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Establishing a relationship between bacteria in the human gut and Complex Regional Pain Syndrome

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ABSTRACT

Complex Regional Pain Syndrome (CRPS) is a serious and painful condition involving the peripheral and central nervous systems. Full comprehension of the disorder's pathophysiology remains incomplete, but research implicates the immune system as a contributor to chronic pain. Because of the impact gastrointestinal bacteria have in the development and behavior of the immune system, this study compares the GI microbial communities of 16 participants with CRPS (5 of whom have intestinal discomforts) and 16 healthy controls using 454 sequencing technology. CRPS subjects were found to have significantly less diversity than their healthy counterparts. Statistical analysis of the phylogenetic classifications revealed significantly increased levels of Proteobacteria and decreased levels of Firmicutes in CRPS subjects. Clustering analysis showed significant separation between healthy controls and CRPS subjects. These results support the hypothesis that the GI microbial communities of CRPS participants differ from those of their healthy counterparts. These variations may hold the key to understanding how CRPS develops and provide information that could yield a potential treatment.

1. Introduction

1.1. Complex Regional Pain Syndrome

Complex Regional Pain Syndrome (CRPS) is a serious and painful condition that generally develops after an inciting event such as injury, illness or surgery and cannot be explained by another medical diagnosis. The array of symptoms that accompany this condition can be classified into four main categories: abnormal pain processing, vasomotor changes, trophic changes, and impaired motor functions (Alexander et al., 2007; Schwartzman et al., 2009; Harden et al., 2010). Each category contains multiple indications which a person may exhibit individually or in concert with other symptoms. While those with CRPS present similar categorical traits, the arrangement and degree to which these symptoms are expressed vary across the population resulting in the creation of disease subsets (Alexander et al., 2012). Additionally, there is no one specific therapy that wholly addresses the effects of this varied condition (Goebel et al., 2010; Alexander et al., 2012). It is estimated that there are between 200,000 and 1.2 million Americans with CRPS and in a recent study, 81% of those affected by CRPS reported pain levels that precluded them from remaining in the workforce (Schwartzman et al., 2009; Mailis, 2003). There is evidence that early recognition and treatment of CRPS increases the chance of recovery though current treatments are largely ineffective (Goebel et al., 2010; Watkins et al., 2007). Although the mechanisms behind CRPS have not been fully identified, research has shown that neurogenic inflammation plays a significant role and appears to be modulated by both the central nervous and immune systems (Marchand et al., 2005; Alexander et al., 2007; Schwartzman et al., 2011; Goebel et al., 2010).

1.2. Gut-brain axis

The gut-brain axis is a bi-directional communication network involving the sympathetic, parasympatheic, and enteric systems (Collins and Bercik, 2009; Cryan and O'Mahony, 2011; Mayer and Tillisch, 2011; Bercik et al., 2012). There is a perpetual flow of information detailing bodily performance that is acquired by the brain and used to maintain homeostatic levels – among them the digestive and "gut-associated immune" systems (Mayer and Tillisch, 2011). Sensory signals are carried by the enteric, spinal,

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and vagal pathways. Of particular note is the vagus nerve which is in contact with processes of dendritic cells that extend through the epithelial layer of the GI tract (Collins and Bercik, 2009; MacDonald and Monteleone, 2005; Round and Mazmanian, 2009). This nerve carries signals to the CNS and is essential in regulating emotion, pain, and immune response, it has been named in the immune-to-brain pathway, and has been associated in the development of sickness responses (MacDonald and Monteleone, 2005; Watkins and Maier, 2005; Collins and Bercik, 2009; Round and Mazmanian, 2009; Mayer and Tillisch, 2011). Molecules involved in the transmission of information include cytokines, hormones, endotoxins, and neuropeptides (Watkins and Maier, 2005; Bercik et al., 2012).

Under healthy circumstances, interoceptive information is not generally perceived consciously. However in persons experiencing functional abdominal pain syndrome, they are keenly aware of this transmission and experience extended pain/discomfort (Mayer and Tillisch, 2011). It has been suggested that these pain states result from dysregulated interactions between the gut lumen and mucosa, the enteric nervous system, and the central nervous system – all culminating in modification of affect, perception, GI motility, and in certain conditions, immune function (Mayer and Tillisch, 2011).

1.3. Importance of bacteria

The GI bacterial community is purported to contain on the order of 10^{13}–10^{14} organisms, harbors approximately 1000 different species, and is considered to have the most diversity among other human-host environments such as the skin and oral cavity (Tancrede, 1992; Hugenholtz and Tyson, 2008; Savage, 1977; Quince et al., 2009; Reeder and Knight, 2010; Van den Abbeele et al., 2011). Bacteria within the gut are vital to nutrient breakdown and absorption; they prevent colonization of pathogens, can metabolize toxins on a scale equal to that of the human liver and provide a structural backbone to a functional immune system (Collado et al., 2009; Kurokawa et al., 2007; MacDonald and Monteleone, 2005; Round and Mazmanian, 2009). Along the GI tract, dendritic cells are directly stimulated by contact with microbes, prompting the body to develop an immune response which includes the release of cytokines (Collins and Bercik, 2009; MacDonald and Monteleone, 2005; Round and Mazmanian, 2009).

