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Serum correlates of the placebo effect in Irritable Bowel Syndrome

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Abstract

In diseases defined primarily by the subjective nature of patient self-report, placebo effects can overwhelm the capacity of randomized controlled trials to detect medication-placebo differences. Moreover, it is unclear whether such placebo effects represent genuine psychobiological phenomena or just shifts in selective attention. Knowledge of predictors of the placebo response could improve the design of clinical trials and the delivery of personalized medical care. Irritable bowel syndrome (IBS) represents an illness model with high placebo rates. In a subset of IBS patients of our previous study that were randomized to placebo treatment (sham acupuncture) or no-treatment group (waitlist), we tested an enriched panel of 10 serum biomarkers at the enrollment and the 3rd week of intervention, using a multiplex electrochemiluminescent immunoassay. More pronounced changes overtime in serum levels of osteoprotegerin (OPG) have been found in patients who received placebo treatment compared to waitlist group ($p=0.039$). Moreover, serum levels of OPG at baseline were found to be higher ($p=0.0167$) in patients who subsequently achieved adequate relief (AR) of their IBS symptoms, independently of their treatment group. Besides, serum levels of TNF-related weak inducer of apoptosis (TWEAK) at baseline were also higher ($p=0.0144$) in patients who reported AR and in particular in those who received the placebo treatment. These two measurable biological parameters associated with placebo, namely serum OPG and TWEAK, provide a proof of principle for discovering putative molecular signatures of placebo response in IBS and perhaps in other illnesses with patient self-reported outcomes.

Keywords

placebo; irritable bowel syndrome; serum; biomarkers; randomized controlled trials

Introduction

Evidence based medicine relies on placebo controlled randomized clinical trials (RCTs) to distinguish the effects of an active pharmacological agent or procedure from the effects of a mimicking placebo treatment.¹ Especially in illnesses defined by patient self-reported outcomes, the placebo response can be substantial and make detection of a medication-

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placebo difference problematic. For example, a review of files from the Food and Drug Administration reported that many known pharmaceutically active medications that have FDA approval-including analgesics, anxiolytics, β -blockers, antidepressants and gastrointestinal motility-modifying drugs-are frequently indistinguishable from placebo in well-designed RCTs.² Given the prevalence and validation of the placebo-controlled RCT model, as described in a 2007 report in the Journal of American Medical Association,³ 36,249 trials from 140 countries have been registered with ClinicalTrials.gov. Being able to predict whether some patients are especially responsive to placebo treatment, based on a well-characterized set of biomarkers, could have a tremendous scientific and economical impact on the successful design and execution of clinical trials. Moreover, at the level of personalized medicine, this knowledge might help with medication selection, dosage adjustment and reduction of side effects.

In recent years, our understanding of the neurobiological mechanisms of the placebo effect has been greatly advanced, but only for particular disease paradigms, including placebo analgesia, Parkinson's disease, depression and various autonomic processes.⁴⁻⁶ Well-controlled studies, using quite sophisticated approaches, have demonstrated that psychosocial cues such as conditioning and expectation elicit quantifiable changes in neurotransmitters, hormones, immune regulators and regionally specific brain activity⁷⁻¹¹. It is believed that these changes could influence disease experience and response to treatment via plausible physiological mechanisms that are most likely disease-specific.⁶ Placebo research has been primarily performed in controlled experimental settings involving durations of rarely more than a few minutes and with research subjects being primarily healthy volunteers. Often the experimental manipulations used to induce placebo effects had little bearing to clinical practice and involved deception. Little research has been performed in chronically ill patients over significant time periods in settings that closely resemble clinical care. Given this shortcoming, we decided to prospectively investigate placebo responses in the relatively long term situation of a randomized controlled trial. We have chosen irritable bowel syndrome (IBS) as our disease model, because numerous RCTs have reported high rates of placebo response in this illness.^{12, 13} It is still unknown whether the placebo response in IBS RCTs (and most other illness with subjective self-rated outcomes) represents a psychobiological phenomenon or purely shifts in selective attention to diffuse symptoms.¹⁴⁻¹⁶ Furthermore, the bulk of information we have concerning placebo effects in chronic diseases like IBS is derived from RCTs, and is usually confounded by the absence of no-treatment controls.¹⁷ Without such controls, the response detected in the placebo arm of an RCT encompasses the "genuine" placebo effect of a pill or procedure and non-specific effects originating from spontaneous remission due to the natural course of the illness, measurement artifacts and the statistical phenomenon of regression to the mean.^{1, 17}

Factors reported to impact placebo responses in IBS include patient and health care provider characteristics, the particular treatment being applied and the experimental setting.¹³ It has been reported that emotion and expectation are central to the effects of placebo in various disease paradigms, including IBS.^{14, 15} Another study identifies frequency of the intervention as a predictor of placebo responses in IBS patients.¹⁸ However, to our knowledge, biological predictors in the placebo response in IBS have not yet been reported. Furthermore, IBS serves as a strong model for exploring biological changes over time in response to placebo treatment because it is considered by many an archetypical "mind-body" illness mediated by complex neuroimmune and neuroendocrine interactions.^{16, 19} Psychological stress is frequently reported to be the cause of both initial presentation and exacerbation of IBS symptoms.^{20, 21} Moreover, psychosocial factors such as history of emotional or physical abuse, loss of a loved one, social stress and maladaptive coping mechanisms influence the illness experience and treatment outcome in IBS.^{22, 23} Additionally, chronic stress has been reported to alter immune function and cytokine

production in many disease paradigms²⁴. For example, negative emotions and anxiety result in the up-regulation of serum interleukin (IL)-6, which in turn further activates the hypothalamic-pituitary-adrenal axis and enhances the release of catecholamines and glucocorticoids. Indeed, increased levels of cortisol, IL-6 and IL-8 have been described in the serum of IBS patients.²⁵ Among the putative biological mediators of stress in IBS, infusion of corticotrophin-releasing factor has been shown to elicit more pain and produce a greater number of descending colon contractions in IBS patients as compared to controls.^{24, 26}

