Centre for Clinical Trials in Rare Neurodevelopmental Disorders

Poster abstracts

The Utility of a Synbiotic and Adjunct Gut-Directed Hypnotherapy in Children with Autism Spectrum Disorder and Comorbid Functional Gastrointestinal Disorders

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Background: Up to 83% of children with Autism Spectrum Disorder (ASD) suffer functional gastrointestinal disorders (FGIDs). Dysfunction of the microbiome-gut-brain (MGB) axis has been implicated in pathogenesis of both ASD and FGIDs. Probiotics and prebiotics can modulate the gut microbiome and research has shown efficacy at improving gastrointestinal (GI) symptoms in children with ASD and neurotypical (NT) children with FGIDs. Gut-directed hypnotherapy (GDH) has shown utility in treating FGIDs in NT children and adults but has not yet been trialled in children with ASD. Given the bidirectional nature of the MGB axis a combined, bidirectional treatment approach may be warranted.

Objective: To compare changes in GI symptom severity pre and post intervention between a combined treatment (synbiotic + GDH) vs single treatment (synbiotic or GDH) approach.

Methods: Children diagnosed with ASD aged 5.00-10.99 years (n=60) will be recruited through community and clinical settings and randomised (1:1:1) to one of three 12-week treatment intervention groups: 1) synbiotic; 2) GDH; or 3) synbiotic + GDH. The primary outcome will be measured using the six-item Gastrointestinal Severity Index (6-GSI). Secondary measures include characterisation of GI and systemic health indicators (gut microbiome, inflammatory and intestinal permeability markers, Bristol stool chart) and changes to ASD severity and anxiety scores.

Conclusion: Current behavioural approaches for ASD therapy do not alleviate the high comorbidity of FGIDs within this population. Targeting therapies to address the dysfunction of the bidirectional MGB axis will likely be more effective than either brain/behavioural or gut-based therapy alone.

Queensland Government

The Interplay between the Endocannabinoid System, Epilepsy and Cannabinoids

Author

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Background: Epilepsy is a neurological disorder that affects approximately 50 million people worldwide. There is currently no definitive epilepsy cure. However, in recent years, medicinal cannabis has been successfully trialled as an effective treatment for managing epileptic symptoms, but whose mechanisms of action are largely unknown. Lately, there has been a focus on neuroinflammation as an important factor in the pathology of many epileptic disorders.

Objective: In this literature review, we considered the links that have been identified between epilepsy, neuroinflammation, the endocannabinoid system (ECS), and how cannabinoids may be potent alternatives to more conventional pharmacological therapies. We reviewed the research that demonstrates how the ECS can contribute to neuroinflammation, and could therefore be modulated by cannabinoids to potentially reduce the incidence and severity of seizures. We particularly scrutinised the cannabinoid cannabidiol (CBD) as it has been reported to have anti-convulsant and anti-inflammatory properties, and is emerging as an effective treatment for epilepsy in minors.

Conclusion: There are a multitude of signaling pathways that involve endocannabinoids, eicosanoids, and associated receptors by which cannabinoids could potentially exert their therapeutic effects. Further research is needed to better characterise these pathways, and consequently improve the application and regulation of medicinal cannabis.

Abstract topics: endocannabinoid system; epilepsy; neurological diseases; cannabinoids; neuroinflammation; biomarkers.

A Dimeric, Luminescent Biosensor for Rapid and Sensitive Detection of DNA In Live Cells

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An extensive arsenal of biosensing tools has been developed based on the clustered regularly interspaced short palindromic repeat (CRISPR) platform, including those that detect the presence of specific DNA sequences both *in vitro* and in live cells. DNA biosensing approaches have traditionally used monomeric fluorescent reporter-based fusion probes; however, such methods typically do not adequately differentiate between unbound and bound forms of the probe and often require tandem arrays to increase signal-to-noise, among other issues. Herein we describe a luminescence-based, dimeric DNA sequence biosensor that provides a sensitive readout for DNA sequences through proximity-mediated reassembly of two independently optimized fragments of NanoLuc luciferase (NLuc), a small, bright reporter. Reconstitution of NLuc becomes more favorable upon binding of two guide RNAs (gRNAs) to two DNA target sites with a defined orientation and spacing. We demonstrate rapid and sensitive detection of as low as 190 amol of transfected target DNA, and single-copy endogenous genomic loci in live cells. These results present a highly novel and widely applicable approach for DNA biosensing.

