The Opioid Receptor Mu 1 (*OPRM1*) rs1799971 and Catechol-O-methyltransferase (*COMT*) rs4680 as genetic markers for placebo analgesia

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Abstract

The placebo effect is considered the core example of mind-body interactions. However, individual differences produce large placebo response variability in both healthy volunteers and patients. The placebo response in pain, placebo analgesia, may be dependent on both the opioid system and the dopaminergic system. Previous studies suggest that genetic variability affect the function of these two systems. The aim of the present study was therefore to address the interaction between the single nucleotide polymorphisms (SNPs) Opioid Receptor Mu 1 (*OPRM1*) rs1799971 and Catechol-O-methyltransferase (*COMT*) rs4680 on placebo analgesia. Two hundred and ninety-six healthy volunteers participated in a repeated measures experimental design where thermal heat pain was used as pain stimuli. Participants were randomized either to a placebo group receiving placebo cream together with information that the cream would reduce pain, or to a natural history group receiving the same pain stimuli as the placebo group without any application of cream or manipulation of expectation of pain levels. The results showed that the interaction between *OPRM1* rs1799971 and *COMT* rs4680 was significantly associated with the placebo analgesic response. Participants with *OPRM1* Asn/Asn combined with *COMT* Met/Met and Val/Met reported significant pain relief after placebo administration, whereas those with other combinations of the *OPRM1* and *COMT* genotypes displayed no significant placebo effect. Neither *OPRM1* nor *COMT* had any significant influence on affective changes after placebo administration. As shown in the present study, genotyping with regard to *OPRM1* and *COMT* may predict who will respond favorably to placebo analgesic treatment.

Keywords: Placebo effect; Pain; Placebo analgesia; experimental pain; genotyping; *OPRM1* rs1799971; *COMT* rs4680; repeated measures.

Introduction

The placebo effect in pain, placebo analgesia, is probably the best-studied example of placebo responses. The placebo analgesic effect is shown to have several biological and psychological correlates [7; 11; 40], but show substantial inter-individual variability [41]. Exclusion of placebo responders from drug-trials might reduce statistical noise [39] and decrease the costs of drug development by allowing for smaller sample sizes [20]. Moreover, recognition of placebo responders may also be important for how health-personnel communicate with patients and for selection of treatments [10; 20]. Unfortunately, earlier studies on predictors for the placebo response in pain have revealed mixed results [22], it has been suggested that genetic factors, which are stable traits across contexts, may be linked to placebo analgesia [20].

Opioid antagonists may reverse placebo analgesia [5; 24; 49], and endogenous opioid activity in cerebral pain-related networks is related to placebo analgesic responses [43]. One genetic factor that may influence on this system is the SNP *OPRM1* A>G rs1799971 in the opioid receptor mu 1 gene. This SNP leads to a substitution of asparagine (Asn) to aspartic acid (Asp) at codon 40 and subsequent removal of a putative N-linked glycosylation site in the receptor [8]. Individuals with Asn/Asn display higher placebo responses compared to those with the Asp/Asp [32]. In addition, the SNP *COMT* A>G rs4680 in the Catechol-O-methyltransferase gene may affect sensory processing [13; 23]. This SNP leads to a substitution of an amino acid i.e, Valine (Val) to methionine (Met) at codon 158 – which reduces the enzyme enzymes activity, i.e., degradation of catecholamines [25]. Thus, SNP rs4680 is associated with sensitivity to experimental pain [13; 48]. Yu and colleagues [47] showed that higher number of *COMT* Met-alleles were linearly associated with higher experimental placebo analgesia, whereas Hall and colleagues [19] found similar results with higher placebo responses in patients with irritable bowel syndrome.

The *COMT* SNP rs4680 may influence the affective components of pain [48] and pain catastrophizing [18]. Generally, reduction of negative emotions is concomitant with placebo analgesia [14]. Previous findings show that *COMT* Met/Met carriers display larger placebo analgesic responses than the Val/Met or Val/Val combinations [19; 47]. Thus, it can be anticipated that Met/Met carriers show larger reductions in negative emotions after placebo administration compared to those with *COMT* Val/Met and Val/Val.

