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ORIGINAL ARTICLE

Therapygenetics in mindfulness-based cognitive therapy: do genes have an impact on therapy-induced change in real-life positive affective experiences?

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Positive affect (PA) has an important role in resilience against depression and has been shown to increase with mindfulness-based cognitive therapy (MBCT). To elucidate the underlying mechanisms of change in PA as well as develop insights that may benefit personalized medicine, the current study examined the contribution of genetic variation to individual differences in change in PA in response to MBCT. Individuals ($n = 126$) with residual depressive symptoms were randomized to either an MBCT group or treatment as usual. PA was assessed using experience sampling methodology (ESM). Single-nucleotide polymorphisms (SNPs) in genes known to be involved in reward functioning were selected. SNPs in the genes for brain-derived neurotrophic factor (*BDNF*), the muscarinic acetylcholine receptor M2 (*CHRM2*), the dopamine receptor D4 (*DRD4*) and the μ1 opioid receptor (*OPRM1*) significantly moderated the impact of treatment condition over time on PA. Genetic variation in the genes for *CHRM2* and *OPRM1* specifically had an impact on the level of PA following MBCT. The current study shows that variation in response to MBCT may be contingent on genetic factors associated with the regulation of PA. These findings contribute to our understanding of the processes moderating response to treatment and prediction of treatment outcome.

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INTRODUCTION

A stronger capacity to experience positive affect (PA) is associated with resilience against major depressive disorder (MDD)[1–4] as well as against general negative emotional experiences[5–7] and other forms of psychopathology.[8–10] Therefore, recent studies have examined to what degree non-pharmacological interventions are able to modify the experience of PA. Several therapies that aim at enhancing PA have been developed. Indeed, randomized controlled trials of mindfulness-based cognitive therapy (MBCT) and Loving Kindness Meditation show increases of positive emotional experience in a variety of samples.[11–14] However, the underlying mechanisms of how psychotherapy elevates PA remain unknown.

Therapygenetic approaches aim to investigate the impact of specific genetic variants on differences in the level of success of psychological therapies.[15,16] Studies investigating PA have yielded heritability estimates between 30 and 50%,[17,18] suggesting that genetic factors underlie, at least in part, individual differences in the ability to experience PA. It can therefore be hypothesized that heterogeneity in treatment outcome in terms of PA can be traced to genetic individual differences in biological systems regulating positive emotional experience.[19,20] Investigating the role of genetic variation in treatment outcome may elucidate the underlying mechanism of change in PA and enhance a personalized medicine approach in treatment.[21]

Two brain reward pathways are considered to be essential for the experience of PA. First, the mesolimbic dopaminergic pathway appears to be at the heart of the brain reward system.[22–25] Second, the opioid pathway is also believed to be associated with reward.[26–28] The opioid system strongly influences dopaminergic reward circuitry as the latter is heavily innervated by endogenous opioid peptide (endorphin and enkephalin) circuits.[29,30] As the brain reward system depends on dopamine and opioid neurotransmission in mesolimbic and frontal areas, we hypothesize that genetic variations of genes coding for dopamine and opioid regulation may contribute to individual differences in the impact of MBCT on positive affective experiences.

Psychotherapy–genetic studies, as opposed to pharmacogenetic studies, constitute a relatively novel area of research that needs further exploration.[31] Furthermore, recommendations for gene–environment interaction studies (G × E) suggest that improvements in the chosen outcome measurement—using intermediate phenotypes instead of clinical outcome measures—may reduce noise and thereby inconsistencies in results.[32] This is because affective disorders are heterogeneous disease classifications and specific representations are likely associated with interactions between polygenetic clusters and the environment.[33] The current study is the first to relate genotype to change in an underlying intermediate phenotype—the experiential expression of PA—which, in addition to reducing noise, may furthermore shed light on the mechanisms by which genes relate to treatment outcome.[33,34]

To elucidate these mechanisms, the current study aimed to examine which genetic variants have an impact on MBCT

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outcome—that is, which genotype is associated with a larger increase in PA following MBCT (boosting effect) compared with other genotypes within several single nucleotide polymorphisms (SNPs). To investigate which SNPs had an impact on MBCT outcome, we performed a randomized controlled trial in which individuals with residual depressive symptoms were randomized to either an 8-week intensive MBCT training group or a control group, and assessed genetic profiles in relation to modification of real-world PA experience. PA was assessed prospectively, repetitively and in-the-moment using experience sampling methodology (ESM),[35] thus avoiding recall bias in the assessment of affect.

MATERIALS AND METHODS

Participants

Participants with residual depressive symptoms and at least one prior episode of MDD were recruited through outpatient mental health-care facilities in Maastricht (The Netherlands) as well as via posters in public places. Residual symptoms were defined as a score $\geqslant 7$ on the 17-item Hamilton Depression Rating Scale for Depression (HDRS[36]). Exclusion criteria included the following: fulfilling criteria for a current depressive episode, schizophrenia or psychotic episodes in the past year, and recent (past 4 weeks) or upcoming changes in on-going psychological or pharmacological treatment. Currently depressed individuals were excluded since, at trial preparation, no evidence existed that currently depressed individuals were able to participate in, or benefit from, MBCT. All study procedures were approved by the Medical Ethics Committee of Maastricht University Medical Centre, and all participants signed an informed consent form. The trial was registered at the Dutch Trial Register (number NTR1084).

Procedure

Potential participants were screened by phone to check for availability during the study period along with the likelihood of meeting inclusion and exclusion criteria. During a second screening, the Structured Clinical Interview for DSM IV-Axis I[37] and HDRS[36] were administered by trained psychologists. All eligible participants were invited for a one-on-one explanation of the ESM procedure followed by saliva collection for DNA extraction, after which they participated in baseline assessment. The baseline assessment consisted of 6 days of ESM in the individual's own environment (see ESM below) and administration of the HDRS interview. Participants were then randomized to either the experimental (MBCT) or control (wait list) arm of the trial (for specific randomization and stratification procedures see Geschwind *et al.*[13]).

