CANNFORMATICS

CANNABIS-RESPONSIVE BIOMARKERS: A NEW TECHNOLOGY TO DETERMINE THE IMPACT OF MEDICAL CANNABIS TREATMENT IN CHILDREN WITH AUTISM SPECTRUM DISORDER

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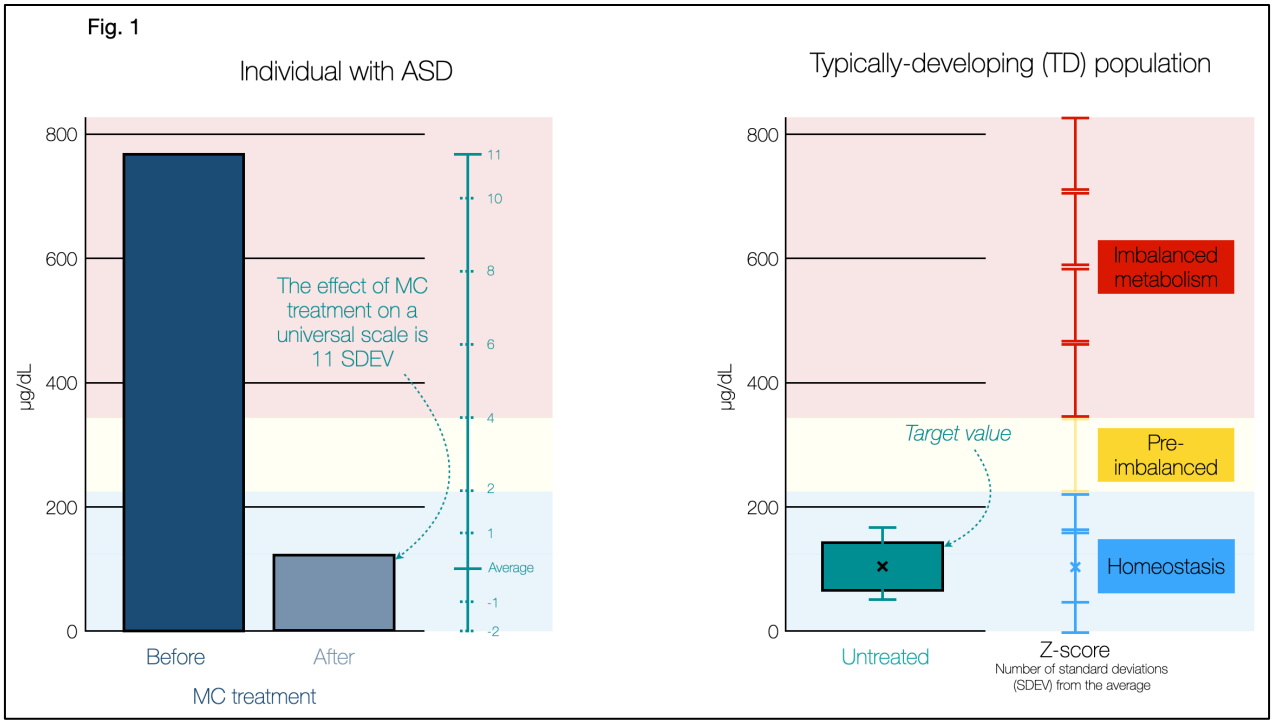
ABSTRACT

Autism spectrum disorder (ASD) is a complex neurodevelopmental group of conditions characterized by a range of heterogeneous clinical symptoms and severity levels. The increasing prevalence of ASD, and the absence of pharmacotherapy specifically designed for ASD, have led to a growing interest in medical cannabis (MC) as a potential alternative or adjunctive therapy. However, to consider MC as a viable therapeutic option for ASD treatment, objective and precise assessment methods and scientific information regarding the effect of each cannabinoid are essential. This White Paper explores the innovative technology of Cannabis-Responsive (C-Res) biomarkers developed by Cannformatics, Inc., a San Francisco-based biotech startup aiming to address this knowledge gap. The Company's mission is to deliver scientifically validated MC treatment guidance to healthcare providers and patients, to improve health and quality-of-life for individuals with ASD and to provide the much-needed scientific data on mechanism of action (MOA) and cellular targets of cannabinoids. This paper summarizes the findings of the Company's FDA/IRB-approved ASD Pilot Study. Cannformatics has published two peer-reviewed papers based on the pilot study, Siani-Rose et al. 2021[1] and Siani-Rose et al 2022[2], both receiving exceptional attention with top 1% attention scores (Altmetric). A third paper is currently under review for publication in the peer-review journal Scientific Reports[3].

