Tuning Elements Task 3- Evaluation of Behavioral Energetic Patches' (BEST) Potential Effects on Pediatric Behavioral Disorders

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In fulfillment of Task Agreement 4- Amended
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BACKGROUND

Autism is a mental disorder, diagnosable from early childhood, characterized by impaired communication and socialization, and repetitive behaviors. Along with the learning difficulties and limitations present in intellectually disabled, these characteristics may pose a large burden on parents of disabled children. While there is no known cure for autism, certain behaviors may be managed by adherence to strict routines, regular consultations with health care professionals, and focus on alleviating the most detrimental symptoms.

Clinical studies have shown a potential link between autism and the microbes making up the population of the gastrointestinal tract. There is emerging evidence for bacterial populations influencing cognition, learning, appetite, and other behaviors through a “gut brain axis.” How these microbes may influence biological functions and behaviors associated with autism is not well characterized. However, in some cases, increases in a pathogenic bacterial species correlated with episodes of self-harm.

Behavioral Energetic Patches™ are silicon based Titanium Salt infused adhesive patches, 4x4 cm in diameter. They have no additives. The BEST has a removable plastic cover and a paper backing. The patches have been shown to be harmless. They are imprinted with an energetic message that is passively transmitted when applied to skin. This is speculated to have potential benefits to children with behavioral disorders. The goal of this study was to evaluate the ability of Behavioral Energetic Patches to alleviate symptoms and behaviors associated with autism, and furthermore to evaluate whether any efficacy might be acting through the gut-brain axis.

METHODS

AUTISM SCORING AND ANALYSIS

Children diagnosed with varying types of behavioral disorder were evaluated weekly by their parents on a variety of behaviors. Initially, children received a basal evaluation on the following attributes:

Focus/Attention
Communication
Mood
Memory
Tantrums
Aggression
Tics (if applicable)
Appetite

The most severe possible score for each attribute was 0, and the best score for each was a 3. Therefore, each child was able to receive between 0 and 21 or 24 points, depending on whether tics comprised a relevant symptom of their disorders.

Following initial scoring, children enrolled in the study were given a patch to wear for a seven-day period. At the end of the first 7 days, behaviors were evaluated as described above and a new patch was applied for another 7 days. This was repeated for 7 weeks. At the end of week 7, the patch was not re-applied. Final evaluations were performed on children after they had gone 7 days without a patch.

Overall scores were calculated through summation of scores for each aspect of the evaluation. These were then compared statistically using SPSS Statistical Software (IBM).

DNA Extraction and DGGE Analysis

Fecal samples were collected from patients prior to the patch application, and again at the conclusion of the treatments. Samples were stowed in OMNIgene*GUT fecal preservative (DNA Genotek Inc., Ottawa, ON) and shipped to CBLS, at which time they were stored at 4°C until extraction.

Samples were centrifuged at 1300 RPM for 5 minutes to separate supernatant from solid feces. The total solid sample was taken and total DNA was extracted using a commercially-available kit designed for the isolation of DNA from stool (Qiagen). DNA concentrations were determined spectrophotometrically. The V3-V4 region of the 16S rRNA gene was amplified using PCR. PCR products were run through denaturant gradient gel electrophoresis (DGGE). Gels were then stained with ethidium bromide and imaged using Carestream MI software and the images analyzed to determine percentage similarity of fecal microbiota between groups, specifically before and after patch application.

RESULTS

BEHAVIORAL SCORES

Initially, a Shapiro-Wilks' test was conducted to evaluate normality of the data. Since the data were non-normal (P= 0.031), non-parametric statistical tests were used.
A Friedman test was performed to test for general significance between time-points. The Friedman test was highly significant (P< 0.001) indicating that there were changes between time points.
To more specifically evaluate the time-points where the changes took place, pairwise comparisons were conducted between each set of time points using a Wilcoxon's Test.

All time points were found to be significant from the basal reading before any patch treatment was used. (P= 0.017, P= 0.018, P= 0.018, P= 0.018, P= 0.018, P= 0.018, P= 0.018, respectively, Fig. 1).

Weeks 2 and 3 showed statistically similar scores (P= 0.061), but weeks 4, 5, 6 and 7 showed significant increases compared to scores following week 1 of treatments. (P= 0.027, P= 0.018, P= 0.018, P= 0.018). Following a week of no patch treatment, week 8 again showed similar scores to week 1 of testing (P= 0.072).