After 8 weeks of MBCT (see Intervention section), or equivalent waiting time, participants once more participated in a 6-day ESM assessment phase and administration of the HDRS. Assessment periods of control participants were matched to those of MBCT participants. All participants received compensation in the form of gift vouchers worth 50 euros. Participants in the control condition were given the opportunity to participate in an MBCT training after post-intervention assessment.

Intervention

The content of the MBCT training sessions followed the protocol of Segal *et al*.[38] The training consisted of eight weekly sessions of 2.5 h each, involving groups of 10–15 participants. Sessions included guided meditation, experiential exercises and discussion. In addition to the weekly group sessions, participants received CDs with guided exercises and were assigned homework exercises (of 30–60 min daily). Sessions were taught by experienced trainers in a centre specialized in mindfulness trainings. Trainers were supervised by an experienced health-care professional who trained with Teasdale and Williams (co-developers of MBCT).[39]

ESM

ESM is a momentary assessment method to assess participants in their daily living environments, thus providing repeated in-the-moment assessments of affect in a prospective and ecologically valid manner with several advantages over retrospective questionnaires.[40] In the current study, participants received both a digital wristwatch and a set of ESM self-assessment forms, the latter organized in six booklets—one for each day. The wristwatch was programmed to emit a signal ('beep') at unpredictable moments, but certainly once every 90 min between 0730 and 2230 hours, on six consecutive days, resulting in 60 beeps per assessment period. After each beep, participants were asked to fill out the ESM self-assessment forms. The forms included reports on current mood and context, all given on a 7-point Likert scale. All reports that were not filled out within 15 min after the beep, and all participants who were with less than 20 valid reports at baseline, were excluded from the analysis because these have been shown to be less reliable.[35]

Measures

PA. At each beep, several ESM mood adjectives were assessed on 7-point Likert scales ranging from 1 (*not at all*) to 7 (*very*). Adjectives were selected based on previous experience of our research group within this field[41,42] in combination with the following considerations: they should reflect state (rather than trait) measures and should show intra-individual variation. Items should, furthermore, preferably load on the same latent factor (PA). Consistent with previous work,[3,13] principal component analysis with oblique rotation was used to generate a factor representing PA. The mood adjectives 'happy', 'satisfied', 'strong', 'enthusiastic', 'curious', 'cheerful', and 'inspired' loaded on the PA factor. The mean levels of PA were then computed by averaging the above items per participant and beep moment, yielding a total of 11 513 PA observations in this sample.

17-Item Hamilton Depression Rating Scale. The HDRS[36] was administered by two trained research assistants with master degrees in psychology. The HDRS is a semistructured interview designed to assess depressive symptoms over the past week. Internal, interrater and retest reliability estimates for the overall HDRS are good.[43]

Genotyping. Genomic DNA was obtained from saliva samples. Saliva was collected in Oragene-DNA Self Collection Kits (DNA Genotek, Ottawa, Ontario, Canada), and DNA was isolated using the AutoGenFlex DNA isolation system (Autgen, Hilliston, MA, USA) according to the manufacturer's instructions. SNPs were determined with Sequenom (Hamburg, Germany) using the Sequenom MassARRAY iPLEX platform at the facilities of the manufacturer. SNPs with a call rate of lower than 90% or that show violation of Hardy–Weinberg equilibrium (GENHWI command in STATA 12.1,[44] $P < 0.01$) were excluded from analyses.

A selection of genetic variations (see Table 1) under study was made based on (i) their involvement in dopamine and/or opioid regulation and/or (ii) previous literature showing associations between the selected variations and mental disorders associated with a deficient reward system, such as MDD.[45–53] Of the selected 32 SNPs,

- Three SNPs could not be included in any of the multiplex assays due to neighbouring SNPs or overlapping sequences (rs11030103 and rs568201086 in the brain-derived neurotrophic factor (*BDNF*) gene and rs1799732 in the dopamine receptor D2 (*DRD2*) gene);
- One SNP was excluded because according information from the Database of Single Nucleotide Polymorphisms (dbSNP; http://www.ncbi.nlm.nih.gov/SNP/) its variation was suspected to be false positive due to artifacts of the presence of paralogous sequence in the genome or because evidence suggested sequencing error or computation artifacts (rs28722151 in *BDNF*);
- One SNP was excluded because it was a multivariate SNP which was unknown at the time of genotyping (rs1799836 in the monoamine oxidase B (*MAO-B*) gene);
- Three SNPs were excluded because they showed no, or minimal, variation: rs12273539 and rs57083135 in *BDNF*; rs6267 in the catechol-*O*-methyltransferase (*COMT*) gene);
- One SNP was excluded because of genotyping failure (that is, call rate < 0.90; rs747302 in the dopamine receptor D4 (*DRD4*) gene).