TECHNOLOGY: ASD C-Res BIOMARKERS

ASD C-Res biomarkers are a new class of molecules found in saliva of individuals with ASD that objectively measure the response before and after MC treatment, and indicate the impact in comparison to targeted values determined in an untreated typically developing (TD) population. Homeostasis, pre-imbalanced and imbalanced metabolism ranges are defined in standard deviations (SDEVs) calculated as z-score from the TD population. Fig. 1 shows the z-score value of a single C-Res biomarker before (dark blue, left panel) and after (light blue, left panel) MC treatment on the universal scale of SDEVs established by the TD group (green, right panel).

Fig. 1



[1] Cannabis-Responsive Biomarkers: A Pharmacometabolomics-Based Application to Evaluate the Impact of Medical Cannabis Treatment on Children with Autism Spectrum Disorder

[2] The Potential of Salivary Lipid-Based Cannabis-Responsive Biomarkers to Evaluate Medical Cannabis Treatment in Children with Autism Spectrum Disorder

[3] Quillet JC, et al "A Machine Learning Approach for Understanding the Metabolomics Response of Children with Autism Spectrum Disorder to Medical Cannabis Treatment" Scientific Reports, (2023) submitted.

CLINICAL FEASABILITY: ASD PILOT STUDY

The FDA/IRB approved Pilot Study included 15 children with ASD who were successfully treated with MC supervised by a physician (Bonni Goldstein, MD - Los Angeles, CA), as permitted by California law, for at least one-year. The MC regimens were previously personalized by Dr. Goldstein based on each child's specific response, with dosages ranging from THC-dominant MC (0.05 mg – 50 mg per dose) in 40% of children to CBD-dominant MC (7.5 mg – 200 mg per dose) in 60% of children. Nine age-and gender-matched untreated children (TD group) served as controls. Saliva samples were collected from the ASD group in the morning before MC treatment (PRE) and approximately 90-minutes after treatment based on parents' report as time of maximal impact (PEAK). Five minutes prior to saliva collection, parents completed a Likert scale survey capturing observational parent reports of frequency and/or severity of pre-identified behaviors and/or social-emotional functioning. A single saliva sample was collected from children in the TD group in the morning. Parents of the ASD group and TD control group completed ABAS-3, BASC-3 and SRS-2 rating forms about their child's social, emotional, and behavioral functioning as a baseline evaluation. Survey design and analysis and ABAS-3, BASC-3 and SRS-2 analysis were conducted by Stephany Cox PhD, a pediatric neuropsychologist from UCSF. Within the ASD group, 11 children exhibited a severe range, 2 children exhibited a moderate range and 2 children exhibited a mild range of social impairment associated with ASD.

SCIENTIFICALLY VALIDATED FINDINGS

In our first published paper Cannformatics used a pharmacometabolomics approach to identify 65 potential C-Res biomarkers in children with ASD. These biomarkers showed a shift towards the physiological levels detected in the TD group, indicating a positive impact of MC treatment at the metabolic level. Notably, 23 biomarkers were associated with anti-inflammation, redox regulation, bioenergy production, mitochondrial function, neural function, and/or categorized as neurotransmitters, amino acids, and endocannabinoids. Also notable, we found N-acetylaspartic acid (NAA), a known ASD neurodegenerative biomarker since 1997[4], spermine, a known pain biomarker, and dehydroepiandrosterone sulfate (DHEA-S), a known aggression biomarker, moved into the physiological range.

Cannformatics' second paper focused on lipidomics analysis of the same saliva samples in which we identified 22 new potential lipid-based C-Res biomarkers that shifted toward the TD physiological levels in children with ASD after MC treatment. These biomarkers were mapped for the first time, distinguishing two C-Res biomarker metabolic networks that are also associated with other neurodegenerative and neurological disorders including: Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, and schizophrenia.

Cannformatics third paper describes the first use of machine learning (ML) technics on the high-resolution C-Res biomarker database to distinguish groups of patients, identify non-cannabinoid plant molecules found in cannabis extract with synergistic (entourage) effects, and to describe a novel ASD-related pathway with specific THC, CBD and CBG metabolic targets that can potentially reduce stress and depression behaviors.

Importantly, ML analysis did not show any evidence of negative impact of MC where biomarkers shifted out the physiological range toward the imbalanced range after successful MC treatment.

C-Res BIOMARKER EXAMPLES

1) Metabolic profile of individual

Figure 2 shows first-ever proof that MC treatment shifts imbalanced metabolic states to homeostasis, in a 12-year-old boy with ASD.

The left panel shows a metabolic panel of 268 C-Res biomarkers analyzed before (PRE) and after MC treatment at time of maximal improvement in behaviors reported by parents (PEAK). The homeostasis range of each C-Res biomarker was determined by 9 TD children that never used cannabis. In this example, the number of C-Res biomarkers found in homeostasis increased from 30% to 83% and the number of C-Res biomarkers found in an imbalanced metabolic states decreased from 54% to 7% after successful MC treatment. The metabolic panel of an age- and gender-matched control (TD) child showing a profile similar to the child with ASD at PEAK is presented in the right panel.