Microbiome derived metabolites associated with autism spectrum disorder negatively impact multiple aspects of neuronal development and functionality

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A strong association between microbiome abnormalities and autism spectrum disorder (ASD) has been demonstrated; however, the pathological mechanisms connecting changes in the gut to those in the brain have yet to be fully elucidated. We hypothesize that bacteria-derived metabolites can disrupt neuronal development and function. To test our hypothesis we focused on 4-ethylphenylsulfate (4-EPS) and indoxyl sulfate (IS), two metabolites known to be elevated in ASD patients. Deficits in myelination, loss of white matter and defects in the function and regulation of synapses have been reported in ASD patients and are believed to be foundational contributors to core ASD behavioral abnormalities. To interrogate these pathologies we leveraged primary cell cultures from rat brain containing either neurons and oligodendrocytes to study neurogenesis and myelination, or hippocampal neurons to study synaptogenesis. Cell cultures were derived from 17-day old rat fetuses. Brain samples were first treated with trypsin to yield a cell suspension. Following further mechanical dissociation neurons and oligodendrocytes, or hippocampal neurons, were isolated and seeded into 96 well-plates pre-coated with poly-L-lysine and laminin, and maintained at 37°C in a humidified incubator during treatment with either 4-EPS of IS. After treatment we adopted an immunostaining approach to identify; oligodendrocyte precursor cells, differentiating oligodendrocytes, mature oligodendrocytes, neurons or synapses. Following appropriate counter-staining images were captured and quantitated using an ImageExpress instrument. Our results demonstrate that IS treatment significantly attenuated ($p < 0.05$); neurite outgrowth, axon formation, proliferation and differentiation of oligodendrocyte precursor cells, oligodendrocyte maturation, axonal myelination and reduced synapse density. Similarly, 4-EPS treatment significantly attenuated neurite sprouting and outgrowth, and axon formation. Interestingly, 4-EPS treatment significantly increased numbers of mature oligodendrocytes, however, this was not associated with a concomitant increase in axonal myelination. Our data demonstrate that exposure to bacteria-derived metabolites such as 4-EPS and IS negatively impact multiple aspects of neuronal development and functionality. These data highlight a novel therapeutic opportunity that targets the microbiome to develop new medicines that address core behavioral symptoms in ASD patients.

Characterization of GI barrier integrity and gut microbiome-derived metabolites in BTBR, Shank3 and Cntnap2 mouse models of ASD, and demonstration of AB-2004 as a potential mitigating therapeutic

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Background: Autism spectrum disorder (ASD) is a complex developmental disability that affects an already large and still increasing proportion of the world population. ASD is predominantly characterized by behavioral abnormalities; however, there is growing evidence that patients also suffer from comorbid symptoms including gastrointestinal dysfunction (Chaidez et al., 2014). Microbiome dysbiosis could play a role in increased intestinal permeability (leaky gut) which is a common condition found in ASD. Dysregulation of bacterial metabolites, such as those generated from metabolism of tyrosine and tryptophan, has been implicated as a pathological driver contributing to the disease symptomology. Specifically, 4-ethylphenylsulfate (4-EPS), a gut microbiota-derived metabolite, is elevated in a pediatric ASD population (Needham, et al., 2018 INSAR #28205).

Objectives: The purpose of the present study was to characterize intestinal permeability as well as microbiota-derived metabolites in three relevant and commonly used animal models of ASD: BTBR, Shank3 and Cntnap2-/- mice.

Methods: Serum FITC-dextran amounts were measured via fluorescence intensity in samples from BTBR, Shank3 and Cntnap2-/- mice. 4-EPS was extracted from urine from Cntnap2-/- mice and measured using LC-MS/MS. To determine the effects of AB-2004, this compound was formulated into chow to a final concentration of either 0, 1 or 5 % and was available ad libitum for the duration of 4 weeks. The same methods were used to evaluate the impact on leaky gut and 4-EPS in Cntnap2-/- mice.