Weeks 1 and 2 showed statistically similar scores (P= 0.088), but weeks 4, 5, and 6 showed significant increases compared to scores following week 1 of treatments. (P= 0.026, P= 0.026, P= 0.017).

Although a general trend of improvement remained, by the third week of patch administration (Week 4 on graph) the changes were no longer significant to the week prior. Weeks 3 through 6 did not show statistical differences to each other at any time point. All of these time points were significant from the initial readings.

The final reading at week 8 was administered following a seven day period of no patch treatment. These scores showed a slight negative trend, but were still statistically different from basal readings. However, they no longer showed statistical significance from scores of children who had been wearing the patches for 1 or 2 weeks. In addition, scores at this time point were significantly decreased from scores at weeks 4, 5, 6 and 7.

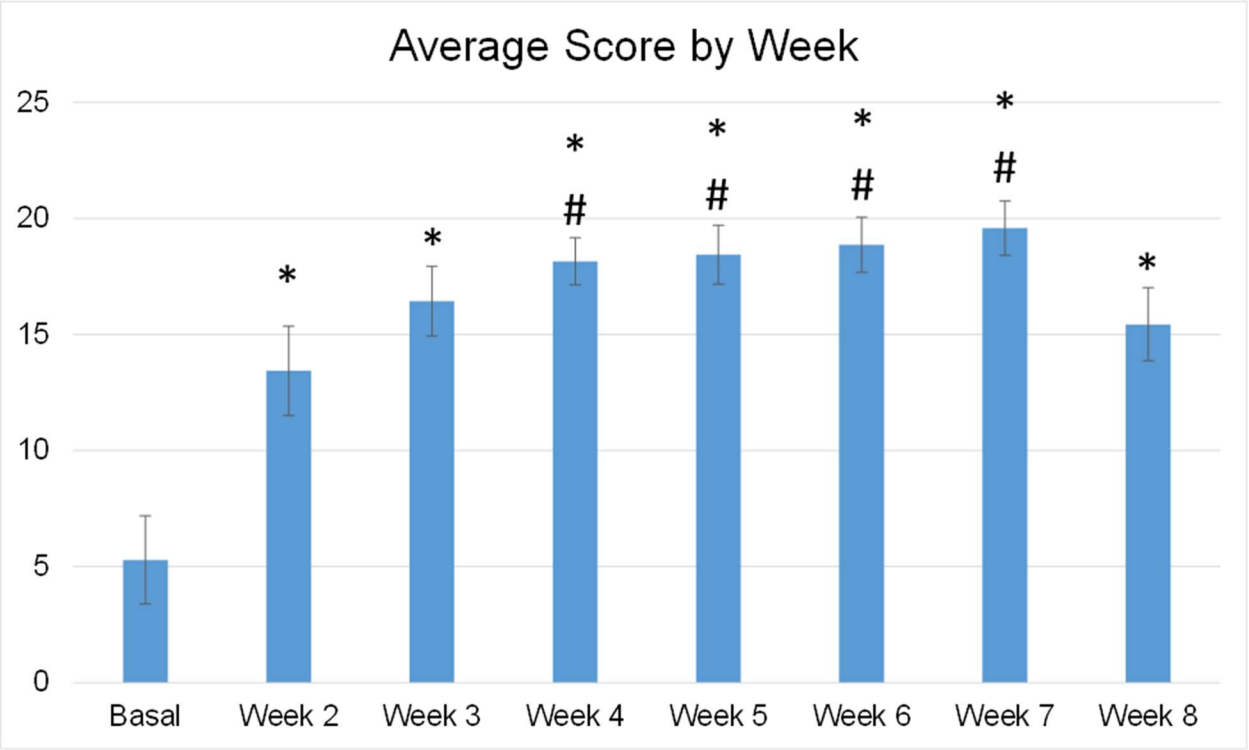

Fig 1. Behavioral scores by week ± SEM. * indicates significance from basal readings, while # indicates significance from the week 2 readings.

MICROBIOME ANALYSIS

Analysis of the microbiota of each sample involves treating each sample as a unique fingerprint. Each fluorescent band represents a bacterial species. Initially, every band was identified in every sample to assess total similarity in the profile of the microbial flora. Percent similarity is evaluated and more similar samples group together on a dendrogram. If a treatment causes a large-scale shift in gut microbial populations, the dendrogram will show a clear delineation between samples before and after the treatment. In this experiment, samples from a single individual tended to be more similar to one another than to the microbial profile of a different patient, regardless of patch application, indicating that if patches cause any changes, higher resolution observation is necessary.

