

Interaction between the *MTHFR* C677T polymorphism and traumatic childhood events predicts depression

A Lok¹, CLH Bockting², MWJ Koeter¹, H Snieder³, J Assies¹, RJT Mocking¹, CH Vinkers⁴, RS Kahn⁴, MP Boks⁴ and AH Schene¹

Childhood trauma is associated with the onset and recurrence of major depressive disorder (MDD). The thermolabile T variant of the *methylenetetrahydrofolate reductase* (*MTHFR*) C677T polymorphism (rs1801133) is associated with a limited (oxidative) stress defense. Therefore, C677T *MTHFR* could be a potential predictor for depressive symptomatology and MDD recurrence in the context of traumatic stress during early life. We investigated the interaction between the C677T *MTHFR* variant and exposure to traumatic childhood events (TCEs) on MDD recurrence during a 5.5-year follow-up in a discovery sample of 124 patients with recurrent MDD and, in an independent replication sample, on depressive symptomatology in 665 healthy individuals from the general population. In the discovery sample, Cox regression analysis revealed a significant interaction between *MTHFR* genotype and TCEs on MDD recurrence ($P = 0.017$). Over the 5.5-year follow-up period, median time to recurrence was 191 days for T-allele carrying patients who experienced TCEs (T + and TCE +); 461 days for T – and TCE + patients; 773 days for T + and TCE – patients and 866 days for T – and TCE – patients. In the replication sample, a significant interaction was present between the *MTHFR* genotype and TCEs on depressive symptomatology ($P = 0.002$). Our results show that the effects of TCEs on the prospectively assessed recurrence of MDD and self-reported depressive symptoms in the general population depend on the *MTHFR* genotype. In conclusion, T-allele carriers may be at an increased risk for depressive symptoms or MDD recurrence after exposure to childhood trauma.

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Introduction

Childhood maltreatment is associated with substantial and long-lasting cognitive and biological effects on the brain including heightened stress sensitivity. Therefore, individuals who have been exposed to childhood maltreatment are predisposed to an unfavorable course of major depressive disorder (MDD) and treatment outcome, as indicated by a recent meta-analysis.¹ However, not all individuals exposed to traumatic stress develop a depression. Therefore, it is important to characterize the gene–environment interplay underlying the effects of traumatic childhood events (TCEs) on depression outcomes.

After a first episode of MDD, ~50% of patients will experience recurrences, which are responsible for considerable disability and impairment.^{2–5} Burcusa and Iacono⁶ stated as an explanation for recurrence in MDD that ‘individuals at high risk for multiple episodes possess the necessary characteristics to make them prone to recurrent depression, and such characteristics exist even before their first episode’. The recurrent type of MDD has a higher heritability than a single episode of MDD.⁷ Furthermore, biological studies in individuals at risk for MDD, or remitted from MDD, as well as their nondepressed family members, showed that pathophysiological disturbances also precede the development of MDD and remain present after remission, suggestive of stable heritable vulnerability traits, that is, endophenotypes.^{8–12} However, a direct identification of candidate genes with recurrence of

MDD has proven to be difficult, presumably as a result of complex interactions between genes and environmental stressors.^{13,14}

A recently emerged pathway potentially underlying susceptibility to onset, symptomatology and recurrence of MDD is folate-mediated one-carbon (1-C) metabolism.^{15,16} The 1-C cycle plays a central role in (1) the regulation of oxidative stress and (2) the generation of methyl groups for methylation of DNA, proteins, phospholipids and neurotransmitters.^{17,18} A crucial enzyme in this pathway is *5,10-methylenetetrahydrofolate reductase* (*MTHFR*). A single-nucleotide polymorphism (SNP) in the *MTHFR* gene (C677T or rs1801133) results in the production of a thermolabile variant of *MTHFR*, which is associated with decreased methylation capacity¹⁹ and increased oxidative stress. This genetically determined variation in 1-C cycle activity associated with increased stress sensitivity may contribute to alterations in neurocognitive functioning and mood regulation.^{20,21}

Nevertheless, results of meta-analyses on data linking polymorphisms in the *MTHFR* gene with MDD have been inconsistent.^{22–26} From these studies it has become apparent that the main genetic effects overall are weak in MDD, whereas gene–environment interactions may provide stronger predictors.²⁷ Investigating genetic susceptibility to stress is of particular relevance in the context of MDD as stress is considered one of the main pathogenic factors involved in depressive symptomatology and MDD recurrence: TCEs,

¹Department of Psychiatry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; ²Department of Clinical Psychology, University of Groningen, Groningen, The Netherlands; ³Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands and ⁴Department of Psychiatry, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands

Correspondence: A Lok, Department of Psychiatry, Academic Medical Centre, Meibergdreef 5, Amsterdam 1105 AZ, The Netherlands.