We furthermore decided not to run genetic analyses on genotypes present in just a small proportion of the sample. We used the cutoff of $n = 30$ as the minimum number of individuals for a genotype to be included in the analyses. Owing to this, five further SNPs were excluded (rs 1800955 in *DRD4*, rs1799971 and rs563649 in the μ1 opioid receptor (*OPRM1*) gene, and rs 6350 and rs6413429 in the dopamine transporter (*SLC6A3*) gene), resulting in a final set of 18 SNPs. An overview is provided in Table 1. Table 2 shows the correlations between SNPs, indicating that the effects of SNPs are mostly independent. Of the SNPs that did correlate highly (that is, $r \geqslant 0.70$) only one SNP was analysed to avoid finding several significant effects that actually come down to one single effect as well as

Table 1. Overview of selected SNPs

SNP	*Genotypes* (n) *or reason of exclusion*	*Gene*	*Location*	*Function*	*Alleles*	*Alternative name(s)*	*Inclusion reference*	*Callrate*	*HW* χ^2
rs6265	G/G (78); A/G (43); A/A (5)	*BDNF*	11p13	Missense	G/A	Val66Met	Licinio *et al.*[44]	1.00	0.262
rs11030101	A/A (36); T/A (63); T/T (27)	*BDNF*	11p13	UTR-5	A/T		Licinio *et al.*[44]	1.00	0.109
rs11030102	C/C (76); G/C (43); G/G (7)	*BDNF*	11p13	NearGene-5	C/G		Licinio *et al.*[44]	1.00	0.004
rs11030103	Design failure	*BDNF*	11p13	NearGene-5	G/A		Licinio *et al.*[44]	/	/
rs12273539	No variation	*BDNF*	11p13	Intronic	T/C		Licinio *et al.*[44]	1.00	/
rs28722151	*Suspected*	*BDNF*	11p13	UTR-5	C/G		Licinio *et al.*[44]	/	/
rs56820186	Design failure	*BDNF*	11p13	UTR-3	G/T		Licinio *et al.*[44]	/	/
rs57083135	Too little variation	*BDNF*	11p13	NearGene-5	C/T		Licinio *et al.*[44]	1.00	0.031
rs4633	C/C (31); C/T (63); T/T (32)	*COMT*	22q11.21	Synonymous	C/T		Diatschenko *et al.*[51]	1.00	0.009
rs4680	G/G (31); G/A (63); A/A (32)	*COMT*	22q11.21	Missense	G/A	Val158Met	Diatschenko *et al.*[51]	1.00	0.009
rs4818	C/C (41); C/G (64); G/G (21)	*COMT*	22q11.21	Synonymous	C/G		Diatschenko *et al.*[51]	1.00	0.362
rs6267	No variation	*COMT*	22q11.21	Missense	G/T	Ala72Ser	Diatschenko *et al.*[51]	1.00	/
rs6269	A/A (39); A/G (65); G/G (22)	*COMT*	22q11.21	NearGene-5	A/G		Diatschenko *et al.*[51]	1.00	0.479
rs324650	A/A (36); T/A (68); T/T (22)	*CHRM2*	7q31-q35	Intronic	A/T		Wang *et al.*[45]	1.00	0.762
rs1824024	T/T (56); G/T (50); G/G (20)	*CHRM2*	7q31-q35	Intronic	G/T		Wang *et al.*[45]	1.00	1.649
rs2061174	T/T (58); T/C (51); C/C (17)	*CHRM2*	7q31-q35	Intronic	C/T		Wang *et al.*[45]	1.00	0.717
rs6276	A/A (63); G/A (54); G/G (9)	*DRD2*	11q23	UTR-3	A/G	A1385G	Kraschewski *et al.*[46]	1.00	0.188
rs6277	T/T (38); C/T (63); C/C (25)	*DRD2*	11q23	Synonymous	C/T	C957T	Kraschewski *et al.*[46]	1.00	0.002
rs1799732	Design failure	*DRD2*	11q23	NearGene-5	−/C	−141C del	Kraschewski *et al.*[46]	/	/
rs747302	Genotyping failure	*DRD4*	11p15.5	NearGene-5	C/G	C616G	Ben Zion *et al.*[43]	0.06	/
rs936461	G/G (58); G/A (53); A/A (15)	*DRD4*	11p15.5	NearGene-5	A/G	A809G	Ben Zion *et al.*[43]	1.00	0.048
rs1800955	Too little variation	*DRD4*	11p15.5	NearGene-5	C/T	C521T	Ben Zion *et al.*[43]	0.93	5.165
rs1799836	Multivariable SNP	*MAO-B*	Xp11.23	Intronic	A/G	B-SNP 13	Balciuniene *et al.*[47]	/	/
rs495491	T/T (65); T/C (48); C/C (13)	*OPRM1*	6q24-q25	Intronic	C/T		Zhang *et al.*[48]	1.00	0.938
rs609148	C/C (75); C/T (44); T/T (7)	*OPRM1*	6q24-q25	Intronic	C/T		Zhang *et al.*[48]	1.00	0.068
rs648893	T/T (75); C/T (44); C/C (7)	*OPRM1*	6q24-q25	Intronic	C/T		Zhang *et al.*[48]	1.00	0.068
rs1799971	Too little variation	*OPRM1*	6q24-q25	Missense	A/G	Asn40Asp 118 A/G	Zhang *et al.*[48]	1.00	0.004
rs3823010	A/A (83); A/G (37); G/G (6)	*OPRM1*	6q24-q25	Intronic	A/G		Zhang *et al.*[48]	1.00	0.484
rs563649	Too little variation	*OPRM1*	6q24-q25	UTR-5	A/G		Shabalina *et al.*[49]	1.00	0.038
rs6347	A/A (68); A/G (49); G/G (9)	*SLC6A3*	5p15.3	Synonymous	A/G	Ex2+159 C>T	Azzato *et al.*[50]	1.00	0.006
rs6350	Too little variation	*SLC6A3*	5p15.3	Synonymous	C/T	−3714G>T	Azzato *et al.*[50]	1.00	0.607
rs6413429	Too little variation	*SLC6A3*	5p15.3	NearGene-5	G/T	Ex9–55A>G	Azzato *et al.*[50]	1.00	0.853