Previous data suggest that the opioid and catecholaminergic systems may influence each other. For example, individuals with reduced enzymatic degradation of the catecholamines display reduced regional release of endogenous opioids during pain [48]. The aim of the present study was therefore to address the interaction between the *OPRM1* rs1799971 and *COMT* rs4680 on placebo analgesia. We hypothesized that subjects with *OPRM1* Asn/Asn in combination with *COMT* */Met reported significantly higher placebo analgesic responses compared to individuals with other combinations of the *OPRM1* and *COMT* genotypes. Furthermore, we hypothesized that reduction in negative emotions after placebo administration mediated the placebo analgesic response and that those with *COMT* Met/Met genotype should display higher reduction in negative emotions compared to participants with the *COMT* */Val genotype.

Methods

Participants

The experiment included a total of 327 healthy Caucasian participants with a mean age of 23 years (SD = 3.3), 200 (61.2%) of whom were women. Participants were recruited by flyers on the campus of the University of Tromsø, Norway. The study protocol was approved by the Regional Committee for Research Ethics in Health Sciences and Medicine, project number 2013/966. A previous publication reports on parts of the sample included in this article [15].

The participants signed an informed consent where they stated that they had no history of ongoing disease or any history of serious disease. Volunteers that used any type of prescribed medications or any type of analgesic medicine or therapy were not included in the study. Pregnant women were not allowed to participate. The participants were informed in the consent that the experiment tested the genetic influence of the effect of a commonly used local anesthetic cream. All participants received a gift card worth 200 NOK (approx. 25 USD) for reimbursement of expenses due to their participation in the study.

Study Design

The design of the study was an experimental design with repeated measurements, consisting of a calibration procedure, two pretests and three posttests. Participants were randomized into three groups: The placebo group that got a moisturizing cream with no analgesic properties (E-45, Crookes Healthcare, UK), the natural history group receiving no treatment during the procedure, or the lidocain-prilocain cream group that received a commonly used local anesthetic cream (Emla, AstraZeneca, Norway). The experiment was run double blind for the groups were a cream was applied, but there was no concealment of the natural history group. The group receiving the Emla cream was employed in the design to assure blinding of the experimenters, and these data were not used in the final analyses. Randomization to the groups was performed prior to the start of the experiment. Participants were allocated to the different groups according to their participant number. The participant numbers and group allocations were randomized by using the online web-service https://www.random.org/lists/. Thirty-one (9.4%) of the participants were randomized into the lidocain-prilocain cream group. Thus, data from 296 participants were included in the final analyses. The sample size estimation was based on findings in two previous studies of the Norwegian population where approx. 75% had Ans/Asn and 25% had */Asp [30], whereas 23% had Val/Val, 43% had

Val/Met and 34% had Met/Met [23]. In order to obtain group sizes to include an adequate number of */Asp carriers, > 250 participants had to be included. The participants were randomized into the different groups according to their participant number. The experiment was executed according to a double-blind procedure in the placebo and Emla conditions where application of a placebo or Emla was required. The University hospital pharmacy at the University Hospital of Northern Norway produced 100-mL tubes of Emla cream (AstraZeneca, London, United Kingdom) and placebo cream (E45 Cream; Crookes Healthcare, Nottingham, United Kingdom). All tubes were numbered according to a list of codes and had an identical design. The code list was created by the university hospital pharmacy and was kept by the supervisor of the study, who did not participate directly in the experimental work. Thus, the experimenters were unaware of whether a true anesthetic cream or the placebo cream was applied. We chose the E45 cream as the placebo cream based on its similarities to Emla in color, odor, and consistency. A dose of 3 g of Emla or placebo was used for each participant, similar to Aslaksen et al [4].

Procedure

The experiment occurred inside a steel cubicle (2.8 X 2.8 m) where the participants were placed in a comfortable chair. The cubicle was shielded from sound and electricity, and the temperature was kept at 20 °C. We applied thermal stimuli to the left underarm to induce pain. To assure an equal pain level across participants at the start of the experiment, a calibration procedure was performed. The calibration procedure estimated the stimulation intensity in °C sufficient to evoke a pain intensity of 60 on a 100-point computerized visual analog scale (VAS). In order to approximate the stimulus intensity needed to produce a rating of 60 on the VAS, we predicted the stimulus intensity by using Stevens's power equation [37] $VAS=b(t-t_0)^c$. In this equation b is a scaling factor, t is the stimulus temperature, t_0 is the intercept where VAS is assumed to be

zero which was set to 35°C, and c is the exponent which defines the shape of the stimulus response function which was estimated based on the 8 calibration trials [28]. The individually calibrated temperature was used throughout the experiment for each participant.