E-mail: a.lok@amc.uva.nl

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recent life events, daily hassles and stress related to previous depressive episodes all pose increased risks for MDD and its recurrences.^{1,28–32} Especially during early life, traumatic stress may result in lifelong programming, potentially through methylation-mediated alterations in expression of genes implicated in MDD.^{27,33–39} As mentioned above, the thermolabile T variant of the *MTHFR* C677T polymorphism is associated with increased vulnerability to oxidative stress and a decreased DNA methylation capacity. Therefore, carriers of the *MTHFR* C677T variant may be particularly vulnerable to long-lasting effects of childhood traumatic stress on depressive symptomatology and MDD.

To study this proposed relation, we investigated the possible gene $\times$ environment interaction between TCEs and the *MTHFR* genotype as a potential predictor for depressive symptoms and recurrence in patients with a high risk of recurrence of depression over a 5.5-year follow-up period. We hypothesized that recurrently depressed patients carrying the T-allele would have a shorter time to MDD recurrence after exposure to TCEs, whereas this association would not be present in T-allele carriers without exposure to TCEs. Because the effects of the *MTHFR* genotype may not only be limited to MDD but also be present in the general population, we examined this gene–environment interaction in an independent population-based sample for replication.

Materials and methods

Study participants. The current study was part of the DELTA study, a randomized clinical trial, investigating the effect of cognitive therapy on recurrence in 172 euthymic patients.^{40,41} In the DELTA study, we sampled a group of patients with recurrent MDD. We considered these patients to suffer from a more biologically pronounced and endogenously determined subtype of MDD with a relatively high recurrence rate.¹² Inclusion criteria of the original trial were: ≥ 2 previous MDD episodes in the past 5 years, as defined by the Structured Clinical Interview for DSM-IV disorders (SCID);⁴² in remission > 10 weeks and < 2 years ago, as defined as a score of < 10 on the 17-item Hamilton Depression Rating Scale (HDRS₁₇)⁴³; and 18–65 years old. Exclusion criteria were: (a history of) bipolar spectrum disorder or any psychotic disorder, organic brain damage, alcohol and/or drug abuse and/or dependency or predominant anxiety disorder, all assessed by the SCID. The background, methodology and procedure of the DELTA study have been described in more detail previously.⁴⁰ At 2 years after baseline, we asked the patients to provide DNA for the current study. After complete description of the study to the subjects, written informed consent was obtained before enrolment. The study was approved by the ethics committee of the Academic Medical Center of the University of Amsterdam (MEC 02/048).

CannabisQuest cohort. Participants in the discovery sample were recruited using a project website launched in 2006 targeted at Dutch young adults and adolescents from age 18 to 25 years (www.cannabisquest.nl).⁴⁴ Strategies to generate traffic on the project website included collaboration with

over a hundred colleges, universities and youth centers, as well as the use of online commercial advertisement products (that is, banners and text links).⁴⁴ The chance to win an Apple iPod or a Nintendo Wii was used as an incentive. Double entries were prevented by exclusion of subjects with an identical e-mail address, surname and date of birth. Anonymous submission of data was not possible. The online assessment included verification questions to protect against random answers, and participants failing to correctly complete the verification questions were subsequently excluded. From the online data ($N = 17\,698$), 1259 participants were included for subsequent genetic assessment in two waves. First, in order to increase power for gene $\times$ environment interactions,⁴⁵ in the context of cannabis and psychosis, we prioritized a sample of 719 participants who belonged to the top or bottom quintile of total scores of psychotic experiences as measured by the Community Assessment of Psychic Experiences (CAPE) score who were either cannabis naive (that is, a lifetime cannabis exposure frequency of < 6 times) or were heavy cannabis users (that is, current expenditure for personal cannabis use exceeded 3€ weekly). Second, an unselected sample of 540 individuals was included. As ascertained with the validated Dutch version of either the SCID⁴² or the MINI International Neuropsychiatric Interview,⁴⁶ healthy controls had no history of any psychotic disorder. For 84 participants, no interview data were available, and for these cases the presence of a psychotic disorder was excluded by the absence of antipsychotic drug use or a history of psychiatric treatment. The possible concomitant use of recreational drugs was assessed with the substance abuse module of the Composite International Diagnostic Interview.⁴⁷ Of the 1259 participants who completed comprehensive assessments and provided blood samples for genetic testing, complete data were available for 665 subjects because of a later implementation of the Childhood Trauma Questionnaire (CTQ)⁴⁸ assessment in the study. All participants provided a urine sample to screen for the presence of recreational drugs in order to verify recent self-reported cannabis use. The study was approved by the ethical review board of the University Medical Center Utrecht and all participants gave written informed consent.