Abbreviations: *BDNF*, brain-derived neurotrophic factor; *CHRM2*, cholinergic receptor muscarinic 2; *COMT*, catechol-*O*-methyltransferase; *DRD2*, dopamine receptor D2; *DRD4*, dopamine receptor D4; ESM, experience sampling methodology; *OPRM1*, μ1 opioid receptor; *SLC6A3*, dopamine transporter; SNP, single nucleotide length polymorphism; UTR, untranslated repeat. Note: call rates and X^2 values of Hardy–Weinberg (HWχ^2) equilibrium (both calculated on $n = 131$; including five participants without ESM data) are indicated. SNPs with a callrate < 0.90 (genotyping failure); no or too little variation (two or more genotypes with less than 30 subjects); or a X^2 value above 10.83 (marked with *, corresponding with a *P*-value < 0.001) were excluded from analyses.

to avoid overcorrecting for multiple testing problems. This decision influenced the analyses of the following SNPs: rs4633, rs4680, rs4818, rs6269 in *COMT* (all $rs \geqslant 0.85$); rs1824024 and rs206174 in *CHRM2* ($r = 0.86$); rs6276 and rs6277 in *DRD2* ($r = 0.71$); and rs495491 and rs3823010 in *OPRM1* ($r = 0.79$). For the *COMT* gene, only the most well-known functional SNP (rs4680) was analysed. From the other highly correlated SNPs only the one with the lowest rs number was analysed. All in all 14 SNPs were analysed. SNPs were coded in 0,1,2 format with 0 being the most frequent homozygous genotype unless it involved a functional SNP, of which the non-risk allele (according to dbSNP) was coded 0.

Statistical methods

ESM data have a hierarchical structure. Thus, multiple observations (Level 1) are clustered within participants (Level 2). Multilevel analyses take the variability associated with each level of nesting into account.[54] The XTMIXED command in STATA 12.1[44] was used to perform multilevel linear regression analyses. The large amount of observations of the outcome measure per participant increases the effective sample size.[55,56] Owing to the multilevel structure of the data, the effective sample size depends on the size of the ICC. For the current data, the ICC is around 0.07, implying that the effective sample size is about $11\,513/(1+0.07\times 99) = 1451$.

To test whether the effect of MBCT on PA depends on genotype, fourteen three-way interactions between SNP, time (baseline vs post assessment) and group (control vs MBCT) on PA were performed, yielding a total of 16 analyses (three analyses for the SNPs rs4633/rs4680, as all three genotypes were included for analysis, and one analysis per SNP for the other SNPs). The MARGINS command[44] was used to calculate estimated marginal means to plot PA levels at baseline and post assessment per combination of genotype and group. For all the SNPs that returned a significant interaction effect, the TEST command, which uses a Wald test,[57] was used to analyse whether (and if so, in which direction) the change in PA within a group differed per genotype.

All analyses were controlled for age and gender. Furthermore, to avoid between-person differences in average set-point of the subjective ratings, analyses were corrected for individual baseline average of PA. In addition, all multilevel models included a random intercept, covariance was set to unstructured. To correct for multiple testing problems, Holm's procedure[58,59] was used. Finally, in order to graphically display the clinical effect associated with the impact of genotype on PA experience, differences in change in HDRS scores as a function of genotype and treatment arm were shown for each significant finding.

RESULTS

Participants

At baseline, there were no significant differences between treatment groups with respect to sociodemographic and clinical characteristics: age, gender, full-/part-time work, illness/unemployment benefits, living with partner/own family, HDRS score, comorbid past/present anxiety disorder, current use of antidepressants or benzodiazepines (all *P*-values >0.05). Furthermore, MBCT compared with CONTROL was associated with significant increases in PA (for details of sociodemographic and clinical

Table 2. Correlation coefficients between SNPs

	rs6265	rs11030101	rs11030102	rs4633	rs4680	rs4818	rs6269	rs324650	rs1824024	rs2061174	rs6276	rs6277	rs936461	rs495491	rs609148	rs648893	rs3823010	rs6347
rs6265	1.00																	
rs11030101	−0.44	1.00																
rs11030102	*−0.29*	**−0.54**	1.00															
rs4633	*−0.10*	*0.10*	−0.08	1.00														
rs4680	*−0.10*	*0.10*	−0.08	**1.00**	1.00													
rs4818	0.07	−0.11	0.08	**−0.85**	**−0.85**	1.00												
rs6269	0.08	−0.12	0.09	**−0.89**	**−0.89**	**0.98**	1.00											
rs324650	−0.01	0.07	*−0.11*	*0.13*	*0.13*	−0.08	*−0.11*	1.00										
rs1824024	0.02	−0.03	−0.09	−0.02	−0.02	0.06	0.06	0.39	1.00									
rs2061174	0.06	−0.02	*−0.14*	−0.03	−0.03	0.05	0.05	0.41	**0.86**	1.00								
rs6276	*−0.14*	−0.02	*0.19*	*0.14*	*0.14*	*−0.11*	*−0.14*	−0.03	*−0.10*	*−0.12*	1.00							
rs6277	−0.04	−0.05	*0.15*	*0.14*	*0.14*	−0.07	−0.08	−0.05	−0.07	*−0.10*	**0.71**	1.00						
rs936461	*−0.16*	0.09	−0.04	0.03	0.03	0.03	0.03	0.16	0.08	0.07	*0.19*	*0.17*	1.00					
rs495491	0.04	0.01	−0.05	−0.03	−0.03	−0.02	0.00	−0.06	0.02	−0.03	−0.06	−0.05	*−0.13*	1.00				
rs609148	−0.06	0.03	0.01	−0.01	−0.01	0.00	0.01	−0.04	*−0.15*	*−0.17*	−0.01	*−0.12*	*−0.09*	−0.31	1.00			
rs648893	−0.06	0.03	0.01	−0.01	−0.01	0.00	0.01	−0.04	*−0.15*	*−0.17*	−0.01	*−0.12*	−0.09	−0.31	**1.00**	1.00		
rs3823010	0.09	0.09	−0.06	*−0.12*	*−0.12*	−0.01	0.02	−0.06	0.04	0.02	−0.06	−0.07	*−0.16*	**0.79**	*−0.25*	*−0.25*	1.00	
rs6347	0.00	*−0.19*	*0.20*	−0.04	−0.04	0.06	0.05	0.01	−0.01	−0.02	−0.01	−0.01	*−0.13*	*−0.13*	*0.16*	*0.16*	*−0.15*	1.00

Note: The size of the Pearson correlation coefficients (that is, small, medium or strong correlation) are indicated as follows: $r = 0.1–0.3$ (small, shown in italics), $r = 0.3–0.5$ (medium, shown as underlined) and $r = 0.5–1.0$ (strong, shown in bold).