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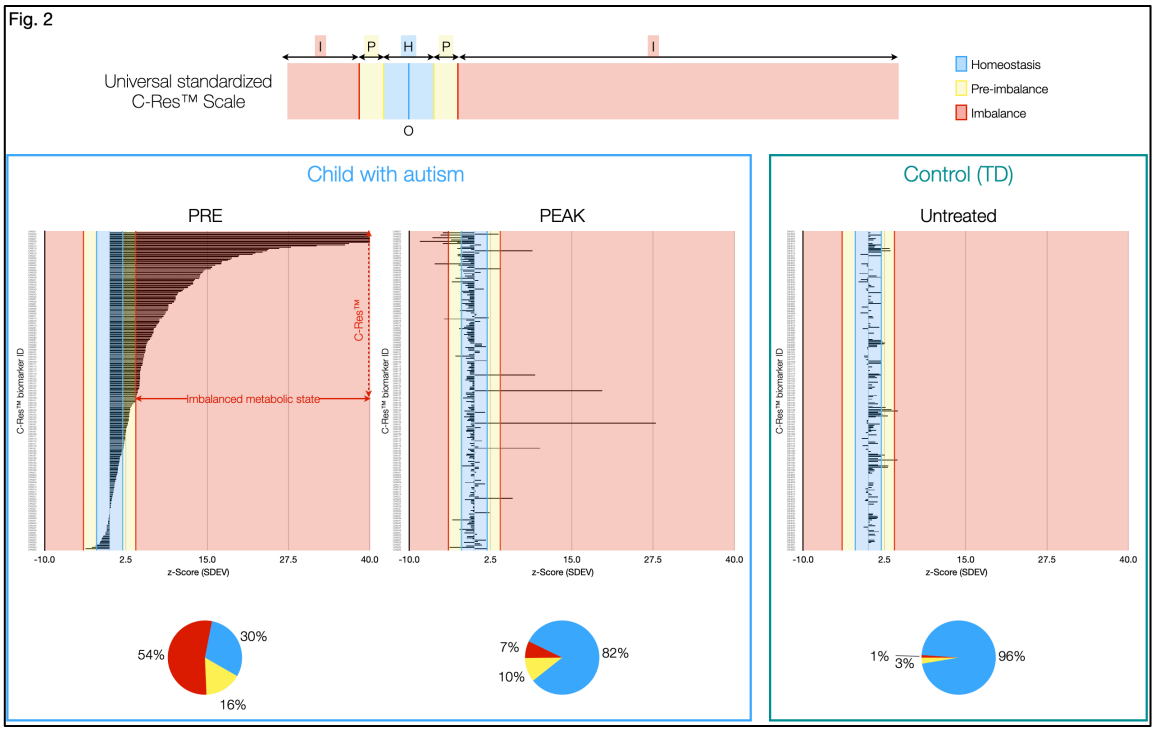
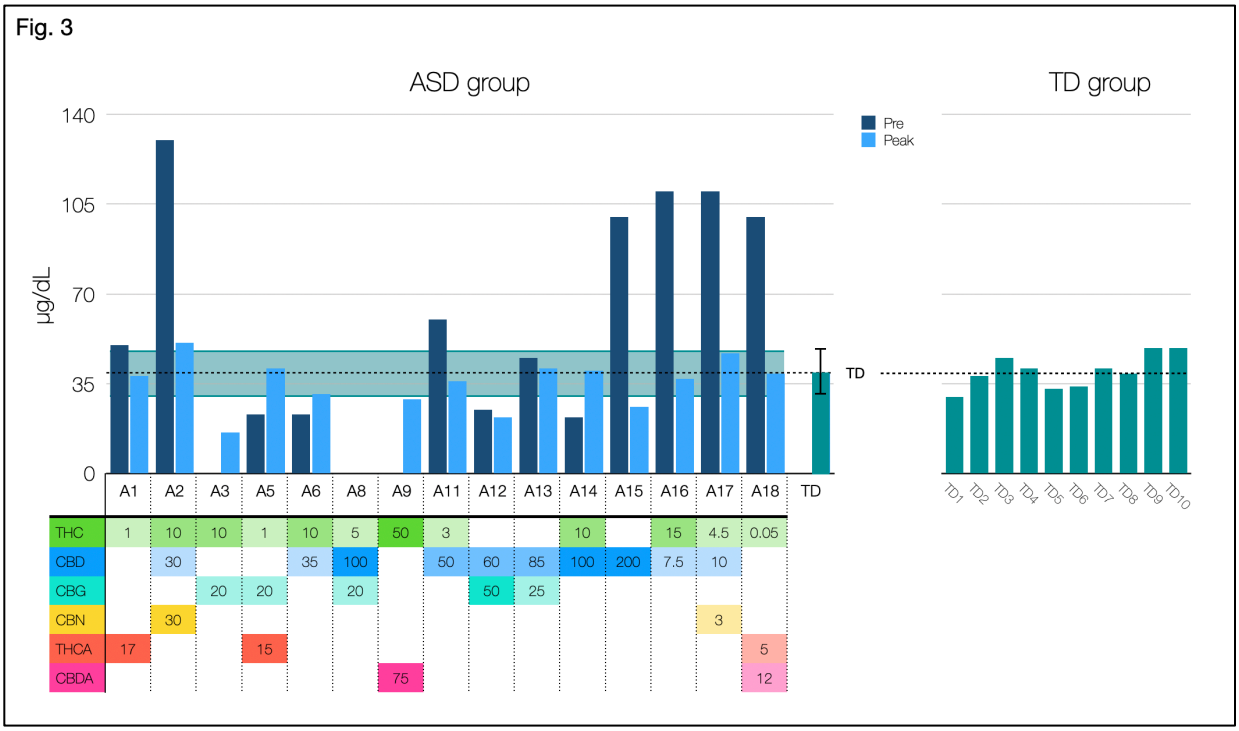
[4] Hashimoto, Toshiaki, et al. "Differences in brain metabolites between patients with autism and mental retardation as detected by in vivo localized proton magnetic resonance spectroscopy." Journal of child neurology 12.2 (1997): 91-96.



