as the nucleus accumbens moderated the same only in combination with IGF-1.

Conclusions: We discuss the significant influence of serological IGF-1 and *BDNF* p.Val66Met variant in terms of neuroplasticity. We interpret the role of changes in memory and reward-related brain circuits as hints towards the localization of the interaction between *BDNF* p.Val66Met variant and IGF-1. Our findings point to a potential mechanism in juvenile depression which differs from actual models in adult depression and could in a further step contribute to targeted therapies for depressive adolescents.

1. Introduction

Adolescent depression is a serious mental health problem with increasing prevalence from childhood to adolescence and an estimated lifetime prevalence up to 20% by late adolescence [1]. Adolescent depression includes a persistent

feeling of sadness and loss of interest in activities. In comparison to adult depression, the loss of interest in adolescent patients is the predominant symptom and has been attributed to a diminished positive affect of leisure activities, i.e. the lack of reward-associated feelings such as fun and satisfaction [2]. The reward circuit in the brain comprises mesocortico-limbic pathways including the nucleus accumbens (NAcc), the amygdala (AMY), orbito-frontal and prefrontal cortex (PFC), e.g. the inferior frontal gyrus (IFG). Whereas the NAcc is the core structure in reward processing and associated with reward-seeking behavior, AMY represents the processing of positive and negative emotions. In contrast, PFC serves as controlling structures and inhibition [3]. For example, fMRI studies showed reduced reactivity in the NAcc during tasks with monetary as well as social reward in adolescent patients [2, 4], which co-occurred with enhanced PFC and increased AMY response [5].

In addition, a recent meta-analysis showed neuropsychological impairments of children and adolescents with depression in various cognitive domains, including verbal memory [6]. Human as well as animal studies found, that the impairments in cognition and memory of adults and juveniles are highly linked to neuronal loss and the lack of neurogenesis in hippocampal areas [7] such as the hippocampus (HC), the parahippocampal gyrus and entorhinal cortex. The HC is the most representative structure for learning and memory. Together with the parahippocampal gyrus and the entorhinal cortex they are involved in spatial (working) memory and memory consolidation (entorhinal cortex), spatial memory encoding and recognition (parahippocampal gyrus). Morphometry studies confirmed structural alterations in adolescent depression in terms of enhanced PFC volumes accompanied by reduced HC and parahippocampal volumes [8].

For adolescent depression, a genetic influence has been described in diverse studies. Beside the influence of serotonergic genes, the neuroplasticity associated *Brain-derived neurotrophic factor* (BDNF) p.Val66Met variant has been identified as playing an important role [9]. The *BDNF* gene, located on chromosome 11p13 encodes the precursor peptide proBDNF from which BDNF dissociates [10]. The substitution of the amino acid valine (Val) to methionine (Met) at codon 66 is described as p.Val66Met variant, substitution of both as p.Met66Met variant. The methionine substitution at codon 66 is considered to diminish intracellular transport and secretion of BDNF. The latter, however, seems to vary with age, as age-related changes in *BDNF* mRNA levels were found. For example, Webster and colleagues (2002) reported an increase of *BDNF* mRNA in the dorsolateral PFC from infancy to adulthood of about 30% with a peak during young adulthood [11]. In addition, adult studies showed associations between BDNF markers, HC function and mood disorders demonstrating that especially the Met allele was associated with reduced HC synaptic activity [12], altered HC and PFC volumes [13], and consequently with deficits in episodic memory [12]. Findings on the influence of *BDNF* p.Val66Met variant on reward-related pathways such as the NAcc [14] as well as the interaction between reward neural circuit and mood disorders [15] stem from animal studies, to date.

Regarding the role of the *BDNF* p.Val66Met variant in mood disorder in general and depression in particular, the Val allele has been reported to be significantly associated with mood disorders. This has been shown for both, adult-onset and childhood-onset mood disorders [9]. Thus, findings on influences of the *BDNF* p.Val66Met variant on serological BDNF levels are inconsistent accross studies (both, increase and decrease) [16, 17]. Insulin-like Growth Factor 1 (IGF-1) is regarded as another key players in neurogenetic pathways and thus in the pathology of depression. The majority of studies reported increased levels of IGF-1 in depressive patients, though findings in total are mixed and may be influenced by gender, age etc. [18]. A recent literature review presented a significant relationship between serological IGF-1, fronto-limbic cognitive dysfunction and depression [18, 19]. IGF-1/IGF-2 increases the synthesis and activity of BDNF, fostering brain plasticity. IGF-1 is produced in the central and peripheral nervous systems, controlling maturation and differentiation of cells as well as metabolic processes and is especially developed e.g. in the olfactory bulb and HC [20]. As IGF-1 can cross the blood-brain barrier, the application of IGF-1 in the periphery enhanced neurogenesis in the HC in an animal model [21], potentially explaining its anti-depressant effects:mice with a selective inactivation of cerebral *IGF-1* gene showed depression-like behavior [22]. In rat, centrally administered IGF-1 has led to higher serotonin-concentration and a decrease in depression symptoms [23], based on its neuroprotective function. Clinical studies on the relationship of peripheral IGF-1 and depression are inconsistent [20]. Most studies, however, report higher levels of peripheral IGF-1 in depressive patients compared with healthy controls [24]. Finally, the pathophysiological pathways of the actors in depression genesis (growth factors, neurogenesis, immunological factors) are still not sufficiently understood. Regarding depression in adolescents, there is even less evidence [25]. Especially, available data leave unexplained, if differences between adolescent and adult depression are determined by different physiological mechanisms. Thereby, adult depression is suggested to depend on immunological factors and not primarily on growth factors. The actual debate remains open if growth factors such as IGF-1 have a significant contribution in the anti-depressive effect of physical activity on depression in adolescents.

In this study, we aimed to reveal, if the *BDNF* p.Val66Met variant influences depression severity, and if yes, whether the growth factor IGF-1 in the serum and/or brain morphology affects this relationship. We collected blood samples to obtain serological IGF-1 and genotyping for the *BDNF* p.Val66Met variant was done by saliva analyses. Participants underwent a structural MRI scan to apply brain morphometry. Based on earlier findings of altered reward processing (reduced feelings of fun and satisfaction [2]) and reduced medio-temporal neurogenesis, we used a region-of interest (ROI)-based approach focusing on volumes of memory-associated regions including the HC, para-hippocampus and entorhinal cortex and reward-based circuits covering the NAcc, AMY, IFG (opercular/triangular/orbital part).