Measurements

Depression

Discovery sample. Using SCID-I, current and past depressive episodes were assessed at baseline, and at 5 follow-up measurements at 3, 12, 24, 36 and 66 months after baseline. With these follow-up assessments, we diagnosed relapses (< 6 months after a previous major depressive episode) or recurrences during follow-up, both further addressed as ‘recurrence’ for clarity reasons. The trained SCID evaluators were blind to treatment condition and subjects were instructed not to reveal their treatment condition to the interviewers (psychologist/research assistants). All interviews were audio taped. Two independent experienced psychiatrists, also blinded to treatment condition, evaluated all occasions of participants meeting the DSM-IV criteria for MDD. In cases of disagreement, we used the ratings of the psychiatrists. The κ -value for inter-rater agreement between the interviewers and psychiatrist on categorization of a

relapse/recurrence or no relapse/recurrence was 0.96, indicating high agreement.⁴⁰

Replication sample. In the replication sample, the Beck Depression Inventory (BDI) was used to assess depressive symptoms. This validated 21-item self-report questionnaire measures current depressive symptoms during the last week. Each question has four possible answer choices ranging in intensity (0–3), resulting in a total BDI score ranging from 0 to 63.

Traumatic childhood events

Discovery sample. We defined TCEs as having experienced one of the following traumatic events before age 16: parental loss, sustained alcohol and/or drug abuse by caregiver, victim of a serious crime, victim of a serious accident and victim of sexual and/or physical abuse. We selected traumatic stress variables and stressors occurring to the self within this age period using the 7 items from the Negative Life Events Questionnaire that indicate traumatic events: items 5 and 8–13.^{49,50} This questionnaire proved to have a good predictive validity, as the number of negative life events predicted MDD symptom severity. We dichotomized the absence or presence of experienced TCEs.

Replication sample. In the replication sample, childhood maltreatment was assessed using the 25-item version of the CTQ.⁴⁸ The CTQ assesses five types of self-report childhood trauma: emotional abuse, physical abuse, sexual abuse, emotional neglect and physical neglect. The validity of the CTQ, including a Dutch translation, has been demonstrated in clinical and community samples.^{48,51} One translated item (*I believe I was molested*) was excluded as this translation was found to be an invalid indicator of childhood sexual abuse in a previous validation study.⁵¹ Childhood maltreatment was used as the continuous sum score divided by the number of completed items. One item of the CTQ was only available for a subset of the replication sample (*My family was a source of strength and support*). Additional analyses in which this item was excluded altogether did not affect the results.

Genotyping procedures and analysis

Discovery sample. We collected 20 ml blood samples at patients' homes by venipuncture. DNA was isolated from blood using a filter-based method (QIAamp DNA Mini Kit, Qiagen, Manchester, UK). The PCR primers were designed using Primer 3 (<http://frodo.wi.mit.edu/primer3/input.htm>). The PCR primer sequence was 5'-GGCAGGTTACCCCAAGGC-3' and 5'-TGGGGTGGAGGGAGCTTATG-3', and the PEX primer sequence was 5'-GAGAAGGTGTCTLCGGAG-3'. Genotyping was done using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer from Bruker Daltonics (Wormer, The Netherlands). All samples were genotyped in duplicate to increase reliability.⁵² Genotyping error rate based on these duplicates was 3.7%.