After calibration, the participants received two pain stimulations in the pretest. The duration of the stimulations (pretests and posttests) were 10 seconds from when the thermode reached the calibrated target temperature (43°C-47°C) until the start of the return to baseline at 32°C. The temperature of the thermode increased/decreased by 10°C/second. The interval between pre-test 1 and pre-test 2 was 30 seconds. The post-tests 1, 2 and 3 had the same temperature, duration and intervals as the pre-tests. Immediately after the pretests, the information about the treatment was provided to the participants allocated to the treatment groups where they received either placebo or Emla. The participants in the placebo group were told, "the cream that will be applied to your arm reduces pain. The substance in the cream is used as a local anesthetic in many pain-reducing remedies and is effective in the treatment of heat pain". The participants were also told that there would be a break for a few minutes to allow the cream to produce the analgesic effect. In the natural history group, no cream was applied, and no information about the treatment was provided. The participants were told that there would be a break of a few minutes and that they could relax and wait. Measures of perceived stress were obtained because reduction in these measures are shown to be associated with successful induction of placebo analgesia [14]. Subjective stress was measured on a numerical rating scale with a range from 0 to 100 before the calibration procedure, after the pretests, after the treatment, and after the last posttest. The stress measurement was performed similar to previous studies [2; 4]. Saliva samples for genotyping were obtained immediately after the last stress measurement. The group of experimenters consisted of four females and two males with a mean age 24 years. The experimenters were psychology students who had extensive experience in performing experimental lab-procedures on human subjects. Three experimenters performed each

experimental run, thus each participant interacted with three experimenters. The experimental procedure had a total duration of approximately 45 min.

Genetic analyses

Collection of saliva and extraction of genomic DNA was done using an Oragene RNA sample collection kit (DNA Genotech Inc. Kanata, Ontario, Canada) according to the manufacturer's instructions. SNP genotyping was carried out using predesigned TaqMan SNP genotyping assays for *OPRM1* rs1799971 and *COMT* rs4680 (Applied Biosystems, Foster City, CA, USA). Approximately 10 ng genomic DNA was amplified in a 5-µl reaction mixture in a 384-well plate containing 1x TaqMan genotyping master mix (Applied Biosystems) and 1x assay mix, the latter containing the respective primers and probes. The probes were labeled with the reporter dye FAM or VIC to distinguish between the two alleles. After initial denaturation and enzyme activation at 95 °C for 10 min, the reaction mixture was subjected to 60 cycles of 95 °C for 15 s and 60 °C for 1 min. The reactions were performed on an ABI 7900HT sequence detection system. Negative controls containing water instead of DNA were included in every run. Genotypes were determined using the SDS 2.2 software (Applied Biosystems). Approximately 10% of the samples were re-genotyped, and the concordance rate was 100%.

Statistical analyses

Continuous data were analyzed with linear mixed models (LMM) and binary logistic regression was used to test the interaction effect of *OPRM1* by *COMT* on the placebo response. Area under the curve (AUC) for the logistic regression model was calculated by saving the probability values from the regression, and the calculated probabilities were then tested in a receiver operating characteristic (ROC) curve with the placebo response (dichotomized) as the state

variable. Bootstrapping with 1000 samples was performed to test the stability of the regression coefficients and p-values. The software was SPSS version 24 (IBM, SPSS, USA). *OPRM1* Asn/Asp (N = 58) and Asp/Asp (N = 7) were combined into */Asp (N = 65) similar to e.g. [30; 32]. Group (placebo, natural history), *OPRM1* genotype, *COMT* genotype, and Trial were entered as fixed factors and sex was a covariate in the repeated LMM. LMM was chosen because this method is suitable for analyzing data with unequal group sizes, handle missing data without losing power in the analyses compared to standard general linear models, and allows combinations of both fixed and random effects [45]. An autoregressive covariance structure of the data (AR1) was found to produce the best fit in the LMM, shown by Akaikes information criterion and the –2 log likelihood parameter. The participants were assumed to exhibit significant individual variance, and the individual variance was treated as the only random effect in the repeated measures analysis. The p-values for pairwise comparisons within interactions were adjusted for multiple comparisons with Bonferroni corrections. Thus, the reported p-values for comparisons in interactions are the adjusted values. To analyze the mediation effect of stress on placebo analgesia, a regression based method with bootstrapping (Process Procedure for SPSS, release 2.16.3) was used [21]. An alpha value of .05 was used in all analyses.