Recently, our team completed a RCT in IBS patients that demonstrated statistically and clinically significant responses to a three week placebo treatment (sham acupuncture), with 53% of patients reporting improvement on the validated IBS outcome of adequate relief (IBS-AR) vs 28% in the waitlist group.²⁷ Expanding the findings of this study and in order to capture the individual sensitivity to placebo response we focus here on identifying serum biomarkers that may predict who is most or least likely to benefit from a placebo response. Furthermore, we sought to generate hypotheses for the mechanisms of placebo effect in IBS by investigating the biological changes of serum markers over time in patients that self-report improvements in their symptoms. Based on previously reported evidence, we targeted 10 pre-identified serum biomarkers, the majority of them proinflammatory cytokines, that best differentiated patients with IBS from other gastrointestinal pathologies and healthy controls²⁸. We reasoned that perhaps some of the inflammatory mediators that might be implicated in the pathogenesis of IBS, might be also correlated to, or affected by, the placebo response in the same patients.

Patients and Methods

Participants-Study population

Participants in this study have been included in our previous single-blinded randomized placebo-controlled trial that was prospectively designed to investigate placebo effects in patients with IBS, and importantly included a no-treatment (waitlist) control group. Briefly, we conducted a three-week single blind randomized clinical trial of 262 patients, ≥ 18 years old (75% women) diagnosed by Rome II criteria for IBS and with a score of ≥ 150 on the IBS Symptom Severity Scale (IBS-SSS).²⁷ Patients with IBS (N=262) were randomized to one of three arms: (a) waitlist, (b) placebo acupuncture ("limited"), or (c) placebo acupuncture plus a supportive patient-practitioner relationship ("augmented"). The placebo treatment was delivered using a validated sham acupuncture device, in twenty minute sessions, twice per week for three weeks.²⁹ During the placebo treatment, the patient sees and feels the placebo acupuncture needle appear to pierce the skin, but unbeknownst to the patient the needle retracts into a hollow tube as it is pressed against the skin. Six to eight placebo needles were placed for twenty minutes over predetermined, non-acupuncture points on the arms, legs, and abdomen. The details and outcomes of the design of the clinical trial are summarized elsewhere.^{27, 29} IBS patients who received either of the two conditions of placebo treatment in the RCT (limited or augmented), were collapsed into one group (placebo) in order to increase statistical power. Thus, patients were finally grouped, to those that received a placebo treatment (sham acupuncture) and to those that received no-treatment (waitlist). Patients in the waitlist served as the control group.

Questionnaires

Questionnaires were administered to the patients at enrollment and at the conclusion of the three-week intervention to assess baseline patient and disease characteristics and changes in symptom severity and psychopathology. IBS-Adequate Relief (IBS-AR) is a self-reported yes/no answer to the question "In the past 7 days have you had adequate relief of your IBS

pain and discomfort?”.³⁰ IBS-AR was used to define the response to placebo treatment, as the primary clinical outcome at week three of the intervention. IBS-Quality of Life (IBS-QOL) is a 35-item scale designed to assess the impact of IBS on eight dimensions of health status. IBS Symptom Severity Scale (IBS-SSS) is a validated integrative symptom questionnaire that addresses severity of a representative group of five symptoms in IBS.³⁰ Anxiety was measured with the Beck Anxiety Inventory (BAI) and Depression was measured using the Maier subscale of the Carroll Depression Scale. Symptom check list (SCL)-90 is a 90 item measure of nine dimensions of psychological distress, including somatization, anger and phobias.

Biomarker analysis

All available sera, that were collected and stored from patients at baseline and at the end of the three-week treatment period, were assayed simultaneously in a multiplexing format on an electrochemiluminescence platform (Meso Scale Discovery (MSD), Gaithersburg, MD). The following analytes, which have been previously linked to intestinal inflammatory processes,²⁸ were included in the panel: Interleukin-1 β (IL-1 β), Macrophage inflammatory protein-3 β (MIP-3 β), Growth-regulated oncogene- α (GRO- α), Tumour necrosis factor-related weak inducer of apoptosis (TWEAK), Neutrophil gelatinase-associated lipocalin (NGAL), Osteoprotegerin (OPG); growth factors: Vascular endothelial growth factor (VEGF), Brain-derived neurotrophic factor (BDNF); and tissue remodeling proteins: Metalloproteinase-9 (MMP-9), and Tissue inhibitor of metalloproteinase-1 (TIMP-1). These 10 blood biomarkers are part of a recently developed diagnostic algorithm aiming to differentiate IBS patients from patients with non-IBS gastrointestinal diseases, and healthy volunteers. A recently published report describes the exact identification process for selecting those biomarkers.²⁸ Briefly, first the authors identified biological pathways implicated in digestive diseases (over 600 pathways/60000 biomarkers); then they identifying biomarkers common across multiple pathways (approximately 2000 biomarkers). From those they selected potential blood-based biomarkers to differentiate IBS from non-IBS (approximately 250 biomarkers), of which 140 biomarkers could be measured using commercially available assays. Subsequently, among them they selected 10 based on results from further testing IBS vs non-IBS samples. The resulting diagnostic test was shown to have a sensitivity of 50% and 88% specificity for IBS.²⁸

Statistical Analysis

Changes in serum biomarkers between the two study arms (placebo treatment vs waitlist control) were evaluated by Man-Whitney U-test. A two tailed t-test Student's *t*-test was used to determine differences between mean values and a chi-square test of independence for proportions was used when comparing differences between responders and non-responders. Potential associations between a particular serum biomarker and patient or disease characteristics were addressed by Spearman correlation tests. Since the current study was hypothesis generating, we did not apply Bonferonni/Dunn correction for multiple comparisons to any of our analyses. Results are expressed as mean $\pm$ SD. A $p < 0.05$ was considered to be statistically significant. Data were analyzed using SPSS version 17.