The next phase of the analysis was to assess whether there were any microbial species that were consistently present in only one of the time points for the experiment. While many species appear and disappear within individuals, none reliably did so in a manner that correlated with patch applications.

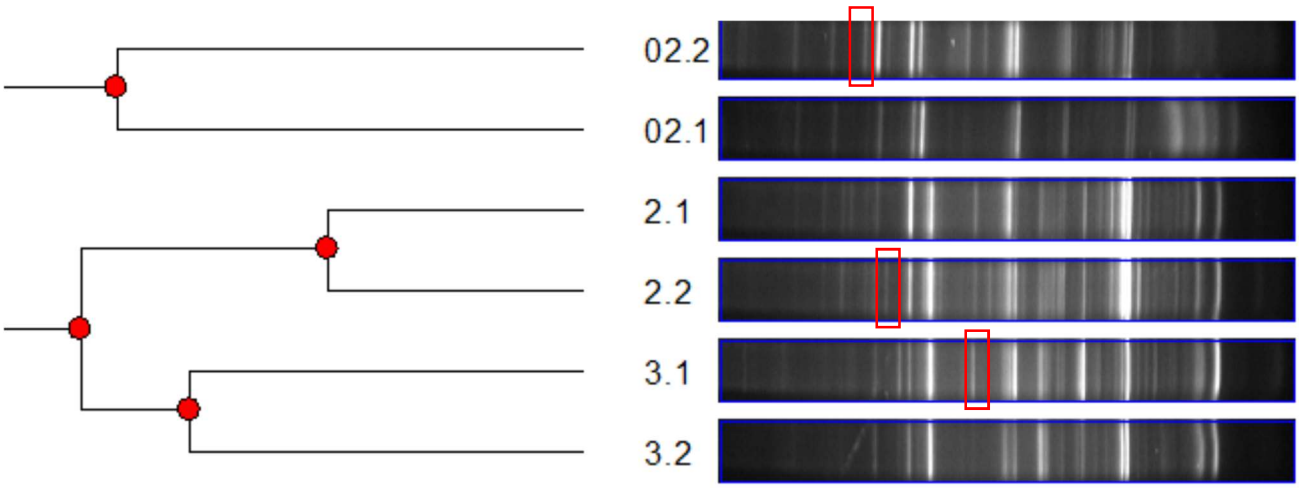

Figure 2. Bacterial profiles tend to cluster based on the sample donor rather than the treatment group. Some species increased or decreased between time points (red boxes), but did not do so consistently across multiple patients.

CONCLUSIONS

Children treated with Tuned Patches showed significant improvement in their behavioral scores following one week of administration. This improvement remained significant from basal levels for the duration of the study. Following the dramatic initial improvement, changes between consecutive weeks tended to be gradual. Weeks 2 and 3 were not significantly different from each other, but weeks 2 and 4 were, for instance.

Behavioral improvements reached a saturation point after 3 weeks of constant patch application. Following this week, the scores showed a trend of improvement up to week 7, but this was non-significant. However, the effects of the final week without the patch were fast, with behavioral scores quickly falling back to levels similar to weeks 2 and 3.

These results are promising for the efficacy of Tuned Patches in treatment of pediatric behavioral disorders. A double-blind crossover study design with a placebo patch would be beneficial in future studies.

While changes in behavior following patch application over several weeks were quite robust, changes in the microbial populations of each patient did not clearly correlate with patch application. Changes may still be taking place, but such changes are likely unique to each individual and so cannot be effectively studied at the level of a whole-population screen such as DGGE. Moreover, human diet and stress/anxiety levels may vary considerably from person to person. For example, even the same person may drastically alter what they eat over time, making it difficult to distinguish natural variability from detrimental or beneficial changes. Submission of samples for next generation sequencing (NGS), a high throughput assay that allows for quantitation and identification of each species present in a DNA sample, might be a useful approach for future studies.

In conclusion, it appears that energetic patch application elicits beneficial changes in behavior as evaluated by parents of the subjects. However, the mechanism by which is does this remains unclear. It cannot be ruled out that patches have an impact on the microbial flora of the gut, but such a change appears to

beyond the detectable resolution of the DGGE technique, which only can identify major changes in bacterial composition. Further studies are warranted to validate the positive behavioral findings.