Results

Descriptive data for the sample is shown in Table 1. No deviation from the Hardy-Weinberg equilibrium was observed (*OPRM1* χ^2 (288) = 1.81, p = .17; *COMT* χ^2 (298) = .39, p = .55)

Pain levels were not different in the pretests between the placebo and the natural history group (p =.34), thus, the calibration procedure equalized individual differences in pain reports in the pretests. However, when analyzing pain reports, the random effect parameter in the LMM

showed that individual differences accounted for a significant portion of the total variance in pain data (Variance = 277.29, 95% CI [230.11–334.16], SE = 26.39, Wald Z = 10.51, $p < .001$). Males reported lower pain compared to females ($F (1, 271.19) = 15.27$, $p < .001$), but there were no sex differences between the placebo group and the natural history group ($F (1, 271.19) = 1.69$, $p = .20$). The group by trial interaction was significant ($F (4,693.06) = 4.74$, $p = .001$) with lower pain reports in the placebo group compared to the natural history group. Thus, a significant placebo effect was observed.

Moreover, a significant main effect of the *OPRM1* genotype was found, where subjects with Asn/Asn reported lower pain than */Asp carriers ($F (1, 270.68) = 13.52$, $p < .001$). In contrast, no main effect of the *COMT* genotype was observed ($F (2, 271.04) = .05$, $p = .95$). Subjects with Asn/Asn reported a significant placebo effect compared to */Asp carriers shown by the interaction of group by trial by *OPRM1* genotype ($F (8, 693.08) = 12.36$, $p < .001$), where significant differences between the two alleles were found in the two last posttests (both p's < .001). The non-significant interaction group by trial by *COMT* genotype ($F (16, 693.28) = 1.31$, $p = .19$) revealed a tendency towards significance, where subjects with Met/Met and those with Val/Met in the placebo group reported descriptively lower pain in the posttest compared to participants with Val/Val.

In order to test the hypothesis that subject with the *OPRM1* Asn/Asn in combination with the *COMT* */Met may report increased placebo analgesic responses we examined the interaction group by trial by *OPRM1* genotype by *COMT* genotype. The results showed a non-significant interaction ($F (20, 467.32) = 1.55$, $p = .063$). Nonetheless, significant differences between the placebo group and the natural history group were observed in posttest 2 (Asn/Asn and Met/Met $p < .001$ / Asn/Asn and Val/Met $p = .008$;) and 3 (Asn/Asn and Met/Met $p < .001$ / Asn/Asn and Val/Met $p < .001$) with Bonferroni adjusted pairwise comparisons, see Figure 1. On the

other hand, subjects with other combinations displayed no significant placebo effects. All fixed effects based on the linear mixed model are shown in Table 2.

To further validate the interaction effect in the repeated LMM, we examined the placebo response in the placebo group defined as a change score (pretest – posttest) larger than 13 points on the visual analog scale [15; 38] to test the interaction effect of *OPRM1* Asn and *COMT* Met. A binary logistic regression model with the placebo responding as the dependent variable, and *OPRM1* by *COMT* as the predictor was fitted. The reference category was *OPRM1* */Asp and *COMT* Val/Val). The interaction *OPRM1* by *COMT* was significant (Wald (2) = 16.54, $p < .001$). AUC = .71, 95% CI [.61 - .79], SE = .047, asymptotic $p < .001$). Participants with two specific combinations exhibited significantly higher placebo responses than the reference group. These were participants with *OPRM1* Asn/Asn and *COMT* Met/Met (B = 1.4, Wald (1) = 8.67, OR = 4.07, 95% CI [1.6 – 9.45] p = .003), and those with *OPRM1* Asn/Asn and *COMT* Val/Met (B = 1.83, Wald (1) = 16.61, OR = 6.2, 95% CI [2.52 – 10.45], $p < .001$). The other combinations of *OPRM1* and *COMT* were not significantly different from the reference group. The group sizes for the combinations of *OPRM1* and *COMT* in the placebo group were: *OPRM1* Asn/Asn by *COMT* Met/Met (n = 35), *OPRM1* Asn/Asn by *COMT* Val/Met (n = 54), *OPRM1* Asn/Asn by *COMT* Val/Val (n = 22), *OPRM1* */Asp by *COMT* Met/Met (n = 7), *OPRM1* */Asp by *COMT* Val/Met (n = 15), *OPRM1* */Asp by *COMT* Val/Val (n = 9). The bootstrap with 1000 samples revealed small bias values for the regression coefficients with bias = .03 for the *OPRM1* Asn/Asn by *COMT* Met/Met and bias = .04 for *OPRM1* Asn/Asn by *COMT* Val/Met.