Results: Based on the results, intestinal permeability, as measured by serum FITC-dextran intensity, was significantly increased in Shank3 and Cntnap2-/- mice but not in the BTBR cohort. Similarly, the levels of urinary 4-EPS were significantly elevated in Cntnap2-/- mice compared to control animals. This data aligns with previously published work in the maternal immune activation (MIA) mouse paradigm of ASD in which elevated 4-EPS levels and increased intestinal permeability were observed (Hsiao, et al., 2013). To further investigate whether intestinal permeability and increased 4-EPS levels could be ameliorated, the effects of AB-2004, an oral gut-restricted experimental therapeutic, were assessed in the Cntnap2-/- model. AB-2004 treatment effectively restored GI barrier integrity and reduced 4-EPS levels in a gut-restrictive manner.

Conclusions: In conclusion, the Cntnap2-/- mouse model recapitulated the leaky gut phenotype and elevated levels of the gut microbiome-derived metabolite 4-EPS that have been reported in ASD patients. Interestingly, treatment with AB-2004 restored GI integrity and normalized elevated 4-EPS. Taken together, these findings identify the Cntnap2-/- model as a promising platform for the development of microbiome-inspired therapies for the effective treatment of GI and behavioral dysfunctions in ASD.

Parental and Carer Perceptions and Observations of the Benefits of Cannabidiol Treatment for Children Diagnosed with Refractory Epilepsy.

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Background: The Compassionate Access Scheme (CAS) being delivered through the Queensland Children's Hospital is designed to allow access to an investigational purified Cannabidiol oral solution to paediatric patients with severe refractory epilepsy.
Objectives: The objectives of this study were to conduct semi-structured interviews to:
1. Understand families' expectations and attitudes surrounding use of an investigational cannabinoid product for their child's seizures;
2. Understand families' perceptions of Cannabidiol's efficacy for their child's seizures; and other aspects of their child's behaviour, quality of life and/or cognition.
Methods: Children aged 6-18 years had been enrolled in, were currently enrolled in, or were under consideration to be enrolled in a compassionate access scheme for Cannabidiol. 20 semi-structured interviews with parents or caregivers of children diagnosed with refractory epilepsy were voice-recorded, transcribed and analysed to generate common themes.
Results: Key themes emerged relating to seizure activity, family and school engagement, drug safety and legal access, efficacy, clinical support and social acceptance of the medication. The use of Cannabidiol was perceived to have benefits in relation to reducing the severity and frequency of seizure activity for some, but not all patients experiencing refractory epilepsy. For other patients, benefits included improved social engagement, wakefulness and a reduction of side effects related to a reduction of conventional medication dosage.
Conclusion: This study provided unique perspectives of families' experiences managing untreatable epilepsy, their experiences with conventional and experimental pharmacological treatments and health services. Whilst families' perceptions showed the use of Cannabidiol did not provide a therapeutic reduction in the seizure activity for all patients diagnosed with refractory epilepsy, it's use as an additional, safe pharmacological agent was perceived to provide other benefits by some patient families. The findings from this study can be used for comparison with electroencephalographic data for further analysis of Cannabidiol efficacy.

Eye gaze tracking as a biometric assessment tool for anxiety: A pilot study of cortisol related anxiety in children (CRAIC)