This is the first study examining differences between *BDNF* p.Val66Met variant genotype groups, serum IGF-1 and brain volumes in a sample with depressive adolescents. Therefore, a detailed analysis of *a priori* differences was

performed regarding to the experimental variables (IGF-1 and brain volumes) as well as general parameters (age and sex distribution, comorbidities and medication).

To address the nature of genetic influence we applied the genetic moderation approach which has been used earlier in the context of how the serotonergic 5-HTTLPR gene moderates the relationship between stress/stressful life events (SLE) and depression (for a meta-analysis of 54 studies see [26]): the *BDNF* p.Val66Met variant was used as independent variable (X), and the Beck Depression Inventory (BDI) as dependent variable (Y). Moderating variables were serum IGF-1, and central parameters in terms of brain volumes of depression-associated pathways. IGF-1 was introduced as moderator (M) based on its crucial role in neuroplasticity and co-acting factor of BDNF (see above). Therefore, we hypothesized that genetic variation in BDNF interacted with this peripheral growth factor. Brain volumes were defined as a second moderating variable (W), assuming that BDNF-induced neuroplasticity manifests itself in concrete brain pathways, namely in the HC as well as further regions of memory- and reward-related circuits.

Regarding the genetic moderation, we assumed that *BDNF* p.Val66Met variant has a direct influence on depression severity with higher BDI scores in Val/Val homozygotes compared to Met allele carriers (direct effect BDNF variant). Likewise, we hypothesized a significant BDNF genotype x IGF-1 interaction (indirect effect of IGF-1, i.e. IGF-1 served as a moderating variable) (**Figure 1**), the way that the protective influence of IGF-1 was higher in Val/Met-allele carriers compared to Val/Val homozygotes.

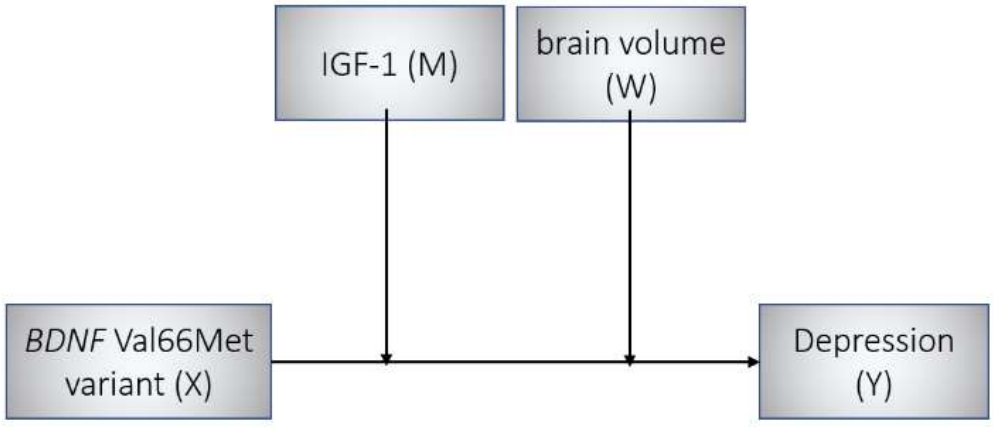


Figure 1: Models of the direct and indirect influence of moderator variables (e.g. IGF-1, brain volume) on the influence of the independent factor X (e.g. *BDNF* Val66Met variant) on the dependent variable Y (depression severity).

Regarding the role of regional brain volume as a second moderating variable, analyses were rather explorative: (i) if brain volumes were significant moderators, brain volume could be seen as depression determining factors rather than secondary effects/consequences of depression; (ii) if brain volumes did not significantly moderate but interacted significantly with IGF-1, brain volumes were understood as the regions, where the interaction of *BDNF* p.Val66Met variant and IGF-1 manifested itself in the brain; (iii) if brain volumes did not moderate significantly and did not interact significantly with IGF-1, depression-specific alterations belong to an independent aspect of the pathological mechanism.

	Total sample [n=40]	**Val/Val [n=28]**	**Val/Met [n=12]**	**Statistics**
Sex m/f	12/28	7/21	5/7	X^2=1.1, p=.292
Age (SD)	15.87 (1.2)	16.18 (1.04)	15.16 (1.15)	$T_{(38,2)}$=2.8, p=.009
BDI (SD)	30.20 (11.65)	32,93 (11,17)	23,83 (10,56)	$T_{(38.2)}$=2.4, p=.022
IGF1	408.08 (87.49)	397.04 (94.09)	433.85 (66.14)	$T_{(38.2)}$=1.2, p=.227
BDNF	32962 (7769)	33450 (7998)	31825 (7412)	$T_{(38.2)}$=.60, p=.551
Comorbidities [y/n]				
Phobia	21/19	15/13	6/6	X^2=.04, p=.836
Panic Disorder	1/39	1/27	0/12	X^2=1.3, p=.545
Generalized anxiety	5/35	4/24	1/11	X^2=1.2, p=.736
Obsessive compulsive	4/36	4/24	0/12	X^2=1.9, p=.386
Medication [y/n]				
SSRI, PRN	7/33	5/23	2/10	X^2=.01, p=.928
Antipsychotics PRN	4/36	3/25	1/11	X^2=.05, p=.818
Sedative, PRN	2/38	1/27	1/11	X^2=.40, p=.527
Benzodiazepine, PRN	1/39	1/27	0/12	X^2=.44, p=.507
SSRI, long-term	4/36	3/25	1/11	X^2=.05, p=.818
Antipsychotics, long-term	1/39	1/27	0/12	X^2=.44, p=.507

Note. $q^*=p_{corrected\ for\ 12\ tests}<.00025$; PRN: pro re nata medication (see s1); SSRI: selective serotonin reuptake inhibitors. Val/Val: carriers of the *BDNF* p.Val66Met Val/Val variant; Val/Met: carriers of the *BDNF* p.Val66Met Val/Met variant and Met/Met variant.

Table 1: Sample description.

