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Biological mechanisms in the background of ruminative response style

PhD Thesis

Nóra Eszlári

Semmelweis University

Doctoral School of Mental Health Sciences

Supervisor: Gabriella Juhász, MD, PhD

Official reviewers: Judit Gervai, CSc
Róbert Urbán, DSc

Head of the Final Examination Committee: Gábor Faludi, MD, DSc

Members of the Final Examination Committee: Edit Buzás, MD, DSc
Zsolt Demetrovics, DSc
Adrien Pigniczkiné Rigó, PhD

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1. Abbreviations

1-C cycle: one-carbon cycle

5-HTTLPR: serotonin-transporter-linked polymorphic region

10-formyl-THF: 10-formyl-tetrahydrofolate

ACC: anterior cingulate cortex

ARQ: Analytical Rumination Questionnaire

BDI: Beck Depression Inventory

BDNF: brain-derived neurotrophic factor

BSI: Brief Symptom Inventory

CBT: cognitive behavioural therapy

CERQ: Cognitive Emotion Regulation Questionnaire

CES-D: Center for Epidemiological Studies-Depression

COMT: catechol-O-methyltransferase

CREB1: cAMP-response element binding protein 1

CRSQ: Children's Response Style Questionnaire

CRSS: Children's Response Styles Scale

CSF: cerebrospinal fluid

CTQ: Childhood Trauma Questionnaire

DLPFC: dorsolateral prefrontal cortex

DRD2: dopamine receptor D2

ECQ: Emotion Control Questionnaire

FDR: false discovery rate

fMRI: functional magnetic resonance imaging

GIRK2: G protein-activated inwardly rectifying potassium channel subunit 2

GxE: gene-by-environment interaction

GWAS: genome-wide association study

HRV: heart rate variability

HTR2A: serotonin receptor 2A

IFG: inferior frontal gyrus

LEIDS-R: Leiden Index of Depression Sensitivity Revised

MDD: major depressive disorder

miRNA: microRNA

MRQ: Multidimensional Rumination Questionnaire

MTHFD1L: mitochondrial monofunctional 10-formyltetrahydrofolate synthetase

MTHFR: 5,10-methylenetetrahydrofolate reductase

PET: positron emission tomography

PFC: prefrontal cortex

PTSD: post-traumatic stress disorder

RIES: Revised Impact of Event Scale

RLE: recent negative life events

RNE: Rumination on a Negative Event

ROS: Rumination on Sadness Scale

RRQ: Rumination Reflection Scale

RRS: Ruminative Responses Scale

RSQ: Response Styles Questionnaire

RTS: Ruminative Thought Style Questionnaire

SAH: S-adenosylhomocysteine

SAM: S-adenosylmethionine

S.E.M.: standard error of mean

SMRI: Scott Macintosh Rumination Inventory

SNP: single nucleotide polymorphism

SSRI: selective serotonin reuptake inhibitor

TCQ: Thought Control Questionnaire

THF: tetrahydrofolate

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2. Introduction

Since we are humans, all of us can think about ourselves, all of us can reflect on our own feelings, thoughts and deeds, all of us can wonder about our own memories and representations of the future. But the points that really matter are how often, in which way and on which content we do so. I am going to substantiate throughout my thesis that we actually differ from each other in these self-reflecting processes, and that these individual differences have plenty of consequences on differences in our health and illness, and that they also have well-definable molecular underpinnings residing in our DNA.

2.1. Rumination

2.1.1. Definition of rumination

2.1.1.1. Response styles theory

The most widely used definition for rumination stems from the response styles theory (1, 2). In that framework, rumination, or in other words, depressive rumination or ruminative response style, is a stable, trait-like mode of the individual to respond to distress (1, 2). It denotes a passive, repetitive, perseverative process of thinking about the person's own feelings, problems, symptoms of distress, and their possible causes and consequences (1). Passivity entails that ruminative thinking prevents active problem solving, it interferes with taking action to change circumstances of the distress symptoms, by the vicious circle that the person remains fixated on the problems and related feelings, with a reduced self-confidence in solutions for problems and a reduced motivation to initiate instrumental behaviour (1). According to the response styles theory, rumination is defined as a process of thinking, not the content itself; however, the content of ruminative thought typically has a negative valence, just like negative cognitive styles, automatic thoughts and schema known from the cognitive theory of depression (1, 3). Nevertheless, rumination is associated with several maladaptive cognitive styles:

pessimism (4), low mastery (5), negative attributional styles, self-criticism, neediness, dependency (6), hopelessness, dysfunctional attitudes, sociotropy and neuroticism (1).

Operationalisation and measurement of rumination, in the framework of response styles theory, can be carried out by the Ruminative Responses Scale (RRS) of the Response Styles Questionnaire (RSQ) (1). RRS consists of 22 items, each of which describes a ruminative thought or behaviour. The participant has to indicate on a Likert scale how often he or she engages in each one when he or she feels sad, blue or depressed. One possible grouping of these 22 items is self-focused, symptom-focused, and focused on the possible causes and consequences of the mood (1). Considering the criticism of some items for the remarkable overlap in content with depressive symptoms themselves, an alternative grouping was introduced and underpinned with factor analyses by Treynor et al, 2003 (7): brooding, reflection and depression subscales. Brooding, with five items, denotes a moody pondering, an anxious and gloomy thinking with self-criticism or criticism of others or fate, passively comparing the person's current situation with an unachieved standard. Reflection encompasses the five items describing engagement in a neutrally valenced contemplation with the purpose of dealing with and attempting to overcome problems. The depression subscale consists of the twelve items criticised for the overlap with items of the Beck Depression Inventory (BDI). These brooding and reflection dimensions have been corroborated in studies worldwide, among adolescents, undergraduate students and major depressive patients (8-15). As for convergent validities, while the whole 22-item RRS rumination scale significantly showed a moderate positive correlation with chronic stress and strain and a moderate negative correlation with a sense of mastery over important life events (5), these results were replicable only for the brooding but not the reflection subscale (7). Moreover, among adolescents, brooding was positively associated with voluntary disengagement strategies in response to stress, such as denial, avoidance or fleeing, whereas reflection was associated positively with coping strategies such as problem solving (changing the stressor) and cognitive restructuring (changing his or her attitude toward the stressor) (15). It is important to note that according to the factor analysis, only half of the variance on the 10-item RRS scale (encompassing the five brooding and the five reflection items) can be explained by the brooding and reflection factors (7), and this value could be replicated among never

depressed and formerly depressed participants (16), so rumination as a whole construct is also worth investigation, besides the two subscales.

2.1.1.2. Other definitions

Apart from the definition in response styles theory, Smith and Alloy, 2009 (2) give a thorough review on the alternative possibilities of conceptualising and operationalising rumination.

Among these alternative definitions, some are closely related to that of the response styles theory: rumination on sadness (17), and rumination on negative inferences associated with stressful life events (18). The Rumination on Sadness Scale (ROS), comprising one single factor, is associated with RSQ rumination and neuroticism (19).

Trapnell and Campbell (1999) (20) separated two types of self-attentiveness, two motivational dispositions of private self-consciousness: the negatively toned rumination and the positively or neutrally toned reflection. According to their definitions and items of their Rumination Reflection Scale (RRQ), rumination can be linked to neuroticism, and reflection is related to openness to experience, among the Big Five personality factors (20).

Watkins (21, 22) differentiated two modes of ruminative self-focus: a maladaptive conceptual-evaluative one, with an analytical focus on discrepancies between current and desired outcomes, and an adaptive experiential one, which means an awareness of the moment's experience, intuitively, non-evaluatively (2).

Other models have grasped either rumination after trauma (23), or a post-event, continued processing of a social interaction recurrently and intrusively, in the framework of social phobia (24), or an intrusive response to traumatic events in the framework of post-traumatic stress disorder (PTSD) (25, 26), or may define intrusiveness as a dimension of rumination (27). The Multidimensional Rumination Questionnaire (MRQ) measures three subtypes of rumination in response to a stressful event: first, emotion-focused rumination, which assesses thinking about depressive symptoms and is associated with neuroticism, second, searching for meaning of negative experiences, and third, instrumental rumination, meaning a thinking about what can be done to change the situation (19, 23). Intrusiveness of thoughts about a recent stressful event can be measured by the intrusion subscale of the Revised Impact of Event Scale (RIES) (19, 26). The

Rumination on a Negative Event (RNE) questionnaire covers different aspects (intrusiveness, frequency, suddenness, voluntariness, dismissability) of ruminative thoughts about a recent negative event, but yields only one coherent factor (termed thus general rumination) out of the emerging two factors (19).

Rumination can also be defined as a self-regulation process in response to goal discrepancy: a discrepancy between actual and desired status (28, 29), or it can be a volitional self-regulation in response to stress (30). The Scott Macintosh Rumination Inventory (SMRI) assesses rumination of failed goal-pursuits, and is constituted by three subscales: emotionality, motivation to accomplish goals, and (although with a poor internal consistency) distraction (19).

Rumination has also been interpreted in the framework of emotional regulation and coping strategies in response to emotions provoked by stress (31, 32). The Thought Control Questionnaire (TCQ) measures coping with intrusive thoughts, and its reappraisal subscale means focusing on and revising thoughts about stressful emotional events, and this subscale is associated with self-consciousness (19).

Emotional intelligence, social and emotional competence have also emerged as a context of defining rumination (33). The Emotion Control Questionnaire (ECQ) assesses inhibition of unwanted thoughts, and its rehearsal subscale, denoting that someone tends to think about negative events over and over, is related to trait anxiety (19, 33).

Brinker and Dozois (34) conceptualised and measured rumination as a broad concept of repetitive, recurrent, intrusive and uncontrollable way of thinking, encompassing negative, positive and neutral thoughts as well, and thoughts oriented towards both the past and the future. Their single-factor scale for its measurement is the Ruminative Thought Style Questionnaire (RTS).

Rumination and worry can be viewed as two types of perseverative cognition, which expands the temporal duration of the stressor beyond the traditional stress response by the extendedly and inflexibly activated mental representation of the stressor (35, 36). In this allostatic load model of stress, perseverative cognition, by prolonging affective and physiological stress response in advance of and following the stressor, is related to an enhanced activity of many physiological (cardiovascular, endocrinological, neurovisceral, immunological) parameters, a chronic pathogenic physiological state, and thus mediates the health consequences of stressors (35-37).

2.1.1.3. Distinguishing rumination from other related constructs

At this point, differences between rumination and worry also have to be discussed. While ruminative thoughts are considered to put more focus on events of the past, themes of worry have a future orientation (2). Worry thoughts are about problem solving, fuelled by the motivation of avoiding worry thoughts themselves, although rumination concerns themes of loss, entails less effort and less confidence in problem solving ability, and is motivated by the need to understand personal relevance of the situation (2). However, a recent study applying structural equation modelling found that rumination and worry were two uncorrelated method factors of one latent factor, rather than two separate factors, so that we can regard them two sides of one coin, repetitive negative thinking (38).

It is also important to distinguish rumination from obsession, which characterises obsessive-compulsive disorder. Depressive rumination, according to the response styles theory, stems from negative affect, whereas obsession generates negative affect (2). Moreover, obsession entails some action, namely compulsion, in order to neutralise this negative affect, but rumination interferes with any instrumental behaviour or problem solving action (2). Thirdly, in contrast to rumination, the content of obsessive thoughts are restricted to six specific areas (2).

2.1.1.4. Potential latent taxonomies in the background of different measurements of rumination

To explore potential latent taxonomies within the diversified conceptualisation and operationalisation of rumination, multivariate statistical methods can be applied.

Siegle et al, 2004 (19) conducted a factor analysis on multiple measures of rumination among undergraduate students. The first factor was assembled by rumination on sadness, worry and other negatively valenced trait ruminations, such as the brooding subscale of the RSQ. The second factor stood for scales referring to a distant-past negative event. The third factor was loaded by scales of either a reappraisal of negative events or a neutrally valenced reflection (RSQ reflection and RRQ reflection). The last, fourth factor encompassed scales representing possible alternate responses to rumination.

Mandell et al, 2014 (39) performed a factor analysis with almost the same measures as the above Siegle et al, 2004 study, but in a sample of depressed patients. It yielded a

first factor termed as experiential rumination and composed by negative emotions, depressive symptoms, repetitive focus and reactivity to negative aspects of the self, such as ECQ rehearsal, RRQ rumination and worry. The second factor, event-related rumination, encompassed measures (such as RIES intrusiveness and RNE general rumination) on intrusiveness and frequency of thoughts related to specific negative events. The third factor, constructive rumination, stood for non-negative or adaptive repetitive cognition, such as TCQ reappraisal and RRQ reflection.

Segerstrom and Stanton (40) measured multiple forms of repetitive thought among students, such as rumination and worry, and their multidimensional scaling revealed two dimensions in their background: emotional valence (positive or negative) of the content, and purpose of the repetitive thought (searching or solving).

In sum, we can conclude that besides considering the appropriate theoretical framework in which we intend to conceptualise and measure rumination, it is also important to know that many, partly overlapping concepts of rumination have emerged and need to be clarified within a study.

2.1.2. Rumination and depression

2.1.2.1. Is depressed mood a precondition of rumination?

As defined by the response styles theory, ruminative thinking is a response to distress and depressed mood, prolonging and exacerbating them in several ways (1). First, it sustains the state of negative affect, making more negative memories get activated and be utilised for interpretation of the person's current situation (1, 41). Second, rumination transforms thinking to a more pessimistic and fatalistic one, thus interfering with effective problem solving and instrumental behaviour, and leading to a vicious circle (1, 42-44). This vicious circle can also be due to the loss of social support because of constant rumination (1).

Besides questionnaire measurement of trait rumination, state rumination can be induced experimentally by the instructions to think about the meanings, causes and consequences of the participant's current feelings, for eight minutes (1, 45). In contrast, distraction induction instructs the participant to focus on non-self-relevant images (1, 45).

Experiments manipulating response styles have revealed that rumination increased dysphoric mood only in those participants being already in a dysphoric mood at the beginning, but it had no effect on mood in the non-dysphoric participants (1, 41, 44, 45). Similarly, distraction induction decreased dysphoric mood only in dysphoric participants, but it had no effect on mood in non-dysphoric participants (1, 41, 44, 45). These findings could be replicated also in clinically depressed participants (1, 46). These findings imply that depressed mood or distress is a precondition of the future depressogenic effect of rumination.

The studies investigating test-retest stability of RRS rumination over one year and finding a test-retest correlation r=0.67 for the whole 22-item rumination scale (comprising brooding, reflection and depression items as well), an r=0.62 for the brooding and r=0.60 for the reflection subscale, got a comparable r=0.60 test-retest correlation for the BDI depression scale over the same one year (5, 7). This means that ruminative tendencies are just as stable as the level of depression, also underlining the stress response nature of rumination. Similarly, Bagby et al, 2004 (47) stated that RRS rumination does not show an absolute stability, since it decreases with the reduction of depressed mood, being the elevation of depressive symptoms a necessary context to evoke rumination. They also reviewed test-retest correlations of RRS rumination in different studies, as an investigation of its relative stability, defined as a stability of individual differences on test scores over time (47). In case of a stable level of depression over time, its test-retest correlation coefficient was 0.66 in inpatients within a four-week interval, and 0.80 in a community sample within a five-month interval (4, 47, 48). However, they found lower test-retest correlations if the level of depressed mood changed over time: r=0.50 in inpatients within four weeks, and between 0.36 and 0.55 in college students within various intervals from six weeks to one year (47-50). Their own results in treated unipolar major depressed outpatients revealed that change in symptom-focused RRS rumination level was significantly associated with change in depression level, however, change in self-focused RRS rumination was unrelated to depression change (47). Symptom-focused and self-focused facets of RRS rumination had been gained by factor analysis on items of the RSQ (51), and self-focused rumination has been considered more or less consistent with brooding and reflection (47). Facets of rumination become important at this point

because we must bear in mind the degree and way of overlap of the rumination construct with depressive symptoms if we are considering its dependency on depression level (2).

All in all, we can state that depressed mood is a precondition, a trigger of depressive rumination, which is a style of response to that stress, and is stable over time only if its trigger, depression is stable. However, when thinking about its stability as a function of depression level, we must not forget that rumination can be decomposed into subscales, each of which has a distinct overlap with depression. So depressive rumination can be viewed as that a ruminative person does not ruminate constantly, but their level of rumination is a stable trait throughout different situations when encountering distress and depressed mood (2).

2.1.2.2. Rumination, concurrent and future depression

According to Treynor et al, 2003 (7), the whole, 22-item RRS rumination scale showed an r=0.48 correlation with concurrent, and an r=0.38 with future (one year later) BDI depression level. Comparable in magnitude to them, the brooding subscale had an r=0.44 with concurrent, and an r=0.37 with future depression level, in contrast to the reflection subscale, which yielded an r=0.12 with concurrent, and r=0.08 with future depression level (7). However, in a structural equations modelling approach on the same data, while the brooding subscale yielded the same positive association with one year later depression, the reflection subscale associated negatively with future depression level, suggesting its potential long-term protective role against depression, perhaps by facilitating effective problem solving (7).

In the meta-analysis of Aldao et al, 2010 (52) including a wide variety of types of sample and measurements, rumination had a large positive association with psychopathologies, with the largest value for depression out of anxiety, depression, eating and substance use symptoms. The association of rumination with psychopathology in general was not moderated by age but was moderated by sample type, with larger effect sizes in studies including clinical samples than in studies with only non-clinical ones (52). Similarly, age did not moderate the association of rumination with depression, but rumination had a larger association with depression in studies including clinical participants than in those without clinical participants (52). Comparable effect sizes

emerged to each other, for the brooding subscale and the non-RSQ rumination measures: medium to large with psychopathology and large with depression (52).

Aldao et al, 2010 (52) also reviewed longitudinal studies, and found that the RSQ rumination predicted an increase in depressive symptoms over three years in children, and an increase in self-rated (but not in clinician-rated or mother-rated) depressive symptoms and new onsets of major depression over one to four years in adolescents (52). Among adults, positive studies have found that RSQ rumination predicted an increase in depressive symptoms over a wide range of time, from a few days across a few weeks to one year, and that it also predicted onset of major depression over one year; and negative studies emerged only on depressive symptoms and with 5-10 week intervals (52). Aldao et al, 2010 (52) found longitudinal studies on depression using measurements of rumination other than the RSQ scarce and contradictory.

Rood et al, 2009 (53) also conducted a meta-analysis regarding rumination and depression including only non-clinical children and adolescent sample studies only on rumination conceptualised by the response styles theory. They found an r=0.44 pooled effect size between rumination and depression in cross-sectional studies, with an r=0.36 within children and an r=0.48 within adolescents, all of which effect sizes showed adequate stability (53). However, in longitudinal studies, by partialling out the baseline depression level they got a significant r=0.07 between rumination and future depressive symptoms, but it has to be interpreted with caution because of stability issues (53).

To conclude, there is a considerable amount of evidence compiled on the remarkable positive association of rumination with both concurrent and future depression, robust and replicable across age groups and sample types (clinical or non-clinical), nevertheless, specificity of rumination subscales and importance of concurrent depression in the longitudinal effect of rumination are worth to be noted.

2.1.2.3. Relationship of rumination and depression, in the context of other related constructs

In the predictive role of rumination for either concurrent or future depression, it is crucial to take other constructs related to rumination or depression into consideration.

In the angle that both rumination and overgeneral autobiographical memory are vulnerability factors for depression, Hamlat et al, 2015 (54) investigated their effects on early adolescents' nine month later depressive symptoms. Their results revealed that

while CRSQ (Children's Response Style Questionnaire) rumination was unrelated to specificity or overgenerality of autobiographical memories, a four-way interaction effect emerged: stressful life events increased depressive symptoms in girls with more overgeneral autobiographical memories and a high level of rumination (54).

Regarding neuroticism, the association between rumination and depression remains significant even after controlling for neuroticism, implying its independent depressogenic effect beyond that of neuroticism (1, 2). On the other hand, among clinically depressed participants, the association between neuroticism and depressive symptoms was partially mediated by RRS rumination, which held true for both the brooding and reflection subscales entered as simultaneous mediators in an another model, and worry was not a significant mediator of the neuroticism-depression association besides rumination or besides brooding and reflection (55).

Regarding potential overlap with negative automatic thoughts, rumination also remains to be related to depression if negative cognitions are controlled for (2). It also maintains its association with depression when controlling for perfectionism or pessimism (1, 4). On the other hand, dysfunctional attitudes, negative inferential styles, self-criticism, neediness and dependency are associated with depression partially or fully mediated by rumination (1, 6).

In conclusion, the depressogenic effect of rumination is wholly or partly independent of the depressogenic effect of overgeneralising memory processes, neuroticism, negative automatic thoughts, dysfunctional attitudes and other negative cognitive styles, and being thus unsubstitutable in its relationship with depression, rumination is undoubtedly worth investigating among risk factors of depression.

2.1.2.4. The third direction: from depression to rumination

Rumination shows the highest scores in currently depressed persons, a lower one in those with only a past history of depression, and the lowest one in the never depressed (47). This difference between ever depressed and never depressed persons could either suggest that rumination in those prone to rumination and thus depression is so stable that it does not vanish with the depressive episode, or that rumination can be a scar of the episode, representing some residual symptoms after recovery (47). Consequently, it is necessary to deal with the third direction: depression and future rumination.

In a multiwave longitudinal study among adolescents, Abela et al, 2011 (56) found that rumination, besides moderating the relationship of negative events with future depressive symptoms and major depressive episodes, was associated with an increased risk of major depressive episodes in the past. Similarly, Gibb et al, 2012 (57) found in children that brooding, besides predicting onset of new depressive episodes over 20 months even after controlling for baseline depression level, also showed a higher level in children with a history of depressive disorders than in children without that.