Replication sample. All participants were of Dutch ancestry. Genotype data were generated on three different array platforms: the Illumina (San Diego, CA, USA) Human Omni Express ($N=576$), the Illumina Human610-Quad Beadchip ($N=768$) and the Illumina HumanHap550 array ($N=34$). For

each SNP platform, quality control procedures were initially performed separately using PLINK V1.07.⁵³ Subjects were excluded based on >5% missing genotypes and gender errors. We used linkage disequilibrium-based SNP pruning to select the most informative SNPs ($R^2 < 0.2$) only for the subsequent quality control step. This resulted in ~67k SNPs for the sets to assess heterozygosity ($F < 3$ s.d.), homozygosity ($F > 3$ s.d.) and relatedness by pairwise identity-by-descent values (PIHAT > 0.15). Data sets were merged with Hapmap Phase 3 individuals to check ethnicity. After these quality control procedures on subjects (excluding in total 101 individuals), quality control on SNPs was performed as follows. All SNPs were filtered on missingness (>2%) and Hardy-Weinberg ($P > 1e^{-6}$) before merging the three data sets. Four duplicates and three related sample pairs were detected in the merged data sets (according to criteria described above) and one outlier after clustering the merged data set. From these data, the *MTHFR* genotype (rs1801133) was extracted.

Cannabis use (replication sample). In the replication sample, cannabis use was defined as current use of more than an equivalent of 3€ per week (roughly equivalent to weekly cannabis use) during the past month or longer. The monetary amount spent on cannabis has been reported as a valid proxy of exposure to Δ^9 -tetrahydrocannabinol.⁵⁴

Statistical analysis

Genotypes. Although significantly different enzymatic activity and thermolability were reported, overlapping profiles for the TT and CT genotypes have been described, with CC remaining as a distinct genotype.⁵⁵ Therefore, and for power reasons, the *MTHFR* C677T polymorphism variable was dichotomized into T-allele carriers (TT and CT combined) and non-T carriers (the wild-type genotype CC) groups in the discovery sample. In the replication sample, genotypes were coded 0, 1, or 2 and modeled as a linear effect (additive genetic model) to account for different genotype distributions because it avoids small subgroup stratification.⁵⁷ Deviation from Hardy-Weinberg equilibrium was tested by allele counting and χ^2 analysis.

Discovery sample. The interaction between the *MTHFR* genotype and TCEs on MDD recurrence was investigated using Cox regression, which takes into account differences in time at risk and censoring (no recurrence during the study period of 5.5 years). Half the study sample was randomly allocated to preventive cognitive therapy (CT: 8 sessions during the first 3 months after inclusion in the DELTA study). Preventive CT has a protective effect on recurrence that increases with the number of previous depressive episodes.⁴⁰ To test whether this intervention modified the relation between *MTHFR* genotype, TCEs and recurrence, we assessed the significance of the four-way interaction of treatment condition by *MTHFR* genotype by TCEs by the number of previous episodes. Because neither the four-way *MTHFR* genotype by TCEs by treatment by previous episodes nor the three-way *MTHFR* genotype by TCEs by treatment interaction terms were significant, patients who were or were not randomized to receive CT were pooled for the Cox regression analyses. *MTHFR*, TCEs and *MTHFR* × TCEs were included as predictors in the model; the

interaction term tests our hypothesis whether the effect of the *MTHFR* C677T variant (T-allele carrier, non-T carrier) is modified by TCEs (present, absent). To ensure that our results were not influenced by initial differences in group characteristics, we also reanalyzed the data adjusted for age, gender and antidepressant use by incorporating these variables as covariates in the Cox regression model. We used PASW statistics 18.0 (IBM SPSS, 2010, Chicago, IL, USA). We considered $P < 0.05$ statistically significant.

Replication sample. To examine the interaction between the *MTHFR* genotype and TCEs on depressive symptoms, the total BDI score was regressed on TCEs, *MTHFR* genotype, their interaction and covariates using the following model: $BDI = \beta_0 + (\beta_1 * \text{covariate}) + (\beta_2 * rs1801133) + (\beta_3 * \text{TCEs}) + (\beta_4 * rs1801133 * \text{TCEs})$. As covariates we included cannabis use (modeled as a dichotomous indicator), age and gender. Analyses were performed in R (www.r-project.org).⁵⁶ Continuous sum scores of the BDI and the CTQ were used.

Results

Sample characteristics. Descriptives of both samples are depicted in Table 1.