Table 3. Unstandardized effect sizes (s.e.) of the group × time × SNP interaction and their *P*-values

SNP	*Gene*	*Effect size (s.e.)*	P-*value*
rs6265	*BDNF*	−0.067 (0.082)	0.415
rs11030101	*BDNF*	0.274 (0.089)	0.002[a]
rs11030102	*BDNF*	0.128 (0.081)	0.112
rs4680[b]	*COMT*	(a) 0.204 (0.097)	0.036
		(b) −0.049 (0.110)	0.656
		(c) −0.254 (0.092)	0.006
rs4818	*COMT*	−0.063 (0.085)	0.461
rs6269	*COMT*	−0.104 (0.086)	0.229
rs324650	*CHRM2*	−0.206 (0.087)	0.018
rs1824024[c]	*CHRM2*	−0.259 (0.083)	0.002[a]
rs6276	*DRD2*	−0.206 (0.079)	0.010
rs6277	*DRD2*	−0.156 (0.090)	0.082
rs936461	*DRD4*	0.426 (0.082)	0.000[a]
rs495491[d]	OPRM1	0.372 (0.081)	0.000[a]
rs609148[e]	OPRM1	0.647 (0.083)	0.000[a]
rs6347	*SLC6A3*	−0.209 (0.081)	0.009

Abbreviations: *BDNF*, brain-derived neurotrophic factor; *CHRM2*, cholinergic receptor muscarinic 2; *COMT*, catechol-O-methyltransferase; *DRD2*, dopamine receptor D2; *DRD4*, dopamine receptor D4; *OPRM1*, μ1 opioid receptor; *SLC6A3*, dopamine transporter; SNP, single nucleotide length polymorphism. Note: the most common homozygotic genotype is the reference category (except for rs4680, of which the homozygote of the non-risk allele is the reference category); (a) tests group 1 to 0 (heterozygotic genotype to homozygote of non-risk allele), (b) tests group 2 to 0 (homozygote of risk-allele to homozygote of non-risk allele), (c) tests group 2 to 1 (homozygote of risk-allele to heterozygotic genotype). [a]Indicates a significant result, that is when *P*-values are ordered from smallest P_1 to largest P_n, $Pi \leqslant \alpha/(n-i+1)$, following Holm's method for correcting for multiple testing.[54,55] [b]rs4680 is perfectly correlated with rs4633 and highly correlated with rs4818 and rs6269. [c]rs1824024 is highly correlated with rs2061174. [d]rs495491 is highly correlated with rs3823010. [e]rs609148 is perfectly correlated with rs648893.

characteristics as well as PA levels per group and Participants flow see Geschwind *et al.*[13]). Of the 130 subjects included, one subject had to be excluded from analysis for not meeting the prespecified number of experience sampling entries for baseline assessment, and three subjects were excluded due to low call rate (that is, call rate < 0.90), resulting in a final sample of 126 participants.

Gene x intervention interactions

PA levels did not differ per genotype at baseline (main effect of SNP on PA; all *P*-values >0.05). When correcting for multiple testing with Holm's method,[58,59] 5 of the 16 SNP × time(baseline − post) × group(MBCT − control) analyses were significant (see Table 3). These were SNPs in *BDNF*, the muscarinic acetylcholine receptor M2 (*CHRM2*) gene, *DRD4* and *OPRM1*. For each of these significant results, two follow-up analyses (to further examine how change in PA within a group differed per genotype) were performed (see Figure 1).

Prominent effects on change in PA in response to MBCT were found for SNPs in *OPRM1* (rs495491, rs609148/rs648893). Compared with the reference genotype, the heterozygotic variant of these SNPs was consistently associated with larger boosts in PA from baseline to post assessment in the MBCT group. For the latter two SNPs (rs609148/rs648893), the same heterozygotic variant, compared with the reference genotype, was furthermore associated with a decrease in PA in the control group. For one SNP (rs1824024 in *CHRM2*), the boosting effect in PA from baseline to post assessment appeared to be greater, compared with the reference genotype, in the MBCT group with the homozygotic variant. Two more SNPs were in significant interaction with group and time: rs11030101 in *BDNF* and rs936461 in *DRD4*. However,