Results

Changes in serum biomarkers overtime as a result of a placebo treatment in patients with IBS

A total of 130 IBS patients who had complete clinical and serum biomarker data, both at baseline and at week 3, were included in the analysis looking for changes in serum biomarkers overtime as a result of the placebo treatment. Patient characteristics are presented in Table 1. At the baseline, there was no difference comparing demographics and

disease characteristics in patients randomized to the placebo treatment vs. waitlist control. Serum biomarker levels also did not differ at baseline between the waitlist and placebo treated patients. At week 3 of the study, changes (value at baseline minus value at week 3) in serum OPG levels were significantly higher in patients who received the placebo treatment compared to those in waitlist ($p=0.039$ by Man–Whitney U-test) (Table 2).

Serum biomarker levels at baseline and clinical improvement based on IBS-AR

A sum of 190 patients who completed the three week intervention and had complete clinical and biomarker data at baseline were included in the comparison of serum biomarker levels between placebo responders and non-responders. Overall, 44% (83/190) of study participants [55% (68/124) in the placebo group and 23% (15/66) in no-treatment control group, $p<0.0001$] reported adequate relief at week three of the intervention, consistent with our previous reports.^{27, 31} In this analysis, such patients are grouped as “placebo responders”, independently of the treatment that they have received. Comparisons of patient demographics and disease characteristics at baseline between placebo responders and non-responders are presented in Table 3. Disease severity measured by IBS-SSS ($p=0.034$) and disease duration ($p=0.036$) were found to be differed between the two groups; however there were no significant differences among the rest of the parameters that we examined.

In addition, we compared baseline levels of serum biomarkers between responders and non-responders. We found that OPG levels were higher in those IBS patients who subsequently reported IBS-AR (563 ± 24 pg/ml vs. 491 ± 18 pg/ml, responders vs. non-responders, respectively; $p=0.0167$), irrespective of the type of intervention that they received (Table 4). Likewise, serum TWEAK levels at baseline, were found to be significantly higher in responders vs. non-responders (1018 ± 34 pg/ml vs. 892 ± 34 pg/ml, responders vs. non-responders, respectively; $p=0.0144$). Furthermore, baseline TWEAK was significantly higher in IBS patients who achieved AR in response to the placebo treatment (1022 ± 41 vs. 871 ± 45 pg/ml, responders vs. non-responders, respectively; $p=0.0157$). As well, patients in the waitlist group who reported AR had at baseline higher levels of serum OPG (610 ± 54 vs. 453 ± 24 pg/ml, responders vs. non-responders, respectively; $p=0.0052$) (Table 4).

Serum biomarker levels at baseline and clinical improvement based on IBS-SSS

IBS patients who received placebo treatment by sham acupuncture had a mean improvement (baseline – week 3) in the IBS-SSS score of 68.3 ± 7.8 points, while patients in wait list had a change of 30.8 ± 7.6 points ($p=0.002$). A positive correlation between serum OPG levels at baseline and clinical improvement based on changes in the IBS-SS score ($R=0.224$, $p=0.0021$ by Spearman correlation test) was evident when all patients were included in the analysis (table 5). In parallel, we examined potential correlations between baseline levels of non-biological predictors of clinical improvement including psychological variables, such as depression, anxiety, somatization; quality of life (QOL), and changes in IBS-SSS score at week 3 of treatment. We found lack of such associations in this particular study. Among the demographic and disease-related parameters that we examined, we found a negative correlation between duration of IBS and clinical improvement (table 5)

Discussion

Although the existence of significant placebo responses (both genuine and non-specific effects) in IBS is well-documented, it remains under debate whether such effects are confined to the brain and represent solely the activation of certain brain circuits that can modulate the cognitive or emotional processing of biological signals.^{10, 14, 32} Our results point to a plausible biological substrate of the placebo effect in patients with IBS. Specifically, we show decrease overtime in the serum levels of OPG in the group of patients

that received the placebo treatment, compared to those in waitlist. Osteoprotegerin (OPG), also known as osteoclastogenesis inhibitory factor or TNFRSF11B, is a secreted glycoprotein and member of the TNF receptor super-family. OPG acts as a decoy receptor for receptor activator of nuclear factor-kappa-B ligand (RANKL/OPGL). In addition to the well documented role of OPG in the regulation of bone resorption, circulating levels of OPG/OPGL have been found to be high in chronic inflammatory and metabolic conditions including rheumatoid arthritis, diabetes, cardiovascular disease and renal failure; the significance of which has just started to emerge. Patients with inflammatory bowel disease have also higher local and systemic levels of OPG and OPGL, respectively, which are normalized in response to treatment.^{33, 34}

As well, we recognized higher levels of OPG and of another serum protein, called TWEAK, in patients who subsequently achieved adequate relief of their IBS symptoms. TWEAK, also a cytokine member of the TNF super-family (TNFSF12), has pleiotropic immunomodulatory effects.³⁵ In at least some cell types it activates pro-inflammatory signaling pathways, including the nuclear factor-kappaB, and is a strong inducer of IL-8. Studies of deficient mice revealed that TWEAK suppresses IFN-gamma and IL-12 production posing a block in the transition of innate immune response to adaptive Th1 immunity.³⁶ Increased serum TWEAK levels have been described in several inflammatory conditions and blocking TWEAK in experimental models of arthritis, encephalomyelitis and colitis resulted in disease attenuation and accelerated tissue repair.³⁷