Discovery sample. Of the 172 patients of the original trial, 137 provided DNA. Of these 35 patients, 15 (8.7%) were lost to follow-up and the remaining patients (11.6%) did not participate because of a diversity of reasons (for example, being afraid of needles, ethical issues concerning genetic study). Five patients could not be analyzed because of *MTHFR* C677T genotyping failure (genotyping success rate = 96.3%). Of the remaining 132 patients, 1 was non-Caucasian, 2 were lost to follow-up and we could not obtain TCEs data for 4 patients. All analyses pertain to the resulting 124 patients. These 124 patients were comparable to the other 48 patients on gender, age, educational level, number of previous episodes and age of onset of first depression, but differed on marital status and antidepressant use. Compared with the 48 excluded patients, the 124 remaining patients comprised fewer singles (37% vs 54%; $\chi^2 = 4.14$, d.f. = 1, $P = 0.042$), and more users of antidepressants (57% vs 35%; $\chi^2 = 6.61$, d.f. = 1, $P = 0.010$).

The *MTHFR* C677T genotype counts and frequencies in the patients were 60 (48.4%) for the CC variant, 49 (39.5%) for the CT variant and 15 (12.1%) for the TT variant. No deviation from Hardy–Weinberg Equilibrium was observed ($\chi^2 = 1.00$, d.f. = 1, $P = 0.317$). T-allele carriers and non-T-allele carriers were largely comparable on demographic and psychopathological characteristics (Table 2), with the exception of educational level, and body mass index. T-allele carriers comprised more persons with medium-level education and had a higher mean body mass index, whereas non-T-allele carriers comprised more individuals with higher-level education.

Replication sample. Table 1 reports the distribution of demographic characteristics, childhood maltreatment, BDI and the *MTHFR* C677T genotype from individuals in the replication sample. Characteristics in the replication sample generalized to the full sample that includes all nongenotyped

Table 1 Characteristics of the discovery and replication samples

Characteristics	Discovery sample	Replication sample
	(n = 124)	(n = 665)
Female %	74	56
Age (range)	44.5 (21–63)	20.5 (18–40)
Depression score ^a	3.6 (0–9)	5.4 (0–34)
Caucasian %	100	100
<i>MTHFR</i> C677T genotype (rs1801133)		
T/T, %	12	10
T/C, %	40	43
C/C, %	48	47
HWE (<i>P</i> -value)	0.32	0.94
Childhood trauma ^b	44	1.4 (1.0–4.3)

Abbreviations: HWE, Hardy–Weinberg equilibrium; *MTHFR*, methylenetetrahydrofolate reductase.

^aDiscovery sample: Hamilton depression rating scale (HDRS) total score; replication sample: Beck Depression Inventory (BDI) total score. ^bDiscovery sample: percent experienced traumatic childhood event(s) (TCE); replication sample: total score Childhood Trauma Questionnaire (CTQ).

Table 2 Demographic and clinical characteristics of the discovery sample (Δ)^a

Characteristics	T-carriers (n = 64)		Non-T-carriers (n = 60)		P-value
	Mean	s.d.	Mean	s.d.	
Sex (M/F)	16/48		16/44		0.832
Age, year	44.0	9.8	45.1	9.7	0.534
Educational level ^b					0.020
Low, %	34		32		
Middle, %	39		20		
High, %	27		48		
Marital status (single), %	42		32		0.226
Received cognitive therapy, %	48		58		0.270
AD use, yes %	56		58		0.815
BMI	28.0	6.0	26.0	4.3	0.045
HDRS ₁₇ score	3.8	3.0	3.5	3.0	0.638
Number of previous episodes	4.5	4.7	7.0	12.0	0.138
Age of first onset, years	29.4	12.7	27.9	12.7	0.510
Psychiatric diseases first relatives (%)	63		75		0.150
TCEs (%)	40.6		46.7		0.498

Abbreviations: AD, antidepressant; BMI, body mass index; F, female; HDRS₁₇, 17-item Hamilton Depression Rating Scale; M, male; TCE, traumatic childhood event.

Group comparisons were calculated using Student's *t*-tests, χ^2 tests or Fisher's exact tests.

^aAll data represent baseline values. ^bEducational level is defined as follows: low, primary education or preparatory middle-level applied education; middle, higher general continued education or middle-level applied education; high, preparatory scientific education, higher applied education or scientific education.

individuals.⁴⁴ Genotyping was successful in all subjects and no departure from Hardy–Weinberg Equilibrium was detected.