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Background: Findings from eye gaze tracking studies have shown eye gaze avoidance as a measure of anxiety and fear learning is consistent in studies of adults and children. As the use of study techniques using eye tracking techniques becomes more commonplace, there is a need to establish its validity in a broader research context to include typically developing children, children diagnosed with neurodevelopmental disorders and children diagnosed with anxiety.
Objectives: The objectives of this pilot study were to conduct assessments to:
1. Determine the validity of, and develop protocols for establishing Eye Gaze Tracking as a reference biomarker for anxiety associated with Autism Spectrum Disorder (ASD) and rare neurodevelopmental disorders with a high prevalence of anxiety (RND- ANX), as a model for Anxiety in the general paediatric population;
2. Conduct clinical trials to comparatively assess eye gaze with other biological stress markers in children diagnosed with ASD and RND-ANX to determine if change in anxiety is measurable and repeatable.
Methods: The pilot study was conducted at the Centre for Clinical Trials in Rare Neurodevelopmental Disorders (CCTRND). Participants were children (n=3) described as typically developing aged 2-12 years. Study instruments were tested after procedures were explained, participant assent and parent consent were obtained. Instruments included lux light meter (Digitech), saliva swab collection (Salivette), heart rate variability (HRV) mini-electrocardiograph (ECG) 3-lead device, HRV finger monitor device (CorSense), Tobii (X3-120) eye gaze tracking and a parent-administered questionnaire for anxiety (the past week, Spence). Data viability analysis included collection, storage and analytical methods to understand assessment timing, limitations and usefulness of assessment instruments.
Results: Each of the assessment instruments had different data storage mechanisms requiring an understanding for each. Software licenses and hardware purchases were required for specialised tools necessitating tailored training and the establishment of clear lab protocols, manuals and training research officers. The data for each instrument was successfully collected. For viable comparison, the data was collected concurrently.
Conclusion: This study provided unique an understanding of the viability of the validation study for participant families engaging in a clinical trial, their experiences with conventional and experimental assessment instruments and health services.
The proposed studies are feasible and will provide evidence for the establishment of a biological marker for the assessment of anxiety in children at high risk of anxiety-related comorbidities impacting their ability to communicate and learn.

Title: Exploring Ketogenic Diet Predictors of Success in Children with Epilepsy

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Background: The ketogenic diet (KD) is an effective treatment used in refractory childhood epilepsy. The extent to which patient and diet-related variables enhance treatment outcomes enabling targeted therapy is poorly understood.
Objective: To evaluate efficacy of KD treatment and to search for child or diet-related factors that can predict its efficacy.
Methods: A retrospective chart audit of 53 children (Mean:4.9±4.1years, Range:1 month to 16years) receiving KDT at a tertiary Queensland hospital between December 2014 and April 2018 was performed. Patient variables (i.e. gender, age, seizure type, anthropometric measures, number of anti-epileptic drugs (AEDs)) and diet-related variables (i.e. diet type, route of administration (ROA), supplement use) were collected at baseline, three, six and 12 months and analysed against seizure control (defined by a >50% reduction in seizure frequency from baseline).
Results: Majority of participants (60%, n=32) had generalised epilepsy and initiated KDT on the more liberal, modified KD (53%, n=28). Of those following the stricter classic KD (n=25), the 3:1 ratio (grams of fat: protein and carbohydrates) was the most utilised at 68% (n=17). Oral ROA 68% (n=36) was more common than tube feeding. Of the remaining KDT participants at three, six and 12 months, 17/40, 13/22 and 12/13 reported attaining seizure control, of which 4/17, 7/13 and 6/12 were seizure free. Gender, age, seizure and diet type, supplement and AED use and anthropometric measures were not significant predictors of seizure control (all $p>0.05$). ROA with respect to seizure control approached statistical significance ($p=0.07$).
Conclusion: The KD is an effective treatment in reducing seizures in children and tube fed patients should be prioritized for the KD. Seizure type or number of anti-epileptic drugs should not preclude a patient being offer the KD or the type of diet used.
This information will improve selection processes, expectations, treatment plans and outcomes.

Does the Ketogenic Diet Change Body Composition?