2. Methods

2.1 Subjects

In this fMRI-study, we examined 64 adolescents with depression, 48 provided saliva sample for genotyping and 53 underwent an MRI scan. In 40 patients, both MRI data and genotype were available (28 females; aged from 13.53 to 17.67 years), suffering from depression (F32.1: n=39, F32.2: n=1). Twenty-nine patients showed comorbidities such as anxiety and obsessive-compulsive disorders (Table 1). Participants were drawn from three out of four inpatient units at the Department of Child and Adolescent Psychiatry Psychosomatic and Psychotherapy at the University Hospital of Cologne.

2.2 Inclusion criteria

All participants were caucasian inpatients or day clinic patients and fulfilled DSM-IV/-5 and ICD-10 criteria of non-psychotic major depressive disorder (MDD), assessed by clinician rating with the Structured Clinical Interview for DSM-IV Axis I disorders, German version (SKID-I) [27]. Additionally, BDI (revised version BDI-II [28]) was rated, which was used as dependent variable in this moderation analysis. The raw-point-scoring in BDI-II was not part of inclusion criteria but was used for the assessment of depression severity. Participants were of normal intelligence (IQ > 70) tested by Kaufman Assessment Battery for Children (K-ABC) [29] or Wechsler Intelligence Scale for Children (WISC), Hamburg-Wechsler-Intelligenztest für Kinder (HAWIK) [30]. Comorbidities were allowed if they were not part of the exclusion criteria.

2.3 Exclusion criteria

Adolescents were excluded when suffering from one of the following conditions: schizophrenia, other psychotic disorders or psychosis in the medical history, including psychotic depression, bipolar I and II disorder, personality disorders, pervasive developmental disorder or current substance abuse. Permanent psychiatric medication (defined as administration daily longer than 3 weeks) or medication with inherent psychotropic effects (anticonvulsants, steroids, methylphenidate, amphetamine, antidepressants, neuroleptics, benzodiazepines, and mood-stabilizer) were not allowed. Intermittent pro re nata (PRN) medication (no daily administration) was no reason for exclusion. Patients on long-term medication were included in the analysis as Intention to treat (ITT). We also excluded patients with Morbus Addison or non-substituted hypothyroidism.

After screening for the inclusion criteria and written adolescent and parental (or legal guardians) consent to participate in the study, the principal investigator or a licensed study clinician conducted the Structured Clinical Interview for DSM-IV Axis I disorders, German version (SKID-I) to confirm diagnosis, to check comorbidities for exclusion criteria, and to evaluate current severity of the depressive disorder [27]. The study was approved by the University of Cologne Ethics Committee and was conducted in accordance to the Declaration of Helsinki in its latest version from 2008. Written informed consent was obtained from all participants and their parents.

2.4 Psychological diagnostic (BDI-II and SKID I)

The Beck Depression Inventory (BDI-II) with an age range from 13 years and over is a multiple-choice self-report inventory. Intensity of depression is assessed with 21 items in alignment with DSM-IV criteria, relating to symptoms of depression (e.g. hopelessness, irritability, guilt, fatigue, weight loss etc.). The test one-week test–retest reliability is Pearson's r =0.93, the internal consistency α=.91. A raw-score between 20 and 28 points was taken as indicator of a moderate depression [31].

The "Klinisches Interview für DSM-IV" (SKID-I) serves to determine and diagnose psychic syndromes and disorders based on definitions of Axis I disorders within the DSM-IV (2000). It is a structured interview lasting approximately 60 minutes [27].

2.5 Genotyping *BDNF*

Genotyping of BDNF was performed using DNA isolated from saliva ORAgene DNA Kits (Ottawa, Kanada). 2 ml saliva of each subject was spit into a test tube. Fully automated extraction of DNA from saliva was performed with the Maxwell 16 Instrument, (Promega, Madison, USA). We performed direct PCR amplification and Sanger DNA sequencing of the region of interest (BDNF gene; OMIM 113505; RefSeq NM_001143807.1; exon 2). Sequence data were analyzed automatically using the program SeqPilot (JSI GmbH; Medical Systems, Germany). BDNF genotypes (rs6265; c.196G>A; p.Val66Met) were defined as following: homozygous Val/Val; heterozygous Val/Met and homozygous Met/Met.

Hardy–Weinberg criteria as determined by the online program DeFinetti (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) were fulfilled for BDNF genotype distribution (Val/Val=28, Val/Met=11, Met/Met= 1, p=0.88). For statistical analyses, only two genotype groups were defined Val/Val homozygotes and Val/Met (the single individual homozygous for Met/Met was included into the Val/Met group).

2.6 Blood serology

Serum samples were drawn from patients by venipuncture in the morning fasting of the measurements clotted at room temperature and transferred to the laboratory of the University Hospital of Cologne. Serologic tests were carried out in accordance with the routine procedures of the central laboratory. Serum samples were analyzed using Onestep-Sandwich-Chemilumineszence-Immunoassay (CLIA). IGF-1 was separated from binding protein by Liaison® autoanalyser (DiaSorin SpA, Saluggia, Italy).

IGF-1 concentrations between 10 ng/ml and 1500 ng/ml can be measured with an intra-assay coefficient of variation (CV) of 4.59 % and a mean of 189, 3 ng/ml (n = 40) and an inter-assay-variation of VC = 4, 3 % with a mean of 202, 6 ng/ml (n = 20) (DiaSorin 2012). Because serological IGF-1 is age-dependent, the reference values, independent of sex, of the central laboratory of University Hospital of Cologne are listed in the legend of **Table s1.**

2.7 MR Data acquisition

MRI data were collected in the neuropediatric ward of the hospital of Meerbusch (Germany) on a 1.5 tesla Siemens Avanto scanner (Siemens Medical Solutions). The scanning protocol included a structural T1-weighted (fl3D) sequence with a voxel size of 1.0x1.0x0.9mm3, sagittal slices, an echo time (TE) of 5.2ms and a repetition time (TR) of 11ms; a T2 (t2tse3dvfl) sequence (voxel size= 1.0x1.0x0.9mm3, sagittal slices, TE=246ms, TR=3200ms) and FLAIR (FLAIR3dvfl) sequence (voxel-size=1.0x1.0x0.9mm3, sagittal slices, TE=234ms, TR=5000ms). Total scan time was 20 min.