Timing of depression and rumination to each other also seems to be important in the factor structure of the RRS. Whitmer and Gotlib, 2011 (16) performed factor analyses on a 20-item RRS scale within three different groups: participants currently in a major depressive episode, only formerly depressed, and never depressed participants, and they got back the brooding and reflection factors only in the formerly and the never depressed group. However, distinction between these two factors got blurred among currently depressed MDD (major depressive disorder) patients (16).

All in all, the relationship between rumination and depression appears to be bidirectional and transactional, with these two constructs constantly and vividly influencing each other, either if investigating them as a stream of processes within one's head or as a statistical decomposition of their variance to parts from which some are accounted for by each other.

2.1.3. Rumination as a potential endophenotype for depression

In the former chapters, I argued that rumination deserves investigation as an unsubstitutable and stable personality trait risk factor for depression, affecting depression in a robust and replicable manner. My next question is whether or not it could also be a potential endophenotype for the disorder.

2.1.3.1. Does rumination fulfil criteria for being an endophenotype?

According to the endophenotype concept, complex disorders such as schizophrenia and depression are too heterogeneous to stem from only one gene, rather they have a multifactorial and polygenic nature (58), so when aiming at exploring the genetic

background of such a disorder, it is useful to decompose it into more specialised and elementary phenotypes, each of which has a more simple and straightforward genetic architecture, a more homogeneous biological background than the disorder itself (58, 59). A more homogeneous, less confused genetic background of the endophenotype would enable us to gain larger genetic effect sizes than for the disorder, and although this assumption has been disproved for certain genes and endophenotypes (60), the endophenotype concept remains to be a useful framework of investigations.

The first criterion in the definition of endophenotype is that it should be associated with the disorder itself (58): we could see throughout chapter 2.1.2 that it is fulfilled by rumination to be an endophenotype for depression. The second criterion of heritability (58) is also met by rumination, moreover, it also meets the criterion of having a common genetic background (59) with depression (see chapter 2.2.1. in detail). The next criterion of state-independency, proposed by Gottesman and Gould, 2003 (58) and denoting the same level of the endophenotype regardless of whether or not illness is active, is not met by rumination, since it has a higher level in currently depressed than in only formerly depressed persons (47). However, the requirement of state-independency has been transformed to a less stringent need, enabling that it can be manifested only after a challenge (60), and rumination fulfils that less stringent form, being stress and depressed mood a necessary trigger to evoke rumination and determine its stability (see chapter 2.1.2.1. for details). The next two criteria, the co-segregation with illness within families, and the higher level in non-affected family members than in the general population (58), have not been investigated with regard to rumination so far. An additional criterion is that the endophenotype should be part of the causal process, the etiopathogenesis by which the disease arises (60), it should lie on the causal pathway between genes and the disorder (59). Rumination seems to fulfil that criterion too. Wilkinson et al, 2013 (61) studied healthy adolescents without any depression history but being at an elevated risk for psychopathology because of stressful life events or the parent's history of psychiatric disease. They entered rumination, depression and anxiety items into a factor analysis and with the items got back from the original scales, they found that elevated rumination predicted elevated levels of depressive symptoms 12 months later, and also onset of depressive disorders within these 12 months, both of them after controlling for baseline depression and anxiety levels (61).

All these results encourage us to propose that rumination is a potential endophenotype for major depression.

2.1.3.2. The endophenotype concept in depression

Flint and Kendler, 2014 (62) review knowledge on the genetic background of major depression, stating that this disorder has a 31-42% heritability and a 21-30% SNP heritability (denoting the disease variability accounted for by single nucleotide polymorphisms), but each common genetic variant (denoting a minor allele frequency of greater than 5%) has such a small effect size on major depression that genome-wide association studies (GWAS) are underpowered to detect these effects. To overcome this problem, one possible solution would be to increase sample size to tens of thousands, and another one would be to identify subtypes of depression that are more homogeneous than the disorder itself and less prevalent compared to the 10% prevalence of major depression (62). In this angle, major depressive disorder can be seen as an undifferentiated phenotype, the final common outcome of diverse processes, in the framework of equifinality, which is a notion of development literature (62).

Thus, from the perspective of major depression, it seems necessary to decompose it into biologically more homogeneous subtypes to facilitate genetic association studies, and rumination may be a candidate mechanism which draws the effect of genes in the direction of depression development.

2.1.4. Cognitive and neurobiological underpinnings of rumination

Having argued that rumination, as an endophenotype, may lie on the causal pathway from genes to major depression, in this chapter I am going to review the cognitive and neurobiological correlates of rumination, with the aim of drawing it closer to the level of biological underpinnings and genes.

2.1.4.1. Cognitive underpinnings

While rumination can also be viewed as a mode of stress response chosen because of positive metacognitive beliefs about its role (2), in the deficit of instructed forgetting of neutral words among undergraduates RRS rumination has been demonstrated to be more

than deliberate re-processing (63), thus the authors regarded rumination as a reduced top-down inhibitory modulation over mnemonic processes, a general memory control deficit not restricted to negatively valenced material. Similarly, Nolen-Hoeksema et al, 2008 (1) align evidence that trait rumination is positively associated with the number of perseverative errors on the Wisconsin Card Sorting Task and with an impaired ability in inhibiting previously useful strategies (rather than impairments in switching to a new strategy) in a set-switching task, both of which associations held true even after controlling for depression level. The positive association of rumination with inhibition difficulties is also corroborated in the instructed inhibition of eye movement to an abrupt peripheral cue (64). Whitmer and Gotlib, 2013 (65), in their attentional scope model of rumination, claim that mood in itself is not enough to generate rumination, but mood-independent individual differences exist in attentional scope, and a narrowed attentional scope will give rise to multiple forms of repetitive thought, such as rumination. When trait ruminators enter a negative mood, their attentional scope will get even narrower, yielding a bias towards negative self-relevant information, which will further fuel rumination (65).

In contrast, other studies argue that rumination, especially brooding, is related to attentional control deficits specific to negatively valenced material (66). Koster et al, 2011 (66) propose that rumination is due to an impaired attentional disengagement from negative self-referent information, and they also point to the longitudinal association between impaired cognitive control and later brooding in response to stress. Nolen-Hoeksema et al, 2008 (1) align that depressed ruminators' biases towards negative information can be measured in tests of basic attention and implicit memory. A converging evidence among adolescents is that rumination did not have a relation to general cognitive flexibility, but it did associate with impaired inhibition of negative information when switching from negative to positive blocks on the Affective Go / No-go task (67). Joorman et al, 2006 (68) also found that neither of brooding or reflection subscales associated with memory bias when controlling for depression level, but brooding was related to an attentional bias for sad faces even when controlling for depression level.

To sum up, either from an angle of general cognition or that of specifically negative information, rumination is consistently correlated with deficits in inhibition of material previously but no longer important.

2.1.4.2. The role of cortisol

Having been repeatedly discussed as a kind of stress response, it is plausible to link rumination to cortisol measurements. Zoccola and Dickerson, 2012 (69), in their review, come to the conclusion that increased cortisol concentrations have been consistently associated with higher state rumination, though inconsistently with trait rumination. Interestingly, whether state or trait rumination, if conceptualised by a stress-related measure, it was almost consistently positively, and if conceptualised by a depression-related measure, it was negatively or not at all associated with cortisol concentration (69). Of most importance within cortisol measurements, stress-related rumination has repeatedly been found to positively associate to cortisol reactivity and delayed recovery in response to stress (69). Moreover, morning cortisol awakening response was positively associated with having been ruminating the day before, but negatively or not at all with rumination in general (69).

Linking the role of cortisol to the association of rumination with cognitive control, Quinn et al, 2014 (70) found in a student sample that executive control training with the n-back task exerted a reducing effect on stress-related cortisol reactivity only case of a high trait rumination level, but it had no effect in case of low rumination.

Thus, we can conclude that the prolonged stress response detailed in the perseverative cognition hypothesis (35, 36) can be underpinned by cortisol correlates only in case of stress-related rumination measures, but rumination seems to play an important role in the association between executive control and cortisol reactivity.

2.1.4.3. Brain regions behind rumination

Among healthy controls, the 10-item RRS has been negatively associated with grey matter volume in left anterior cingulate cortex (ACC), bilateral mid-cingulate cortex and bilateral inferior frontal gyrus (IFG), from which ACC and bilateral IFG volume reduction results reside close to those identified by meta-analysis in depressed patients (71). Moreover, among these volume reduction results for rumination, ACC and right IFG also showed a negative resting state activity association with rumination (71). Moreover,

Nolen-Hoeksema et al, 2008 (1) argue that regardless of depression status, rumination score has been negatively associated with rostral ACC activity when attempting to inhibit negative distracters.

Regarding additional fMRI (functional magnetic resonance imaging) findings, Mandell et al, 2014 (39) conducted a factor analysis on 17 subscales of 10 self-report rumination measures in current MDD patients, along with BDI to control for depression level, and an fMRI task of alternating emotion processing and cognitive control. The three rumination factors derived were correlated with increased sustained amygdala reactivity, and if controlling for amygdala reactivity, specific dimensions of rumination were associated with distinct activity patterns in hippocampus (39). The positive association between trait rumination and amygdala reactivity has also been corroborated in Nolen-Hoeksema et al's review (1), in tasks requiring response to negative stimuli or appraisal of negative photographs in a way that would increase negative affect. Prefrontal cortex (PFC) also seems to be important in ruminative processes, since rumination is negatively associated with anterior medial PFC activity during a rumination task, and positively associated with both anterior and posterior medial PFC activity during a distraction task (1). Subjects with a high level of rumination also had a higher activity in the medial PFC when instructed to simply look at negative photos compared to when instructed to change the negative affect in response to these photos (1, 72). The authors interpret these results on elevated medial PFC activations among high ruminators as a chronic recruitment of regions associated with negative self-referential processing even when simply looking at photos and a sustained self-referential processing even when the task is to distract (1). Activity of the left ventrolateral PFC has also been found to be positively associated with rumination when looking at negative photos without instructions for emotion regulation (1, 72).

To summarise findings within the imaging literature, amygdala, ACC, medial prefrontal cortex and IFG have a repeatedly consistent association with rumination.

2.1.4.4. Integrating cognitive and neurobiological underpinnings along the pathway to depression

Linking cognitive and neurobiological factors into one integrative framework to explain vulnerability for recurrent depression, De Raedt and Koster, 2010 (73) differentiate between attentional control measured by experimental tasks, as a process,

and rumination captured by questionnaires, as a product of the process. In their model, HPA (hypothalamic-pituitary-adrenal) axis, with cortisol at its endpoint, is impaired following hypercortisolism in depressive episodes, leading to a dysregulation also in the serotonergic system, which in turn leads to decreased dorsolateral PFC (DLPFC) activity (73). Decreased DLPFC activity entails a prolonged amygdala activity in response to stress, at the biological level, and a diminished inhibitory attentional control at the cognitive level, both of which aspects will contribute to maintained attention for negative material and impaired ability to stop negative elaborating (such as ruminative thinking) of negative schemas activated by stress, producing a finale of sustained negative affect (73).

As we have seen in this chapter, cognitive, hormonal and neurobiological underpinnings of rumination, such as inhibition deficits, cortisol response and amygdala reactivity, can not only pave the way from genes to this endophenotype, but can also reside on the causal pathway from rumination to depression.

2. 2. Genetic background of rumination

After delineating evidence that rumination can be investigated not only as a stable and unsubstitutable risk factor for major depression but also as a cognitive endophenotype, a biologically and genetically more homogeneous construct than depression itself, in this section I will discuss in detail the genetic associations identified with regard to rumination so far. First of all, it has to be noted that an evolutionary advantage of rumination has been interpreted in the framework of depression, stating particularly that depression is an evolved response to solving complex social problems, and rumination is adaptive in understanding the causes and consequences of the problem, enabling that the person can avoid it in the future (74-76). Although this assumption would be another reason for that the genetic background of depression can be explored by investigating the genetic background of rumination, the RRS questionnaire seems inappropriate to capture this adaptivity. The Analytical Rumination Questionnaire

(ARQ), designed to measure this adaptive analytical function of rumination, comprises two factors, causal analysis and problem-solving analysis, and neither RRS subscale was related to its problem-solving factor (77). Nevertheless, I will argue that depression and RRS rumination share a considerable proportion of genetics.

2.2.1. Twin studies to reveal heritability

Chen and Li, 2013 (78) conducted a twin study among Chinese adolescents, and got a 24% heritability for CRSQ rumination. Moreover, genetic correlations accounted for 68% of the phenotypic correlation (r=0.41) between self-reported rumination and depressive symptoms, and 77% of the phenotypic correlation (r=0.22) between self-reported rumination and parent-reported depressive symptoms (78). Moore et al, 2013 (79), with adolescent twins from the United States and the 10-item RRS, got a 21% heritability for the brooding and a 37% for the reflection subscale. While reflection did not have a considerable phenotypic correlation with depressive symptoms (r=0.14; but it was significant), brooding correlated with depression to a significant r=0.47; 62% of which phenotypic correlation could be explained by common genetic effects (79).

Among young adults twins from the United States, a latent rumination variable composed of RRS brooding, RRS reflection and RRQ rumination, had a heritability of 40% or 41% in males (depending on the type of model chosen) and a 34% or 37% in females (80, 81). In men, 50% of the phenotypic correlation between this latent rumination variable and CES-D (Center for Epidemiological Studies-Depression) depression could be explained by the genetic correlation between them, and in women this proportion is 45% (81).

From these results, Johnson et al, 2014 (81) conclude that the heritable proportion of rumination increases from early adolescence to young adulthood, but this holds only partly true for women and is not true for RRS reflection. Nevertheless, this moderate heritability of rumination entails the justification of candidate gene studies (81), and, along with findings that the half or even two third of common variance between rumination and depression can be explained by common genetics, fulfils criteria (58, 59) of being an endophenotype for depression.

On the other hand, factors other than genetics have also to be delineated when discussing the generation of rumination. Although the role of parent modelling has not been proved in the socialisation for ruminative response style, parents' reactions to the child's sadness or problem has been demonstrated to affect adolescent rumination, with harmful effects of unsupportive and magnifying reactions, or disengagement suggestions (82, 83). Overprotective or over-controlling parenting, and a negative-submissive expressivity within the family have also been proved risks, along with a highly reward-dependent temperament of the child (84, 85). For the roles of over-controlling parenting and emotional and sexual maltreatment in detail, see chapter 2.5.1. In girls, the inverse association of positive maternal behaviour and adolescent depression was mediated by adolescent rumination (86). Socialisation for a feminine gender role has been found to exert a longitudinal effect on a high level of rumination (87, 88). Age also has a robust impact on rumination, since it increases from childhood to adolescence (89), and even more to adulthood (90), but declines gradually throughout adulthood (91-94). The gender difference in rumination, with a female predominance, appears in early adolescence and keeps constant during adulthood (89, 94, 95).

After outlining some examples on the role of environment and time in the emergence of rumination, in the next chapter I will continue to discuss the role of genetics, with particular candidate genes.

2.2.2. Candidate gene studies

After reviewing evidence that rumination has a considerable proportion residing in genes, I move on to the details of candidate gene studies performed on rumination so far.

2.2.2.1. Glucocorticoid and mineralocorticoid receptors

Straightforward from biological findings on the role of cortisol (see chapter 2.1.4.2 for details), glucocorticoid receptor co-chaperone gene *FKBP5* has been investigated along with stressors in determining rumination. Among school-aged children, attachment security was negatively associated with CRSQ rumination only in those with the *FKBP5* rs3800373 CC genotype (96). Moreover, among adolescents, a high level of childhood trauma was associated with high CERQ (Cognitive Emotion Regulation Questionnaire)

rumination only in carriers of the CATT haplotype composed of *FKBP5* rs9296158, rs3800373, rs1360780 and rs9470080 (97).

The mineralocorticoid receptor also mediates the effects of cortisol in stress response, and an activity-enhancing haplotype of its gene *NR3C2*, has been associated with decreased LEIDS-R (Leiden Index of Depression Sensitivity Revised) rumination among only female undergraduates but not in males (98).

2.2.2.2. Serotonergic system

Role of the serotonergic system has also emerged in the comprehensive framework of De Raedt and Koster, 2010 (73), integrating rumination into the complex system of multiple biological and cognitive factors determining recurrent depression (see chapter 2.1.4.4 for details). Among serotonergic genetic candidates, the extensively investigated functional length polymorphism, *5-HTTLPR* (serotonin-transporter-linked polymorphic region), residing in the promoter region of the serotonin transporter gene *SLC6A4*, has also been widely studied regarding rumination. Its association with rumination has been proven to be a function of life stress: the short/short genotype was a risk on LEIDS-R rumination among undergraduates only in case of high childhood emotional maltreatment (99); the genotype moderated the association of life stress with RRS rumination among healthy adults, being the short allele a risk for their positive association (100); and the short allele conferred a risk for 10-item RRS rumination only in case of a high level of adverse events among healthy undergraduates if covarying BDI depression level (101). However, *5-HTTLPR* did not exert its effect in the absence of stress factors, neither among healthy undergraduates on 10-item RRS rumination (101, 102), or among children on RRS brooding (57) or CRSS (Children's Response Styles Scale) brooding rumination (103).

2.2.2.3. Dopaminergic system

C957T polymorphism of the *DRD2* gene encoding dopamine receptor D2 protein, has also been investigated in the background of rumination. CC homozygotes had a higher level of RRS brooding only in the clinically depressed group, but not in the never-depressed controls (104).

COMT gene encoding the catechol-O-methyltransferase enzyme has also been in focus of investigation. Among females in a community sample, the functional Val158Met

(rs4680) polymorphism was not associated with RRS brooding (105). Similarly, among adults, the 10-item RRS rumination was not associated to the rs4680 polymorphism, however, it was associated with *COMT* haplotypes composed of rs933271, rs740603, rs4680 and rs4646316 variants (106).

2.2.2.4. Neuronal plasticity

Importance of synaptic plasticity in the pathway leading to rumination has been demonstrated by a gene-gene interaction effect of rs2070995 residing in exon 3 of the *KCNJ6* gene, encoding the GIRK2 (G protein-activated inwardly rectifying potassium channel subunit 2) protein, and rs2253206 within the promoter region of the *CREB1* gene of cAMP-response element binding protein 1, on 10-item RRS rumination in two independent samples of community adults (107). Moreover, Juhasz et al, 2011 (108) proved a negative association between *CREB1* rs2253206 A allele and 10-item RRS rumination in a partly overlapping sample of these community adults.

The *BDNF* gene encoding the brain-derived neurotrophic factor protein has widely been in focus of seeking genetic associations with rumination, especially its Val66Met (rs6265) polymorphism yielding an amino acid change from valine to methionine. In children, Val66Met was associated neither with RRS brooding (57, 103), nor with CRSS brooding rumination (103). In adolescents however, Val/Val genotype was associated with higher CRSS brooding rumination, but unrelated to RRS brooding (103). Similarly, among adolescent girls, the Val/Val genotype was related to higher CRSQ rumination, moreover, rumination mediated the association of this genotype with a higher level of depressive symptoms (109). Pointing to the same direction of effect also among adults, Juhasz et al, 2011 (108) got a negative association between the Met allele and 10-item RRS rumination, besides the negative association of rumination with a *BDNF* haplotype comprising also the Met allele of Val66Met but otherwise composed of the rs12273363, rs962369, rs988748, rs7127507 and rs1519480 single nucleotide polymorphisms (SNPs). However, other studies with adults have found the Met allele as a risk for higher rumination. Hilt et al, 2007 (109) found that while the Val66Met polymorphism was unrelated to 22-item RRS rumination among never-depressed adult females, in females with adult-onset depression the Val/Met genotype conferred a risk for higher rumination, which association, like that of the Val/Val genotype in adolescent girls, mediated the

association of Val/Met with a higher level of depression. Similarly, among healthy undergraduates, the Val/Met group, compared to the Val/Val group, had a higher level of 10-item RRS rumination, which could be replicated in case of the reflection subscale but was only a trend in case of the brooding subscale (102). Finally, among healthy undergraduates and covarying BDI depression level, the Val/Met group had a higher level of 10-item RRS rumination than the Val/Val group, moreover, the Met/Met group was found to have a higher rumination than the Val/Val group as adverse events increased (101).

To sum up, as a cognitive endophenotype of major depression, rumination has a considerable variation residing in genetics, and some candidate genes, including: *FKBP5*, *5-HTTLPR* polymorphism of *SLC6A4*, *CREB1* and *BDNF*, have already been replicably found to account for this heritability.

In the next section, I am going to delineate a potential new direction of candidate gene studies in rumination: the folate metabolism.

2.3. Folate metabolism in depression and cognition

2.3.1. Methylation and oxidative stress in depression and cognition

Besides genetics, epigenetic regulation, such as methylation, of the relevant genes is also of crucial importance in the background of psychiatric disorders. The universal methyl group donor S-adenosylmethionine (SAM), derived from the one-carbon (1-C) cycle, plays an important role in the expression of key genes influencing cognition, learning, memory and behaviour, and showing an altered expression pattern in psychiatric patients (110). In the 1-C cycle, the amino acid homocysteine is the key intermediate, because, on the one hand, in the transmethylation pathway, with the aid of vitamin B_{12} it can be transformed to SAM, and on the other hand, in the transsulfuration pathway, with the aid of vitamin B_6, it can be catabolised to glutathione, the most important intracellular antioxidant (110). Thus, the 1-C cycle is an integrator, regulating not only methylation processes but also oxidative stress response, and Assies et al, 2014 (110) propose a model

in which oxidative stress induces a shift from the transmethylation to the transsulfuration pathway, entailing a limited bioavailability of methyl groups. In detail, see ***Figure 1***, based on references (110-113). Oxidative stress has been shown to be an important feature not only in major depression but also in cardiovascular disorders (110), providing an additional link between these two disorders besides the perseverative cognition, such as rumination, viewed as a prolonged stress response (35, 36). In both psychiatric and cardiovascular disorders, the key 1-C cycle components show a specific alteration pattern, reflecting the switch from methylation processes to the oxidative stress response: increased homocyteine and glutathione, and decreased folate, vitamin B_{12} and SAM (110). Thus, the 1-C cycle may optimally handle oxidative stress at the expense of proper epigenetic regulation of genes in a pattern that would be necessary in certain functions of cognition.