Higher baseline levels of pro-inflammatory cytokines in patients who subsequently report clinical improvement, as described here, seems somehow counterintuitive. Based on the current analysis and also published reports, IBS patients with more severe disease are less likely to respond to treatment.³¹ However, the more severe cases were also expected to have higher levels of presumably pro-inflammatory cytokines in their sera, given what is known so far about the pathogenesis of IBS.^{25, 38-41} Moreover, we found that the placebo treatment was more effective in reducing serum OPG levels at three weeks compared to the waitlist group. Taken together, these findings suggest that patients with IBS who mount a stronger immune response are more likely to benefit from a placebo treatment.

IBS is often described as a “brain-gut” disorder.^{42, 43} Mind-body interactions, like those taking place during a placebo response, can originate from a regionally specific brain activity and modulate peripheral disease manifestations, such as intestinal visceral sensitivity, motility, secretion, and blood flow.^{44, 45} Potential soluble mediators of such effects include hormones (CRH, ACTH, prolactin, melatonin, vasopressin, oxytocin, somatostatin), neuropeptides (VIP, NPY, endogenous opioids, α MSH) and several cytokines (IL-1 β , IL-6, IL-10, TNF α , IFN γ).⁴⁶⁻⁴⁸ Our biological screening focused on immunological factors based on epidemiologic and laboratory evidence suggesting a certain degree of immune dysregulation in IBS.⁴⁹ In approximately 10% of patients, IBS develops following an episode of viral or bacterial gastroenteritis.³⁸ A reduced frequency of a polymorphism in the IL-10 gene that is associated with lower IL-10 (an anti-inflammatory cytokine) production, and increased frequency of a high TNF α -producing allele has been reported in IBS patients.³⁹ Other studies describe lymphocytic aggregates in the myenteric plexus of patients with severe intractable IBS symptoms; and proximity of mast cells to intestinal nerves correlates with severity of abdominal pain.^{50, 51} Moreover, an altered IL-10/IL-12 balance that favors Th1 responses, and higher secretion of TNF-alpha, IL-1beta, IL-5, IL-6, and IL-13 all pro-inflammatory cytokines, from blood cells has been reported in IBS patients, as well as an abnormal activation of B-cells.^{40, 41, 52, 53}

The concept that placebo works in IBS patients via modulating components of the immune system is not as far-fetched as it may seem. An intriguing possibility is that medical

conditions that involve activation of the innate immune system in their mechanisms of pathogenesis are the ones that are more likely to respond to a placebo treatment.⁵⁴ For example, a placebo ultrasound therapy resulted in a significant reduction of facial swelling and pain along with reductions in serum C-reactive protein, an acute phase protein associated with the inflammatory response.⁵⁵ In further support of the findings of the current study, a role for the immune system in mind-body interactions has been previously documented in another disease paradigm. A study of long-term mindfulness-based stress reduction intervention in patients with early stage breast or prostate cancer resulted in improvements in quality of life and decreased stress symptoms. Those clinical responses were associated with increased IL-4 and decreased IFN γ and IL-10 production from specific T-cell subsets.⁵⁶

An equally important issue is whether the biological predictors of placebo responses are distinct from the ones associated with placebo-like effects that represent forms of non-specific healing such as disease natural history, regression to the mean and report bias. The fact that the potential predictors we identified for those processes belong to the same superfamily (TNF) seems to suggest that they can overlap. Nevertheless, it is quite possible that there might be as many placebo effects as types of disease, each elicited by complex psychobiological interactions.⁶ For example, a recent study of 25 subjects with social anxiety reports an association between a polymorphism in the tryptophan hydroxylase-2 gene and increased susceptibility to a placebo treatment.⁵⁷

The economical burden attributed to IBS, in terms of direct health care costs, resource utilization and loss of productivity is quite significant.⁵⁸ IBS is a chronic disease that affects 10–15% of the population in industrialized countries with no satisfactory treatment so far. These facts emphasize the need for a vigorous testing of new medications and therapeutic modalities. The existence of biological predictors of placebo responses in IBS, as suggested by our findings, provides a potential paradigm shift that has implications for the design of RCTs. One could consider using a set of serum biomarkers as a screening tool to identify IBS patients who are more likely to respond to the placebo and revise inclusion criteria accordingly. This strategy would potentially enhance the feasibility of clinical trials in IBS, substantially reduce their costs and accelerate the discovery of new treatments.

There are several strengths of the current analysis, among them the inclusion of a no-treatment group as the control for the placebo treatment, the study of a large number of patients with a particular disease instead of healthy volunteers, the study of sustained placebo effects over a period of three weeks and collection of data at two different time points. However, there are also several limitations. One of the potential criticisms of the present study is the lack of objective measurements of placebo treatment outcomes, and of IBS itself. Thus, clinical improvement in our analysis is based on a self-reported outcome (IBS-AR). It is quite possible that the introduction of objective clinical endpoints in IBS RCTs might dampen the placebo effect. A novel form of placebo treatment (sham acupuncture) was used in this study, which might elicit higher placebo responses⁵⁹ Furthermore, it is likely that different illnesses have different biological mind-body interactions and thus the current notion supports a “spectrum” of placebo effects.⁶ For example, placebo responses in pain conditions often involve endogenous opioid release and activation of rostral anterior cingulate and nucleus accumbens⁶⁰, while in Parkinson's disease placebo responses have been associated with dopamine release in the striatum⁴. Hence, it is unclear at this point whether the specific biomarkers that we identified in the present analysis would generalize to other forms of placebo treatment (such as pills) or to additional diseases known to be associated, like IBS, with high placebo responses.²

In summary, the design of this study and its robust outcome provide the first, to our knowledge, evidence that genuine placebo effect in IBS results in measurable changes in the serum proteome, beyond non-specific effects (i.e., disease natural history, regression to the mean and report bias). From a public health perspective, our findings represent a proof-of-principle for identifying, using high throughput screening approaches and advanced bioinformatics tools, biological signatures associated with placebo responses in clinical trials.