2.8 MRI Data processing

Structural MRI data was analyzed using the FreeSurfer (versions 5.3) software [32]. Analysis and quality-control protocols of the ENIGMA consortium were applied including the recon-all -all stream and the segmentations of 68 (34 left and 34 right) cortical gray matter regions based on the Desikan–Killiany atlas. Data of two whole-hemisphere measures were visually inspected and statistically evaluated for outliers following standardized ENIGMA protocols (http://enigma.ini.usc.edu/protocols/imaging-protocols). According to our hypotheses (stated in the introduction) and after quality control, regional volumes were extracted for memory-associated (i.e. HC, parahippocampal gyrus, entorhinal cortex [8]) as well as reward-related brain circuits (NAcc, AMY, IFG [2, 3]). Regional volume scores were corrected for global brain volume in terms of % of ICV and entered statistical ROI analyses.

2.9 Statistical analyses

For the testing of a priori differences between BDNF p.Val66Met variant groups ANOVA models were defined with the BDNF p.Val66Met variant as independent variable (Val/Val versus Val/Met), and BDI (clinical measure of depression severity), IGF-1 plasma levels, and regional brain volumes (16 regions of interest representative for the main structures of the reward / declarative memory systems) as dependent variables. Results were defined as significant when they passed the threshold of $p<0.05$ FDR-corrected for multiple comparisons, reported in terms of the corrected threshold q*.

In a next step, moderation analyses were performed. These analyses were performed using the PROCESS macro for SPSS (http://www.processmacro.org/index.html) with BDNF genotype as the independent factor X, BDI as dependent variable Y, serum IGF-1 as moderator variable M, and regional brain volume as moderating variables W (Figure 1). Sex and comorbidity were used as nuisance variables. Post-hoc power analyses were performed using G*Power (http://gpower.hhu.de/) with the parameters: F tests - Linear multiple regression: Fixed model, R^2 deviation from zero, Analysis: Post-Hoc: Compute achieved power, Input: Effect size $f^2 = 0.30$ α err prob = 0.05 Power (1-β err prob) = 0.80 Number of predictors = 2. A power $\geq .80$ was generally considered desirable.

3. Results

3.1 A priori differences between BDNF p.Val66Met variant groups

A priori analyses did not reveal significant main effects of BDNF p.Val66Met variant groups on dependent variables. In the ROI-based analysis of memory-related and reward-associated structures, individuals with the BDNF p.Val66Met Val/Val variant had significantly reduced volumes in the right parahippocampal gyrus (puncorrected=.004) in combination with a significantly enhanced right AMY volume (puncorrected<.001) (for an overview see table s1). There were no significant effects of sex, comorbidity and medication on regional brain volume (Table s2).

3.2 Moderation analyses

Post-hoc power analysis for moderations revealed a power of 0.85, a critical F = 3.25 and df=37, 2.

3.2.1 Direct influence of the *BDNF* p.Val66Met variant on BDI: Moderator analyses revealed that the BDNF p.Val66Met variant had a significantly direct influence on depression severity as measured by the BDI in the models with IGF-1 and all memory-related models as well as IGF-1 and five reward-associated models comprising right/left AMY, right/left NAcc and the right IFG pars opercularis (Table 2).

M	W	R^2_{BDI}	F_{BDII}	F_{int1}	F_{int2}	F_{both}
IGF-1	r_HC	0.43	3.1*	7.2*	0.1	3.6*
IGF-1	l_HC	0.43	3.1*	7.0*	0.1	3.6*
IGF-1	r_paraHC	0.45	3.3*	6.5*	0.1	3.3*
IGF-1	l_paraHC	0.43	3.0*	5.6*	0.1	3.5*
IGF-1	r_ENTO	0.44	3.1*	6.9*	0.4	3.8*
IGF-1	l_ENTO	0.45	3.2*	7.5*	0.1	3.8*
IGF-1	r_AMY	0.47	3.5*	6.7*	0.4	3.4*
IGF-1	l_AMY	0.47	3.5*	7.9*	0.1	4.0*
IGF-1	r_NAcc	0.49	3.5*	8.0*	1.4	4.0*
IGF-1	l_NAcc	0.46	3.5*	6.3*	0.3	3.4*
IGF-1	r_IFG_op	0.43	3.1*	5.1*	0.1	2.9

*: >critical F=3.25, IGF-1: insulin-like-growth-factor 1; HC: hippocampus; paraHC: parahippocampal gyrus; ENTO: entorhinal cortex; AMY: amygdala; NAcc: nucleus accumbens; IFG_op: inferior frontal gyrus, pars opercularis; IFG_tri: inferior frontal gyrus, pars triangularis; IFG_orb: inferior frontal gyrus, pars orbitalis.

Table 2: Significant results from moderation analyses.

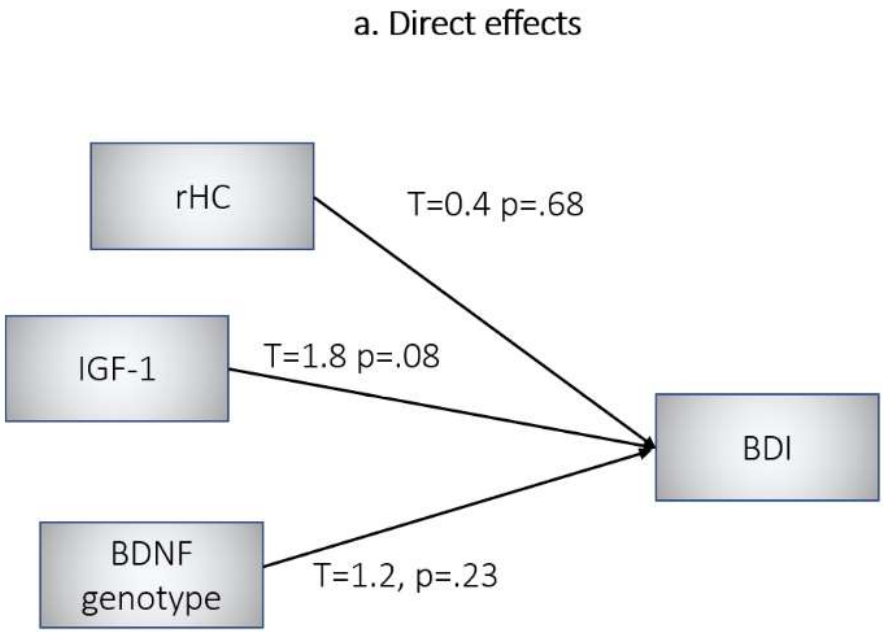
3.2.2 Inclusion of IGF-1 as a moderating variable of *BDNF* p.Val66Met variant effects on BDI: Furthermore, in the same models, IGF-1 significantly moderated the influence of the BDNF p.Val66Met variant on depression