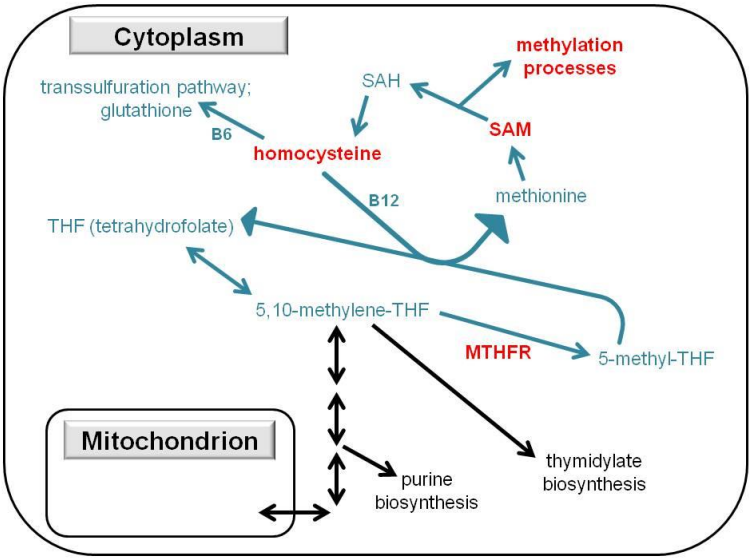

***Figure 1*. One-carbon metabolism, and its role (marked with blue) in methylation and oxidative stress response, based on references (110-113). B12: vitamin B_{12}; B6: vitamin B_6; MTHFR: 5,10-methylenetetrahydrofolate reductase enzyme; SAM: S-adenosylmethionine; SAH: S-adenosylhomocysteine; THF: tetrahydrofolate. The most important components are in red.**

2.3.2. Homocysteine and folate in depression and cognition

Corroborating the postulated model, homocysteine level has been associated positively with depressive symptoms (110), and found to be elevated in major depression (114). Plasma homocysteine level has also been positively associated with risk of depression among older adults in a meta-analysis (115). In bipolar patients, homocysteine level had a consistent negative correlation with executive functioning defined by cognitive flexibility and measured with the Trail Making Test and the Wisconsin Card Sorting Task (114). Moreover, serum homocysteine level was negatively related to performance on Stroop test among healthy older adults, both concurrently and 2.3 years later, but unrelated to verbal learning or dementia score (116). Similarly, in a cross-sectional study with older subjects, serum homocysteine level was negatively related to executive functioning but unrelated to memory performance (117). On the contrary, in patients with geriatric depression, serum homocysteine had a positive association with language processing performance and processing speed (118). Nevertheless, Moustafa et al, 2014 (114), in their review, conclude that among older subjects, homocysteine level has been negatively associated with information processing speed, overall cognitive performance, episodic memory performance and executive functioning, but has an inconclusive association with working memory.

In the central nervous system, capacity of homocysteine metabolism is largely dependent on supplies of folate and vitamin B_{12} (113). Consistent with this finding, homocysteine level had a negative correlation with folate and vitamin B_{12} levels in depressed patients, and folate level was associated negatively with either depression severity, or duration of the depressive episode, or length of hospitalisation (114). Similarly, vitamin B_{12} deficiency was more common in depressed than healthy subjects, and was also associated with a higher risk of developing depression (114). Nevertheless, it has to be noted that in the Framingham Study, strength of the negative association between plasma folate and plasma homocysteine depended on whether or not grain products were fortificated with folic acid in the United States (119).

Reynolds, 2002 (120) gives a review on the importance of folic acid in all ages. In neonates, infants, children or adolescents born with errors in folate transport and metabolism, many syndromes can be detected, such as developmental delay, cognitive deterioration, behavioural and psychiatric symptoms (120). Among healthy elderly

participants, decreased serum vitamin B_{12} and especially folate level were associated with a specific pattern a cognitive impairments resembling normal ageing: they had detectable effects on attention, working memory, cognitive shift and flexibility, visuospatial functioning and phonemic search, although only marginal effects on primary memory, category fluency and spatial orientation (120). Moreover, in the healthy elderly, deficiency in folate or vitamin B_{12} conferred a risk for developing Alzheimer's disease in the future (120). As for psychiatric patients, folate deficiency was present in up to one third of them (120). Regarding depression, those depressed patients who had folate deficiency could be characterised by higher depression scores, higher affective morbidity, lower drive level and poorer response to standard antidepressant treatment (120). According to a systematic review of longitudinal studies in adults, folate consumption is negatively associated with risk of unipolar depression (121). Another systematic review (122), composed of mainly cross-sectional and case-control studies, also demonstrated that low folate status confers a risk for depression.

To conclude, high homocysteine and low folate levels have been consistently associated with an increased level or risk of depression and a specific pattern of cognitive deficits, namely deficits of executive functions and cognitive flexibility. Although these findings have been reported mostly among the elderly, and contradictory results have emerged for other cognitive domains, the relationship of rumination (an endophenotype for depression characterised by inflexible cognition) with homocysteine and folate levels would be a thoroughly underpinned hypothesis to test.

2.3.3. The role of *MTHFR* gene in folate metabolism, depression and cognition

As maybe the most important gene in folate metabolism, *MTHFR* encodes the 5,10-methylenetetrahydrofolate reductase MTHFR enzyme protein (***Figure 1***). Its most widely investigated polymorphism, C677T or rs1801133, entails an alanine (C allele) to valine (T allele) amino acid substitution, with a reduced enzyme activity in case of T allele carriers (123). T allele is also associated with a lower erythrocyte folate level, and T/T genotype is related to lower plasma folate and vitamin B_{12} levels and an increased plasma homocysteine level (113, 123). However, this genotype-homocysteine association is stronger in case of low plasma folate level (113). Similarly, T/T genotype has been found

to be related to a lower DNA methylation level only in case of a reduced plasma folate level (123, 124). These findings outline a complex relationship network of *MTHFR* rs1801133 genotype, folate, homocysteine and methylation (***Figure 1***), in which folate correlates with genotype on the one hand, and interacts with it on the other hand. Methylation patterns are of crucial importance because, during key periods of development, methyl donor deficiency may have a huge impact on epigenetic remodelling, and *MTHFR* genotype has been associated with methylation status of neurodevelopmental genes (125).

MTHFR rs1801133 has been extensively investigated with regard to depression. T allele carriers having experienced childhood trauma had a shorter time of recurrence of major depression compared to those without childhood trauma (114, 126). Meta-analyses have proven the risk conferred by the rs1801133 T allele to depression (125, 127-130), however, this association may be restricted to Asian, but not Caucasian populations (125, 128, 131). Moreover, these association findings with rs1801133 and depression are contradictory in case of geriatric depression, since some studies did not find the association (125), while a meta-analysis did find a risk in case of T/T genotype (115).

Similarly to the findings with depression, the T allele of *MTHFR* rs1801133 proved to be a risk for Alzheimer's disease only in East Asians but not in Caucasians in a meta-analysis (132).

Regarding other cognition domains, *MTHFR* rs1801133 had no association with any memory performance (visual and verbal aspects of working memory, short-time and long-time memory) in undergraduates (133). Similarly, in the elderly, it was related to neither executive functioning, nor memory (117). In contrast, among elderly males without psychiatric disorders or dementia, *MTHFR* rs1801133 C/T heterozygotes performed better than both homozygous groups on both short-term memory and concentration-mental manipulation, but they showed no difference on a digit span task (134). In healthy adults, *MTHFR* rs1801133 was not related to any cognitive performance: cognitive flexibility, planning, working memory, processing speed, or verbal learning (135). To make the picture even more complicated, Durga et al, 2006 (136) found in 50-70 year old participants that *MTHFR* rs1801133 T/T genotype was associated with a better sensorimotor speed, moreover, in subjects with low erythrocyte folate status, it was also associated with a better cognitive flexibility, switching ability.

Linking depression, cognition and methylation together, in gene-gene interaction studies with Val158Met (rs4680) of *COMT* gene encoding the COMT enzyme breaking down dopamine and being a major methyl donor (125), it is interesting that while major depression risk was related to the simultaneous presence of *COMT* Val/Met and *MTHFR* rs1801133 C/T (137), a higher number of perseverative errors on Wisconsin Card Sorting Task among schizophrenic patients was related to the simultaneous *COMT* Val/Val and *MTHFR* T carrier genotypes (114), and a worse performance on Symbol Digit Modalities Test among elderly participants was related to carrying both *COMT* Val and *MTHFR* T alleles (138). Sugden, 2006 (139) argues that the COMT methyltransferase enzyme stemming from a Val/Val genotype consumes four times as much SAM for its methylation process as the one from Met/Met genotype, and that stress may elevate the need for methylation of biogenic amines.

In conclusion, the association of *MTHFR* rs1801133 with depression and Alzheimer's disease seems to be consistent only in Asian populations, and its association with different domains of cognitive performance is contradictory, pointing to the importance of other factors to consider, such as its interactions with folate level and other genes.

2.3.4. Another possible candidate for cognition within the folate pathway: the *MTHFD1L* gene

We could see from the previous chapters that since high homocysteine and low folate levels have been associated with both inflexible cognition and increased depression, and since rumination is a potential depression endophenotype characterised by inflexible cognition, homocysteine and folate metabolism is highly relevant regarding rumination. We could also see that if trying to seek in terms of genetics, while *MTHFR* rs1801133 could be a proper candidate in that its T allele has been associated with high homocysteine and low folate, its associations with homocysteine, DNA methylation, depression and inflexible cognition are not so straightforward.

Another relevant gene within folate metabolism and the 1-C cycle is the *MTHFD1L* gene, encoding the human mitochondrial monofunctional 10-formyl-tetrahydrofolate synthetase (C_1-THF synthase) protein (MTHFD1L), which is located at the matrix side of the inner mitochondrial membrane (112). It catalises the conversion of 10-formyl-

tetrahydrofolate (10-formyl-THF) into formate (***Figure 2***), both in the embryonic (111) and adult (112) mitochondria. This formate then fluxes out to the cytoplasm, and is indispensable in purine and thymidylate biosyntheses, besides methylation processes through SAM (see ***Figure 1*** and ***Figure 2***) (111).

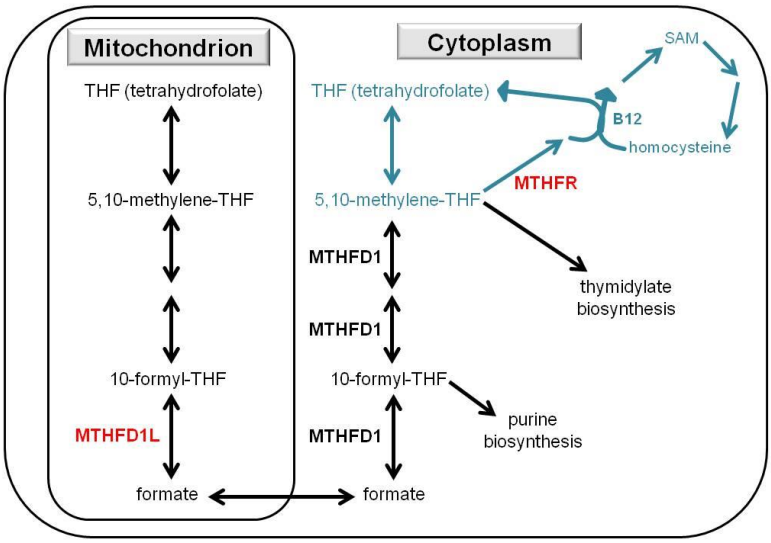

Figure 2. **One-carbon metabolism, and the role of two distinct enzymes (marked with red) in it, modified from (140). B12: vitamin B_{12}; MTHFR: 5,10-methylenetetrahydrofolate reductase enzyme; MTHFD1L: mitochondrial monofunctional C_1-tetrahydrofolate synthase enzyme; MTHFD1: cytoplasmic trifunctional C_1-tetrahydrofolate synthase enzyme; SAM: S-adenosylmethionine; THF: tetrahydrofolate. Blue colour denotes the same processes as those marked with blue in *Figure 1*.**

Considering genetics, the A allele of *MTHFD1L* rs11754661 has been associated with a high plasma homocysteine level, after controlling for levels of plasma folate and vitamins B_6 and B_{12} (141).

This same A allele has also been found as a risk for Alzheimer's disease in a GWAS, surviving the correction for genome-wide multiple testing (142). This association has been replicated among Han Chinese (143) and Northern Han Chinese participants (144).

However, there was no association between rs11754661 and Alzheimer's disease among Spanish participants (145).

Although and because other cognitive phenotypes have not been investigated with regard to *MTHFD1L* genotype, it would be an exciting new field to test.

2.4. Folate metabolism and monoamine levels

In chapters 2.1.4 and 2.2.2. I detailed cognitive, neurobiological and genetic candidates in the background of rumination. In this section I am going to link folate metabolism into one of these candidates, hypothesised in the integrative model of De Raedt and Koster, 2010 (73), and replicably underpinned by genetic association studies for rumination (99-101): the serotonergic system.

Firstly, rats with hyperhomocysteinemia had lower levels of serotonin and dopamine in brain cortex and a lower level of BDNF in cerebral spinal fluid (CSF) than controls (146), linking homocysteine metabolism also to these two other candidates of rumination: dopaminergic system and BDNF (see chapters 2.2.2.3 and 2.2.2.4).

Moreover, Bottiglieri et al, 2000 (147) found that the subgroup of severely depressed patients having a raised plasma homocysteine level, had lower levels of serum, erythrocyte and CSF folate, and lower levels of SAM and metabolites of serotonin, dopamine and noradrenaline in CSF. These findings corroborate rodent results with the importance of serotonin and dopamine in the hyperhomocysteinemic subgroup of depressive patients on the one hand, and point to the possible etiological mechanism of methylation, on the other hand. Indeed, Reynolds, 2002 (120) argue that SAM and folate exert a similar effect on mood, and that both SAM and folate have an impact on monoamine metabolism, a candidate drug target in depression. In contrast, while children with an inborn subnormal CSF level of folate had lower CSF levels of serotonin and dopamine metabolites, children with inborn subnormal CSF level of SAM did not show these alterations in monoamine metabolism (148).

Nevertheless, consistently with the necessity of methylation processes in maintaining mental health (110), methyl donor SAM has been found to be an effective antidepressive agent (114, 149, 150).

Moreover, in rat, an acute single administration of SAM elevated serotonin levels in various brain regions, from which the effect in hippocampus had the longest, two-hour long duration, and the authors suggested the probable methylation of a serotonergic receptor behind these effects (151).

Whether or not this effect depends on methylation processes, folate seems to be involved in the synthesis of serotonin, dopamine and noradrenaline (113, 152). Moreover, antidepressant treatment with fluoxetine, sertraline, nortriptyline or imipramine has been found to be less effective in case of folate deficiency (113, 153-155).

To summarise, high homocysteine and low folate levels are consistently associated with an altered metabolism of serotonin and dopamine, whether or not these effects are mediated by SAM and altered methylation processes. Consequently, the serotonergic system has not only been proven to be a consistent candidate in the genetics of rumination, but it can also be linked to homocysteine and folate metabolism, an emerging new candidate.

2.5. New candidates in the relationship between the serotonergic system and rumination

In this section, I will focus on the serotonergic system with regard to rumination and the possibilities of extending former candidate gene findings detailed in chapter 2.2.2.2.

2.5.1. Serotonergic system, childhood maltreatment and rumination

As we could see in chapter 2.2.2.2., the risk for high rumination conferred by the short allele of the *5-HTTLPR* polymorphism within the serotonin transporter gene, emerges only in case of a high level of stress, but the polymorphism is silent in the absence

of stress (57, 99-103). Among these findings, Antypa and Van der Does, 2010 (99) got their gene-by-environment interaction results with childhood maltreatment as the stressor.

Childhood maltreatment is indeed important in the generation of rumination. In a more general perspective, negative parenting has been associated with cognitive vulnerabilities, such as negative attributional style, dysfunctional attitudes, and a selective attention to angry faces (156). Moreover, peer rejection and victimisation can lead to the exacerbation of cognitive vulnerabilities (156). Regarding specifically rumination, in undergraduates, over-controlling parenting and childhood emotional (and in women also sexual) maltreatment were positively associated with RSQ rumination, even if controlling for BDI depression level and negative cognitive styles (157).

The second important step is that cognitive vulnerability is one mediator through which childhood emotional abuse raises depression level (156). The same is true for specifically rumination, since, even if controlling for negative cognitive styles, it fully mediated the relationship of over-controlling parenting and partially mediated the relationship of childhood emotional maltreatment with the number of major depressive episodes during the 2.5 year follow-up period among undergraduates (157). These findings are of crucial importance because of corroborating the depression endophenotype nature of rumination on its own, above and beyond other negative cognitive styles, and childhood maltreatment seems to be important in the endophenotype concept since we could see that it is useful to incorporate stressful life events into genetic explanation models.

However, it is important that this mediation of the depressogenic effect of childhood maltreatment is exclusively restricted to the brooding but not the reflection subscale. This fact has been proven in cross-sectional studies with RRS rumination, BDI depression and childhood emotional abuse measured by the Childhood Trauma Questionnaire (CTQ), both among undergraduate students, yielding a partial mediative role of brooding when controlling for reflection (158), and among pregnant women, also yielding a partial mediation of brooding and the result that reflection was not correlated with childhood maltreatment at all (159). Moreover, this partial mediation by brooding has also been proven in a longitudinal study with adolescents, in that CRSS brooding partially mediated the association of former emotional abuse by either parents or peers with CES-D depressive symptoms measured later than brooding, but there was no such mediation in

case of reflection (160). In contrast, in a study among adolescents, though childhood maltreatment was positively associated with RRS brooding, brooding itself did not predict changes in internalising symptoms over time (161).

To summarise, childhood maltreatment, interacting with candidate genes, may become a necessary component in the endophenotype nature of rumination, paving the way to depression, but may be important only in the gene-by-environment background of brooding, not reflection.

2.5.2. 5-HT_{2A} and cognitive vulnerabilities for depression

After proving the crucial role of childhood maltreatment in the generation of rumination not only by moderating the effect of the serotonergic candidate gene polymorphism *5-HTTLPR* but also in general, in this chapter I will argue that another candidate within the serotonergic system, the serotonin receptor 5-HT_{2A}, is also worth investigating with regard to rumination.

Tryptophan depletion in humans provoked deficits both in a reversal learning task measuring a form of cognitive flexibility, and in a go / no-go response task measuring inhibitory control, and the deficit in reversal learning was corroborated also in monkeys and rats, by serotonin depletion (162). These results are relevant with regard to rumination, because, as we could see in chapters 2.1.1.2 and 2.1.4.1., rumination is a form of inflexible cognition and has been consistently associated with inhibition deficits.

Linking these effects to the 5-HT_{2A} receptor, Macoveanu et al, 2013 (163) found in healthy adults with an adapted no-go paradigm that a successful no-go response inhibition was related to an increased activation of the right IFG, moreover, acute tryptophan depletion provoked a larger no-go response in the right IFG in those subjects who had a low 5-HT_{2A} receptor availability (operationalised by ^{18}F-altanserin steady-state binding measurements in positron emission tomography, PET) in that right IFG region, but it reduced this no-go response in those with high 5-HT_{2A} availability there. This means that serotonin deficiency exerts its deleterious effect on inhibitory control via an increased availability of 5-HT_{2A} receptors in right IFG, suggesting that serotonergic deficiencies may affect also rumination via 5-HT_{2A} receptors of right IFG, since IFG and inhibition

deficits have replicably been found relevant in rumination (see chapters 2.1.4.1 and 2.1.4.3 for details).

5-HT_{2A} receptor binding in the medial PFC has been shown to be inversely associated with amygdala reactivity, which points to the role of 5-HT_{2A} in regulating the feedback inhibitory control of the medial PFC on amygdala reactivity (164). In chapter 2.1.4.3., we could see that rumination associated positively to amygdala reactivity while increasing negative affect, and negatively to anterior medial PFC activity while ruminating (1), which implies that we would expect a lower level of 5-HT_{2A} binding in case of high rumination within the medial PFC. De Raedt and Koster, 2010 (73) also depict rumination as a product of prolonged amygdala activity, but they derive it not from medial but dorsolateral PFC deficit.

With regard to depression itself, Baeken et al, 2012 (165) found that treatment-resistant depressed patients had a lower 5-HT_{2A} receptor binding in the ACC and dorsal PFC, compared to healthy controls and antidepressant-naïve first-episode depressed patients. These results would again emphasise dorsal PFC instead of medial PFC, but also underline ACC, found to be replicably associated with rumination (see chapter 2.1.4.3 for details). Consistently with Baeken et al, 2012's results, in major depressive inpatients, after 4 weeks of treatment with paroxetine, 5-HT_{2A} receptor binding in the frontal cortex was higher in the remitted than in the nonresponder patients (166).

In major depressed patients, dysfunctional attitudes were positively associated with 5-HT_2 receptor binding in the cortex, especially in Brodmann's area 9 (167). Similarly, in unmedicated, recovered unipolar depressed patients, dysfunctional attitudes were positively correlated with 5-HT_{2A} receptor binding in cortex (168).

Arguing again for involvement of the dorsal PFC, Baeken et al, 2014 (169) found in healthy subjects that 5-HT_{2A} receptor binding in dorsal PFC was positively associated with harm avoidance, and, binding in left dorsal PFC, particularly with its anticipatory worry subscale.