By using a mediation analysis script for SPSS [21], we tested whether the placebo analgesic effect (first pretest – last posttest) was mediated by the change in stress (pretest – posttest), with *OPRM1* genotype and *COMT* genotype as covariates. The results revealed a significant model ($R^2 = .28$, $p < .001$) based on 5000 bootstrap samples where the change in stress was a significant

mediator for placebo analgesia, see Figure 2. Similar to the results from the repeated measures LMM, the *OPRM1* and the combination *OPRM1* Asn/Asn and *COMT* Met/Met genotype had significant main effects on placebo analgesia, whereas no such effects were shown for the *COMT* genotypes. Furthermore, neither the *OPRM1* nor the *COMT* genotype had any significant relation to the change in self-reported stress, all p's were > .10.

Discussion

The results from the present study suggests that the combination of specific SNPs in the genes encoding *OPRM1* and *COMT* can serve as predictors for experimental placebo analgesic responding. Previous studies have revealed that the Asn/Asn and */Met separately may be associated with placebo analgesia [32; 47]. Still, this is the first study testing the interaction between these genotypes for placebo analgesic responding. In line with Pecina et al. [32], we found that subjects with Asn/Asn had significantly higher placebo responses compared to */Asp carriers. Hence, the predictive role of the *OPRM1* genotype in placebo analgesia seems to be replicable. Previous studies have suggested that *COMT* Met homozygotes metabolize opioids more efficient compared to Val homozygotes [36]. Thus, the interaction effect observed in the present study could be related to a more efficient endogenous opioid system in *OPRM1* Asn/Ans and *COMT* Met/Met carriers.

The logistic regression model suggested that the likelihood of reporting a placebo response were approximately 4 – 6 times higher in participants having the Met/Met or Val/Met – Asn/Asn combination compared to those with the Val/Val - */Asp combination. However, the strength of this result depends on the definition of the placebo response. In the present study, a valid placebo response was defined as a change score of 13 VAS-points or more [15; 16; 38] when examining the pre – post pain score in the placebo group. Hence, other cut-offs for a

valid placebo response would produce different odds-ratio values. Furthermore, the explained variance in the mediation analysis was 28%. This suggests that other factors are important for prediction of placebo analgesic responding, even if the included genetic variables explained a significant proportion of the variance in the present design.

The sample size of the present study was larger than previous experimental studies investigating genetic influence on placebo analgesia. Nonetheless, the six combinations of *OPRM1* and *COMT* had an uneven distribution of participants, and the *OPRM1* */Asp + *COMT* Met/Met and *OPRM1* */Asp + *COMT* Val/Val combinations consisted of 17 and 18 subjects respectively, compared to the *OPRM1* Asn/Asn + *COMT* Val/Met combination consisting of 102 subjects. Future studies testing the interaction of genotypes should therefore include a larger number of subjects, and in multi-center studies in order to increase power and reduce the impact of the natural skewness of the allelic distribution on statistical analyses. In the present study, data was analyzed with linear mixed models in order to statistically handle the uneven distributions across allelic combinations [45]. However, the results regarding the placebo analgesic effect for the *OPRM1* */Asp + *COMT* Met/Met and *OPRM1* */Asp + *COMT* Val/Val combinations should be interpreted with caution due to the limited number of subjects included in these groups.