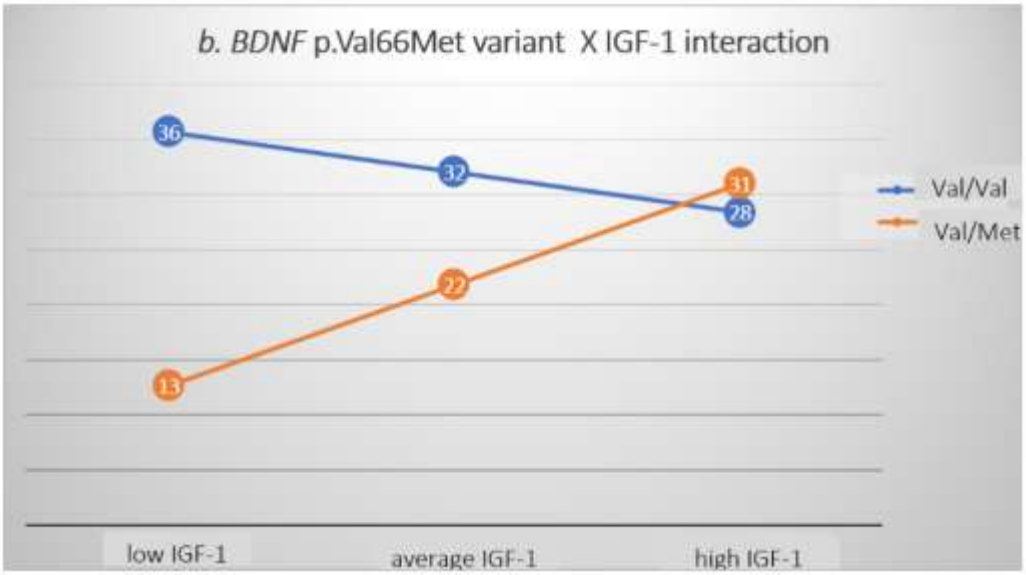
severity, revealing a significant interaction between central and peripheral neurotrophic factors. In addition, when looking at the conditional effects, we found that genetic moderation was strongest when IGF-1 was low to average. These results suggest a robust moderation of the influence of the BDNF p.Val66Met variant on depression severity by the IGF-1 (Table 3, Figure 2).

W	**<1SD$_{M,W}$**	**M$_M$ / <1SD$_W$**	**>1SD$_M$ / <1SD$_W$**	**<1SD $_M$ / M$_W$**	**M$_{M,W}$**	**>1SD$_M$ / M$_W$**	**<1SD $_M$ / >1SD $_W$**	**M$_M$ / >1SD $_W$**	**>1SD$_M$ / >1SD $_W$**
r_HC	-22.9*	-10.1	2.7	-23.1*	-10.2*	2.6	-23.2*	-10.4	2.4
l_HC	-24.5*	-11.8*	5.5	-23.1*	-10.4*	0.4	-21.8*	9.1	3.4
r_paraHC	-23.6*	-11.4*	0.7	-22.1*	-10.0*	2.2	-20.7*	-8.5	3.6
l_paraHC	-20.1	-7.8	4.6	-24.0*	-11.6	0.8	-30.1	-17.7	-5.4
r_ENTO	-25.7*	-13.2*	-0.6	-23.2*	-10.6*	1.9	-20.6*	-8.0	4.5
l_ENTO	-25.1*	-12.1	0.9	-23.4*	-10.4*	2.6	-21.6*	-8.6	4.4
r_AMY	-19.0*	-6.3	5.5	-22.1*	-9.9*	2.3	-25.2*	-13.0*	-0.8
l_AMY	-22.7*	-9.6	3.4	-24.0*	-10.9*	2.1	-25.3*	-12.3*	0.8
r_NAcc	-19.2*	-5.3	8.5	-24.2*	-10.3*	3.6	-29.1*	-15.3*	-1.4
l_NAcc	-20.7*	-7.5	5.8	-23.5*	-10-3*	3.0	-26.3*	-13.1*	0.2
r_IFG_op	-23.1	-10.8	1.6	-22.8*	-10.4	1.9	-22.4*	5.6	2.2

*: >critical F=3.25, IGF-1: insulin-like-growth-factor 1; HC: hippocampus; paraHC: parahippocampal gyrus; ENTO: entorhinal cortex; AMY: amygdala; NAcc: nucleus accumbens; IFG_op: inferior frontal gyrus, pars opercularis;

Table 3: Conditional effect of BDNF p.Val66Met variant on BDI at values of the moderators M=IGF-1 and W=regional brain volume.


a. Direct effects
rHC
T=0.4 p=.68
IGF-1
T=1.8 p=.08
BDI
BDNF genotype
T=1.2, p=.23


b. BDNF p.Val66Met variant X IGF-1 interaction
36
32
31
28
22
13
Val/Val
Val/Met
low IGF-1
average IGF-1
high IGF-1

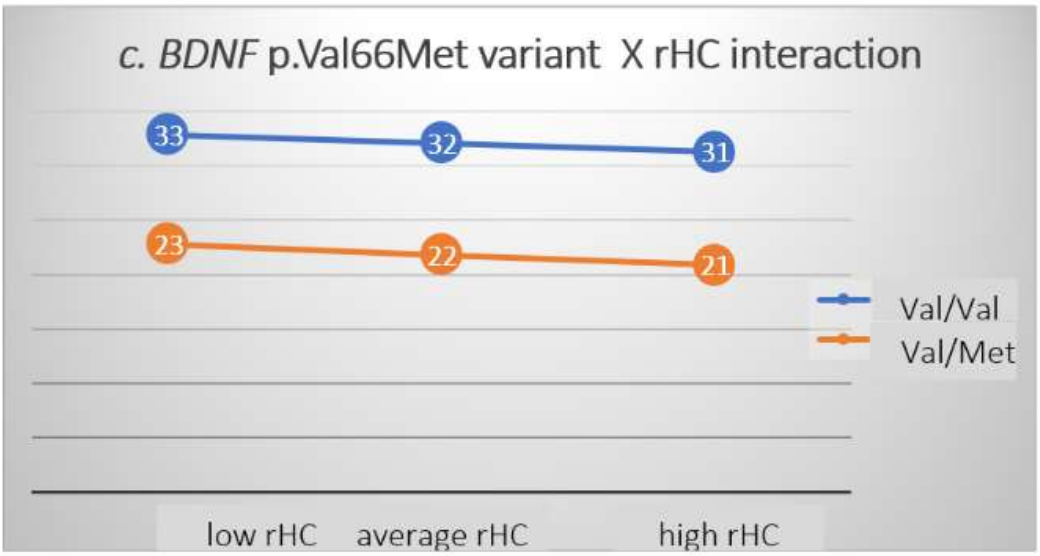


Figure 2: Presents at the example of the right hippocampus (rHC) the direct and interaction effects of the genetic moderation; In (a): Direct effects were reported with the statistical scores T and p; In (b and c): line diagrams show the interaction effects for both moderating variables.