Recurrence in the discovery sample. Overall, 98 patients (79.0%) experienced relapse/recurrence at least once over the 5.5 years. Mean time to recurrence was 750 days (s.e. = 61.7 with a median of 493 days (range 20–2056 days)). The *MTHFR* C677T by TCE interaction predicted time to recurrence ($P = 0.017$). This indicates that the predictive effect of TCEs on MDD recurrence was modified by *MTHFR* genotype (T-allele carriers; Table 3). This result

Table 3 Effect of *MTHFR* modified by TCE in the discovery sample

	β	s.e. $_{\beta}$	Wald	d.f.	P-value	RR
MTHFR	-0.081	0.284	0.080	1	0.777	0.923
TCE	0.368	0.296	1.544	1	0.214	1.445
MTHFR $\times$ TCE ^a	0.979	0.410	5.713	1	0.017	2.663

Abbreviations: *MTHFR*, methylenetetrahydrofolate reductase; TCE, traumatic childhood event; RR, relative risk.
Cox regression analysis.
MTHFR: T-allele carrying patients versus non-T-allele carrying patients, with non-T-allele carriers as the reference category.
TCE: experienced TCE yes/no, with no TCE as the reference category.
^a $P=0.016$ after adjustment for age, gender and antidepressant (AD) use.

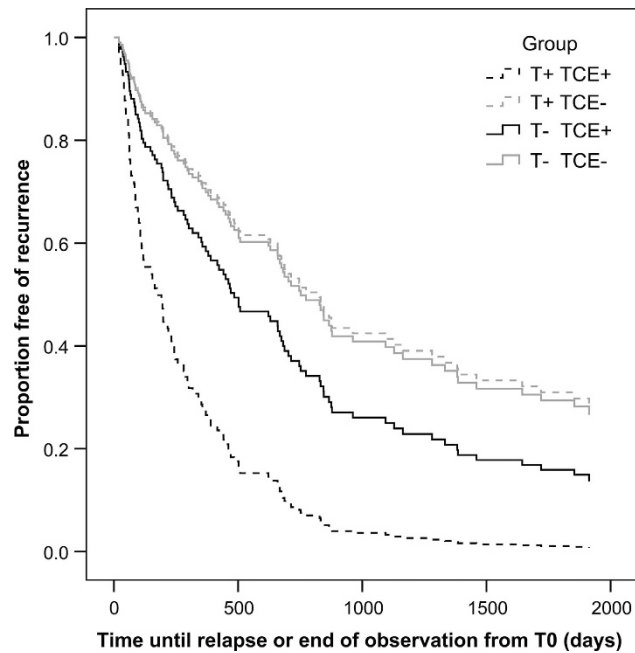


Figure 1 The effect of the gene–environment interaction between *methylenetetrahydrofolate reductase* (*MTHFR*) and traumatic childhood events (TCEs) on time to recurrence in 124 euthymic patients with recurrent major depressive disorder (MDD) over 5.5 years. T+ TCE+ vs T- TCE- = 3.55 ($P<0.001$); T+ TCE- vs T- TCE- = 0.92 ($P=0.78$); and T- TCE+ vs T- TCE- = 1.45 ($P=0.21$). Relative risk for recurrence of MDD calculated with Cox regression analysis. T- , non-T-allele carriers; T+ , T-allele carriers; TCE- , no experience of TCEs; TCE+ , experienced TCEs.

did not change after adjusting for age, gender and antidepressant use ($P=0.016$).

The extent of the effect modification can be seen by comparing the risk for recurrence between the four *MTHFR* C677T by TCE combination groups (T+ and TCE+ $N=26$; T+ and TCE- $N=38$; T- and TCE+ $N=28$; T- and TCE- $N=32$). We found a significantly higher hazard for the T+ and TCE+ groups as compared with the T- and TCE- groups (hazard ratio 3.55; Wald 17.7, d.f. = 1, $P<0.001$). For patients who experienced TCEs, the hazard for recurrence in T-allele carriers was 2.4 times higher than in non-T-allele carriers ($P=0.002$; Figure 1). This corresponds to the observed differences in median time till recurrence that were respectively 191 days for T+ and TCEs+ patients; 461 days

Table 4 The effects of childhood maltreatment and the *MTHFR* genotype and their interaction on depressive symptoms in the replication sample

Sample	β	s.e.	t-Value	P-value
Intercept	-9.00	2.98	-3.02	0.0026**
Gender	-1.06	0.50	-2.10	0.036*
Age	-0.023	0.091	-0.26	0.80
Cannabis	1.25	0.56	2.22	0.027*
Childhood maltreatment	11.08	1.66	6.66	5.7×10^{-11} ***
<i>MTHFR</i> genotype	4.13	1.48	2.79	0.0054**
Maltreatment $\times$ <i>MTHFR</i> genotype	-3.19	1.06	-3.03	0.0026**

Abbreviation: *MTHFR*, methylenetetrahydrofolate reductase.
* $P<0.05$, ** $P<0.01$, *** $P<0.001$.