To conclude, on the one hand, we would expect that rumination would be negatively associated with 5-HT_{2A} receptor binding within the medial PFC, dorsal PFC and / or ACC, based on results with amygdala reactivity and treatment-resistant depression, and on the other hand, we would expect a positive association between rumination and 5-HT_{2A} binding within Brodmann's area 9 and again dorsal PFC, based on results with

dysfunctional attitudes and harm avoidance. This may be contradictory, but can also be resolved by the possibility that other factors within the serotonergic system may moderate the effect of 5-HT_{2A} binding, such that a high level of binding can be beneficial in itself, but in this case the decisive dependence of a given process (such as inhibitory control) on 5-HT_{2A} can backfire in case of a serotonin deficiency stemming from another source, as we could see in case of tryptophan depletion and right IFG.

2.6. Gap in the knowledge

I have argued that rumination can be regarded as an unsubstitutable endophenotype for major depression, I have detailed candidate genes proven in its background so far, and I have delineated two new directions of candidate gene studies: the folate metabolism on the one hand, and new candidates with regard to the serotonergic system: childhood maltreatment and 5-HT_{2A} receptor, on the other hand.

According to this scope, for candidate genes to test I chose two SNPs from two folate genes: rs1801133 from the *MTHFR* gene, because of its contradictory associations with both depression and cognitive performance, and rs11754661 from the *MTHFD1L* gene, because it has been investigated neither with depression nor with cognitive vulnerability yet. My hypotheses were to test their associations with ruminative response style measured by the most widely used RRS.

As an additional candidate, I chose rs3125 from *HTR2A* gene encoding the 5-HT_{2A} receptor protein. Rs3125 resides in a microRNA (miRNA) binding site (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html), and binds five different miRNAs (https://snpinfo.niehs.nih.gov/cgi-bin/snpinfo/mirna.cgi?2_rs3125) (170). From these miRNAs, miR-539 is bound by the G allele of rs3125, and shows decreased expression in ACC in an animal model of chronic neuropathic pain (171). This is interesting because ACC volume and resting state activity had an inverse association with rumination (see chapter 2.1.4.3 for details), and ACC had a reduced 5-HT_{2A} receptor binding in treatment-resistant depressed patients (see chapter 2.5.2 for details). Moreover, the C allele of rs3125 has been proven to be a risk for depressive symptoms in cardiac patients (172),

and one possible way through which cardiovascular disorders may be associated with depression is a form of perseverative cognition: rumination (35, 36).

Besides miRNA binding, another epigenetic modification, methylation, has also been revealed with respect to the functioning of the *HTR2A* gene, linking environmental impacts to psychiatric or cognitive outcomes. Methylation level of the promoter region of *HTR2A* gene within placenta was positively associated with the newborn infants' attention score and negatively with quality of movement score (173), which scores predict medical and behavioural problems later in childhood, making the precise timing of epigenetic regulation processes inevitable to consider in the background of adult psychiatric disorders (174). In the promoter region of *HTR2A*, a cytosine at position -1439 is methylable only if the allele is G at the adjacent polymorphic -1438 A/G (rs6311) site (173, 175, 176). Investigating the frontal lobe, schizophrenic and bipolar patients showed a reduced *HTR2A* expression compared to controls, and, correspondingly, this -1439 position was found to be more methylated in them than in controls (175). Moreover, antipsychotic use, regardless of whether typical or atypical antipsychotics, was related to a lower methylation level than that of drug-free patients (175). In addition and being consistent also with gene expression levels, methylation level at -1439 decreased with age in individuals with C/C genotype, but this was true only in controls, but not in schizophrenic and bipolar patients (175). (Note that this nomenclature stands for the complementary strand to the one in the -1438 A/G name.) Regarding rs6311 genotype in itself, Fiocco et al, 2007 (177) found that rs6311 G/G (C/C on the other strand) genotype was related to higher levels of depression, neuroticism, emotion-based coping strategies, and cortisol response following a psychosocial stressor, all of which can be associated to rumination (see chapters 2.1.1.1., 2.1.2. and 2.1.4.2 for details). Consequently, I also chose rs6311 from the promoter region of *HTR2A* to test in association with rumination, expecting the G allele (C allele on the other strand) to be the risk. Thus, I could tag two distinct epigenetic regulatory mechanisms within *HTR2A*: miRNA binding and methylation.

Based on the literature reviewed in chapter 2.5.1, when testing the association of these two *HTR2A* SNPs with RRS rumination, I also considered childhood adversity and the two rumination subscales: brooding and reflection.

With regard to all SNP-rumination associations, in the perspective of the endophenotype concept, I also tested whether or not these possible findings can be related to depression.

To test the possibility of robustness, I also tested replicability of the findings within the two subsamples of my study sample: the one recruited in Budapest, Hungary and the one in Manchester, United Kingdom.

Regarding rs6311 and depression, moderating effects of different types of stress have been demonstrated to be contradictory. Dressler et al, 2016 (178) found that rs6311 A/A genotype (T/T on the other strand) denoted a risk for higher CES-D depression only in case of a high level of childhood adversity, but this interaction effect on depression was entirely mediated by cultural consonance in family life. It means the degree to which the individual incorporates salient cultural models into own beliefs and behaviours (178). A allele (T allele on the other strand) of rs6311 has been positively related to either seasonal affective disorder or specifically to its winter-type, but not to seasonality itself (179, 180), but negative results have emerged with regard to seasonal affective disorder, too (181). These contradictory results with rs6311 may be explained by gene x gene interactions, specifically, G allele (C allele on the other strand) decreases in vitro and ex vivo promoter activity in function of genotypes on other *HTR2A* promoter SNPs (182). Thus, in case of finding no significant association between rs6311 and depression in any of our models significant for a rumination phenotype, we test its association with a complex depression-anxiety phenotype in a complex Bayesian model, to further seek its possible role in depression, if not through rumination. Since a third type of stress, recent stress has been found to have a huge impact on depression (183), this complex model will include recent stress, besides six other polymorphisms previously related to depression: *HTR1A* rs6295 (184), *SLC6A4 5-HTTLPR* (185), *BDNF* rs6265 (108), *GALR2* rs8836 (186), *CNR1* rs7766029 (187) and *P2RX7* rs7958311 (188).

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3. Objectives

Based on the literature review (section 2), my objectives were the following.

A) Genetics of folate metabolism in the background of rumination

3.A.1. Is rs1801133 polymorphism of the *MTHFR* gene related to ruminative response style?

3.A.2. Is rs11754661 polymorphism of the *MTHFD1L* gene related to ruminative response style?

3.A.3. In case of a significant SNP-rumination association, is it mediated by depression?

3.A.4. In case of a significant SNP-rumination association, does it mediate the SNP-depression association?

3.A.5. In case of a significant SNP-rumination association, can it be replicated in the separate Budapest and Manchester subsamples?

3.A.6. Can the findings regarding the mediation of the SNP effect be replicated in the separate Budapest and Manchester subsamples?

B) Serotonin receptor gene *HTR2A* and childhood adversity in the background of rumination

3.B.1. Is rs3125 polymorphism of the *HTR2A* gene related to ruminative response style?

3.B.2. Is rs6311 polymorphism of the *HTR2A* gene related to ruminative response style?

3.B.3. In case of a significant SNP-rumination association, is it dependent on rumination subscale, and / or childhood adversity level?

3.B.4. In case of a significant SNP-rumination association, is it mediated by depression?

3.B.5. In case of a significant SNP-rumination association, does it mediate the SNP-depression association?

3.B.6. In case of a significant SNP-rumination association, can it be replicated in the separate Budapest and Manchester subsamples?

3.B.7. Can the findings regarding the mediation of the SNP effect be replicated in the separate Budapest and Manchester subsamples?

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3.B.8. If we cannot find any significant association of rs6311 with depression, can it be detected in a complex model, taking recent stress and six other depression-related polymorphisms into consideration?

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4. Methods

4.1. General aspects

This work was carried out as part of the NewMood (New Molecules in Mood Disorders) study funded by the European Union (LSHM-CT-2004-503474, Sixth Framework Program of the European Union). It was performed in accordance with the Declaration of Helsinki, and it was approved both by the Scientific and Research Ethics Committee of the Medical Research Council (ETT-TUKEB, Budapest, Hungary), and the North Manchester Local Research Ethics Committee (Manchester, United Kingdom). All participants provided written informed consent.

4.2. Participants

As a population sample, adult participants were recruited via advertisements and general practices in Budapest, Hungary, and via advertisements, a website and general practices in Greater Manchester, United Kingdom. All of our participants were between 18 and 60 years old, they were of European white ethnic origin, without any relative participating in the study. They provided DNA with a genetic saliva sampling kit for genotyping, and filled out the NewMood questionnaire pack in Hungarian or English, as appropriate.

4.2.A. Genetics of folate metabolism in the background of rumination

For study A: *Genetics of folate metabolism in the background of rumination*, 2204 subjects (n=895 in Budapest and n=1309 in Manchester) provided information about rumination, gender and age, and were genotyped for *MTHFR* rs1801133. Among those providing information about rumination, gender and age, 2120 participants (n=862 in Budapest and n=1258 in Manchester) were genotyped for *MTHFD1L* rs11754661.

4.2.B. Serotonin receptor gene *HTR2A* and childhood adversity in the background of rumination

For study B: *Serotonin receptor gene HTR2A and childhood adversity in the background of rumination*, 1501 participants (n=470 in Budapest and n=1031 in Manchester) provided information about rumination, gender and age, and were genotyped for *HTR2A* rs3125. Among them, 1498 subjects (n=468 in Budapest and n=1030 in Manchester) provided information about childhood adversity, and 1486 subjects (n=469 in Budapest and n=1017 in Manchester) were genotyped for *HTR2A* rs6311. 1483 participants (n=467 in Budapest and n=1016 in Manchester) possess both childhood adversity and rs6311 data.

To further test the role of rs6311 on a complex depression-anxiety phenotype, using a model based on Bayesian relevance analysis, 1682 participants provided data on gender, age, recent stress, *HTR2A* rs6311, *HTR1A* rs6295, *SLC6A4 5-HTTLPR*, *BDNF* rs6265 (Val66Met), *GALR2* rs8836, *CNR1* rs7766029, *P2RX7* rs7958311, anxiety and the two depression phenotypes.

4.3. Genotyping

Participants provided buccal mucosa cells, from which genomic DNA was extracted using a validated protocol (189). Genotyping was carried out by the Sequenom MassARRAY technology (www.sequenom.com), by the Centre for Integrated Genomic Medical Research at the University of Manchester, with the ISO 9001:2000 quality management requirements and blinded regarding phenotype.

4.4. Questionnaires

The NewMood questionnaire pack contained, first of all, some questions with regard to background information, including gender, age and lifetime depression. The question on lifetime depression (“Have you ever had… depression”) had been validated with face-

to-face diagnostic interviews within a subsample in Manchester (108), and yields a dichotomous variable.

Rumination and its two subtypes, brooding and reflection, were measured with the 10-item Ruminative Responses Scale (RRS) (7), five items of which pertain to the brooding subscale of rumination, five items to the reflection subscale of rumination, with brooding score and reflection score adding up to rumination score itself.

Current depressive symptoms were measured by the depression items plus additional items of the Brief Symptom Inventory (BSI) (190). We also used the anxiety scale of BSI to measure current anxiety symptoms for the Bayesian relevance analysis.

Each of rumination, brooding, reflection, BSI depression and BSI anxiety scores was calculated as a continuous weighted score: the sum of individual item scores divided by number of the completed items of the scale.

Childhood adversity was measured with the sum of scores on items related to physical and emotional neglect and abuse, derived from the Childhood Trauma Questionnaire (CTQ) (191), and items about parental loss. This childhood adversity measure had been validated with the Childhood Trauma Questionnaire in Juhasz et al, 2011 (108).

Recent stress was operationalised by the number of negative life events having occurred within the last year, measured with the List of Threatening Experiences (192).

4.5. Statistical analyses

4.5.1. Descriptive statistics

Plink v1.07 (http://zzz.bwh.harvard.edu/plink/) was used to calculate the Hardy–Weinberg equilibrium test and the allele frequency for each of the four SNPs, and to test gene-environment correlation in linear regression models on childhood adversity score as the outcome, with either *HTR2A* rs3125 or *HTR2A* rs6311, gender, age (and, in the combined sample, also population) as predictors. For calculation of all other descriptive

statistics, and for visualisation of the results in figures, SPSS 20 or SPSS 24 was used (193).

4.5.2. Testing genetic associations with rumination

Plink v1.07 (http://zzz.bwh.harvard.edu/plink/) was used to build linear regression models in the combined Budapest + Manchester sample, with the aid of scripts written individually in R (194).

4.5.2.A. Genetics of folate metabolism in the background of rumination

In the linear regression models, rumination score was the outcome, and main effect of either of the two SNPs (*MTHFR* rs1801133 or *MTHFD1L* rs11754661), population (Budapest or Manchester), gender and age were the predictors. Additive, dominant and recessive models were run, except with *MTHFD1L* rs11754661, because of the low number of participants homozygous for the minor A allele (see ***Table 1*** for the descriptive statistics). These result in a total of five regression models to answer hypotheses 3.A.1 and 3.A.2., on the association of *MTHFR* rs1801133 and *MTHFD1L* rs11754661 with rumination.

4.5.2.B. Serotonin receptor gene *HTR2A* and childhood adversity in the background of rumination

Linear regression equations were run in an additive, dominant and recessive model on each of rumination score, brooding score and reflection score as the outcome. In those models testing a genetic main effect, the predictors were the SNP (*HTR2A* rs3125 or rs6311), population, gender, age, and, in case of a rumination subscale as the outcome, the other subscale. In the interaction models, the predictors were population, gender, age, the main effects of the SNP and childhood adversity, the SNP x childhood adversity interaction term, and, in case of a subscale as the outcome, the other subscale. These add up to a total of 36 regression models to answer hypotheses 3.B.1., 3.B.2 and 3.B.3., on the associations of rs3125 and rs6311 with rumination, and dependency of these associations on subscale and childhood adversity level.

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4.5.3. Correction for multiple testing

4.5.3.A. Genetics of folate metabolism in the background of rumination

In study A: *Genetics of folate metabolism in the background of rumination*, we applied Bonferroni correction, a strict method to correct for performing multiple tests simultaneously, since the investigated SNPs reside in separate genes. Dividing the nominal $p \leq 0.05$ significance threshold by the five SNP-rumination models described in 4.5.2.A yields a Bonferroni-corrected $p \leq 0.010$ significance threshold.

4.5.3.B. Serotonin receptor gene *HTR2A* and childhood adversity in the background of rumination

Since the investigated SNPs reside within the same gene, in study B: *Serotonin receptor gene HTR2A and childhood adversity in the background of rumination*, we applied an alternative method for correction of multiple testing on the 36 SNP-rumination models described in 4.5.2.B: q-values of false discovery rate (FDR). We used QVALUE v1.0 (195) to calculate q-values, without robust method, with λ in a range of 0-0.99 (by 0.05), and applying a bootstrap method to estimate π_0 (the overall proportion of true null hypotheses). We consider q-values ≤ 0.05 as significant.

4.5.4. Power analyses

Power analysis was performed with Quanto v1.2 (http://biostats.usc.edu/Quanto.html) for each SNP-rumination model described in 4.5.2.

4.5.4.A. Genetics of folate metabolism in the background of rumination

Considering a genetic main effect, an explained variance of $R_G^2=1\%$ was assumed for it in the power analysis of each model described in 4.5.2.A, with a type I error rate of 0.010, corresponding to the Bonferroni-corrected significance threshold.

4.5.4.B. Serotonin receptor gene *HTR2A* and childhood adversity in the background of rumination

Among the SNP-rumination models described in 4.5.2.B, for those testing the main effect of a *HTR2A* SNP, an R_G^2=1% was assumed for the genetic main effect. For the interaction models, the assumed explained variance rates were the following: R_{GE}^2=0.5% for the gene-by-environment interaction, R_G^2=0% for the genetic main effect, and R_E^2=0.066 on rumination, 0.0756 on brooding and 0.0276 or 0.0282 (corresponding to the respective sample size) on reflection for the environmental main effect. Variances explained by the environmental factor were calculated by the squared correlation coefficients (coefficients of determination) between childhood adversity score and the respective rumination, brooding or reflection scores (detailed in ***Table 12*** for the combined Budapest + Manchester sample). In the power analysis of all models, a type I error rate of 0.05 was considered.

4.5.5. Post hoc tests regarding the role of depression

To answer hypotheses 3.A.3, 3.A.4, 3.B.4 and 3.B.5 on the mediative roles of depression and rumination in each other's genetic associations, post hoc tests were run in the combined Budapest + Manchester sample, with the models proven to be significant after correction for multiple testing, among the SNP-rumination models described in 4.5.2. Preconditions of this post hoc testing are that both depression phenotypes should be associated with the respective rumination scale on the one hand, and with the respective SNP in the same Plink model as the significant SNP-rumination association's is, on the other hand. To answer questions 3.A.3 and 3.B.4 on the role of depression in the SNP-rumination association, the significant SNP-rumination association's model was run on the respective rumination scale with both depression phenotypes as additional covariates. To answer questions 3.A.4 and 3.B.5 on the role of rumination in the SNP-depression association, each SNP-depression association model was run with the respective rumination scale as an additional covariate. For all of these post hoc tests, a nominal significance threshold of $p \leq 0.05$ was applied.

4.5.6. Post hoc tests regarding the replicability in the separate Budapest and Manchester subsamples

To answer hypotheses 3.A.5 and 3.B.6 on the replicability of the SNP-rumination association within the subsamples, post hoc tests were run in the separate Budapest and Manchester subsamples, with the models proven to be significant after correction for multiple testing, among the models described in 4.5.2. To answer hypotheses 3.A.6 and 3.B.7 on the replicability of mediations, post hoc tests were run in the separate Budapest and Manchester subsamples, with the models proven to be significant according to the nominal $p \leq 0.05$ significance threshold among the models described in 4.5.5. For feasibility of testing replicability of the mediative roles, the same preconditions must be fulfilled as described in 4.5.5. In all these post hoc models testing replicability within the subsamples, population was not included as covariate, and for all of them, a nominal significance threshold of $p \leq 0.05$ was applied.

4.5.7. Further testing of rs6311 in a complex model for depression

To answer hypothesis 3.B.8, we applied a Bayesian network based Bayesian multi-level analysis of relevance. This complex Bayesian model included the joint analysis of gender, age, *HTR2A* rs6311, *HTR1A* rs6295, *SLC6A4 5-HTTLPR*, *BDNF* rs6265 (Val66Met), *GALR2* rs8836, *CNR1* rs7766029, and *P2RX7* rs7958311 on the target variable, separately in those subjects having experienced a low (0-1), a moderate (2) and a high (3 or more) number of recent negative life events. In the space of directed acyclic graphs, our analysis performed a random walk by a Markov Chain Monte Carlo sampling method (196). Bayesian model averaging (197) then yielded a posterior probability of relevance of each variable with respect to the target variable composed of lifetime depression, BSI depression and BSI anxiety, which complex depression-anxiety phenotype had been detailed in previous studies (186, 198). We define a variable relevant if it has a posterior probability of relevance higher than 0.50 in the respective model.

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5. Results

A) Genetics of folate metabolism in the background of rumination

5.A.1. Descriptive statistics

Descriptive statistics for the two folate SNPs, rumination, gender, age and the two depression phenotypes can be seen in detail in ***Table 1***. The Budapest and Manchester subsamples differ significantly in age, *MTHFD1L* rs11754661 genotype, rumination and the two depression phenotypes, so replicability of findings of the combined sample gains even more importance.

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Table 1. **Descriptive statistics for study A: *Genetics of folate metabolism in the background of rumination*. S.E.M.: standard error of mean; BSI: Brief Symptom Inventory; χ^2: Pearson chi-square.**

		Budapest	**Manchester**	**Budapest + Manchester**	**Difference between Budapest and Manchester**
gender	female (%)	624 (69.7%)	916 (70%)	1540 (69.9%)	χ^2=0.017; p=0.897
	male (%)	271 (30.3%)	393 (30%)	664 (30.1%)	
age (mean +/- S.E.M.)		31.26 (0.355)	34.04 (0.284)	32.91 (0.224)	t=-6.153; p<0.001
***MTHFR* rs1801133**	TT (%)	122 (13.6%)	154 (11.8%)	276 (12.5%)	χ^2=1.717; p=0.424
	TC (%)	400 (44.7%)	602 (46%)	1002 (45.5%)	
	CC (%)	373 (41.7%)	553 (42.2%)	926 (42%)	
***MTHFD1L* rs11754661**	AA (%)	1 (0.1%)	10 (0.8%)	11 (0.5%)	χ^2=9.914; p=0.007
	GA (%)	75 (8.7%)	148 (11.8%)	223 (10.5%)	
	GG (%)	786 (91.2%)	1100 (87.4%)	1886 (89%)	
rumination score (mean +/- S.E.M.)		1.94 (0.016)	2.25 (0.017)	2.13 (0.012)	t=-13.104; p<0.001

Table 1 (continued). **Descriptive statistics for study A: *Genetics of folate metabolism in the background of rumination*. S.E.M.: standard error of mean; BSI: Brief Symptom Inventory; χ^2: Pearson chi-square.**

		Budapest	**Manchester**	**Budapest + Manchester**	**Difference between Budapest and Manchester**
BSI depression score (mean +/- S.E.M.)		0.56 (0.023)	1.07 (0.028)	0.86 (0.020)	t=-13.954; p<0.001
lifetime depression	reported (%)	192 (21.5%)	734 (56.1%)	926 (42%)	χ^2=261.521 ; p<0.001
	not reported (%)	703 (78.5%)	575 (43.9%)	1278 (58%)	

For *MTHFR* rs1801133, the minor allele is T (with an allele frequency of 0.3512), and for *MTHFD1L* rs11754661, A (with an allele frequency of 0.0576). This means that the direction of effect of the Plink regression results have to be interpreted for these alleles. P-values of the tests of Hardy–Weinberg equilibrium are the following: for rs1801133, p=0.384 in Budapest, p=0.670 in Manchester and p=0.852 in the combined Budapest + Manchester sample; and for rs11754661, p=1 in Budapest, p=0.064 in Manchester and p=0.112 in the combined Budapest + Manchester sample. Thus, both SNPs are in Hardy–Weinberg equilibrium.