3.2.3 Regional brain volumes served as moderators in interaction with IGF-1 only: Regional brain volumes interacted within these models, as shown by the significance of the model, but predominantly in combination with IGF-1 (r_HC: F_{both}=3.6, R^2_{model}=0.43; r_HC: F_{both}=3.6, R^2_{model}=0.43, l_paraHC: F_{both}=.3.5, R^2_{model}=0.43; r_ENTO: F_{both}=3.8, R^2_{model}=0.44; l_ENTO: F_{both} =.2.8, R^2_{model}=0.45; r_AMY: F_{both}=3.4, R^2_{model}=0.47; l_AMY F_{both} =4.0, R^2_{model}=0.47; r_NAcc: F_{both} =4.0, R^2_{model}=0.49; l_NAcc: F_{both} =3.4 R^2_{model}=0.46).

4. Discussion

According to our hypotheses we found a significant interaction between BDNF p.Val66Met variant and IGF-1, the way that IGF-1 significantly moderated the direct influence of BDNF p.Val66Met variant on depression severity. In addition, we found, that the manifestation of this influence was located in regional brain volumes of memory-related regions (i.e. right/left HC, right/left entorhinal cortex, left parahippocampus) and in regions of the reward system (i.e. bilateral NAcc and AMY).

4.1 IGF-1 as significant moderator

In our sample of depressive adolescents, we found that the lower the peripheral IGF-1 concentration, the higher the BDI score. This effect was also valid in some models in patients with average IGF-1 levels. Our results are contradictory to the predominant findings in literature where most studies depicted an increase of peripheral IGF-1

in MDD [24]. Following the meta-analysis from Levada and Troyan (2017), studies about peripheral IGF-1 levels in depressive adults are still contradictory, because of confounding factors (e.g. age, sex, course of the disease, ongoing therapy or general health conditions, [18]). Our results are in line with a study by Sievers and colleagues (2014), finding an association between low levels of peripheral IGF-1 and depressive symptoms in women [33]. In our sample, 70% were females, so this might have influenced the present results. The results of Chigogora (2016) are also in line with our outcomes. In their study men and women with a very high or very low level of IGF-1 had higher risk for MDD (resulting in a "U"-shaped pattern, [34]). Interestingly, our findings of low peripheral IGF-1 agree with animal models that low serum IGF-1 levels lead to decreased adult hippocampal neurogenesis [35]. A lack of hippocampal neurogenesis is considered to be a pivotal factor in the genesis of depression [7]. As IGF-1 crosses the blood-brain barrier, the result of low peripheral IGF-1 levels could also be explained by a compensation of low central IGF-1 level [36]. The application of IGF-1 in the peripheral and central system results in enhanced neurogenesis in the HC and in a decrease of depressive symptoms [18, 20]. High central IGF-1 could upregulate BNDF secretion, depending on the BDNF-gene-variant [e.g. 37]. Moreover, low peripheral IGF-1 could be specific for adolescent depression. Therefore, further studies are needed to understand the influence of IGF-1 as moderator in adolescent depression.

4.2 Regional brain volume- manifestation of BDNF p.Val66Met genotype *IGF-1 influence

Moderation analyses revealed that the volume of relevant structures did not serve as significant moderators in the influence of BDNF p.Val66Met variant on depression. Thus, for example, AMY volume, even though it was enhanced in individuals with BDNF p.Val66Met Val/Val variant compared to BDNF p.Val66Met Val/Met variant, did not directly influence the interaction between BDNF p.Val66Met variant and depression. However, as IGF-1 and regional volumes together significantly moderated the influence of BDNF p.Val66Met variant on depression, volumes seemed to play a certain role. Therefore, these regions reflect the localization where the neurotrophic impact of both BDNF p.Val66Met variant and IGF-1 is established in the brain.

Earlier studies reported that BDNF genes and IGF-1 determine neural plasticity and thus, brain volume. For example, Webster and colleagues (2002) discussed their findings of an increase in BDNF mRNA in the PFC in the context of frontal maturation. They argued that BDNF p.Val66Met variant might "act as a regulator on neuronal morphology and synaptic pruning throughout postnatal development and during the adolescent period, the higher level of expression that occurs in the mature cortex might be necessary for the maintenance of connectivity and for synaptic plasticity the way that the BDNF p.Val66Met variant, which is produced in the cortical neurons, might be required by the neurons that provide connections, including for example thalamocortical or ipsilateral cortico-cortical projections." [11]. In this line, BDNF has been described as a 'target-derived' neurotrophic factor produced in the cortical neurons, that might be required by the neurons to provide connections, including for example the thalamocortical and ipsilateral cortico-cortical projections [38]. Target regions of the BDNF p.Val66Met variant in medio-temporal regions such as the HC and the AMY have been described in the human adolescent [39] and adult

brain [40] and in the animal adolescent [41] and adult brain [42]. The same is valid for the PFC [43] and ventral and dorsal striatum [44]. Finally, BDNF is a neurotrophin, known for multiple functions in central and peripheral nerve growth, differentiation, survival and long-term potentiation [45]. Some authors see a pivotal role of BDNF protein in the complex mechanisms of neuroplasticity, influencing the pathways of cytokines and other growth factors like e.g. IGF-1 [46]. IGF-1, in return, is a more widespread growth factor, involved in the regeneration of nerve cells and white matter tracts in the whole brain [47] with recent hints toward impact on the HC and the PFC [35]. Thus, it might be possible, that within these regions, interaction between BDNF p.Val66Met variant and IGF-1 might contribute to a triggering of neuroplastic changes.