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Background: The ketogenic diet (KD) is a high fat, low carbohydrate and nutrient deficient treatment used in refractory childhood epilepsy. Little is known about the effect of the KD on a child's body composition, which is a useful marker of nutritional status.
Objective: For children on the KD to compare
1) Body composition with healthy controls,
2) Resting energy expenditure (REE) with predictive equations
Methods: Children on the KD aged 5years or older were recruited from a Queensland children's hospital. The cross-sectional study measured anthropometry; body cell mass index (BCMI) from total body potassium; fat mass index (FMI) and bone mineral density (BMD) by dual X-ray absorptiometry (DXA). Variables were converted into z-scores and compared to healthy controls. Measured resting energy expenditure (REE) was compared to predictive equations.
Results: Fifteen subjects were recruited mean age 8.93years ± 2.97years along with 15 age- and sex-matched controls. There was no significant difference in BMI z-score ($p=0.68$) between the 2 groups. The study group was more malnourished ($p=0.02$) based on BCMI z-score, had a significantly higher fat mass based on FMI and lower BMD ($p<0.001$) compared to the control group. Measured REE was 62.7% on average higher than the predictive equations indicating moderate to good reliability.
Conclusion: These results indicate that children on the KD have altered body composition and poor bone health, which may not be detected via simple nutritional measures. Detection of body composition changes will enable improvement in clinical practice to help prevent the deterioration of nutritional status.

Preclinical development of cannabis-derived treatments for Dravet syndrome

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Dravet syndrome is a severe epileptic encephalopathy. Children with Dravet syndrome typically present with seizures during the first year of life, often in association with fever, with eventual progression to spontaneous, recurrent seizures. Following the onset of seizures, Dravet syndrome patients develop severe comorbidities including cognitive impairment, social deficits, ataxia and psychomotor dysfunction and have an increased risk of sudden unexpected death in epilepsy. Because these children respond poorly to currently available anticonvulsants, many families in Australia to resort to using illegal cannabis products, as there have been numerous reports of cannabis reducing seizures in Dravet syndrome. Cannabis, however, is a complex mixture containing hundreds of cannabinoids so the active constituent(s) need to be elucidated. Cannabidiol (CBD), a major component of cannabis, was FDA-approved for the treatment of Dravet syndrome. Here we screened novel cannabinoids in the *Scn1a*$^{+/-}$ mouse model, which mimics the hallmark features of Dravet syndrome, including spontaneous seizures, thermally-induced seizures, premature death and behavioral abnormalities. We evaluated the anticonvulsant potential of cannabinoids against hyperthermia-induced seizures in *Scn1a*$^{+/-}$ mice. Similar to the effect observed with CBD, CBDVA and CBGVA treatments increased the temperature threshold for thermally-induced seizures. CBDV, CBGA and THC treatments also increased the threshold but with much greater potency than CBD. Surprisingly, THCV treatment appeared to have proconvulsant effects, exacerbating hyperthermia-induced seizures in *Scn1a*$^{+/-}$ mice. The *Scn1a*$^{+/-}$ mouse model has been used to identify cannabinoids, other than CBD, that could provide therapeutic leads for the treatment of Dravet syndrome.

The establishment of the Global Angelman Syndrome Registry

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Background: Angelman Syndrome (AS) is a rare neurodevelopmental disorder affecting between 1 in 15,000 and 1 in 24,000 individuals. The condition results in severe developmental and expressive language delays, motor impairments, and a unique behavioural phenotype consisting of excessive laughter, smiling and sociability. The Global Angelman Syndrome Registry was developed in response to a need for large scale longitudinal studies to advance research and therapeutics for this rare syndrome.

Method: A parent/ caregiver driven process was utilised to develop the Global Angelman Syndrome Registry. The registry consists of 12 modules which cover patient demographics; developmental, diagnostic, medical and surgical history, communication, behaviour and development, epilepsy, medications and interventions, and sleep. In September 2016 the registry was deployed via the Rare Disease Registry Framework (RDRF) developed by the Centre for Comparative Genomics (CCG) at Murdoch University. Parents and caregivers of individuals with Angelman Syndrome were invited by the registry team and syndrome organisations to submit data to the registry via a secure internet connection.

Results: Since its launch, 1329 individuals with AS have been signed up to the registry worldwide: 54% are from North America, 23% are from Europe, 14% are from the Asia Pacific region, 8% are from South America, and 1% are from the Middle East or Africa. The majority of registrants were children: 7.5% are aged <3 years, 14% are aged 3-5 years, 18% are aged 5-8 years, 21% are aged 8-12 years, and 14.5% are aged 13-18 years. Just under a quarter (24.5%) are aged over 18 years. Most participants indicated a chromosome deletion (68%), with fewer participants indicating a mutation (16%), paternal uniparental disomy (UPD;8.9%) or imprinting defect (ICD; <5%).