5.A.2. Association of rumination with *MTHFR* rs1801133 and *MTHFD1L* rs11754661 in the combined Budapest + Manchester sample

Considering the Bonferroni-corrected p≤0.010 significance threshold, results of Plink linear regression models in ***Table 2*** demonstrate that rs1801133 has no association

with rumination in any of the three models, whereas the A allele of rs11754661 is significantly associated with a higher rumination score in both additive and dominant models. For the visualisation of dominant models with the two SNPs, see ***Figure 3*** and ***Figure 4***. Results of power analyses (***Table 2***) let us deem our findings true negatives and true positives, respectively.

Table 2. **Effects of the two folate SNPs in linear regression models for rumination score as the outcome, with either of the SNPs, population, gender and age as predictors. The minor allele is T in case of *MTHFR* rs1801133; and A in case of *MTHFD1L* rs11754661. Statistical power of the analyses is 98.34% and 97.93%, respectively. SNP: single nucleotide polymorphism; S.E.: standard error of beta; p: nominal p-value. Significant findings are marked with bold.**

	***MTHFR* rs1801133**				***MTHFD1L* rs11754661**			
Model	**Beta**	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**
additive	-0.023	0.017	-1.358	0.175	**0.112**	**0.035**	**3.182**	**0.001**
dominant	-0.043	0.024	-1.825	0.068	**0.122**	**0.038**	**3.228**	**0.001**
recessive	-0.002	0.035	-0.058	0.954				

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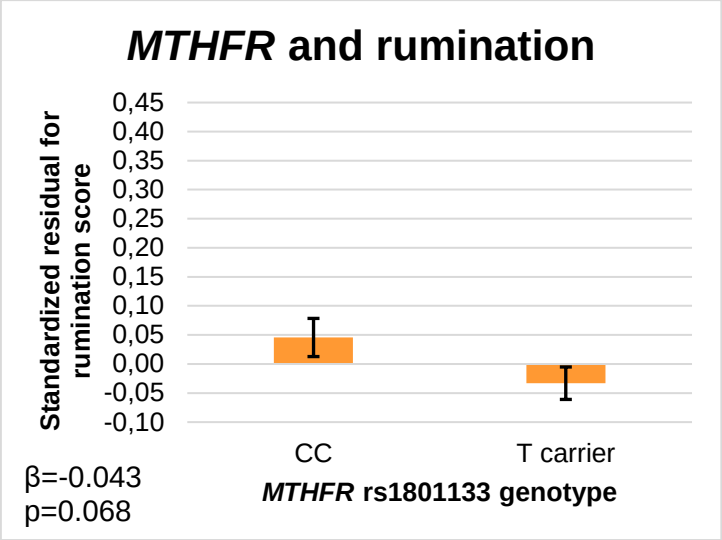

Figure 3. **Means and standard errors of rumination score (having been controlled for population, gender and age in a previous regression) in function of *MTHFR* rs1801133 genotype, in the combined Budapest + Manchester sample.**

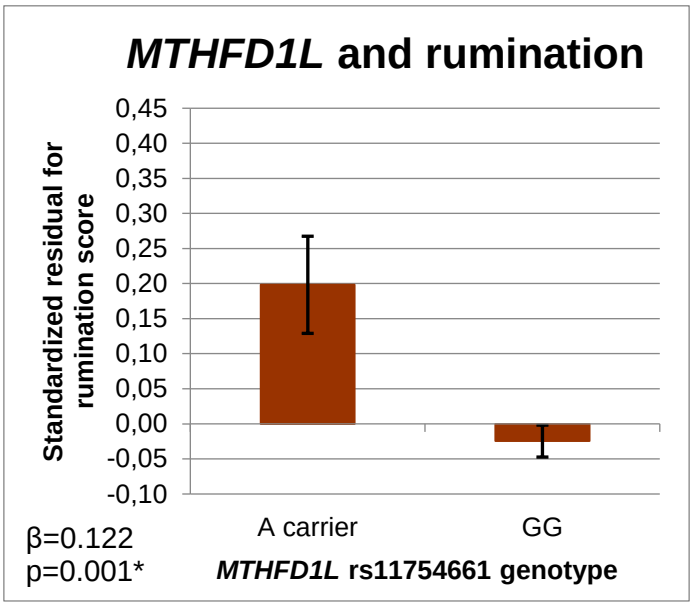

Figure 4. **Means and standard errors of rumination score (having been controlled for population, gender and age in a previous regression) in function of *MTHFD1L* rs11754661 genotype, in the combined Budapest + Manchester sample, based on reference (140).**

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5.A.3. The role of depression in the rs11754661-rumination association, in the combined Budapest + Manchester sample

Preconditions of testing the role of depression in the rs11754661-rumination association are fulfilled. First, because rumination score has a positive association with both depression phenotypes. Namely, rumination score has a significantly (n=2120; t =-22.022; p<0.001) higher mean (2.429±0.019) in those who did report lifetime depression than in those who did not (1.909±0.014); and it has a significant (n=2117; p<0.001) Pearson correlation coefficient of r=0.581 with BSI depression score. Second, like in the models for rumination, the *MTHFD1L* rs11754661 A allele has a positive relation to both depression phenotypes (n=2120 in lifetime depression, and n=2117 in BSI depression models) nominally, either significantly ($p \leq 0.05$) or as a trend ($0.05 < p \leq 0.1$) (***Table 3***).

***Table 3*. Effects of the *MTHFD1L* rs11754661 A allele on lifetime depression in logistic regression models and on BSI depression and rumination in linear regression models. Population, gender and age were covariates in all analyses, and in those for rumination, the two depression phenotypes were also covariates. OR: odds ratio; S.E.: standard error of OR or beta; BSI: Brief Symptom Inventory.**

	additive				**dominant**			
	OR	**S.E.**	**t**	**p**	**OR**	**S.E.**	**t**	**p**
lifetime depression	1.354	0.1395	2.173	0.030	1.405	0.1497	2.271	0.023
	Beta	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**
BSI depression score	0.098	0.058	1.695	0.090	0.118	0.062	1.897	0.058
rumination score (controlling for depression)	0.070	0.029	2.388	0.017	0.072	0.031	2.309	0.021

If entering lifetime depression and BSI depression score as additional covariates in the regression equations discussed in 5.A.2 and ***Table 2*** for rumination score, the positive association of the rs11754661 A allele with rumination remains nominally significant in both additive and dominant models (***Table 3***), pointing out that the rs11754661-rumination association is not only due to depression. For its visualisation in the dominant model, see ***Figure 5***.

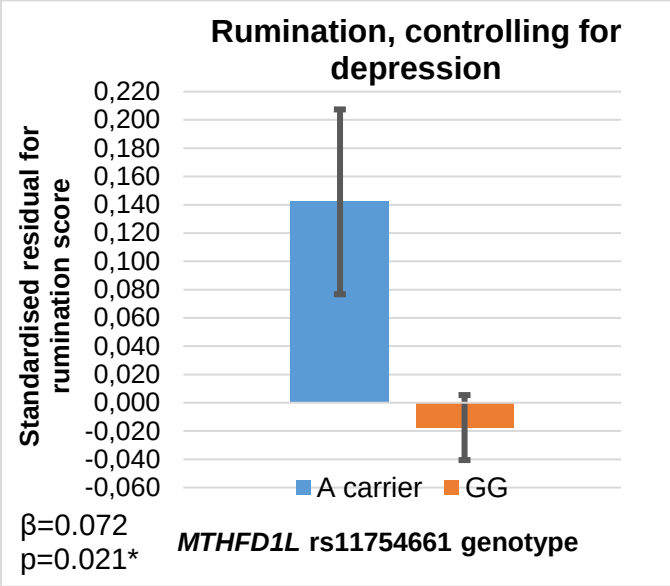

Figure 5. **Means and standard errors of rumination score (having been controlled for population, gender, age, lifetime depression and BSI depression score in a previous regression) in function of *MTHFD1L* rs11754661 genotype, in the combined Budapest + Manchester sample. BSI: Brief Symptom Inventory.**

5.A.4. The role of rumination in the rs11754661-depression association, in the combined Budapest + Manchester sample

Including rumination score as an additional covariate in the regression equations discussed in ***Table 3***, the rs11754661 A allele of *MTHFD1L* loses its significant positive association with both lifetime depression and BSI depression score in both additive and dominant models (***Table 4***). Thus, we can conclude that the rs11754661-depression association is entirely due to rumination. Visualisations of the dominant models are displayed in ***Figure 6*** for lifetime depression and in ***Figure 7*** for BSI depression.

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Table 4. **Effects of the *MTHFD1L* rs11754661 A allele on lifetime depression in logistic regression models and on BSI depression score in linear regression models, with population, gender, age and rumination score as covariates. OR: odds ratio; S.E.: standard error of OR or beta; BSI: Brief Symptom Inventory.**

	additive				**dominant**			
	OR	**S.E.**	**t**	**p**	**OR**	**S.E.**	**t**	**p**
Lifetime depression	1.198	0.152	1.189	0.234	1.235	0.162	1.301	0.193
	Beta	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**
BSI depression score	-0.001	0.049	-0.018	0.986	0.010	0.052	0.193	0.847

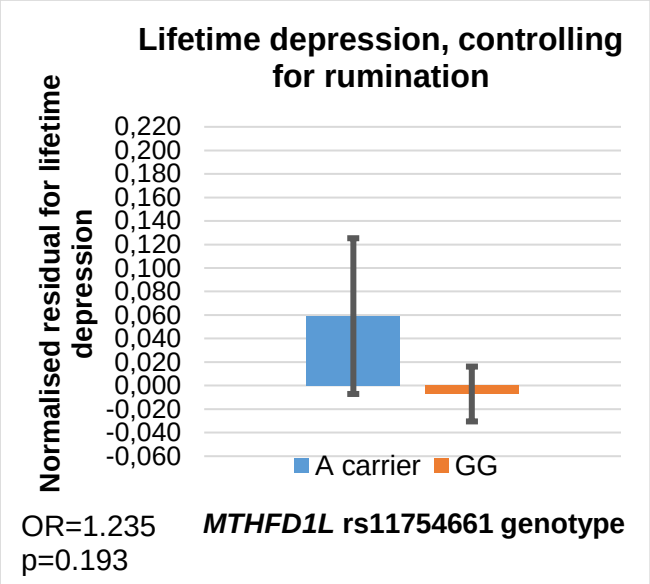

Figure 6. **Means and standard errors of normalised residual for lifetime depression (having been controlled for population, gender, age and rumination score in a previous regression) in function of *MTHFD1L* rs11754661 genotype, in the combined Budapest + Manchester sample. OR: odds ratio.**

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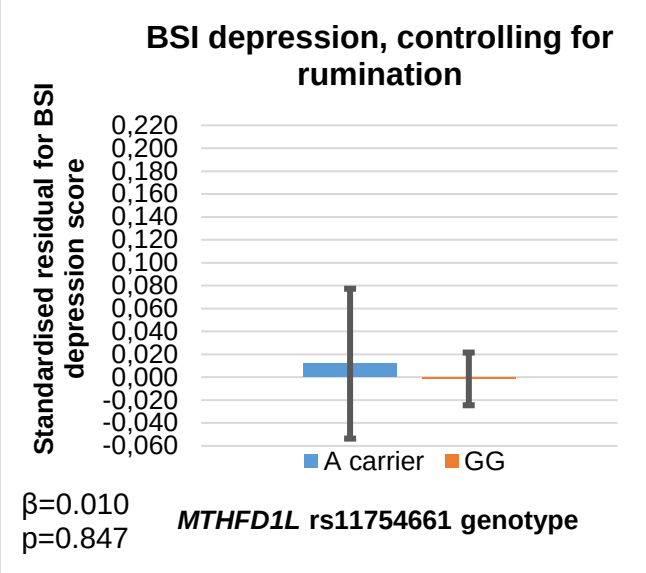

Figure 7. **Means and standard errors of BSI depression score (having been controlled for population, gender, age and rumination score in a previous regression) in function of *MTHFD1L* rs11754661 genotype, in the combined Budapest + Manchester sample. BSI: Brief Symptom Inventory.**

5.A.5. Replicability of the rs11754661-rumination association in the separate Budapest and Manchester subsamples

The positive association of the A allele of *MTHFD1L* rs11754661 with rumination score can be replicated at a nominally significant level in both Budapest and Manchester, in both additive and dominant models (***Table 5***). Visualisations of the dominant models are displayed in ***Figure 8*** for Budapest, and ***Figure 9*** for Manchester.

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***Table 5*. Effect of the *MTHFD1L* rs11754661 A allele on rumination score separately in Budapest and Manchester, in linear regression models with gender and age as covariates. S.E.: standard error of beta.**

	Budapest				Manchester			
Model	**Beta**	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**
additive	0.158	0.054	2.915	0.004	0.095	0.046	2.049	0.041
dominant	0.157	0.055	2.828	0.005	0.107	0.050	2.120	0.034

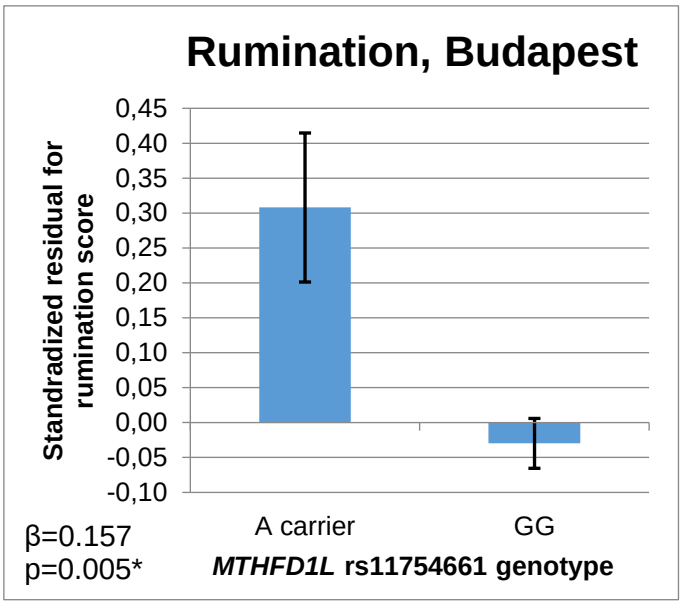

***Figure 8*. Means and standard errors of rumination score (having been controlled for gender and age in a previous regression) in function of *MTHFD1L* rs11754661 genotype, in the Budapest subsample, based on reference (140).**

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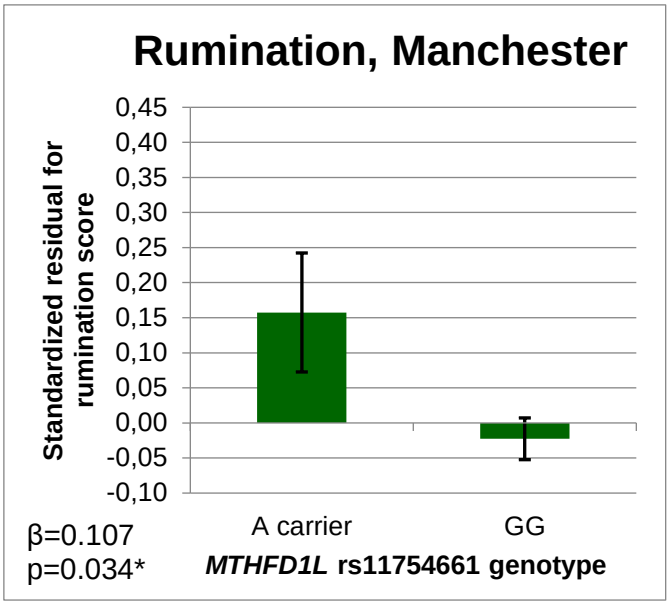

***Figure 9*. Means and standard errors of rumination score (having been controlled for gender and age in a previous regression) in function of *MTHFD1L* rs11754661 genotype, in the Manchester subsample, based on reference (140).**

5.A.6. Replicability of the asymmetry of mediative roles of rumination and depression in the association with rs11754661, in the separate Budapest and Manchester subsamples

Despite the fact that the rs11754661-rumination association can be replicated in the separate Budapest and Manchester subsamples (see section 5.A.5), rs11754661 does not exert an effect on any of the depression phenotypes in Manchester (***Table 6***) (n=1258 in all models), so testing the replicability of mediative roles of rumination and depression is impossible in this subsample.

Table 6. **Effects of the *MTHFD1L* rs11754661 A allele on lifetime depression in logistic regression models and on BSI depression score in linear regression models, in Manchester, with gender and age as covariates. OR: odds ratio; S.E.: standard error of OR or beta; BSI: Brief Symptom Inventory.**

	additive				**dominant**			
	OR	**S.E.**	**t**	**p**	**OR**	**S.E.**	**t**	**p**
lifetime depression	1.230	0.162	1.276	0.202	1.289	0.178	1.426	0.154
	Beta	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**
BSI depression score	0.085	0.078	1.088	0.277	0.107	0.085	1.252	0.211

However, mediation analyses can be implemented in Budapest, since rs11754661 A allele has a positive association with both depression phenotypes in this subsample (***Table 7***) either nominally significantly or as a trend (n=862 in lifetime depression models, and n=859 in BSI depression models). Moreover, the mean of rumination score is significantly higher (n=862; t =-9.603; $p<0.001$) in those participants reporting lifetime depression (2.226±0.035) than in those who did not report it (1.866±0.017), and rumination has a significant (n=859; $p<0.001$) r=0.536 Pearson correlation coefficient with BSI depression score in Budapest.

***Table 7*. Effects of the *MTHFD1L* rs11754661 A allele on lifetime depression in logistic regression models and on BSI depression and rumination in linear regression models, in Budapest. Gender and age were covariates in all analyses, and in those for rumination, the two depression phenotypes were also covariates. OR: odds ratio; S.E.: standard error of OR or beta; BSI: Brief Symptom Inventory.**

	additive				**dominant**			
	OR	**S.E.**	**t**	**p**	**OR**	**S.E.**	**t**	**p**
lifetime depression	1.775	0.258	2.226	0.026	1.737	0.265	2.088	0.037
	Beta	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**
BSI depression score	0.141	0.081	1.734	0.083	0.151	0.083	1.813	0.070
rumination score (controlling for depression)	0.094	0.046	2.051	0.041	0.090	0.047	1.921	0.055

In Budapest, including depression phenotypes as additional covariates in the regression models described in ***Table 5*** in 5.A.5., the positive effect of the rs11754661 A allele on rumination remains significant in the additive model, and a trend in the dominant one (***Table 7***). This means that as in the combined Budapest + Manchester sample (see 5.A.3.), the rs11754661-rumination association in Budapest is not only due to depression.

Findings of the combined sample described in 5.A.4. can also be replicated in the Budapest subsample, since rumination score as an additional covariate in the regression models of ***Table 7*** totally abolishes the rs11754661-depression associations (***Table 8***), leading us to the conclusion that these associations are entirely due to rumination also in Budapest.

Table 8. **Effects of the *MTHFD1L* rs11754661 A allele on lifetime depression in logistic regression models and on BSI depression score in linear regression models, with gender, age and rumination score as covariates, in Budapest. OR: odds ratio; S.E.: standard error of OR or beta; BSI: Brief Symptom Inventory.**

	additive				**dominant**			
	OR	**S.E.**	**t**	**p**	**OR**	**S.E.**	**t**	**p**
lifetime depression	1.471	0.271	1.425	0.154	1.443	0.278	1.322	0.186
	Beta	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**
BSI depression score	0.014	0.069	0.210	0.834	0.025	0.070	0.361	0.719

B) Serotonin receptor gene *HTR2A* and childhood adversity in the background of rumination

5.B.1. Descriptive statistics

Descriptive statistics for *HTR2A* rs3125 and rs6311, rumination and its two subscales, childhood adversity, gender, age and the two depression phenotypes are displayed in ***Table 9***. The Budapest and Manchester subsamples significantly differ in all variables except for rs6311 genotype frequencies, thus it is crucial to test replicability of findings of the combined sample within each subsample.

C is the minor allele of *HTR2A* rs3125, with an allele frequency of 0.1289. It is in Hardy-Weinberg equilibrium, with the following p-values: p=0.9087 in the combined sample, p=0.6156 in Budapest, and p=0.6074 in Manchester. For *HTR2A* rs6311, the minor allele is T, with an allele frequency of 0.4078. It yields a Hardy-Weinberg equilibrium p=0.1631 in the combined sample, a p=0.7035 in Budapest, and a p=0.0508 in Manchester.