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References

1. Kaptchuk TJ. Powerful placebo: the dark side of the randomised controlled trial. *Lancet*. 1998; 351:1722–5. [PubMed: 9734904]
2. Temple R, Ellenberg SS. Placebo-controlled trials and active-control trials in the evaluation of new treatments. Part 1: ethical and scientific issues. *Ann Intern Med*. 2000; 133:455–63. [PubMed: 10975964]
3. Zarin DA, Ide NC, Tse T, Harlan WR, West JC, Lindberg DA. Issues in the registration of clinical trials. *Jama*. 2007; 297:2112–20. [PubMed: 17507347]
4. de la Fuente-Fernandez R, Ruth TJ, Sossi V, Schulzer M, Calne DB, Stoessl AJ. Expectation and dopamine release: mechanism of the placebo effect in Parkinson's disease. *Science*. 2001; 293:1164–6. [PubMed: 11498597]
5. Petrovic P, Kalso E, Petersson KM, Ingvar M. Placebo and opioid analgesia-- imaging a shared neuronal network. *Science*. 2002; 295:1737–40. [PubMed: 11834781]
6. Benedetti F. Mechanisms of placebo and placebo-related effects across diseases and treatments. *Annu Rev Pharmacol Toxicol*. 2008; 48:33–60. [PubMed: 17666008]
7. Zubieta JK, Stohler CS. Neurobiological mechanisms of placebo responses. *Ann N Y Acad Sci*. 2009; 1156:198–210. [PubMed: 19338509]
8. Klosterhalfen S, Enck P. Neurophysiology and psychobiology of the placebo response. *Curr Opin Psychiatry*. 2008; 21:189–95. [PubMed: 18332669]
9. Price DD, Finniss DG, Benedetti F. A comprehensive review of the placebo effect: recent advances and current thought. *Annu Rev Psychol*. 2008; 59:565–90. [PubMed: 17550344]
10. Enck P, Benedetti F, Schedlowski M. New insights into the placebo and nocebo responses. *Neuron*. 2008; 59:195–206. [PubMed: 18667148]
11. Wager TD, Rilling JK, Smith EE, Sokolik A, Casey KL, Davidson RJ, Kosslyn SM, Rose RM, Cohen JD. Placebo-induced changes in FMRI in the anticipation and experience of pain. *Science*. 2004; 303:1162–7. [PubMed: 14976306]
12. Dorn SD, Kaptchuk TJ, Park JB, Nguyen LT, Canenguez K, Nam BH, Woods KB, Conboy LA, Stason WB, Lembo AJ. A meta-analysis of the placebo response in complementary and alternative medicine trials of irritable bowel syndrome. *Neurogastroenterol Motil*. 2007; 19:630–7. [PubMed: 17640177]
13. Bernstein CN. The placebo effect for gastroenterology: tool or torment. *Clin Gastroenterol Hepatol*. 2006; 4:1302–8. [PubMed: 17081807]
14. Price DD, Craggs J, Verne GN, Perlstein WM, Robinson ME. Placebo analgesia is accompanied by large reductions in pain-related brain activity in irritable bowel syndrome patients. *Pain*. 2007; 127:63–72. [PubMed: 16963184]