Conclusion: Findings indicate a need to target recruitment towards parents and caregivers of older children and adults, and families from non-English speaking backgrounds. The registry team is currently working on recruitment strategies and translation of materials into multiple languages towards this end.

The Impact of Seizure and Gastroesophageal Reflux History on Sleep and Behaviour in Angelman Syndrome

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Background: Angelman Syndrome (AS) is a rare neurodevelopmental disorder affecting 1 in 15,000 to 1 in 24,000 individuals. The condition results in severe delays in development and expressive language, and motor impairments. The Global Angelman Syndrome Registry was developed by families to facilitate longitudinal studies to advance research and therapeutics. This study describes preliminary clinical and behavioural outcomes.

Methods: Caregivers completed the Sleep Disturbance Scale for Children (SDSC; N=211) and a 29-item behavioural scale developed for Angelman Syndrome (N=243). Relationships between seizure and gastroesophageal history, and behaviour and sleep were explored via Spearman's correlations.

Results: A history of seizures was associated with higher levels of spontaneous laughter and smiling (*Spearman's r* = .216), behaviour dysregulation (e.g. hitting, *r* = .160), and repetitive behaviours (*r* = .149). Seizures were also associated with lower levels of initiating sleep disorders (*r* = -.154), and higher levels of excessive somnolence (*r* = .164) and breathing disorders during sleep (*r* = .151). While gastroesophogeal reflux impacted nearly 70% of individuals, more severe forms of reflux were associated with higher levels of disorders of sleep and breathing (*r* = .327). Constipation was associated with spontaneous laughter and smiling (*r* = .224) and repetitive behaviours (*r* =.204), with more severe forms of constipation associated with self injury (*r* = .172) and spontaneous laughter and smiling (*r* = .231). Constipation was also associated with more frequent experiences of sleep disorders related to sleep and breathing (*r* = .165) and excessive somnolence (*r* = .230). Vomiting with feeds was linked to higher incidence of repetitive behaviours (*r* = .151) and disordered sleep in relation to breathing, hyperhidrosis, and sleep-wake transitions (*r* = .190-240). Gagging was associated with a number of behaviours including appropriate laughter and smiling (*r* = .139), anxiety (*r* = .214), behaviour dysregulation (*r* = .157) and repetitive behaviours (*r* = .157). In addition, gagging was associated with sleep disorders related to breathing, excessive somnolence, hyperhidrosis, and sleep wake transitions (*r* = .177 - .230).

Conclusion: Repetitive behaviours, spontaneous laughter and self-injury may represent seizure activity or efforts to communicate discomfort associated with gastrointestinal disorders. Excessive somnolence, breathing disorders and reduced sleep onset disorders may be a side effect of seizure activity or anti-epileptics. Gastrointestinal conditions are also linked to a range of sleep disturbances.

Modelling social anxiety symptoms in Fragile X Syndrome using the Fmr1 knockout mouse

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Fragile X Syndrome (FXS), a form of intellectual disability in humans and the most prevalent monogenetic cause of Autism Spectrum Disorder (ASD), is caused by a single gene mutation in the FMR1 gene. Mutations in this gene cause loss of function to the fragile X mental retardation protein (FMRP) which plays a key role in regulating the expression of a wide range of proteins important in healthy cognitive development. Like humans with the *fmr1* loss of function mutation, $Fmr1^{(-/-)}$ mice have pronounced deficits in social interaction and motivation as well as locomotion, repetitive grooming and anxiety-like behaviours. Interestingly and given the high co-morbidity with ASD, social anxiety and avoidance is the most prevalent social deficit in patients with Fragile X Syndrome, present in as many as 75% of males. Until recently however, behavioural phenotyping of sociability has been limited in the ability to isolate aspects of social interaction from social fear. Using a social fear conditioning (SFC) task, we pair a mild foot shock with conspecific social interaction and examine fear extinction and recall profiles, so we can isolate social fear. In preliminary analysis, we have been able to show that whilst unconditioned wild type and $FMR1^{(-/-)}$ mice show the same levels of sociability as WT mice, conditioned $FMR1^{(-/-)}$ mice show more pronounced social fear and avoidance than wildtype mice (*p = 0.044, current n = 7 – 9, target n = 16*). This is thus the first ever work to properly model social anxiety symptoms in Fragile X Syndrome in the $FMR1^{(-/-)}$ mice. Having established our model, we plan to investigate potential pharmacological therapeutics most notably, phytocannabinoids CBD and CBDV.