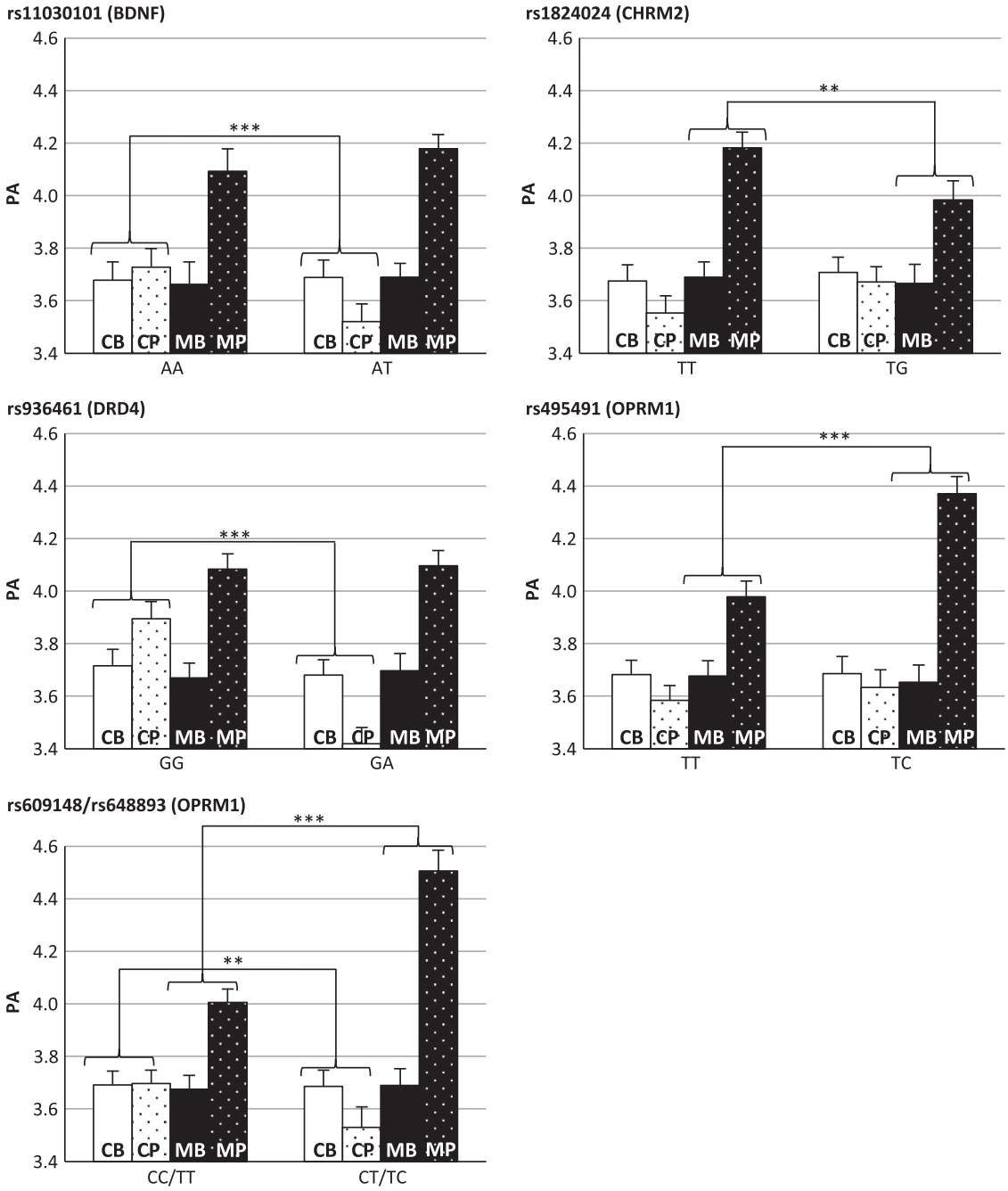

Figure 1. Standardized predicted vales of PA (*y* axis) per combination of group (control/MBCT), assessment time (baseline/post) and SNP genotype for significant interaction effects only; controlled for the mean baseline PA, gender and age. Follow-up analyses were performed to test whether the change in PA from baseline to post intervention within a group (control/MBCT) differed per genotype: $*P \leqslant 0.05$; $**P \leqslant 0.01$; $***P \leqslant 0.001$. CB, baseline measurement in control group; CP, post assessment in the control group; MB, baseline measurement in the MBCT group; MP, post assessment in the MBCT group; PA, positive effect.

the effect in these SNPs was driven by differences in the control group rather than the MBCT group. In the control group the heterozygotic variants of these SNPs were associated with a decrease in PA from baseline to post assessment, compared with stable or increasing PA levels from baseline to post assessment in participants with the homozygotic variant. The increase in PA in the MBCT group for these two SNPs was similar for heterozygotic and homozygotic genotypes. This indicates that people with a certain genotype would show a decrease in PA (that is, deteriorate) if left untreated, and that this deteriorating effect could be prevented with MBCT.

In order to visually relate these findings to clinical changes in depressive symptoms, Figure 2 shows, for each of these SNPs, the HDRS total score per combination of SNP genotype and group over time. Findings show that the difference between genotypes in change in HDRS scores is on average about 2 points. Change in HDRS scores, furthermore, generally mirrors the change in PA—that is, whenever the average PA increases in the MBCT group for a certain genotype, the average HDRS total score decreases as well.

A *post hoc* analysis was conducted to investigate whether the differential effect of the significant SNPs on PA might be mediated