15. Vase L, Robinson ME, Verne GN, Price DD. The contributions of suggestion, desire, and expectation to placebo effects in irritable bowel syndrome patients. An empirical investigation. *Pain*. 2003; 105:17–25. [PubMed: 14499416]
16. Mayer EA, Naliboff BD, Chang L. Basic pathophysiologic mechanisms in irritable bowel syndrome. *Dig Dis*. 2001; 19:212–8. [PubMed: 11752839]
17. Hrobjartsson A, Gotzsche PC. Is the placebo powerless? An analysis of clinical trials comparing placebo with no treatment. *N Engl J Med*. 2001; 344:1594–602. [PubMed: 11372012]
18. Pitz M, Cheang M, Bernstein CN. Defining the predictors of the placebo response in irritable bowel syndrome. *Clin Gastroenterol Hepatol*. 2005; 3:237–47. [PubMed: 15765443]
19. Arebi N, Gurmany S, Bullas D, Hobson A, Stagg A, Kamm M. Review article: the psychoneuroimmunology of irritable bowel syndrome--an exploration of interactions between psychological, neurological and immunological observations. *Aliment Pharmacol Ther*. 2008; 28:830–40. [PubMed: 18637004]
20. Talley NJ, Weaver AL, Zinsmeister AR, Melton LJ 3rd. Onset and disappearance of gastrointestinal symptoms and functional gastrointestinal disorders. *Am J Epidemiol*. 1992; 136:165–77. [PubMed: 1415139]
21. Mayer EA. The neurobiology of stress and gastrointestinal disease. *Gut*. 2000; 47:861–9. [PubMed: 11076888]
22. Nicholl BI, Halder SL, Macfarlane GJ, Thompson DG, O'Brien S, Musleh M, McBeth J. Psychosocial risk markers for new onset irritable bowel syndrome--results of a large prospective population-based study. *Pain*. 2008; 137:147–55. [PubMed: 17928145]
23. Jarcho JM, Chang L, Berman SM, Suyenobu B, Naliboff BD, Lieberman MD, Ameen VZ, Mandelkern MA, Mayer EA. Neural and psychological predictors of treatment response in irritable bowel syndrome patients with a 5-HT(3) receptor antagonist - a pilot study. *Aliment Pharmacol Ther*. 2008
24. Tache Y, Martinez V, Million M, Rivier J. Corticotropin-releasing factor and the brain-gut motor response to stress. *Can J Gastroenterol*. 1999; 13 A:18A–25A.
25. Dinan TG, Quigley EM, Ahmed SM, Scully P, O'Brien S, O'Mahony L, O'Mahony S, Shanahan F, Keeling PW. Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? *Gastroenterology*. 2006; 130:304–11. [PubMed: 16472586]
26. Fukudo S. Role of corticotropin-releasing hormone in irritable bowel syndrome and intestinal inflammation. *J Gastroenterol*. 2007; 42 17:48–51. [PubMed: 17238026]
27. Kaptchuk TJ, Kelley JM, Conboy LA, Davis RB, Kerr CE, Jacobson EE, Kirsch I, Schyner RN, Nam BH, Nguyen LT, Park M, Rivers AL, McManus C, Kokkotou E, Drossman DA, Goldman P, Lembo AJ. Components of placebo effect: randomised controlled trial in patients with irritable bowel syndrome. *Bmj*. 2008; 336:999–1003. [PubMed: 18390493]
28. Lembo AJ, Neri B, Tolley J, Barken D, Carroll S, Pan H. Use of serum biomarkers in a diagnostic test for irritable bowel syndrome. *Aliment Pharmacol Ther*. 2009; 29:834–42. [PubMed: 19226291]
29. Conboy LA, Wasserman RH, Jacobson EE, Davis RB, Legedza AT, Park M, Rivers AL, Morey EB, Nam BH, Lasagna L, Kirsch I, Lembo AJ, Kaptchuk TJ, Kerr CE. Investigating placebo effects in irritable bowel syndrome: a novel research design. *Contemp Clin Trials*. 2006; 27:123–34. [PubMed: 16427366]
30. Camilleri M, Mangel AW, Fehnel SE, Drossman DA, Mayer EA, Talley NJ. Primary endpoints for irritable bowel syndrome trials: a review of performance of endpoints. *Clin Gastroenterol Hepatol*. 2007; 5:534–40. [PubMed: 17428741]
31. Passos MC, Lembo AJ, Conboy LA, Kaptchuk TJ, Kelly JM, Quilty MT, Kerr CE, Jacobson EE, Hu R, Friedlander E, Drossman DA. Adequate relief in a treatment trial with IBS patients: a prospective assessment. *Am J Gastroenterol*. 2009; 104:912–9. [PubMed: 19293784]
32. Berman SM, Naliboff BD, Suyenobu B, Labus JS, Stains J, Ohning G, Kilpatrick L, Bueller JA, Ruby K, Jarcho J, Mayer EA. Reduced brainstem inhibition during anticipated pelvic visceral pain correlates with enhanced brain response to the visceral stimulus in women with irritable bowel syndrome. *J Neurosci*. 2008; 28:349–59. [PubMed: 18184777]

33. Franchimont N, Reenaers C, Lambert C, Belaiche J, Bours V, Malaise M, Delvenne P, Louis E. Increased expression of receptor activator of NF-kappaB ligand (RANKL), its receptor RANK and its decoy receptor osteoprotegerin in the colon of Crohn's disease patients. *Clin Exp Immunol*. 2004; 138:491–8. [PubMed: 15544627]
34. Bernstein CN, Sargent M, Leslie WD. Serum osteoprotegerin is increased in Crohn's disease: a population-based case control study. *Inflamm Bowel Dis*. 2005; 11:325–30. [PubMed: 15803021]
35. Winkles JA. The TWEAK-Fn14 cytokine-receptor axis: discovery, biology and therapeutic targeting. *Nat Rev Drug Discov*. 2008; 7:411–25. [PubMed: 18404150]
36. Maecker H, Varfolomeev E, Kischkel F, Lawrence D, LeBlanc H, Lee W, Hurst S, Danilenko D, Li J, Filvaroff E, Yang B, Daniel D, Ashkenazi A. TWEAK attenuates the transition from innate to adaptive immunity. *Cell*. 2005; 123:931–44. [PubMed: 16325585]
37. Dohi T, Borodovsky A, Wu P, Shearstone JR, Kawashima R, Runkel L, Rajman L, Dong X, Scott ML, Michaelson JS, Jakubowski A, Burkly LC. TWEAK/Fn14 pathway: a nonredundant role in intestinal damage in mice through a TWEAK/intestinal epithelial cell axis. *Gastroenterology*. 2009; 136:912–23. [PubMed: 19109961]
38. Spiller R, Campbell E. Post-infectious irritable bowel syndrome. *Curr Opin Gastroenterol*. 2006; 22:13–7. [PubMed: 16319671]
39. van der Veek PP, van den Berg M, de Kroon YE, Verspaget HW, Masclee AA. Role of tumor necrosis factor-alpha and interleukin-10 gene polymorphisms in irritable bowel syndrome. *Am J Gastroenterol*. 2005; 100:2510–6. [PubMed: 16279907]
40. O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, O'Sullivan GC, Kiely B, Collins JK, Shanahan F, Quigley EM. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology*. 2005; 128:541–51. [PubMed: 15765388]
41. Liebrechts T, Adam B, Bredack C, Roth A, Heinzel S, Lester S, Downie-Doyle S, Smith E, Drew P, Talley NJ, Holtmann G. Immune activation in patients with irritable bowel syndrome. *Gastroenterology*. 2007; 132:913–20. [PubMed: 17383420]
42. Drossman DA, Camilleri M, Mayer EA, Whitehead WE. AGA technical review on irritable bowel syndrome. *Gastroenterology*. 2002; 123:2108–31. [PubMed: 12454866]
43. Jones MP, Dille J, Drossman D, Crowell MD. Brain-gut connections in functional GI disorders: anatomic and physiologic relationships. *Neurogastroenterol Motil*. 2006; 18:91–103. [PubMed: 16420287]
44. Mayer EA, Derbyshire S, Naliboff BD. Cerebral activation in irritable bowel syndrome. *Gastroenterology*. 2000; 119:1418–20. [PubMed: 11185466]
45. Colloca L, Benedetti F. Placebos and painkillers: is mind as real as matter? *Nat Rev Neurosci*. 2005; 6:545–52. [PubMed: 15995725]
46. Ader R. On the development of psychoneuroimmunology. *Eur J Pharmacol*. 2000; 405:167–76. [PubMed: 11033324]
47. Blalock JE. The immune system as the sixth sense. *J Intern Med*. 2005; 257:126–38. [PubMed: 15656872]
48. Besedovsky HO, Rey AD. Physiology of psychoneuroimmunology: a personal view. *Brain Behav Immun*. 2007; 21:34–44. [PubMed: 17157762]
49. Barbara G, De Giorgio R, Stanghellini V, Cremon C, Salvioli B, Corinaldesi R. New pathophysiological mechanisms in irritable bowel syndrome. *Aliment Pharmacol Ther*. 2004; 20:2:1–9. [PubMed: 15335408]
50. Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, Pasquinelli G, Morselli-Labate AM, Grady EF, Bunnett NW, Collins SM, Corinaldesi R. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology*. 2004; 126:693–702. [PubMed: 14988823]
51. Tornblom H, Lindberg G, Nyberg B, Veress B. Full-thickness biopsy of the jejunum reveals inflammation and enteric neuropathy in irritable bowel syndrome. *Gastroenterology*. 2002; 123:1972–9. [PubMed: 12454854]