Safety, Tolerability, and Pharmacokinetics of "Pharmaceutical Grade Full Spectrum Cannabidiol" Administered as Single Sublingual Wafer and Oil Solution in Health Volunteers

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Background: Cannabidiol (CBD) is the non-psychoactive component of the cannabis plant that has shown therapeutic effect for multiple conditions. There is limited information on CBD's pharmacokinetics (PK) in human.

Objective: To determine the safety, tolerability, and PK of Bod Proprietary CBD Extract after oral administration of single sublingual wafer and oil in healthy volunteers and to compare the PK profiles of CBD with Sativex oromucosal spray.

Methods: The study was open-label, four-way crossover in 12 healthy volunteers randomised to receive a sequence of four different single doses of CBD as WaferiX 25 or 50 mg CBD, oil solution 50 mg/mL CBD and eight actuations of Sativex (20 mg CBD and 21.6 mg THC).

Results: The CBD Extract, when administered in the form of wafer and oil, was generally well-tolerated by participants. The most common related adverse events (AE) were somnolence, sedation, and mood altered. All AEs were mild or moderate in severity. All participants completed the study. Administration of the Bod Proprietary CBD Extract in the form of wafer and oil solution, lad to bioavailable CBD and provided high plasma concentrations of CBD. Following administration of wafer and oil solution, the maximum concentrations of CBD occurred after 4-5 hours, with the terminal elimination half-life of 6 hours. The plasma exposure of CBD increased in a dose-proportional manner. The CBD Extract WaferiX 25 mg CBD had 25% greater exposure (AUC) than the equivalent dose of Sativex.

Conclusions: Both wafer and oil solution formulations were well tolerated and demonstrated safe and efficient delivery of CBD. The PK profile of CBD was fully characterised following a single dose of Bod Proprietary CBD Extract.

An Exploration of Single- Session Music Therapy to Reduce Clinic- Related Anxiety in Children with 22q11.2 Deletion Syndrome

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Background: 22q11.2 Deletion Syndrome is a rare genetic neurodevelopmental disorder caused by the microdeletion of genetic material from chromosome 22 at the Q11.2 band. High prevalence rates of depression and anxiety recorded in this population may be exacerbated during appointments based in a medical setting. Previous research that has explored effective ways to manage anxiety in clinical settings has found supporting evidence for single- sessions of Music Therapy (MT) in reducing stress and anxiety symptoms in children(Barrera, Rykov, & Doyle, 2002).

Objective: Therefore, this project aimed to explore whether a single session of MT is effective in reducing 22q11.2 DS children and adolescent's stress and anxiety levels, exacerbated by clinical environments during clinic- related health appointments.

Methods: To assess the effects of music therapy on clinic-related anxiety, a combination of objective and subjective measures were utilised including: electrocardiographs to assess heart rate; Visual Analogue Scale to measure perceptions of anxiety by participants and family members (pre-post); Observational analysis of patient anxiety-type behaviours, bodily and facial expressions gleaned from audio-visual recordings of the music therapy session.

Results: Emerging results indicate that immediately after the music therapy session there were decreases in heart rate, anxious eye contact, facial expression and body behaviours. There was also an increase in the duration of non-anxious behaviours at the end of the session. Parent rated scales also showed a significant positive change both immediately after the music therapy session and for the remainder of the day after the music therapy session.

Conclusion: Based on the current data, the hypothesis that clinic related anxiety will decrease immediately after a single music therapy session is supported. The data also supports that the effects of music therapy in reducing anxiety extend post session.