The importance of gastrointestinal (GI) bacteria and their ability to influence human health has been the focus of many human microbiome studies. To date, many of these studies have attempted to ascertain the constituents of a healthy GI microbial community or to describe community differences in the face of diet differences, obesity, antibiotic usage, colon cancer, or inflammatory bowel diseases (IBDs) (Eckburg et al., 2005; Ley et al., 2006; Manichanh et al., 2006; Dethlefsen et al., 2008; Sobhani et al., 2011). While there is still some debate as to what constitutes the 'core' GI microbial community, the prevailing belief is that a GI system housing an unbalanced microbial community leads to health issues involving inflammation (Round and Mazmanian, 2009). It seems intuitive that GI bacteria can be correlated to GI maladies, however there are a number of studies that have found a link between GI bacteria and behavior, stress, depression, sickness response, and pain perception (Collins and Bercik, 2009; Forsythe et al., 2010; Bravo et al., 2011; Finegold et al., 2010; Watkins and Maier, 2005; Quan and Banks, 2007). In particular, Amaral demonstrated that GI bacteria could influence how rodents perceived pain that was located at their extremities (Amaral et al., 2008).

1.4. Targeting bacteria

In order to identify the bacterial communities of each sample, this study targeted the 16S rRNA (small subunit ribosomal RNA) gene. The gene is comprised of approximately 1600 nucleotides, with conserved and variable regions (Petrosino et al., 2009). The conserved areas allow researchers to design primers that will recognize all bacteria while the areas of variability provide valuable phylogenetic information (Hamady and Knight, 2009; Petrosino et al., 2009). For this investigation, bacterial sequences were procured with 454 sequencing technology using primers directed at the V2 conserved region of the 16S rRNA gene.

2. Methods

2.1. Subjects

The study was undertaken at Drexel University College of Medicine (DUCOM) in Philadelphia PA and was approved by the Internal Review Board (IRB). Sixteen CRPS subjects and 16 healthy controls were enrolled in this study. Informed consent was obtained from all subjects prior to their participation. As CRPS is approximately four times more prevalent in women than men, only women were recruited for this investigation (Schwartzman et al., 2009; de Mos et al., 2007). Subjects with CRPS (CRPS_All $n = 16$) were recruited from the DUCOM Pain Clinic and met the International Association for the Study of Pain (IASP) diagnostic criterion for CRPS (Harden et al., 2010). Of the 16 CRPS subjects, 14 of them were classified as having Type I CRPS. A subset of the CRPS population (CRPS_GI $n = 5$) experienced gastrointestinal discomforts (e.g. pain, constipation, diarrhea) but did not have a diagnosis of IBD or other GI disorders. Participation was offered to patients using the following inclusion criteria: (1) female; (2) 20–55 years of age; (3) physician diagnosis of CRPS I (no demonstrable nerve lesion) or II (identifiable nerve lesion); (4) willingness to complete a self-administered written survey; (5) a willingness to provide a fecal sample; (6) reside within the Philadelphia region; and (7) exercise non-vegetarian eating habits. Exclusion criteria included: (1) the inability to complete the questionnaire; (2) other serious medical conditions; (3) use of antibiotics, narcotics or colon cleansing within 3 months prior to sample collection; and (4) women who had hysterectomies, were pregnant or were on hormone replacement regiments. The control subjects ($n = 16$) consisted of healthy females with no medical conditions and with the exception of having a pain diagnosis, adhered to same participation requirements as the CRPS population. In addition to collecting a fecal sample, all participants completed a standardized questionnaire constructed for this study that captured self-reported demographic information, a history of medical diagnoses (including age of onset of their symptoms and age of their diagnosis by a physician), medication and/or nutritional supplement usage, and a synopsis of their typical food and drinking habits.

2.2. Sample collection and transport, DNA extraction and sequencing

In order to assure our study was comparable to other studies, this study followed the protocol employed by the Human Microbiome Project (Peterson et al., 2009; Gevers et al., 2012). Fecal samples were collected by study participants in their homes. Collected samples were placed in a stool specimen container and immediately placed under anaerobic conditions, stored in a cooler at −4 °C and transported at 4 °C to the laboratory within 24 hours. Once in the laboratory the samples were weighed, partitioned into shipping and storage tubes and stored in a freezer at −80 °C. Analysis samples were shipped overnight express with dry ice to the Research Testing Laboratories (Lubbock, TX) for sequencing. Methods for DNA extraction, amplification, and 454 sequencing follow those referenced in a recent publication by Finegold et al. (2010).