Table 9. **Descriptive statistics for study B: *Serotonin receptor gene HTR2A and childhood adversity in the background of rumination*. S.E.M.: standard error of mean; BSI: Brief Symptom Inventory; χ^2: Pearson chi-square.**

		Budapest + Manchester		Budapest		Manchester		Difference between Budapest and Manchester	
		Frequency	%	Frequency	%	Frequency	%	χ^2	p
Gender	Male	371	24.7%	88	18.7%	283	27.4%	13.209	0.00028
	Female	1130	75.3%	382	81.3%	748	72.6%		
Lifetime depression	Not reported	824	54.9%	370	78.7%	454	44.0%	156.889	<0.00001
	Reported	677	45.1%	100	21.3%	577	56.0%		
HTR2A rs3125	CC	24	1.6%	6	1.3%	18	1.7%	8.678	0.01305
	CG	339	22.6%	85	18.1%	254	24.6%		
	GG	1138	75.8%	379	80.6%	759	73.6%		
HTR2A rs6311	TT	234	15.7%	83	17.7%	151	14.8%	2.589	0.27405
	TC	744	50.1%	223	47.5%	521	51.2%		
	CC	508	34.2%	163	34.8%	345	33.9%		
		Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	t	p
Age		32.823	0.2747	30.315	0.4925	33.967	0.3249	-6.244	<0.00001
Rumination score		2.174	0.0151	1.986	0.0209	2.259	0.0192	-9.601	<0.00001
Brooding score		2.197	0.0178	1.954	0.0250	2.308	0.0224	-10.574	<0.00001
Reflection score		2.150	0.0172	2.019	0.0267	2.210	0.0217	-5.543	<0.00001
Childhood adversity score		3.392	0.0895	2.801	0.1365	3.660	0.1136	-4.838	<0.00001
BSI depression score		0.900	0.0244	0.540	0.0283	1.063	0.0319	-12.268	<0.00001

As for possible gene-environment correlations regarding rs3125 (with n=1498 in the combined sample, n=468 in Budapest, and n=1030 in Manchester), we can see in ***Table 10*** that except for the recessive models, the C allele of rs3125 is in a significant positive association with childhood adversity score both in the combined Budapest + Manchester

sample and in Manchester. However, there is no such gene-environment correlation in Budapest. Nevertheless, in case of a possible gene-by-environment interaction (GxE) finding with rs3125 in the combined sample, these gene-environment correlations make even more crucial to test its replicability in the Budapest subsample.

With regard to rs6311 and childhood adversity score (n=1483 in the combined sample, n=467 in Budapest, and n=1016 in Manchester), no significant gene-environment correlation can be detected in any model, neither in the combined sample or in any subsample (***Table 11***).

Table 10. **Effect of *HTR2A* rs3125 (with C as the minor allele) on childhood adversity score in a linear regression model, with gender, age (and, in the combined sample, also population) as covariates. S.E.: standard error of beta.**

	Budapest + Manchester				**Budapest**				**Manchester**			
Model	**Beta**	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**
additive	0.422	0.187	2.264	0.024	0.182	0.312	0.582	0.561	0.494	0.230	2.147	0.032
dominant	0.544	0.206	2.643	0.008	0.275	0.343	0.801	0.424	0.629	0.254	2.473	0.014
recessive	-0.338	0.703	-0.481	0.630	-0.677	1.206	-0.561	0.575	-0.286	0.860	-0.333	0.739

Table 11. **Effect of *HTR2A* rs6311 (with T as the minor allele) on childhood adversity score in a linear regression model, with gender, age (and, in the combined sample, also population) as covariates. S.E.: standard error of beta.**

	Budapest + Manchester				**Budapest**				**Manchester**			
Model	**Beta**	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**
additive	0.021	0.130	0.165	0.869	-0.122	0.193	-0.630	0.529	0.102	0.168	0.603	0.547
dominant	0.048	0.187	0.257	0.797	-0.483	0.285	-1.697	0.090	0.302	0.239	1.267	0.205
recessive	-0.006	0.243	-0.026	0.979	0.338	0.356	0.949	0.343	-0.174	0.318	-0.548	0.584

Table 12 shows the correlation of childhood adversity with rumination and its two subscales, in the combined Budapest + Manchester sample (n=1498), Budapest (n=468) and Manchester (n=1030). All associations are significantly positive, except for the one with reflection in Budapest, which is not significant. This means that except for this sole association, testing a GxE effect on rumination phenotypes would shed light on the moderating role of *HTR2A* rs3125 or rs6311 genotype in the potential of childhood adversity to intensify rumination.

***Table 12*. Pearson correlation coefficient (and its p-value) of childhood adversity score with rumination, brooding and reflection scores, respectively, in the combined sample and the two subsamples.**

	Budapest + Manchester		**Budapest**		**Manchester**	
	r	**p**	**r**	**p**	**r**	**p**
rumination	0.257	<0.001	0.109	0.019	0.275	<0.001
brooding	0.275	<0.001	0.104	0.025	0.299	<0.001
reflection	0.166	<0.001	0.073	0.115	0.178	<0.001

The Pearson correlation coefficients between the brooding and reflection subscales of rumination are the following: r=0.487 in the combined sample (n=1501; p<0.001), r=0.308 in Budapest (n=470; p<0.001), and r=0.521 in Manchester (n=1031; p<0.001). These results underline the importance of including the other subscale as a covariate in the regression equations for a subscale as the outcome.

5.B.2. Association of *HTR2A* rs3125 and rs6311 with rumination and its two subtypes in function of childhood adversity level, in the combined Budapest + Manchester sample

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As we can see in ***Table 13***, only three models survive the correction for multiple testing. Namely, rs3125 is associated only with brooding, and only in the function of childhood adversity score, both in additive and dominant models. Moreover, rs6311 is associated only with rumination, and, similarly, only in function of childhood adversity level, in an additive model. Results of power calculations (***Table 13***) validate our results as true positives and true negatives, respectively.

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***Table 13*. Effect of *HTR2A* rs3125 (with C as the minor allele) or rs6311 (with T as the minor allele) in linear regression models for each rumination phenotype, with population, gender and age as covariates. Additional covariates were: the other subscale (in case of a subscale outcome), and the main effects of the SNP and childhood adversity (in case of interaction models). Statistical power of the analyses is between 97.16%-97.28% for the main effect models and 78.98%-81.34% for the interaction models. SNP = single nucleotide polymorphism; p: nominal p-value. Findings surviving the correction for multiple testing (having a q≤0.05) are marked with bold.**

			Additive model			Dominant model			Recessive model		
		SNP	**Beta**	**P-value**	**Q-value**	**Beta**	**P-value**	**Q-value**	**Beta**	**P-value**	**Q-value**
Main effect of SNP	**Rumination**	**rs3125**	0.041	0.180	0.114	0.061	0.068	0.085	-0.133	0.245	0.116
		rs6311	0.001	0.953	0.212	0.011	0.724	0.176	-0.014	0.727	0.176
	Brooding	**rs3125**	0.010	0.747	0.176	0.014	0.686	0.176	-0.020	0.871	0.199
		rs6311	0.020	0.367	0.124	0.030	0.348	0.124	0.019	0.640	0.176
	Reflection	**rs3125**	0.035	0.278	0.116	0.053	0.132	0.114	-0.127	0.291	0.116
		rs6311	-0.019	0.400	0.124	-0.018	0.570	0.166	-0.034	0.404	0.124
SNP x childhood adversity interaction	**Rumination**	**rs3125**	0.011	0.173	0.114	0.011	0.203	0.116	0.020	0.582	0.166
		rs6311	**0.015**	**0.013**	**0.035**	0.017	0.046	0.073	0.022	0.042	0.073
	Brooding	**rs3125**	**0.028**	**0.001**	**0.006**	**0.030**	**0.001**	**0.006**	0.036	0.345	0.124
		rs6311	0.008	0.227	0.116	0.010	0.282	0.116	0.010	0.394	0.124
	Reflection	**rs3125**	-0.015	0.091	0.091	-0.017	0.075	0.085	-0.013	0.729	0.176
		rs6311	0.009	0.147	0.114	0.010	0.286	0.116	0.016	0.186	0.114

Figure 10 displays visualisation of the rs3125 x childhood adversity interaction on brooding in the dominant model, showing that carrying the minor C allele is protective against brooding rumination only in case of a low level of childhood adversity, but it becomes a risk for higher brooding in case of high childhood stress.

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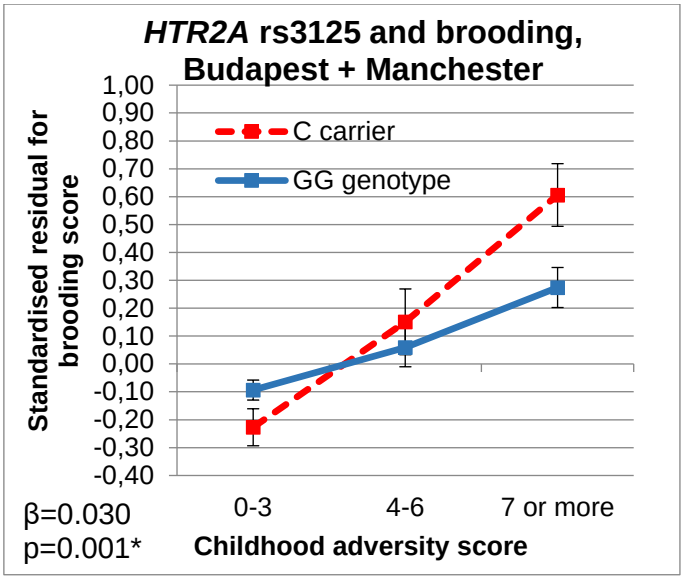

***Figure 10*. Means and standard errors of brooding score (having been controlled for population, gender, age and reflection in a previous regression), in function of childhood adversity score and *HTR2A* rs3125 genotype, in the combined Budapest + Manchester sample, based on reference (199).**

Figure 11 displays visualisation of the rs6311 x childhood adversity interaction on rumination in the additive model. Although standard error bars for the distinct genotypes do not separate clearly from each other by this grouping of childhood adversity, we can see that the minor T allele can protect against rumination in case of a low level of childhood adversity, but may confer a proneness to high rumination in case of a high level of childhood adversity. Number of the carried T allele(s) also seems to matter in this interaction effect.

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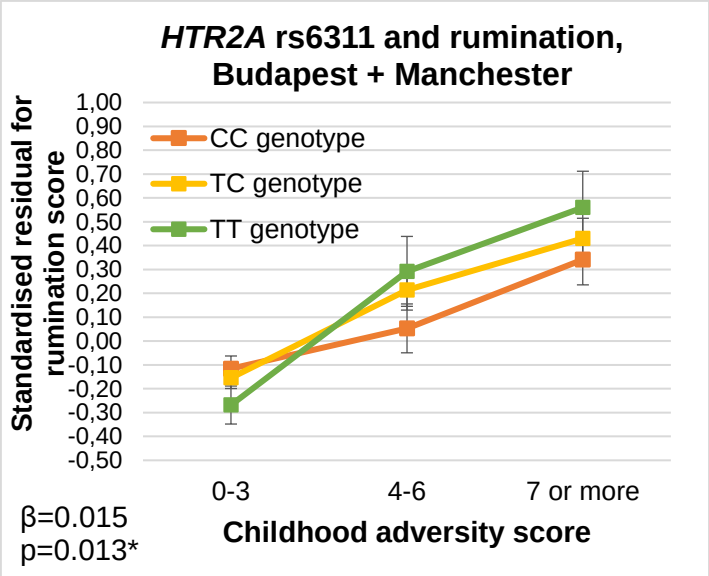

***Figure 11*. Means and standard errors of rumination score (having been controlled for population, gender and age in a previous regression), in function of childhood adversity score and *HTR2A* rs6311 genotype, in the combined Budapest + Manchester sample.**

5.B.3. The role of depression in the rs3125 x childhood adversity interaction effect on brooding, in the combined Budapest + Manchester sample

Preconditions of testing the mediative role of depression in the found rs3125 x childhood adversity interaction are fulfilled, since brooding score has a significant positive association with BSI depression score (n=1500; Pearson r=0.620; p<0.001), and it has a significantly (n=1501; t=-18.970; p<0.001) higher mean in those reporting lifetime depression (2.536±0.026) than in those who did not report it (1.919±0.019). Moreover, ***Table 14*** displays the nominal trend in the positive associations of the rs3125 x childhood adversity interaction term with both lifetime depression (n=1498) and BSI depression score (n=1497).

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Table 14. **Interaction of *HTR2A* rs3125 and childhood adversity on lifetime depression in logistic regression models and on BSI depression and brooding in linear regression models. Population, gender, age, rs3125 and childhood adversity were covariates in all analyses, and in those for brooding, the two depression phenotypes and reflection were also covariates. OR: odds ratio; S.E.: standard error of OR or beta; BSI: Brief Symptom Inventory.**

	additive				**dominant**			
	OR	**S.E.**	**t**	**p**	**OR**	**S.E.**	**t**	**p**
lifetime depression	1.081	0.043	1.813	0.070	1.087	0.045	1.851	0.064
	Beta	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**
BSI depression score	0.024	0.013	1.834	0.067	0.026	0.014	1.881	0.060
brooding score (controlling for depression)	0.018	0.008	2.438	0.015	0.019	0.008	2.382	0.017

As we can see in ***Table 14***, including the two depression phenotypes as covariates in the rs3125 x childhood adversity interaction model on brooding (5.B.2, ***Table 13***), the interaction term remains nominally significant in both additive and dominant models. This means that the association of rs3125 with brooding, dependent on childhood adversity level, is not exclusively due to depression.

5.B.4. The role of brooding in the rs3125 x childhood adversity interaction effect on depression, in the combined Budapest + Manchester sample

Table 15 demonstrates that including brooding as an additional covariate in the rs3125 x childhood adversity interaction models described in ***Table 14*** in 5.B.3, the interaction term loses its trend association with both lifetime depression and BSI depression score in both additive and dominant models. These findings suggest that the childhood stress-dependent association of *HTR2A* rs3125 with depression is exclusively due to brooding.

***Table 15*. Interaction of *HTR2A* rs3125 and childhood adversity on lifetime depression in logistic regression models and on BSI depression in linear regression models, with population, gender, age, rs3125, childhood adversity and brooding as covariates. OR: odds ratio; S.E.: standard error of OR or beta; BSI: Brief Symptom Inventory.**

	additive				**dominant**			
	OR	**S.E.**	**t**	**p**	**OR**	**S.E.**	**t**	**p**
lifetime depression	1.035	0.045	0.757	0.449	1.038	0.047	0.790	0.430
	Beta	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**
BSI depression score	0.005	0.011	0.440	0.660	0.006	0.012	0.524	0.600

5.B.5. Replicability of the rs3125 x childhood adversity interaction effect on brooding in the separate Budapest and Manchester subsamples

Table 16 demonstrates that the rs3125 x childhood adversity effect on brooding (***Table 13*** in 5.B.2.) can be replicated at a nominally significant level in both the Budapest and the Manchester subsamples, in both additive and dominant models. Visualisations of the dominant models are displayed in ***Figure 12*** for the Budapest, and ***Figure 13*** for the Manchester subsample. We can see that as in the combined sample (***Figure 10***), carrying the C allele is protective against brooding in case of low childhood adversity but turns into a risk factor for brooding in case of a high level of childhood adversity also in

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Manchester (***Figure 13***). However, in the Budapest subsample, we can detect only the risk conveyed by the C allele in case of high childhood stress, but it is not protective in those with a low level of childhood adversity (***Figure 12***).

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Table 16. **Interaction of *HTR2A* rs3125 and childhood adversity on brooding, separately in Budapest and Manchester, in linear regression models with gender, age, rs3125, childhood adversity and reflection as covariates. S.E.: standard error of beta.**

	Budapest				Manchester			
Model	**Beta**	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**
additive	0.042	0.019	2.231	0.026	0.024	0.010	2.414	0.016
dominant	0.042	0.019	2.185	0.029	0.026	0.011	2.411	0.016

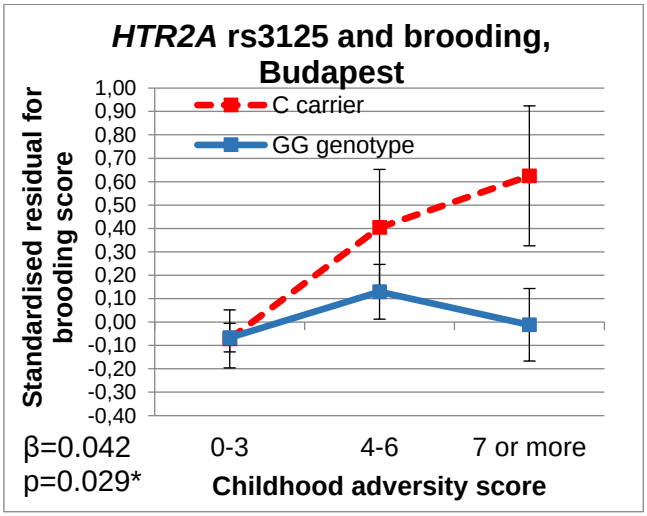

Figure 12. **Means and standard errors of brooding score (having been controlled for gender, age and reflection in a previous regression), in function of childhood adversity score and *HTR2A* rs3125 genotype, in the Budapest subsample, based on reference (199).**

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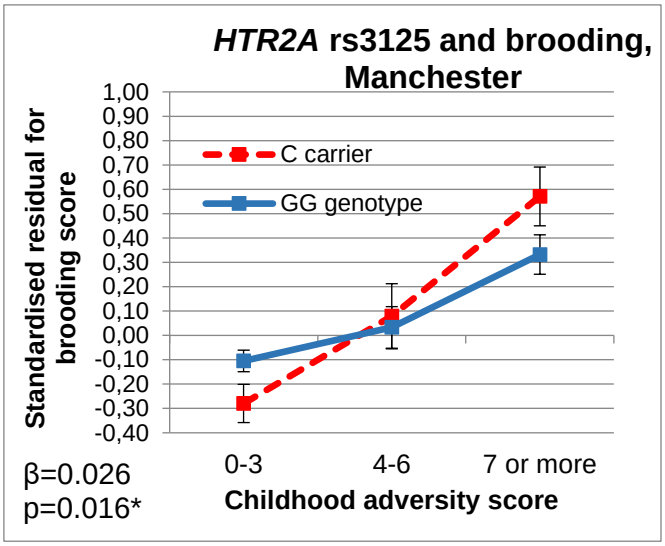

***Figure 13*. Means and standard errors of brooding score (having been controlled for gender, age and reflection in a previous regression), in function of childhood adversity score and *HTR2A* rs3125 genotype, in the Manchester subsample, based on reference (199).**

5.B.6. Replicability of the asymmetry of mediative roles of brooding and depression in the rs3125 x childhood adversity interaction, in the separate Budapest and Manchester subsamples

Preconditions of testing the mediative roles of brooding and depression phenotypes on each other in the rs3125 x childhood adversity interaction are not met in either subsample, since the interaction term does not exert a significant effect on any of the depression phenotypes in any of the regression models, neither in Budapest (***Table 17***; n=468 for lifetime depression and n=467 for BSI depression), nor in Manchester (***Table 18***; n=1030).

Table 17. **Interaction of *HTR2A* rs3125 and childhood adversity on lifetime depression in logistic regression models and on BSI depression in linear regression models, in Budapest. Gender, age, rs3125 and childhood adversity were covariates in all analyses. OR: odds ratio; S.E.: standard error of OR or beta; BSI: Brief Symptom Inventory.**

	additive				**dominant**			
	OR	**S.E.**	**t**	**p**	**OR**	**S.E.**	**t**	**p**
lifetime depression	1.108	0.083	1.236	0.217	1.098	0.084	1.102	0.270
	Beta	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**
BSI depression score	0.017	0.022	0.781	0.435	0.018	0.023	0.812	0.417

Table 18. **Interaction of *HTR2A* rs3125 and childhood adversity on lifetime depression in logistic regression models and on BSI depression in linear regression models, in Manchester. Gender, age, rs3125 and childhood adversity were covariates in all analyses. OR: odds ratio; S.E.: standard error of OR or beta; BSI: Brief Symptom Inventory.**

	additive				**dominant**			
	OR	**S.E.**	**t**	**p**	**OR**	**S.E.**	**t**	**p**
lifetime depression	1.071	0.052	1.332	0.183	1.082	0.055	1.439	0.150
	Beta	**S.E.**	**t**	**p**	**Beta**	**S.E.**	**t**	**p**
BSI depression score	0.020	0.016	1.255	0.210	0.022	0.017	1.274	0.203

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5.B.7. The role of depression in the rs6311 x childhood adversity interaction effect on rumination, in the combined Budapest + Manchester sample

Preconditions of testing the role of depression in the rs6311 x childhood adversity interaction effect on rumination are not fulfilled, since the same rs6311 x childhood adversity interaction in the same additive model (covarying population, gender, age and main effects of rs6311 and childhood adversity) does exert a significant effect on neither depression phenotype (on lifetime depression, n=1483; OR=1.022; S.E.=0.027; t=0.808; p=0.419; and on BSI depression score, n=1482; β=-0.001; S.E.=0.009; t=-0.13; p=0.894).

5.B.8. Replicability of the rs6311 x childhood adversity interaction effect on rumination in the separate Budapest and Manchester subsamples

The rs6311 x childhood adversity interaction on rumination according to an additive model, proven to be significant in the combined sample (***Table 13*** in 5.B.2), loses its significance in the Budapest subsample (n=467; β=0.009; S.E.=0.009; t=1.019; p=0.309), but remains significant in the Manchester subsample (n=1016; β=0.015; S.E.=0.007; t=1.962; p=0.050). Gender, age, and main effects of rs6311 and childhood adversity were the covariates in these models.

5.B.9. Relevance of rs6311 in a complex depression-anxiety phenotype, taking recent stress and six other depression-related polymorphisms into consideration

Our Bayesian multi-level analysis of relevance revealed that, treated within the same model with gender, age, *HTR1A* rs6295, *SLC6A4 5-HTTLPR*, *BDNF* rs6265, *GALR2* rs8836, *CNR1* rs7766029 and *P2RX7* rs7958311, the rs6311 SNP of *HTR2A* does not prove to be relevant with respect to the complex depression-anxiety phenotype composed of lifetime depression, BSI depression and BSI anxiety, in case of any level of recent stress exposure (***Figure 14***).