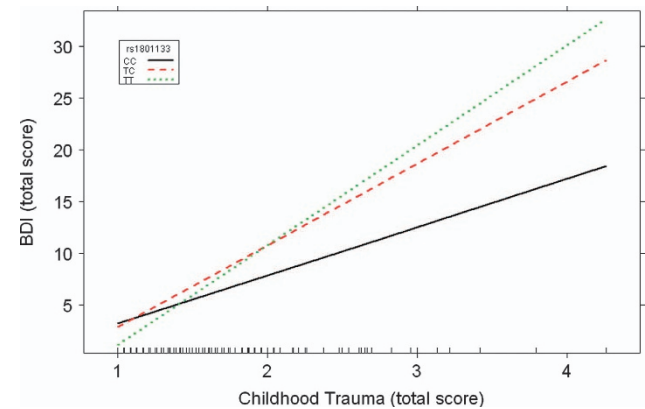


Figure 2 The gene–environment interaction between *methylenetetrahydrofolate reductase* (*MTHFR*) genotype and traumatic childhood events (TCEs) on depressive symptoms in 665 individuals from the general population ($P=0.0027$). 0, T/T genotype; 1, C/T genotype; and 2, C/C genotype.

for T- and TCE+ patients; 773 days for T+ and TCE- patients and 866 days for T- and TCE- patients.

Depressive symptoms in the replication sample. Table 4 shows the results of the linear regression model in the replication sample. Significant effects on depressive symptoms were present for childhood maltreatment ($\beta=11.08$, $P=5.7 \times 10^{-11}$), gender ($\beta=-1.06$, $P=0.036$), cannabis use ($\beta=1.25$, $P=0.027$) and the *MTHFR* genotype ($\beta=4.13$, $P=0.0054$). Moreover, there was a significant interaction between childhood maltreatment and the *MTHFR* genotype ($\beta=-3.19$, $P=0.0027$). For individuals carrying the TT genotype, childhood maltreatment resulted in increased levels of depressive symptoms (Figure 2).

To ensure that cannabis use in the replication sample did not influence the interaction between the *MTHFR* genotype and childhood maltreatment, additional stratified analyses were carried out in noncannabis users ($N=478$) and cannabis users ($N=187$).

In both subsamples of the replication sample, the *MTHFR* $\times$ maltreatment interaction was present with a similar directionality ($P=0.050$ and $P=0.056$). Moreover, cannabis use did not interact with the *MTHFR* genotype in the replication sample ($P=0.85$). Therefore, it is unlikely that

cannabis use affected the interaction between childhood maltreatment and the *MTHFR* genotype.

In both the discovery and replication samples, no significant association was found between the genotype and childhood maltreatment, making the presence of gene–environment correlations unlikely.

Discussion

This study shows that remitted recurrently depressed patients with the thermolabile variant of the *MTHFR* genotype who have experienced TCEs have a poor prognosis over a follow-up period of 5.5 years, in terms of recurrence of depression. This finding supports existing evidence on the specific role of gene–environment interactions in recurrent depression, especially when TCEs are examined²⁷ and the overall reported unfavorable course in patients with childhood trauma.¹ It may explain part of the vulnerability for recurrences in MDD. In support, we also found a significant interaction between childhood trauma and the *MTHFR* genotype on depressive symptoms in an independent sample from the general population, underscoring the overall importance of *MTHFR* as a genetic risk factor for depression in the context of early-life stress.