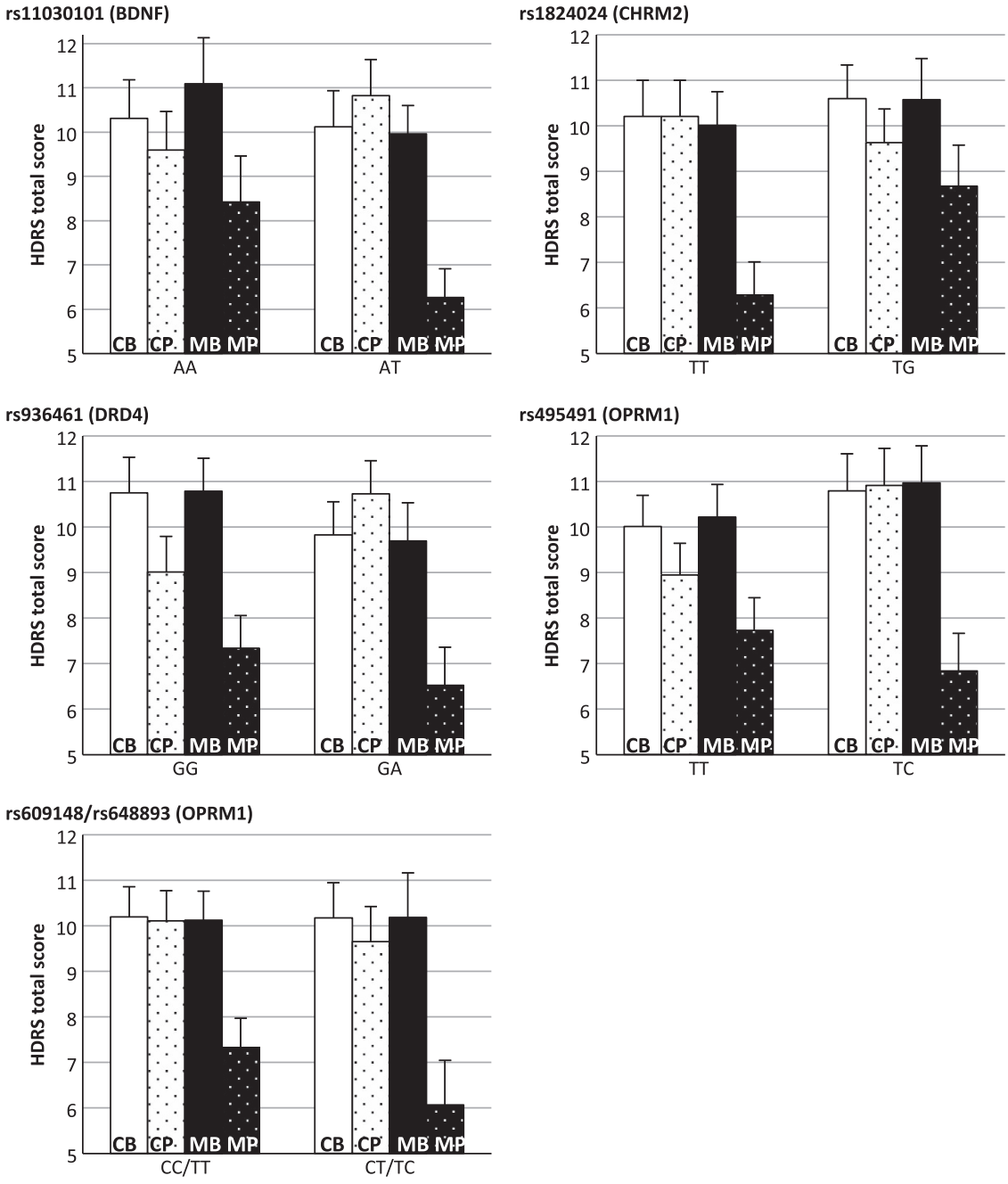

Figure 2. Standardized predicted vales of 17-item Hamilton Depression Rating Scale (HDRS) score (*y* axis) per combination of group (control/MBCT), assessment time (baseline/post) and SNP genotype; controlled for gender and age. CB, baseline measurement in control group; CP, post assessment in the control group; MB, baseline measurement in the MBCT group; MP, post assessment in the MBCT group.

by differences in behavioural engagement during the MBCT training. The analysis revealed that, after correction for multiple testing, none of the SNPs significantly predicted the total amount of minutes that participants practiced. Therefore, it does not seem likely that the effect of SNPs is transferred through differences in behavioural engagement during the MBCT training.

DISCUSSION

To the best of our knowledge, this is the first study combining assessment of DNA and ESM in a randomized controlled trial with MBCT. The study aimed at investigating the impact of SNPs on MBCT outcome in terms of PA. It was hypothesized that genetic variations in a selection of genes related to dopamine and opioid regulation and/or that have associations with mental disorders that have been linked with a deficient reward system may contribute to individual differences in the impact of MBCT on positive affective experiences. The results suggest that SNPs in *CHRM2* and *OPRM1* moderate the positive impact of MBCT in the sense that change in PA was on average more pronounced in participants with certain variants of these SNPs (boosting effect). An additional effect that was not hypothesized, but revealed to be associated with SNPs within *BDNF* and *DRD4*, can best be described as a deteriorating effect in the control group. The average increase in PA in participants in the MBCT group did not vary with variations within these SNPs. The MBCT intervention therefore can be conceived as counteracting a natural liability to worsen.

The impact of genetic variation in the context of previous literature

One SNP in *OPRM1* that showed a boosting effect in the MBCT group additionally showed the deterioration effect in the control group, thus showing that this SNP moderates susceptibility to environmental influences in a for-better-and-for-worse manner.[60] Indeed, it has been found previously that another SNP in the *OPRM1* gene was associated with differential susceptibility to the environment, with one genotype making individuals simultaneously more vulnerable to the negative consequences of lower levels of maternal care (as reflected by highest levels of fearful attachment) and more responsive to the benefits of higher levels of maternal care (as reflected by lowest levels of fearful attachment).[61]

It is noteworthy that the interaction effect between MBCT and both analysed SNPs in the *OPRM1* gene on PA turned out to be very significant, despite the fact that these SNPs were not highly correlated. Hence, the opioid system may be strongly involved in reward expression in the flow of daily life. Initially, 'reward' was conceptualized as if it were a single psychological process or a unitary feature of a reinforcing stimulus, and dopamine in the nucleus accumbens was nominated as the 'pleasure neurotransmitter'.[62,63] However, with the separation of component processes of reward ('liking' and 'wanting'),[64] a more complex model was debated.[63,65,66] Our results coincide with recent literature showing that the perception of hedonic properties of rewards ('liking') may be mediated more by opioids rather than dopamine.[64,67]

Previous literature showed that *BDNF* has an important role in the mesolimbic system and specific areas that are centrally involved in the regulation of reward, such as the Ventral Tegmental Area and the Nucleus Accumbens.[68–70] In line with this research, the current study found a significant effect of variation in *BDNF*. This effect was not characterized by a boosting effect in the context of MBCT but was brought about by differential ability to maintain stable levels of PA in the waiting list control group.