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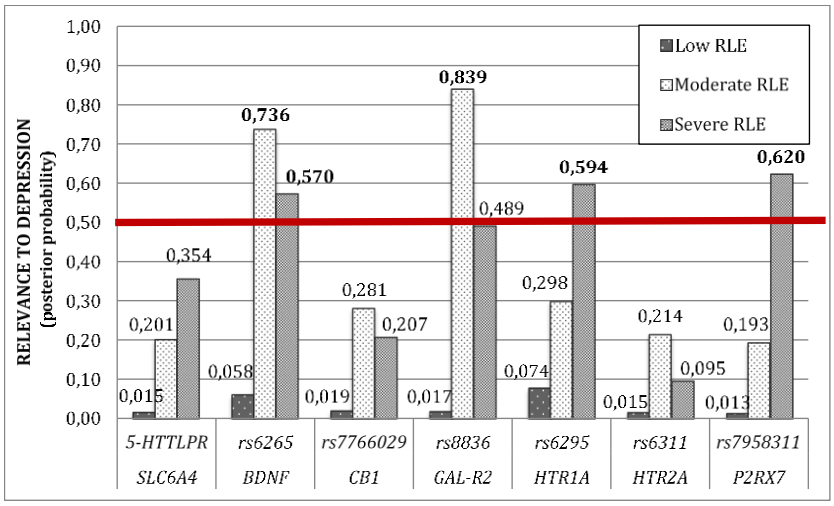

Figure 14. **Posterior probability of relevance of the seven polymorphisms with respect to the complex depression-anxiety phenotype, separately in those with a low, a moderate and a high number of negative life events in the past year, based on reference (200). A posterior probability of relevance can be interpreted as high if > 0.50. RLE: recent negative life events; low: 0-1; moderate: 2; severe (high): 3 or more negative life events in the past year.**

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6. Discussion

6.1. Ruminative response style is positively related to the A allele of *MTHFD1L* rs11754661, but unrelated to *MTHFR* rs1801133 in the combined Budapest + Manchester sample

In chapter 5.A.2, answering hypotheses 3.A.1 and 3.A.2, we could see that the widely investigated *MTHFR* rs1801133 polymorphism had no effect on rumination, whereas the scarcely investigated *MTHFD1L* rs11754661 was associated with it.

In chapter 2.3.3, we could see that regarding the contradictory picture of *MTHFR* rs1801133, the T allele has proven to be the risk for depression, but only in Asians and not in Caucasians. However, while the SNP failed to show any association with performance on a wide variety of cognitive domains among undergraduates, healthy adults and elderly participants (see chapter 2.3.3 for details), it is challenging to interpret that heterozygote elderly males showed a better performance on some working memory tasks than both homozygotes (134), and that in the elderly the T/T genotype predicted a better sensorimotor speed and, in those with a low erythrocyte folate level, also a better cognitive flexibility (136). Durga et al, 2006 (136) interpreted their results as that although the T/T genotype entails a reduced methylation capacity, it also leads to a higher concentration of 10-formyl-THF and a possibly enhanced capacity of thymidylate synthesis (***Figure 1*** and ***Figure 2***). While chapters 2.3.1 and 2.3.3 have demonstrated how important methylation processes are in depression and cognition, on the other hand 10-formyl-THF promotes mitochondrial integrity by preventing citotoxicity and apoptosis, and an enhanced capacity of thymidylate synthesis protects against genomic and mitochondrial DNA damage, pointing to two advantages of the T/T genotype, being mitochondria also important in depression and cognition (136, 201). Though we did not get any association between rumination and rs1801133, these interpretations can explain our discrepancy found in the effects of the two SNPs. Particularly, the reason may be that the MTHFR enzyme can support either SAM synthesis and thus methylation, by its enhanced activity, or thymidylate biosynthesis and 10-formyl-THF production, by its reduced activity (see ***Figure 1*** and ***Figure 2***), at the expense of each other, and since rs1801133 affects the level of enzyme activity, there may be no “good genotype”. In

contrast, we can see in ***Figure 2*** that the MTHFD1L enzyme can support syntheses of 10-formyl-THF, thymidylate and SAM with the same direction of activity, so effects of polymorphisms within the *MTHFD1L* gene may be more straightforward than those of *MTHFR*. We also know that the A allele of rs11754661, found a risk for higher rumination in our study, has been associated with a high plasma homocysteine level (141), suggesting that it would also entail a decrease in methylation and thymidylate and 10-formyl-THF syntheses (***Figure 1*** and ***Figure 2***).

A second, related reason for the discrepancy in the effects of the two SNPs might be the different subcellular localisation of the two encoded enzymes (***Figure 2***), corroborated by a recent review (201) suggesting the simultaneous importance of mitochondrial dysfunctions and cognitive symptoms in a subset of depressed patients, within a framework of the endophenotype concept.

A third reason for the discrepancy may be the difference between the two enzymes in their sensitivity to other external and / or internal factors, such as folate levels. Durga et al, 2006 (136) got their *MTHFR* rs1801133 results on the cognitive flexibility phenotype only among participants with a low erythrocyte folate level, being these results only trend in case of a low serum folate level. Erythrocyte folate level reflects a long-term folate status, whereas serum folate is a marker of the short-term folate intake by diet, and it is important to note that these participants were not exposed to any folic acid fortification (136), which had proven to moderate the association of plasma folate and homocysteine levels with each other (119), so all of these findings underscore the complexity of impacts exerted by external and internal factors on genetic effects themselves. As we saw in chapter 2.3.3, results have a similar interactional nature on homocysteine concentration, since homocysteine-elevating effect of the T/T genotype is present only in case of low folate intake or a low level of plasma folate, but not in case of high folate intake or a high level of plasma folate (113, 202-205), or, at least, it is stronger in case of a low level of plasma folate (141). A similar *MTHFR* rs1801133 x folate interaction has been demonstrated on DNA methylation level, in that the T/T genotype was related to a lower methylation status of genomic DNA only in case of a low level of plasma folate (124). However, it is important to note in this complicated pattern of variables that despite the fact that the effects of *MTHFR* rs1801133 on cognitive flexibility, homocysteine concentration and DNA methylation all depend on folate status,

its T/T genotype has been associated with a lower level of plasma folate in case of both a low and a high folate intake (202), which entails a gene-environment correlation.

On the other hand, the risk for a higher plasma homocysteine level conferred by the A allele of *MTHFD1L* rs11754661, is significant even after controlling for plasma folate level (141). Although we already know that consistently the same A allele denotes the risk for Alzheimer's disease, with only one negative study out of four (see 2.3.4 for details), and for high rumination in our study, it would be crucial to test dependence of these genotype-phenotype associations on folate levels. It would also be necessary to test whether or not these effects of *MTHFD1L* rs11754661 are mediated by methylation processes, since another SNP within *MTHFD1L*, rs1738574, has been associated with a marker of genome-wide DNA methylation if controlling for plasma folate level (141). Both rs11754661 (https://genome.ucsc.edu/cgi-bin/hgc?hgsid=650891075_4qYEgyt1sdt4NIGMHpWXKxMAPcAX&c=chr6&l=151206827&r=151207328&o=151207077&t=151207078&g=snp150Common&i=rs11754661) and rs1738574 (https://genome.ucsc.edu/cgi-bin/hgc?hgsid=650891569_csfGusGox84ObHgCQ3yBQOpsMXx7&c=chr6&l=151286719&r=151287220&o=151286969&t=151286970&g=snp150Common&i=rs1738574) are intronic variants within *MTHFD1L*, therefore the mechanisms by which they exert their effects on the phenotypes should also be clarified in the future.

Testing dependency of the rs11754661-rumination association on folate levels would also yield implications on the possibilities of folate "therapy" in high rumination. Papakostas et al, 2012 (206) found in MDD outpatients resistant to SSRI (selective serotonin reuptake inhibitor) treatment that keeping SSRI dosages constant, an adjunctive L-methylfolate (the biologically active form of folate, and the only one crossing the blood-brain barrier) at 15 mg per day proved to be an effective and safe adjuvant therapy compared to SSRI plus placebo. Taylor et al, 2004 (207) in their meta-analysis conducted on three randomised controlled trials, also came to the conclusion that folate as an adjuvant therapy in the treatment of MDD would decrease depression score, besides its safety and acceptability. On the contrary, Bedson et al, 2014 (208), performing a randomised controlled trial with a folic acid augmentation of antidepressant medication in moderate to severe depressed patients, got no evidence on the effectiveness of folic acid. Nevertheless, it may be attributed to that folic acid is biologically inactive, thus

needs to be converted, and may compete with the biologically active methylfolate for the transport across the blood-brain barrier, implicating that methylfolate would be a better candidate for the augmentation of antidepressant treatment (208). If the risk conveyed by the rs11754661 A allele on rumination vanishes due to supplementation with some form of folate, maybe methylfolate, like the challenging associations conveyed by the T/T genotype of rs1801133 on multiple phenotypes vanish, then we can somewhat compensate the genetic risk by dietary or treatment augmentation factors, and mitigate the part of rumination attributable to the *MTHFD1L* gene, either in ruminating depressed patients or in mentally healthy people with this endophenotype and thus at risk for the disorder.

If this rs11754661-rumination association will be revealed to be due to methylation dysfunctions, another therapeutic possibility emerge: SAM, which has been shown to have antidepressant properties and to improve cognitive functioning in demented patients (149), and is used as an effective adjuvant therapy in depression in some countries (114), being the most effective for the particular symptoms of depressed mood, activity, interest, psychomotor retardation, guilt and suicidal tendencies, but it may induce a quick and frequent switch to euthymic or hypomanic phase in bipolar patients (150). In these findings with SAM, predominance of cognitive, affective and motivational symptoms in depression and that of cognition in dementia suggest its usefulness in the highly ruminating subgroup of depressed patients.

To summarise and fuel further studies, our results on the discrepancy in the effects of the two SNPs within two folate enzyme genes may either stem from their distinct biochemical roles, or from their distinct subcellular localisation, or from their distinct sensitivity to other internal or external factors, such as folate status. Dependence of the *MTHFD1L*-rumination association on folate status is worth testing not only because of investigating robustness and replicability of the genetic effect, but also from a therapeutic angle: we may define a subgroup within depressed patients, characterised by mitochondrial dysfunctions, predominance of cognitive symptoms and a high level of rumination, for whom a folate or SAM augmentation of antidepressant medication would be more effective than for other subgroups of depressed patients. Further strengthening the endophenotype concept of rumination, we should test whether or not a high

rumination treated and alleviated successfully with folates (or even with SAM) would even prevent the emergence of MDD.

6.2. Effects of *HTR2A* polymorphisms on rumination phenotypes are function of childhood adversity level, in the combined Budapest + Manchester sample

I detailed in chapter 5.B.2, answering to hypotheses 3.B.1 and 3.B.3, that *HTR2A* rs3125 is related to any of the rumination phenotypes, but this association is restricted to the brooding subtype and is detectable only in function of childhood adversity level in our study.

McCaffery et al, 2009 (172) found the minor C allele of rs3125 a risk for higher BDI depression score in French Canadian patients with coronary artery disease. McAuley et al, 2009 (209) revealed in Australians of British or Irish descent that C allele is present in both the protective and the risk haplotype in relation to bipolar disorder, but the minor allele was G in their study, with a comparable allele frequency to that of C in McCaffery et al, 2009 (172) and in our results (chapter 5.B.1), so I propose that given that all three studies involved European participants, McAuley et al, 2009 (209) must have called the complementary strand of DNA when genotyping rs3125. Our results have revealed the minor C allele as a risk for higher brooding only in case of high childhood adversity, which is in line with the positive finding of McCaffery et al, 2009 (172), proven also within a high-risk population, even if the stress was not a distant one there but a current illness. Our results are also in line with the negative finding of McAuley et al, 2009 (209), where rs3125 genotype does not matter in itself with regard to bipolar disorder, without considering any stress factor. This stress-dependence in the effect of rs3125 may be attributable to that it is a miRNA binding site, transmitting epigenetic impacts provoked by environmental factors. As detailed in chapter 2.6, the G allele (measured on the negative strand) of rs3125 binds miR-539 (https://snpinfo.niehs.nih.gov/cgi-bin/snpinfo/mirna.cgi?2_rs3125), which shows a decreased expression in ACC in chronic neuropathic pain (171). (UCSC Genome Browser also defines the minor C and major G alleles on the negative strand: https://genome.ucsc.edu/cgi-

bin/hgc?hgsid=651088545_3C7HDGS6sx9OSueYkzaaMM6SmE5J&c=chr13&l=47408600&r=47409101&o=47408850&t=47408851&g=snp150Common&i=rs3125). Based on literature (see chapters 2.1.4.3 and 2.5.2 for details), we would expect a lower 5-HT_{2A} concentration in ACC in relation to higher brooding, which could be due to an enhanced miRNA binding, nevertheless we got the non-binding minor C allele as the risk, so the exact mechanism of action by which the C allele exerts its effect on brooding should be clarified in the future.

As detailed in chapter 5.B.2, to answer hypotheses 3.B.2 and 3.B.3, *HTR2A* rs6311 is associated only with rumination, and, similarly to the rs3125 results with brooding, only in function of childhood adversity level. In chapter 2.6, regarding results with newborn infants, we could see that timing of methylation within the *HTR2A* promoter can be crucial in a long-lasting impact on psychiatric phenotypes, and our results with insignificant effect of the methylation site rs6311 but its significant effect in interaction with childhood adversity on rumination also underline this assumption. Former results with regard to rs6311 have yielded contradictions, but these may be resolved if taking stress level into consideration. C/C (G/G on the other strand) genotype has been positively associated with rumination-related phenotypes (177), but in case of high childhood adversity T/T (A/A on the other strand) genotype was the risk for depression, and this latter effect was fully mediated by cultural consonance (178) (see chapter 2.6 for details). Consistently with that, T/T genotype was associated with a reduced heart rate variability (HRV, a measure of parasympathetic activity) only in those healthy participants perceiving a high level of stress within the past month, but not in those with a low level of stress (210), and HRV had been negatively associated with brooding (105). Although inconclusively (see chapter 2.6 for details), also the T allele (A allele on the other strand) denoted a risk for seasonal affective disorder, and seasons can represent a certain type of stressors. Our results with rumination are in line with all these former results, since T allele became a risk only in case of a high level of childhood adversity, but it may protect against rumination in the lack of childhood stress (see chapter 5.B.2). Being the T allele of rs6311 the expression-enhancing allele in vitro and ex vivo (182, 210), we can hypothesise that an increased expression of *HTR2A* and thus an increased level of 5-HT_{2A} receptors in either ACC, medial or dorsal PFC (see chapter 2.5.2 for details) can make the person's rumination level more dependent on 5-HT_{2A}-mediated serotonergic

transmission, and thus this 5-HT_{2A}-dependent rumination more sensitive to environmental impacts. On the other hand, persons with a C/C genotype may have a decreased density of 5-HT_{2A} receptors, thus they may be less sensitive to serotonergic deficiencies, childhood maltreatment or other environmental impacts influencing rumination level in that critical period of development. Our gene-by-environment interaction result can thus give a possible answer to the contradictions detailed in chapter 2.5.2 with regard to the expected association of rumination and 5-HT_{2A} binding.

Nevertheless, our rs6311 x childhood adversity interaction result cannot be replicated within the separate Budapest and Manchester subsamples (see chapter 5.B.8, answering hypothesis 3.B.6), implying that effects of methylation may be influenced by other, population-specific environmental impacts.

To conclude, our negative results with the main effect of both *HTR2A* SNPs and positive results with their interaction effect underscore the importance of involving stress measurements as possible moderators in the investigation of those *HTR2A* polymorphisms that establish epigenetic modifications evoked by environmental effects.

Our finding that only brooding but not reflection is associated with the interaction of rs3125 and childhood adversity, also explaining totally the association of this interaction term with depression, is in line with former results on the role of only brooding but not reflection in partially mediating the depressogenic effect of childhood abuse (see chapter 2.5.1 for details). Therefore, we can conclude that while childhood abuse confers a risk for depression not only through the endophenotype of brooding, its depressogenic effect in interaction with the miRNA binding site SNP rs3125 is entirely due to brooding.

However, our rs3125 results have an important limitation that warns us to draw any conclusion with caution. We could see in chapter 5.B.1, as a proof of gene-environment correlation in the combined sample and the Manchester subsample that the C allele of rs3125 has a positive association with childhood adversity in those additive and dominant models that yield the significant interaction terms for brooding. Yet, including the main effects of both childhood adversity and rs3125 in each interaction model may control for this potential confounding factor in the regression equations.

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6.3. Significant SNP-rumination associations fully explain and go beyond the SNP-depression associations in the combined Budapest + Manchester sample

We could see in case of both the folate-related *MTHFD1L* rs11754661 (in chapters 5.A.3 and 5.A.4, answering hypotheses 3.A.3 and 3.A.4, respectively) and *HTR2A* rs3125 interacting with childhood adversity level (in chapters 5.B.3 and 5.B.4, answering hypotheses 3.B.4 and 3.B.5, respectively), and for both SNPs in both additive and dominant models, that there is an asymmetry in the mediative roles of the rumination phenotype and depression in each other's association with the SNP. Particularly, the SNP-rumination association entirely accounts for the SNP-depression association, but the SNP-depression association only partially accounts for the SNP-rumination association, entailing the assumption that parts of the rumination endophenotype constituted by these two SNPs pave the way for depression but do not stop there, going above and beyond this disorder.

Rumination has indeed been found to predict psychopathologies other than depression. Although Aldao et al, 2010 (52), reviewing longitudinal studies, revealed that RSQ rumination conflictingly predicted anxiety symptoms and alcohol abuse problems, there are convincing results as well. Among disorders, rumination has been related to an increased risk for social phobia (211, 212), PTSD (212-214), substance abuse (215) and premenstrual disorders (216). Regarding symptoms, it has been associated with symptoms of alcohol abuse (217, 218), bulimia nervosa (binge eating) (215), and aggressive behaviour (219). Within a disorder, it can also be associated with specific characteristics and symptom profiles, such as, with generalised anxiety symptoms, obsessive-compulsive symptoms and borderline personality disorder traits in unipolar depressed patients (220), and with depression, hypomania and anxiety symptoms in bipolar patients (221). It also showed a higher level in psychotic patients with current persecutory delusions than in controls (222).

Besides being a potential endophenotype for numerous mental health problems, rumination can also be related to various aspects of physical health: migraine (223), shorter sleep duration (224) and decreased subjective sleep quality (225). The role of

rumination as a form of perseverative cognition, in cardiovascular, immunological, endocrinological and neurovisceral consequences of stressors (35-37), has been detailed in chapter 2.1.1.2.

Rumination not only denotes a transdiagnostic risk factor for all of these disorders, but it can be a potential common endophenotype for them, accounting for a common genetic background behind them.

Increased homocysteine and decreased folate levels have been linked to cardiovascular disorders and first-episode psychosis (110), and increased homocysteine to chronic schizophrenia, negative symptoms within schizophrenia, and bipolar disorder (114). Regarding genetics, another intronic SNP within *MTHFD1L*, rs6922269, has been revealed to be related to coronary artery disease (226, 227) and myocardial infarction (228), with unreplicable but positive results on mortality after acute coronary syndrome (229, 230), and yielding inconsistent (231) and negative (232-235) results also on coronary heart disease itself. Although these findings are heavily contradictory, it must be noted that Angelakopoulou et al, 2012 (236) revealed that rs6922269 associated with coronary heart disease risk without associating with any of its biomarkers, risk factors or intermediate phenotypes, suggesting an unsuspected mechanism of the genetic effect. Moreover, Prasannan et al, 2003 (237) demonstrated that human tissue expression of *MTHFD1L*, while highest in placenta, thymus and brain, is barely detectable in heart. All these results with *MTHFD1L*, along with our ones and with the link between rumination and cardiovascular disorders, let me hypothesise that the variability of rumination explained by this gene denotes an endophenotype that is on the causal pathway not only to depression but also to coronary heart disease.

With regard to rumination-associated disorders and our other candidate, *HTR2A*, its rs6313 SNP, but not rs6311, was found to be related to alcohol dependence or abuse in the meta-analyses of Cao et al, 2014 (238). Borderline personality disorder was not associated with any of four SNPs (rs6313, rs4941573, rs2296972 and rs6314) from *HTR2A* (239), and in females rs6311 in itself showed no association with either heroin dependence or borderline personality disorder within it (240). Nonetheless, in this female sample, in interaction with two other polymorphisms from monoamine oxidase A *MAOA* and serotonin transporter *SLC6A4* genes, rs6311 was related to the co-morbidity of heroin dependence and borderline personality disorder (240). Rs2296972 was associated with

binge eating, without associating with MDD and without the moderating role of MDD in the association (241). Two comprehensive meta-analyses have revealed the association of *HTR2A* also with obsessive-compulsive disorder (242, 243). In contrast, none of rs6311, rs6313 or rs6314 from *HTR2A* has shown association with migraine (244). Based on this literature, I hypothesise that the part of the rumination endophenotype accounted for by *HTR2A* can denote an endophenotype not only for depression, but also for alcohol and substance abuse, borderline personality disorder, binge eating and obsessive-compulsive disorder. However, rumination may confer a risk for only a subgroup of migraine patients, or may lead to migraine by involving other pathways than that of *HTR2A*. It is also crucial to note that *HTR2A* was not enough in itself to predict either heroin dependence or borderline personality disorder, but its impact on their co-occurrence was influenced by two other genes of the serotonergic system, underscoring the need for interaction studies in the endophenotype concept of *HTR2A*.