5. Limitations

One limitation in this study is the small sample size. Findings from a sample of this small size, in our point of view, can mainly be rectified by its high homogeneity of subjects and its high external validity. For example, in accordance with findings of the preferential transmission of the Val allele in mood disorders [9], we found in our sample, that carriers of the BDNF p.Val66Met Val/Val variant highly outnumber Val/Met variant carriers. In that line, carriers of the BDNF p.Val66Met Val/Val variant Val/Val homozygotes had higher BDI scores compared to Val/Met variant carriers, i.e. had more severe depression [48]. Also, on brain level BDNF p.Val66Met Val/Val variant in our sample presented the reported alterations in the reward systems in terms of reduced NAcc together with enhanced AMY volume [2]. Based on a high overlap between findings from earlier studies and our sample, we decided to elaborate this data.

A second limitation is the focus on biological moderators in this analysis despite the body of evidence of environmental factors such as SLE [49], other genes such as serotonergic ones or both [50]. Hosang et al. reported in their systematic review a significant interaction between the BDNF p.Val66Met variant, SLE and depression [51]. Kaufman et al. (2006) showed the influence of the Met variant and sexual abuse on depression in children in the concomitant case of certain variants in the Serotonin-transport-gene [50]. Chen et al. reported higher depression scores after SLE in adolescents with BDNF p.Val66Met Val/Val variant compared to the Val/Met variant [49]. We did not consider these factors in this study.

In summary, we found that serological IGF-1 significantly moderated the influence of the BDNF p.Val66Met variant in juvenile depression, whereas regional brain volume seemed to play a more indirect role as revealed by moderation analyses. The reported findings point to a potentially specific mechanism in juvenile depression and could in a further step contribute to targeted diagnostic and therapies for this clientele. We think that the present results should be published although the number of participants was relatively low, because the actual data suggests an interaction between central and peripheral parameters as important markers in the context of adolescent depression. Therefore, the present data might influence the planning of studies with larger samples in the future and contribute to the further development of an endophenotype of adolescent depression.

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Disclosures

All authors declare that they have no conflicts of interest, neither biomedical financial interests, nor other conflicts of interest, financial or otherwise.

Declarations

All authors declared no conflict of interest.

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Ethics Approval

The study was approved by the University of Cologne Ethics Committee and was conducted in accordance to the Declaration of Helsinki in its latest version from 2008.

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Supplementary Tables

	Val/Val [n=28]	Val/Met [n=12]	Statistics
depressive symptoms			
BDI	32.93 (11.17)	23.83 (10.56)	$T_{(38,2)}$=2.4, p=.02
peripheral parameter [ng/ml]			
IGF-1	396.63 (95.86)	442.44 (75.38)	$T_{(38,2)}$=1.3, p=.20
central parameters [regional brain volume as %/ICV]			
r_HC	0.261[a] (.004)	0.270[a] (.007)	$F_{(38,2)}$=2.5, p=.097
l_HC	0.266[a] (.006)	0.263[a] (.009)	$F_{(38,2)}$=3.1, p=.055
r_ENTO	0.102[a] (.005)	0.095[a] (.008)	$F_{(38,2)}$=0.3, p=.750
l_ENTO	0.093[a] (.008)	0.105[a] (.012)	$F_{(38,2)}$=0.3, p=.720
r_paraHC	0.123[a] (.003)	0.116[a] (.004)	$F_{(38,2)}$=6.4, p=.004
l_paraHC	0.159[a] (.021)	0.132[a] (.032)	$F_{(38,2)}$=0.3, p=.770
r_AMY	0.089[a] (.002)	0.094[a] (.003)	$F_{(38,2)}$=10.7, p=.001
l_AMY	0.086[a] (.002)	0.093[a] (.009)	$F_{(38,2)}$=3.0, p=.063
r_NAcc	0.028[a] (.001)	0.027[a] (.002)	$F_{(38,2)}$=3.4, p=.040
l_NAcc	0.029[a] (.001)	0.030[a] (.002)	$F_{(38,2)}$=2.9, p=.065
r_IFG_op	0.187[a] (.006)	0.199[a] (.010)	$F_{(38,2)}$=0.6, p=.560
l_IFG_op	0.212[a] (.008)	0.208[a] (.012)	$F_{(38,2)}$=0.4, p=.690
r_IFG_orb	0.071[a] (.007)	0.056[a] (.011)	$F_{(38,2)}$=0.7, p=.520
l_IFG_orb	0.049[a] (.003)	0.052[a] (.005)	$F_{(38,2)}$=0.3, p=.710
r_IFG_tri	0.167[a] (.008)	0.167[a] (.012)	$F_{(38,2)}$=0.4, p=.700
l_IFG_tri	0.170[a] (.007)	0.165[a] (.011)	$F_{(38,2)}$=0.1, p=.900

Note *: $q^*=p_{corrected\ for\ 16\ tests}<.00045$; BDI: Beck Depression Inventory; IGF-1: insulin-like-growth-factor 1; [a]: corrected for intracranial volume; HC: hippocampus; paraHC: parahippocampal gyrus; ENTO: entorhinal cortex; AMY: amygdala; NAcc: nucleus accumbens; IFG_op: inferior frontal gyrus, pars opercularis; IFG_tri: inferior frontal gyrus, pars triangularis; IFG_orb: inferior frontal gyrus, pars orbitalis. Age-specific reference values for serological IGF-1 are for 13-year-olds 221-891 ng/ml, 14 year-olds 188-782 ng/ml, 15 year-olds 236-912 ng/ml 16 year-olds 226-829 ng/ml, and 17-year-olds 197-676 ng/ml. In the group of individuals with Val/Met variant one individual with Met/Met variant was included. Val/Val: carriers of the *BDNF* p.Val66Met Val/Val variant; Val/Met: carriers of the *BDNF* p.Val66Met Val/Met variant and Met/Met variant.

Table s 1: BDNF-related group differences.