52. Kindt S, Van Oudenhove L, Broekaert D, Kasran A, Ceuppens JL, Bossuyt X, Fischler B, Tack J. Immune dysfunction in patients with functional gastrointestinal disorders. *Neurogastroenterol Motil.* 2009; 21:389–98. [PubMed: 19126184]
53. Ohman L, Lindmark AC, Isaksson S, Posserud I, Strid H, Sjovall H, Simren M. B-cell activation in patients with irritable bowel syndrome (IBS). *Neurogastroenterol Motil.* 2009; 21:644–50. e27. [PubMed: 19222763]
54. Evans D. Suppression of the acute-phase response as a biological mechanism for the placebo effect. *Med Hypotheses.* 2005; 64:1–7. [PubMed: 15533601]
55. Hashish I, Harvey W, Harris M. Anti-inflammatory effects of ultrasound therapy: evidence for a major placebo effect. *Br J Rheumatol.* 1986; 25:77–81. [PubMed: 2417648]
56. Carlson LE, Specia M, Faris P, Patel KD. One year pre-post intervention follow-up of psychological, immune, endocrine and blood pressure outcomes of mindfulness-based stress reduction (MBSR) in breast and prostate cancer outpatients. *Brain Behav Immun.* 2007; 21:1038–49. [PubMed: 17521871]
57. Furmark T, Appel L, Henningsson S, Ahs F, Faria V, Linnman C, Pissiota A, Frans O, Bani M, Bettica P, Pich EM, Jacobsson E, Wahlstedt K, Orelund L, Langstrom B, Eriksson E, Fredrikson M. A link between serotonin-related gene polymorphisms, amygdala activity, and placebo-induced relief from social anxiety. *J Neurosci.* 2008; 28:13066–74. [PubMed: 19052197]
58. Masion-Bergemann S, Thielecke F, Abel F, Bergemann R. Costs of irritable bowel syndrome in the UK and US. *Pharmacoeconomics.* 2006; 24:21–37. [PubMed: 16445300]
59. Kaptchuk TJ, Stason WB, Davis RB, Legedza AR, Schnyer RN, Kerr CE, Stone DA, Nam BH, Kirsch I, Goldman RH. Sham device v inert pill: randomised controlled trial of two placebo treatments. *Bmj.* 2006; 332:391–7. [PubMed: 16452103]
60. Zubieta JK, Bueller JA, Jackson LR, Scott DJ, Xu Y, Koeppe RA, Nichols TE, Stohler CS. Placebo effects mediated by endogenous opioid activity on mu-opioid receptors. *J Neurosci.* 2005; 25:7754–62. [PubMed: 16120776]

Abbreviations

IBS	irritable bowel syndrome
RCT	randomized controlled trial
IBS-AR	IBS-adequate relief
IBS-SSS	IBS-symptom severity scale
IBS-QOL	IBS-quality of life
TNF	tumor necrosis factor
OPG	Osteoprotegerin
TWEAK	TNF-related weak inducer of apoptosis
SSRI	selective serotonin reuptake inhibitors

Table 1

Demographics, disease characteristics and psychopathology of IBS patients randomized to placebo arm (sham acupuncture) or control arm (waitlist).