Nevertheless, it has to be noted that, in contrast to our GxE findings with *HTR2A* rs3125, our *HTR2A* rs6311 x childhood adversity interaction was not associated with depression at all (see chapter 5.B.7, answering hypotheses 3.B.4 and 3.B.5). Consequently, rs6311 can only contribute to the part of the rumination endophenotype that denotes a risk for disorders other than depression: such as the co-occurrence of heroin dependence and borderline personality disorder, but not migraine, alcohol dependence or abuse (see above). Moreover, our results detailed in chapter 5.B.9, answering hypothesis 3.B.8, revealed that *HTR2A* rs6311 is not relevant in a complex phenotype characterised by depression and anxiety, even if involving recent stress and six other depression-related polymorphisms in the model. Instead, other SNPs, *HTR1A* rs6295, *BDNF* rs6265, *GALR2* rs8836, and *P2RX7* rs7958311 proved to be relevant within the same model, in case of moderate or high exposure to recent stress (***Figure 14***). Consequently, if *HTR2A* rs6311 has an effect on depression, it is neither exerted through rumination in function of childhood adversity level, nor on a complex depression-anxiety phenotype in function of recent stress exposure and genotypes of SNPs highly relevant in depression. Rather, it can be exerted through cultural consonance, in function of childhood adversity level (178), or on seasonal affective disorder (179, 180).

The putative transdiagnostic endophenotype nature of rumination entails the possibility of prevention of these numerous rumination-related disorders by targeting

rumination with psychotherapeutic or other techniques. We saw in chapter 2.1.3.1 that rumination does not fulfil the stringent endophenotype criterion of independency of illness state (58), but this also means that it can be reduced by therapeutic interventions. Based on our results, I proposed a possibility of folate or SAM supplementation in mitigating rumination in chapter 6.1, but psychotherapeutic techniques have been widely applied with this purpose.

Querstet and Cropley, 2013 (245) in their systematic review, come to the conclusion that mindfulness-based and cognitive behavioural interventions proved to be effective in alleviating rumination and worry, treatments in which a more concrete and specific thinking style or a disengagement from emotional response to rumination or worry can be acquired, and they also point to the effectiveness of a specifically rumination-focused cognitive behavioural therapy (CBT). Indeed, in residually depressed patients after an acute treatment, a cognitive behavioural group treatment targeting depressive rumination, compared to the control condition has been found to reduce depressed mood, rumination and dysfunctional metacognitive beliefs, and to improve the perceived control over rumination, even at a one-year follow-up (246). Similarly, in patients with MDD and / or generalised anxiety disorder, an internet-delivered CBT, compared to the control condition, reduced frequency of and positive beliefs about repetitive negative thinking, with gains even after a 3-month follow-up, and these reductions mediated a reduction in depression (247). Decreases of rumination and worry in a mindfulness-based CBT with relapse prevention purposes in patients with a recurrent depression history have been attributed to a regular and consistent practice (248).

Regarding mindfulness-based techniques, whereas highly ruminating undergraduates did not show difference in rumination and depression decreases between the two intervention groups of brief mindful meditation and deep breathing control condition (249), remitted depressed outpatients showed decreased rumination and depression levels due to a formal, but not to informal, mindfulness practice (250). In participants with elevated symptoms of depression, a mindful acceptance training reduced maladaptive beliefs about rumination, compared to a reappraisal training or no training (251). As in case of CBT, mindfulness techniques of alleviating rumination can be useful not only in depression but also in anxiety disorders, since, in college students, rumination was found

to mediate both the effect of number of trauma types on trauma symptomatology and the inverse association between mindfulness and trauma outcomes (252).

In the above detailed literature (and also in chapter 2.1.4.1), we can notice the importance of metacognitive beliefs about rumination, and Korn et al, 2014 (253) indeed suggested the superiority of metacognitive therapy over CBT in alleviating rumination. Albeit divorced depressed women's RRS rumination level did not differ between the two intervention groups of metacognitive therapy and life skills training (254), a group metacognitive therapy in patients with generalised anxiety disorder reduced repetitive negative thinking and emotional distress (255), further corroborating the importance of targeting rumination in disorders other than depression.

Another putative way of mitigating high rumination could be the training of cognitive control, plausible based on the literature detailed in chapters 2.1.4.1, 2.1.4.3 and 2.1.4.4. While a transcranial direct stimulation applied to the DLPFC yielded a faster switching in working memory, and working memory training yielded a HRV increase, neither of them had an effect on self-reported state rumination (256). However, in MDD outpatients, Siegle et al, 2014 (257) revealed that compared to the treatment-as-usual only group, an adjunctive cognitive control training, which denotes attention training exercises requiring prefrontal activity, reduced RSQ rumination and brooding but not reflection, and these responses were the strongest in those with physiological correlates of task engagement, measured before the treatment by pupillary oscillations at the task frequency. Nevertheless, reductions of rumination and depression did not correlate with each other, and cognitive control training had no effect on depression reduction (257).

To summarise therapeutic possibilities of mitigating rumination, while findings with CBT are conclusive, results with mindfulness-based, cognitive control training and metacognitive approaches are conflicting, but we can see that application of these different techniques is not restricted to rumination within MDD, but can be extended to the frameworks of PTSD and generalised anxiety disorder. Mennin and Fresco, 2013 (258) point to the potential of the endophenotype nature of rumination, along with worry, other forms of negative self-referential processing and an intense emotionality, in defining a subgroup of patients with a worsened clinical picture and treatment resistance, with the aim of developing personalised treatments for this subgroup by targeting negative self-referential processes.

To conclude, our genetic associations with the rumination phenotypes can constitute parts of the rumination endophenotype that are on the causal way for not only depression but for other disorders related to rumination, and these parts can yet be targeted by therapeutic interventions, also maybe with preventive purposes.

To place our results in the context of other replicable genetic underpinnings of rumination, see ***Figure 15***. We could see in chapter 2.2.1 that 21-41% (depending on age, gender and rumination subtype) of the variability of rumination resides in heritable factors, establishing its endophenotype nature. Chapter 2.2.2 demonstrated that *FKBP5*, *5-HTTLPR*, *BDNF* and *CREB1*, like our present results, account for a robust, replicable part of the endophenotype nature of rumination. Regarding *FKBP5*, its association with depression was either not measured (97), or not mediated by rumination (96), and not investigated as a mediator of the *FKBP5*-rumination association (96). However, the association of *5-HTTLPR* with rumination was proven to remain significant if controlling for depression (99, 101), while the same genetic association with depression was either not significant (99) or not measured (101). In case of the investigated *CREB1* polymorphism, its association with depression was entirely mediated by rumination (108), although the role of depression was not revealed in either association study for rumination (107, 108). For *BDNF*, an asymmetry similar to the one in our results emerged, since its association with depression was entirely mediated by rumination on the one hand (109), and its association with rumination remained significant after controlling for depression, on the other hand (101, 103). The crucial role of gene-by-gene and gene-by-environment interactions in the establishment of the endophenotype is also worth noting (***Figure 15***). Moreover, it has to be underlined that while I propose here the importance of the endophenotype part of rumination in its transdiagnostic relevance, its role in these disorders may also be due to its proportion attributable to factors other than genetics (***Figure 15***).

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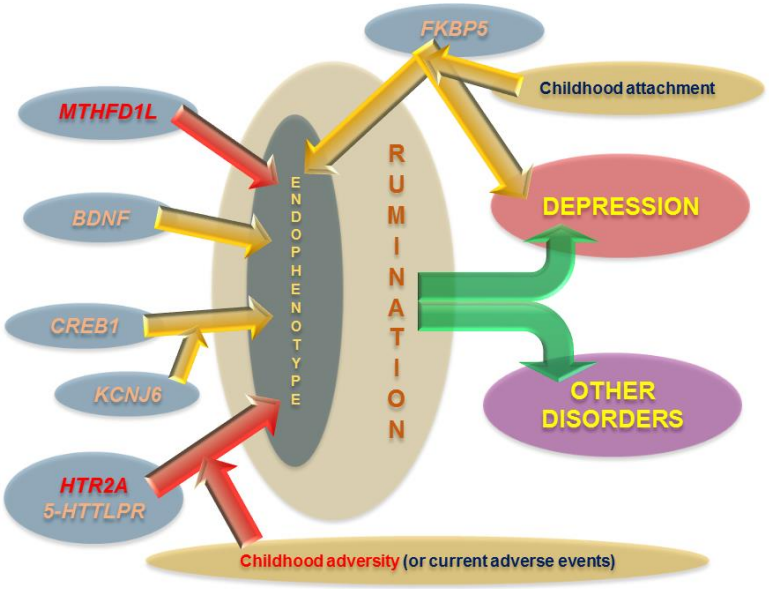

***Figure 15*. Replicable genetic associations for rumination, and the putative transdiagnostic relevance of the endophenotype they establish, based on references (96, 99, 101, 103, 108, 109) and our present results (marked with red). *FKBP5*: glucocorticoid receptor co-chaperone gene; *MTHFD1L*: mitochondrial monofunctional 10-formyltetrahydrofolate synthetase gene; *BDNF*: brain-derived neurotrophic factor gene; *CREB1*: cAMP-response element binding protein 1 gene; *KCNJ6*: gene of the G protein-activated inwardly rectifying potassium channel subunit 2 protein GIRK2; *HTR2A*: serotonin receptor 2A gene; *5-HTTLPR*: serotonin-transporter-linked polymorphic region.**

6.4. While SNP-rumination associations can be replicated, SNP-depression associations cannot be replicated in the separate Budapest and Manchester subsamples

Testing replicability of our findings within the two separate subsamples is of crucial importance in our study. First because the Budapest and Manchester subsamples differed in most of the variables in study A: *Genetics of folate metabolism in the background of rumination* (see chapter 5.A.1), and in study B: *Serotonin receptor gene HTR2A and childhood adversity in the background of rumination* (see chapter 5.B.1). Second, it is

even more important in study B to be able to replicate significant GxE findings of rs3125 particularly in Budapest, since this was the only subsample in which no significant gene-environment correlation emerged (see chapter 5.B.1, ***Table 10***).

Except for the rs6311 x childhood adversity interaction on rumination (chapter 5.B.8, answering hypothesis 3.B.6), significant SNP-rumination association findings could be replicated within each subsample, in case of both the effect of *MTHFD1L* rs11754661 on rumination (detailed in chapter 5.A.5, answering hypothesis 3.A.5), and that of *HTR2A* rs3125 in interaction with childhood adversity on brooding (detailed in 5.B.5, answering hypothesis 3.B.6), yielding a cross-cultural robustness in parts of the endophenotype of rumination comprised by these two SNPs, and also legitimating the GxE finding with rs3125, in spite of the gene-environment correlation observed in the combined sample and in Manchester.

However, the SNP-depression associations have not proven to be replicable. *MTHFD1L* rs11754661 was associated with depression only in Budapest but not in Manchester (detailed in chapter 5.A.6, answering hypothesis 3.A.6). The *HTR2A* rs3125 x childhood adversity interaction on depression was not significant in either subsample (detailed in chapter 5.B.6, answering hypothesis 3.B.7).

I propose that this discrepancy in replicability, particularly the replicable genetic associations on rumination but the unreplicable nature of the same genetic effects on depression, may be stem from that rumination denotes an endophenotype for depression. Although Flint and Munafo, 2007 (60) argue that a more homogeneous and straightforward genetic architecture of the endophenotype than that of the disorder itself would be manifested in a higher genetic effect size and thus a smaller sample size required to detect the same genetic effect in case of an endophenotype compared to the disorder, I hypothesise that this homogeneity and straightforwardness in genetic effects may be captured in their replicability across different populations and cultures. Accordingly, depression may not only be genetically heterogeneous in a given population, and thus making it challenging to find the enormous number of genetic variants associated with it, each by a small effect size, but it may also encompass distinct weights of the same genetic components or even distinct genetic components within distinct populations, thus being genetically heterogeneous across distinct populations. On the other hand, an endophenotype, being closer to the biological and genetic levels and thus less sensitive to

cultural and societal impacts than the disorder itself, may possess a genetic architecture more robust and generalisable across populations and cultures than that of the disorder.

Whether or not this discrepancy in replicability of the genetic effects between rumination and depression phenotypes can be due to the endophenotype nature of rumination, the replicability of SNP-rumination findings provides a strong argument for the cross-cultural importance of *MTHFD1L* and *HTR2A* genes in the endophenotype, at least in European populations.

6.5. Limitations

The most important limitation of our study is that its cross-sectional nature does not allow us to draw conclusions on the real causal effects of childhood adversity on adulthood rumination and depression phenotypes, neither on the causal effects of rumination and depression phenotypes on each other. Without a longitudinal design, we can interpret all of our associations simply as associations.

Another limitation is that we investigated only one or two SNPs per each gene. Tagging each of *MTHFR*, *MTHFD1L* and *HTR2A* with SNPs representing each of their haplotype blocks would yield a more definite picture about the effect of each gene. Extending our scope to rare variants and / or types of variants other than SNPs (such as structural variants, copy number variants or other length polymorphisms) would open new fields in the investigation of genetic effects on rumination.

A third limitation is that lifetime occurrence of depression was measured by self-report. However, this measurement had been validated with diagnostic interviews within a subsample (see chapter 4.4 for details). Moreover, assessing and utilising also a current depression score could allow us to overcome reporting bias for past depressive episodes to some extent. Nevertheless, current depression score was also measured by self-report (see chapter 4.4 for details).

Another limitation is the one stemming from the young average age of study participants (see ***Table 1*** and ***Table 9***), entailing an unawareness of possible depressive disorders with a later onset within a subset of never-depressed participants.

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7. Conclusions

Based on all of the previous chapters, I draw the following conclusions.

7.1. Our study demonstrated that the widely but inconclusively investigated *MTHFR* rs1801133 polymorphism is not associated with rumination in an adult general population sample.

7.2. Our study was the first to reveal that the A allele of *MTHFD1L* rs11754661, already established as a risk factor for Alzheimer's disease in previous genome-wide association studies, is associated with higher rumination score in an adult general population sample.

7.3. The fact that the association of *MTHFD1L* rs11754661 with rumination was replicable within the Budapest and Manchester subsamples suggests that *MTHFD1L* represents part of the rumination endophenotype that is generalizable across European populations.

7.4. The association of *MTHFD1L* rs11754661 with rumination was not exclusively explained by depression, but fully mediated the same genetic association with depression, suggesting that the association of this genetic variant with rumination goes beyond this one disorder, implicating *MTHFD1L* as a contributor in the rumination endophenotype possessing transdiagnostic relevance.

7.5. In spite of the replicable genetic association with rumination, *MTHFD1L* rs11754661 was associated with depression only in Budapest but not in Manchester, which discrepancy in replicability may be a clue that an endophenotype has a biological background more homogeneous and less sensitive to cultural and societal impacts than the disorder itself.

7.6. Our results were the first to demonstrate that *HTR2A* rs3125 exerts an effect only on the maladaptive brooding subtype of rumination, and that this effect is a function of childhood adversity exposure, which finding entails the compelling importance of involving gene-by-environment interaction models in the endophenotype concept, especially in case of polymorphisms transmitting epigenetic impacts, such as alterations of microRNA binding.

7.7. Methylation site *HTR2A* rs6311 was associated only with rumination, and, similarly to the effect of microRNA binding site rs3125, this association was also a

function of childhood adversity exposure, but, in contrast to the rs3125 effect, it could be replicated only in Manchester but not in Budapest, implicating the possible role of the epigenetic mechanism itself in the robustness of these *HTR2A* x childhood adversity interaction findings.

7.8. As the association with *MTHFD1L* rs11754661, the *HTR2A* rs3125 x childhood adversity interaction also contributed to rumination endophenotype in a generalizable manner across European populations.

7.9. As in case of the *MTHFD1L*-rumination association, the *HTR2A* rs3125 x childhood adversity interaction on brooding was not exclusively mediated by depression, but fully accounted for the same interaction results on depression, suggesting that this effect also goes beyond this one disorder, implicating this GxE interaction as a risk factor for rumination endophenotype possessing transdiagnostic relevance.

7.10. As the associations with *MTHFD1L*, the *HTR2A* rs3125 x childhood adversity interaction results also corroborate the biologically homogeneous endophenotype nature of rumination, in that this GxE effect is replicable on brooding but unreplicable on depression.

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8. Summary

8.1. Summary in English

The main objective of the research was to investigate genetic risk factors of rumination, defined as an inflexible thinking style in response to and about the person's own depressed mood that denotes a risk factor and an endophenotype for depression.

With the aim of enrichment of candidate gene studies for rumination, among adults from the general population of Budapest and Manchester we investigated the association of rumination with two polymorphisms of two folate genes: with *MTHFR* rs1801133 in 2204, and with *MTHFD1L* rs11754661 in 2120 subjects. Furthermore, we investigated the role of childhood adversity and rs3125 and rs6311 polymorphisms of the serotonin receptor gene *HTR2A* in rumination and its two subtypes among 1501 subjects. In both studies, we also aimed at revealing the role of depression in the putative associations, and the replicability of the found associations in the separate Budapest and Manchester subsamples.

Our results demonstrated that while *MTHFR* rs1801133 has no association with rumination, the A allele of *MTHFD1L* rs11754661 is related to a higher rumination level. This discrepancy can be either due to the distinct biochemical roles of the two encoded enzymes, or their distinct subcellular localisation, or even their distinct sensitivity to folate status. Future testing of the influence of folate status on our found genetic association may have therapeutic implications. Our results also demonstrated that *HTR2A* rs3125 is associated only with brooding, and rs6311 is associated only with rumination, both of them only in function of childhood adversity level, underscoring the need of involving stress factors in the endophenotype concept of rumination, especially in case of polymorphisms transmitting epigenetic impacts. We revealed for both *MTHFD1L* rs11754661 and *HTR2A* rs3125 that while its association with rumination is only partly due to depression, its association with depression is totally due to rumination, pointing to the possibility that these polymorphisms, as parts of the rumination endophenotype, may pave the way for not only depression, but also for other disorders. We also found for both polymorphisms that while its association with rumination is replicable within the Budapest and Manchester subsamples, its association with depression is not, which discrepancy may stem from the endophenotype nature of rumination.

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8.2. Összefoglalás (summary in Hungarian)

A kutatás célja a rumináció, vagyis a személy saját depresszív hangulatán való rugalmatlan gondolkodási stílus, genetikai hátterének vizsgálata, melynek jelentőségét az adja, hogy a rumináció a depresszió rizikófaktora és endofenotípusa.

A rumináció kandidáns gén vizsgálatainak gazdagítását célozva, kutatásaink során budapesti és manchesteri általános felnőtt populációban vizsgáltuk a rumináció összefüggését a folátmetabolizmus két génjének egy-egy polimorfizmusával: az *MTHFR* rs1801133 polimorfizmusával 2204, az *MTHFD1L* rs11754661 polimorfizmusával pedig 2120 résztvevő körében. Ezen kívül a gyermekkori rossz bánásmód és a *HTR2A* szerotoninreceptor-gén rs3125 és rs6311 polimorfizmusainak szerepét is vizsgáltuk a ruminációban és két altípusában 1501 résztvevő körében. Mindkét vizsgálatban célunk volt továbbá a depresszió szerepének feltárása a feltételezett asszociációkban, valamint a talált összefüggések replikálhatóságának tesztelése külön-külön a budapesti és manchesteri almintákban.

Eredményeink szerint, míg az *MTHFR* rs1801133 nem mutat összefüggést a ruminációval, az *MTHFD1L* rs11754661 A allélja magas ruminációval függ össze. Terápiás jelentőségűek lehetnek azon jövőbeli vizsgálatok, amelyek a folátstátusz szerepét tárják fel az általunk talált genetikai asszociációban. Eredményeink arra is rámutattak, hogy a *HTR2A* rs3125 csak a brooding altípussal függ össze, az rs6311 pedig csak a ruminációval, és mindkét összefüggés a gyermekkori rossz bánásmód mértékének függvénye, kiemelve a stresszfaktorok bevonásának szükségességét a rumináció endofenotípus-koncepciójába. Mind az *MTHFD1L* rs11754661, mind a *HTR2A* rs3125 esetében kimutattuk, hogy míg a ruminációval való összefüggése csak részben köszönhető a depressziónak, a depresszióval való összefüggése teljes mértékben a ruminációnak köszönhető, ezzel arra a lehetőségre rámutatva, hogy ezek a polimorfizmusok, a rumináció endofenotípusának részeiként, nem csupán a depresszióhoz vezető utat alapozzák meg, hanem egyéb betegségek kialakulásában is szerepet játszhatnak. Hasonlóképpen mindkét polimorfizmus esetében, azt találtuk, hogy míg a ruminációval való összefüggés replikálható külön-külön a budapesti és manchesteri almintában, a depresszióval való összefüggés nem, amely diszkrepancia eredhet magából a rumináció endofenotípus természetéből.

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10. Publications

10.1. Original publications related to the thesis

- **Eszlari N**, Kovacs D, Petschner P, Pap D, Gonda X, Elliott R, Anderson IM, Deakin JF, Bagdy G, Juhasz G. (2016) Distinct effects of folate pathway genes MTHFR and MTHFD1L on ruminative response style: a potential risk mechanism for depression. *Transl Psychiatry*. 1:19. **IF: 4.730**
- Gonda X, Hullam G, Antal P, **Eszlari N**, Petschner P, Hökfelt TG, Anderson IM, Deakin JFW, Juhasz G, Bagdy G. (2018) Significance of risk polymorphisms for depression depends on stress exposure. *Sci Rep*. 8:018-22221. **IF: 4.259**

10.2. Original publications not related to the thesis

- Juhasz G, **Eszlari N**, Pap D, Gonda X (2012) Cultural differences in the development and characteristics of depression. *Neuropsychopharmacol Hung*. 14:259-265.
- Juhasz G, Hullam G, **Eszlari N**, Gonda X, Antal P, Anderson IM, Hokfelt TG, Deakin JF, Bagdy G. (2014) Brain galanin system genes interact with life stresses in depression-related phenotypes. *Proc Natl Acad Sci U S A*. 111:24. **IF: 9.674**
- Kovacs D, Gonda X, Petschner P, Edes A, **Eszlari N**, Bagdy G, Juhasz G. (2014) Antidepressant treatment response is modulated by genetic and environmental factors and their interactions. *Ann Gen Psychiatry*. 13:13-17.
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