This impact of the combination of early childhood trauma and C677T *MTHFR* polymorphism on onset of depressive symptomatology and recurrences in MDD gives rise to hypotheses about the underlying pathophysiological pathways. The thermolabile variant of the *MTHFR* gene may represent a genetic vulnerability factor for limited defense against (oxidative) stress, because it results in a reduction of methyl donors for essential methylation processes, for example, glutathione production and synthesis of neurotransmitters.¹⁸ This vulnerability becomes exposed when triggered by enhanced environmental stress such as childhood trauma.³⁷ This could be the result of long-lasting trauma-induced epigenetic changes. These changes include DNA methylation and chromatin modifications, patterns that are inherited but responsive to environmental shifts such as stress, and especially vulnerable during development.⁵⁸ McGowan *et al.*⁵⁹ showed altered methylation of the promoter region of the glucocorticoid receptor gene in hippocampus tissue from suicide victims with a history of childhood abuse. Interestingly, Shalev *et al.*⁶⁰ recently reported stress-related accelerated telomere erosion already in childhood; compared with their counterparts, children who experienced two or more kinds of violence exposure showed significantly more telomere erosion. The authors suggest that these effects are mediated by oxidative stress. Heim *et al.*⁶¹ proposed that many of the biological changes thought to be characteristic of MDD might, in fact, be secondary to early-life trauma and represent the risk of developing MDD. Moreover, Nanni *et al.*¹ revealed that childhood maltreatment was associated with lack of response (or remission) during treatment for MDD. Hypothetically, TCEs disrupt the physiological response to stress, the overactivation of which may lead to detrimental consequences in stress-sensitive systems, namely the nervous, immune, metabolic and endocrine systems.^{61,62} The resulting cumulative biological ‘weight’ might determine

poor prognosis in terms of recurrences in MDD. However, thus far, prospective studies in recurrent MDD were lacking.

The observed gene–environment interaction is of clinical importance, as the burden of MDD is mainly because of its lifelong recurrent nature.⁶³ The T – and TCE – patient groups remained on average recurrence free for 1.85 years longer than the T + and TCE + patient groups. This suggests that MDD patients with a childhood trauma history and carriers of the thermolabile variant of the *MTHFR* gene constitute a subgroup of patients who may particularly require tailored interventions. Those interventions have to combat both MDD recurrence and the consequences of childhood trauma.^{1,64,65} The gene–environment interaction in two independent samples suggests benefit from the integration of two types of therapeutic approaches: on one hand, psychotherapeutic interventions specifically aimed at the consequences of TCEs (including psychotherapeutic treatment of trauma-related problems) that, on the other hand, could be combined with interventions aimed at the 1-C cycle; (oxidative) stress may be corrected by improving antioxidant defenses through dietary modification and (add-on) exercise.⁶⁶ These interventions should be investigated further with randomized controlled trials in specific high-risk groups.

The limitations of our study include the assessment of TCEs with a self-report questionnaire rather than an interview. Those questionnaires may be subject to recall bias through the effects of depressive symptomatology, and therefore the validity of such an approach may be reduced.³⁸ However, this effect may be limited in the discovery sample because at baseline all participants were euthymic. Moreover, in the replication sample, all individuals were healthy. Furthermore, other constructs of TCEs (such as parental neglect, bullying) than those represented in the used questionnaire could play a role. In addition, the questionnaire provides no information regarding the specific timeframe in which the TCEs took place and how prolonged they were. It could be that the effect of TCEs is modified by these time-dependent factors. Another limitation in the discovery sample is the possibility of selection bias because the final sample contained 124 patients of the 172 patients from the original trial. We lost 8.7% to follow-up and 19.2% to a diversity of reasons (for example, genotyping failure, being afraid of needles, ethical issues concerning genetic study). However, the patients of the final sample did not differ on the main clinical variables from those excluded from the analyses. This makes it unlikely that this selection of 124 patients out of the original sample induced any additional selection bias. Similarly, in the replication sample, we cannot exclude that recruitment strategies have resulted in a sample that is not completely representative of the general population with regard to age and educational level, and therefore we cannot be sure that the findings in the replication sample can be generalized to the general population.

In spite of these limitations, our study is unique in providing the opportunity to investigate the role of the interaction between genes and environment on prospectively assessed recurrence over 5.5 years. In addition, this was investigated in a specific sample of highly recurrent depressed patients, which can be considered characteristic for those patients particularly causing the large MDD-associated burden of disease.⁶³ By including specific recurrently depressed

patients, we were able to investigate a clinically highly relevant sample. Our replication of the interaction in a population sample supports the robustness of our findings and suggests that this genetic vulnerability is relevant in the broader context of depression.

In summary, the results of the present study indicate that an interaction between *MTHFR* C677T and TCEs increased risk of recurrence in recurrent MDD patients over 5.5 years of follow-up and is associated with depressive symptoms in the general population. More attention to specific at-risk individuals, that is, patients who experienced TCEs and genetic alterations, including *MTHFR* C677T, could help to improve treatment strategies to prevent depression and recurrences. However, the exact nature of the connection between *MTHFR* C677T, TCEs and the course of recurrence in MDD remains to be clarified. Future, preferably prospective, studies are warranted to replicate these findings.

Conflict of interest

The authors declare no conflict of interest. AL had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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