	Waitlist N=47	Placebo N=83	p- value
Demographics			
Age (years)(SD)	38.6 (14)	39.0 (15)	0.887
Women (%)	80.8	78.3	0.730
Whites (%)	91.4	83.1	0.445
Type of IBS (%)			0.792
Diarrhea	25.5	24.1	
Constipation	21.3	16.9	
Alternating	53.2	59.0	
Duration of IBS (%)			0.792
<1 year	6.4	9.6	
1-5 years	27.7	28.9	
5-10 years	25.5	28.9	
10-20 years	19.1	19.3	
>20 years	21.3	13.3	
Disease severity scores			
IBS-SSS (SD)	289 (60)	296 (62)	0.508
IBS-QOL (SD)	84.1 (23)	85.0 (24)	0.829
Psychopathology scores			
Depression (SD)	3.3 (3.1)	3.7 (3.7)	0.539
Anxiety (SD)	11.7 (8.4)	13.3 (9.4)	
Somatization (SD)	2.4 (2.1)	2.2 (2.0)	0.741
SSRI treatment (%)	21.3	21.7	0.956

SSRI: selective serotonin reuptake inhibitors

Table 2
Changes from baseline in serum biomarkers in response to a three-week placebo treatment in patients with IBS.

Changes in biomarkers	Mean change from baseline (SD)	Min	Max	Median	p-value
OPG pg/ml					0.039
waitlist	3.6 (145)	-484.7	300.9	-4.6	
placebo	53.1 (147)	-483.0	395.0	44.1	
TWEAK pg/ml					0.771
waitlist	103.1 (236)	-331.5	728.3	90.0	
placebo	98.3 (239)	-461.4	762.6	48.9	
MIP3beta pg/ml					0.444
waitlist	38.3 (89)	-347.1	300.1	39.5	
placebo	61.9 (183)	-435.8	1268.6	44.9	
GroA pg/ml					0.443
waitlist	24.5 (124)	-144.8	735.3	-2.5	
placebo	11.8 (85)	-199.8	346.7	4.2	
NGAL ng/ml					0.335
waitlist	36.9 (58)	-186.7	160.1	40.1	
placebo	34.9 (50)	-92.4	222.0	33.1	
TIMP1 ng/ml					0.972
waitlist	-3.2 (70)	-236.5	205.7	2.6	
placebo	4.6 (83)	-273.3	234.6	-1.4	
MMP9 ng/ml					0.397
waitlist	25.6 (206)	-506.8	674.8	28.9	
placebo	18.5 (247)	-652.2	723.6	-12.8	
BDNF pg/ml					0.729
waitlist	884.1 (5581)	-10306	16288	276.6	
placebo	251.5 (6612)	-18337	23389	-48.9	
VEGF pg/ml					0.569
waitlist	60.6 (191)	-348.1	800.1	53.8	
placebo	79.2 (201)	-722.4	780.3	65.1	

Changes in biomarkers	Mean change from baseline (SD)	Min	Max	Median	p-value
IL1beta fg/ml					0.455
waitlist	171.3 (1087)	158.6	7415.0	0.0	
placebo	-126.1 (989)	108.6	428.1	0.0	

Table 3

Comparisons of baseline characteristics between responders and non-responders, based on IBS-AR at week-3 of intervention, and according to treatment.

	IBS- Adequate Relief at week-3		
	Non-responders N=107	Responders N=83	p-value
Demographics			
Age (years)(SD)	39.8 (14)	37.6 (13)	0.286
Women (%)	70.1	80.7	0.094
Whites (%)	86.9	87.9	0.831
Type of IBS (%)			0.861
Diarrhea	25.2	24.1	
Constipation	17.7	19.3	
Alternating	57.0	56.6	
Duration of IBS (%)			0.034
< 1year	6.5	7.2	
1-5 years	25.2	30.1	
5-10 years	26.2	31.3	
10-20 years	18.7	25.3	
>20 years	23.4	6.0	
Baseline disease severity			
IBS-SSS (SD)	281 (66)	260 (70)	0.036
IBS-QOL (SD)	85.5 (25)	83.0 (24)	0.047
Psychopathology scores			
Depression (SD)	3.7 (3.5)	3.7 (3.7)	0.991
Anxiety (SD)	11.4 (9.4)	12.9 (9.4)	0.270
Somatization (SD)	2.3 (1.9)	2.2 (2.2)	0.827
SSRI treatment (%)	22.4	14.4%	0.667

SSRI: selective serotonin reuptake inhibitors

Table 4

Differences in serum biomarker levels at baseline in responders vs. non-responders at week three of intervention based on IBS-AR and according to their treatment.

	N (%)	OPG pg/ml (SD)	TWEAK pg/ml (SD)
All groups	190		
IBS-AR no	107 (56)	491 (192)	892 (350)
IBS-AR yes	83 (44)	563 (217) ^a	1018 (342) ^b
Placebo	124		
IBS-AR no	56 (45)	524 (200)	871 (341)
IBS-AR yes	68 (55)	552 (218)	1022 (344) ^c
Wait list	66		
IBS-AR no	51 (77)	453 (176)	915 (361)
IBS-AR yes	15 (23)	610 (212) ^d	995 (348)

^a
p=0.0167

^b
p=0.0144

^c
p=0.0157

^d
p=0.0052

Table 5

Baseline biological and psychological predictors of clinical improvement, irrespectively of treatment arm, based on changes in the IBS-SSS score

Changes in IBS-SSS (baseline-week 3)		
	R	P value
OPG (pg/ml)	0.22	0.0021
delta OPG	0.22	0.012
TWEAK	0.08	NS
delta TWEAK	0.152	0.085
Age	0.04	NS
Type of IBS	0.13	NS
Duration of IBS	-0.12	0.033
IBS-QOL	-0.07	NS
Depression	0.03	NS
Anxiety	0.05	NS
Somatization	-0.05	NS

NS: not statistically significant