	Sex [12/28]	Phobia [21/19]	GAD/PD [6/34]	OCD [6/34]	Medication [11#/29]
BDI	$T_{(38,2)}$=3.1, p=.01	$T_{(38,2)}$=0.05, p=.96	$T_{(38,2)}$=2.34, p=.03	$T_{(38,2)}$=0.34, p=.72	$T_{(38,2)}$=1.0, p=.31
IGF1	$T_{(38,2)}$=1.8, p=.08	$T_{(38,2)}$=0.06, p=.96	$T_{(38,2)}$=0.78, p=.44	$T_{(38,2)}$=1.62, p=.12	$T_{(38,2)}$=0.09, p=.93
r_HC	$T_{(38,2)}$=0.88, p=.39	$T_{(38,2)}$=0.56, p=.58	$T_{(38,2)}$=0.14, p=.89	$T_{(38,2)}$=1.48, p=.15	$T_{(38,2)}$=0.10, p=.92
l_HC	$T_{(38,2)}$=.59, p=.13	$T_{(38,2)}$=0.54, p=.60	$T_{(38,2)}$=0.97, p=.34	$T_{(38,2)}$=1.04, p=.31	$T_{(38,2)}$=0.78, p=.44
r_ENTO	$T_{(38,2)}$=0.41, p=.68	$T_{(38,2)}$=0.67, p=.51	$T_{(38,2)}$=0.91, p=.37	$T_{(38,2)}$=2.34, p=.02	$T_{(38,2)}$=2.14, p=.04
l_ENTO	$T_{(38,2)}$=0.18, p=.86	$T_{(38,2)}$=0.35, p=.73	$T_{(38,2)}$=0.20, p=.85	$T_{(38,2)}$=0.93, p=.36	$T_{(38,2)}$=0.45, p=.66
r_paraHC	$T_{(38,2)}$=3.0, p=.01	$T_{(38,2)}$=0.31, p=.76	$T_{(38,2)}$=2.99, p=.01	$T_{(38,2)}$=2.42, p=.02	$T_{(38,2)}$=2.14, p=.04
l_paraHC	$T_{(38,2)}$=1.0, p=.32	$T_{(38,2)}$=0.91, p=.37	$T_{(38,2)}$=2.08, p=.05	$T_{(38,2)}$=0.27, p=.79	$T_{(38,2)}$=1.35, p=.17
r_AMY	$T_{(38,2)}$=3.02, p=.01	$T_{(38,2)}$=0.34, p=.74	$T_{(38,2)}$=1.15, p=.26	$T_{(38,2)}$=1.98, p=.06	$T_{(38,2)}$=1.46, p=.15
l_AMY	$T_{(38,2)}$=0.78, p=.44	$T_{(38,2)}$=1.06, p=.30	$T_{(38,2)}$=1.30, p=.20	$T_{(38,2)}$=1.64, p=.11	$T_{(38,2)}$=0.71, p=.48
r_NAcc	$T_{(38,2)}$=1.47, p=.15	$T_{(38,2)}$=1.17, p=.25	$T_{(38,2)}$=0.78, p=.44	$T_{(38,2)}$=1.71, p=.10	$T_{(38,2)}$=2.03, p=.05
l_NAcc	$T_{(38,2)}$=1.39, p=.17	$T_{(38,2)}$=0.61, p=.54	$T_{(38,2)}$=0.,22 p=.83	$T_{(38,2)}$=2.14, p=.04	$T_{(38,2)}$=1.03, p=.31
r_IFG_op	$T_{(38,2)}$=1.17, p=.25	$T_{(38,2)}$=0.15, p=.88	$T_{(38,2)}$=0.49, p=.63	$T_{(38,2)}$=0.59, p=.56	$T_{(38,2)}$=0.53, p=.60
l_IFG_op	$T_{(38,2)}$=0.38, p=.71	$T_{(38,2)}$=0.22, p=.82	$T_{(38,2)}$=0.23, p=.82	$T_{(38,2)}$=0.36, p=.72	$T_{(38,2)}$=1.08, p=.29
r_IFG_orb	$T_{(38,2)}$=0.68, p=.50	$T_{(38,2)}$=0.82, p=.42	$T_{(38,2)}$=2.60, p=.01	$T_{(38,2)}$=0.41, p=.69	$T_{(38,2)}$=2.31, p=.03
l_IF G_orb	$T_{(38,2)}$=1.06, p=.30	$T_{(38,2)}$=1.13, p=.27	$T_{(38,2)}$=0.53, p=.60	$T_{(38,2)}$=0.19, p=.85	$T_{(38,2)}$=1.13, p=.27
r_IFG_tri	$T_{(38,2)}$=0.57, p=.57	$T_{(38,2)}$=1.38, p=.18	$T_{(38,2)}$=0.07, p=.95	$T_{(38,2)}$=1.66, p=.11	$T_{(38,2)}$=2.63, p=.01
l_IFG_tri	$T_{(38,2)}$=0.07, p=.94	$T_{(38,2)}$=1.12, p=.27	$T_{(38,2)}$=0.80, p=.43	$T_{(38,2)}$=1.50, p=.14	$T_{(38,2)}$=2.62, p=.02

Note. GAD: generalized anxiety disorder; PD: panic dicorder, OCD: obsessive-compulsive disorder; [#]: 11= permant medication (N=4) + PRN (N=7); BDI: Beck Depression Inventory; IGF-1: insulin-like-growth-factor 1; [a]: corrected for intracranial volume; HC: hippocampus; paraHC: parahippocampal gyrus; ENTO: entorhinal cortex; AMY: amygdala; NAcc: nucleus accumbens; IFG_op: inferior frontal gyrus, pars opercularis; IFG_tri: inferior frontal gyrus, pars triangularis; IFG_orb: inferior frontal gyrus, pars orbitalis. FDR-correction for 20 comparisons p_{q*}=0.0025.

Table s2: Influence of sex, comorbidities and medication on regional brain volume [%/ICV], revealed via two sample t-tests.

Citation: Heidrun Lioba Wunram, Susanne Neufang, Friederike Körber, Raoul Heller, Serjosha Blick, Gebhart Malchau, Wilhelm Bloch, Andreas Dabringhaus, Matthias Kraemer, Theresia Christina Clajus, Alischa Ziemendorff, Fabian Abuhsin, Andrea Steffen, Eckhard Schönau, Jörg Dötsch, Stefan Bender, Oliver Fricke. Insulin-like-Growth Factor 1 moderates the influence of the BDNF p.Val66Met variant on depression severity in adolescent depression. Journal of Psychiatry and Psychiatric Disorders 4 (2020): 235-255.