The significant interaction of rs1824024 in *CHRM2* in the current study is in line with a study replicating the finding of our inclusion article that this SNP was one of the most important susceptibility SNPs for alcohol dependence and affective disorders.[71] Cohen-Woods *et al*.[72] could not replicate this association in a sample of participants with recurrent unipolar depression not comorbid with substance misuse. Nevertheless, the SNP was found to be significantly associated with the severity of alcohol dependence in a Korean population,[73] and more generally muscarinic receptor binding in the frontal cortex was found to be decreased in MDD subjects.[74] Even though rs1824024 and *CHRM2* have not been investigated extensively, their reported association with reward-related disorders is in accordance with our finding.

Significant associations have also been found between several functional polymorphisms in *DRD4* genotypes and reward-associated outcomes, such as heroin addiction and schizophrenia,[75] and smoking, alcohol use and food craving, as reviewed in Stice *et al*.[76] These findings coincide with our significant result regarding variation in *DRD4*. However, the specific SNP in *DRD4* that we found to significantly moderate the effect of MBCT on PA (rs936461) has not specifically been investigated with regard to reward.

Therapygenetics

Therapygenetics is a relatively new continuation of the more established research area of pharmacogenetics.[77] Although the field is still in its infancy, the initiative of previous studies (see Lester and Eley[31] for an overview of therapygenetic research in affective disorders) to extend the examination of the moderating role that genetic variations have in therapeutic outcome to non-pharmacological therapies is promising. Some of the previous work concurs in providing evidence that interactions between genetic variation and directly manipulated (positive) environmental experiences (G × E) can influence remission of mental disorder outcomes.[15,78–80]

Overall, however, previous therapygenetic results have been somewhat mixed.[31,81] As discussed in the introduction, the specific approach in the current study aimed to continue and extend methodological developments within this field. In using PA as the main outcome measure, instead of clinical outcome measures, we directly tapped into the expression of reward, an important underlying intermediate phenotype for depression. This approach helped to reduce noise and increase power to detect G × E effects.[33,34] Several significant interactions were found. Furthermore, to examine how clinically relevant the current results were, HDRS total scores were rendered visually. The finding that changes in PA indeed coincided with changes in HDRS validates and supports the current approach. Future research may adopt similar approaches to examine whether the current results will be more consistent than previous findings.

Even though it has been suggested that treatment research identifying genetic variants that predict therapeutic outcome may advance personalized treatment in psychiatry,[33,82] to date no genetic predictor examined has sufficient predictive power to warrant their use as a clinical biomarker.[31] This may change once there is more knowledge about specific networks of genetic variants that correlate with treatment outcome and more complicated statistical analytical methods, such as gene-network analysis[83] are applied. In addition, machine-learning algorithms in which (epi-)genetic predictors are combined with clinical, demographic, neuroimaging and other predictors may be valuable to aid clinical judgement in the future. However, until all the relevant data for these options are available, it is important to systematically specify the mechanisms of recovery. The experience of PA during the flow of daily life has been shown to have a central role in the recovery of depressive symptoms.[84–86] Our results add to these findings in that the effect of experiential PA in recovery seems to be associated with biological mechanisms associated with reward-processing. These biological mechanisms need further examination, as they will provide us with a deeper understanding of biological processes underlying resilience and recovery, which eventually may aid in the improvement of therapeutic interventions.

Methodological issues

One possible limitation of the current study is the relatively small number of participants for testing genetic variants. However, given that we used ESM to assess our outcome variable (PA), we could collect many observations per participant, yielding an effective sample size[55] of about 1451 rather than 126, which is considerable. Nevertheless, replication of these findings in further studies is important.

Furthermore, the current study did not differentiate between high and low motivational intensity of PA items. Harmon-Jones *et al*.[87] show that only affective states low in motivational intensity (that is, the urge to move toward/away from a stimulus) broaden cognitive scope. This suggests that only affective states low in motivational intensity could attribute to a positive upward spiral as described in the broaden-and-build theory.[88] However, in the data of the current study the positive emotional states all loaded on one factor. Empirically, therefore, we have no argument to separate the two constructs in our analyses. Future research may look into what the inclusion of additional PA items such as 'relaxed' or 'calm' would do to the factor structure of PA as well as the effect of a potential 'low-motivational intensity PA factor' on the results as described in the current paper.

In addition, it is important to appreciate that the homogeneity of the sample in this study imposes limitations on the

generalizability of the findings. Replication in other—for example, more severely depressed—samples is required in order to determine whether the effects of SNPs is in the same direction as well as large enough to be picked up in these samples as well.

Furthermore, the current study is limited by the fact that included SNPs were selected in 2009 based on available information at that time. Research in and knowledge about genetics, however, is evolving rapidly. Therefore, we were not able to examine newly discovered SNPs with relevance to reward functioning in this paper (for example, *PER2* or *GABRA2*[89,90]). In addition, as the majority of our selection of SNPs is either non-coding or non-functional, biological plausibility at the level of molecular mechanisms cannot be offered. Future research can elucidate the role of genetic variations in the genes that were currently under investigation.

Lastly, the importance of gene–gene interaction (epistasis) in predisposing toward complex syndromes, such as MDD, has been emphasized previously[91] and may very well account for some impact on reward-processing and changes herein following treatment. Unfortunately, this area of research is still in progress and up to now no clear *a priori* hypotheses for specific gene–gene interactions in this respect could be formulated. Future research might be able to explicate these mechanisms.

In conclusion, the current results indicate that changes in daily life-positive affective experiences through a non-pharmacological intervention may be moderated by individual differences in biological mechanisms regulating reward. These findings contribute to our understanding of the processes underlying response to treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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