2) ASD-related biomarker

Figure 3 shows first-ever proof that MC treatment shifts the imbalanced metabolic levels of the known brain neurochemical ASD biomarker NAA to homeostasis levels in children with ASD[5].

NAA levels in all the children with ASD before MC (dark blue, left panel) are shifting (increasing or decreasing) towards the physiological range (light blue, left panel), reaching levels similar to those detected in the TD group (green, right panel). The unique cannabinoid concentrations (mg) of MC treatment of each child are indicted.



[5] Chugani DC, et al. "Evidence of altered energy metabolism in autistic children." Progress in neuro-psychopharmacology & Biological Psychiatry 23.4 (1999): 635-641.

CONCLUSION

Cannformatics' studies suggest that C-Res biomarkers can successfully quantify the benefits of MC treatment at the metabolic level, personalize MC treatment, indicate a possible MOA for cannabinoids, highlight ASD-related metabolic pathways and targets for MC pharmacotherapy and determine the entourage effect of phytochemicals without the need to test each one separately. Additional large-sample-size studies to develop a large, statistically-robust database of C-Res biomarkers, combined with bioinformatics and ML techniques, are needed to scale up these findings for clinical applications.

C-Res BIOMARKER ADVANTAGES FOR ASD TREATMENT

Objective	Universal standardized values - one scale for all the biomarkers in all patients and controls.
Dynamic	Each individual is tested before and after treatment at statistically-dependent multiple time points.
Rich feature	Sample from each individual tested can provide up to 1100 potential biomarkers.
High Resolution	The detection range between the levels of the highest biomarker to the lowest biomarker is 19,000-fold.
Physiological relevance	Show improvement at the metabolic level, taking into consideration genetic and environmental factors.

C-Res BIOMARKER CLINICAL APPLICATIONS

Predict	Indicate the likelihood an individual with ASD will benefit from MC treatment, without trying it first.
Guide	Suggest initial personalized MC treatment (cannabinoid content and dosage) for individual with ASD, without trying it first.
Monitor	Evaluate the impact of MC treatment and optimize dosing over time, in response to patient changes (physical, mental, environmental, etc.)
Categorize	Stratification of groups based on metabolic function of: endocannabinoid system (ECS), lipids, neuroactivity, steroid activity, etc.
Describe	Provide insights for the mechanism of action (MOA) of THC, CBD, CBG and other major and minor cannabinoids based on an array of MC treatments (different cannabinoid compositions and dosage), without the need to treat with a single cannabinoid.
Identify	Determine the general and ASD-specific cellular targets of cannabinoids.

PROSPECTIVE USES OF C-Res BIOMARKERS

C-Res biomarkers are opening up a new precision medicine paradigm in ASD treatment. As the Cannformatics database of C-Res biomarkers and clinical assessments grows, it will enhance the accuracy of treatment prediction and optimization. Through collaborative efforts between Cannformatics, healthcare professionals, and regulatory entities the integration of C-Res biomarker-driven approaches into clinical practice can bring us closer to personalized, evidence-based MC treatment for individuals with ASD. In the future, we will expand the application of C-Res biomarkers to other medical conditions characterized by ECS dysfunction including: Alzheimer's disease, PTSD, neuropathic pain, general anxiety disorder, amyotrophic lateral sclerosis, Parkinson's disease and multiple sclerosis.