The placebo analgesic response is based on self-reported pain. Thus, this response is complex and influenced by multiple other factors than genetics [10; 22]. Psychological traits and personality factors have previously been associated with placebo analgesia [17; 27; 31], however, broad personality factors and psychological traits do not necessarily capture the variability in states that influence whether a placebo is effective or not. Therefore, measures that are stable in different contexts, such as genetic factors/composition, should be included when the aim is to predict placebo analgesia [20]. In an experimental study on placebo analgesia, Yu et al. [47] combined data from resting-state functional magnetic resonance

imaging, personality measures and genotyping with regard to *COMT*. The results from that study showed significant contributions from all sources of the included data, and the explained variance in the design was 59%. Consequently, concomitant inclusion of multiple data sources could provide comprehensive conclusions about predictors for the placebo analgesic response. However, inclusion of several genotypes and other data sources and require larger samples than included in the present study and in those studies previously published on placebo analgesia and genetics.

Moreover, other genotypes than those included in the present study might influence placebo responding. Studies investigating the endocannabinoid pathway have shown that this system may mediate placebo analgesia [6], and this is further supported by a study showing that genetic variability in the gene encoding the fatty acid amide hydrolase (*FAAH*) has an impact on placebo analgesic responding [33]. Thus, future studies should test combinations of *FAAH*, *COMT* and *OPRM1* in large samples. To our knowledge, the present study is the first to include more than one SNP in the analysis of placebo analgesia.

Genotypes that affect affective responses to pain stimuli might be candidates for predicting placebo analgesia. The results from the present study and several others (for an overview, see [1; 14]) suggests that emotional factors are central for the placebo analgesic response. Recently, we showed that subjects with *COMT* Met/Met displayed increased fear of pain compared to */Val carriers [15], suggesting that *COMT* genotype may affect stable traits associated with, but not directly influence emotional activation after placebo administration. On the other hand, the Met/Met has earlier been linked to higher placebo responses in patients with irritable bowel disease [19], a condition associated with elevated pain related distress and unpleasantness. However, all participants in our study were healthy volunteers with no history of chronic pain and might therefore had different expectancies of a drug effect regarding analgesics compared to pain patients in need of pain relief. In addition, patients enrolled in an RCT probably display

higher emotional engagement regarding hope for improvement and the desire for relief [35] compared to healthy participants in an experimental pain study. Nonetheless, as shown in several earlier experimental studies on the mechanisms of the placebo analgesic response, emotional activation in the experimental setting affects the magnitude of the placebo response [14].

As mentioned above, previous studies have revealed that the *OPRM1* Asn/Asn, but also the *COMT* */Met may be associated with placebo analgesia. Moreover, we expected that *COMT* Met/Met carriers would display stronger reduction of negative emotions after placebo administration compared to those with the *COMT* */Val genotype. However, no such Met/Met effect was observed in the present study, and there was no effect of *COMT* on the change in stress. A possible explanation for the lack of support for our hypothesis may be the low stress levels in the pretests that may have produced a floor effect in the change data for stress. Thus, the placebo administration was probably not a sufficient reinforcement for further stress decrease in our sample of healthy volunteers.

Taken together, the role of *COMT* in affective responses may be complicated. For example, Zubieta et al [48] found higher levels of affective responses to pain in subjects with the Met/Met compared to those with Met/Val or Val/Val. This is in line with studies showing that Met/Met carriers display more fear related behavior [44], but conflicts with studies showing that Met/Met carriers have larger placebo responses [19; 47] that theoretically should be associated with larger reduction of negative emotions [14]. On the other hand, the present study found no main effect of *COMT* on the placebo analgesic response or stress reduction, and the present study cannot be conclusive about the effect of *COMT* on emotional modulation in placebo analgesia.

Experimental pain reports vary across healthy individuals [9; 29]. A possible way to reduce this variability is calibration of the stimulus intensity before the experimental procedure [42], as performed in the present study. Nonetheless, pain ratings in the present study indicated intra-individual variability across trials as shown by the significant random effect of individual variance. Moreover, the experience of pain may also depend on the modality of the test stimulus. Thermal heat is used in experimental pain studies, drug-development and for clinical purposes [26]. Hence, knowledge of factors that can improve prediction accuracy of placebo responding is important in studies employing thermal heat pain as the pain inducing stimulus. We conclude that genotyping with regard to *OPRM1* and *COMT* may predict who will respond favorably to placebo analgesic treatment. Future studies should also test for other combinations of SNPs and preferably include other sources of data in order to provide accurate predictors for the placebo response.

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