ICNC-0509  Early detection of autism spectrum disorder (ASD) by applying specific preferential-looking behavior

Purpose: It is well known that children with autism spectrum disorder (ASD) prefer to look at geometric figures rather than people. Based on this specificity, we conducted a cohort study to detect ASD at 18 months old. In this study, our aim was to identify the most sensitive image.

Subjects and Methods: The subjects were 107 high-risk infants suspected to have developmental disorders at health examinations at 18 months old. Forty-eight age-matched normative infants served as controls for the examination of the differences in preferential-looking behavior. Using an eye gaze detector (JVC KENWOOD Corporation, Japan), we determined the most sensitive image among seven images of people and geometric figures. Each infant was examined for two minutes.

Results: Ninety-nine (92.5%) infants in the high-risk group and 45 (93.9%) in the control group completed the examination. Among the seven images, we identified the most sensitive image; the high-risk group significantly preferred a geometric figure while the control group preferred an image of people (p<0.05, respectively). There were no significant differences in the six other images.

Conclusion: We demonstrate that eye gaze examination is feasible to identify the most sensitive images. Our results show that examination eye gaze behavior could be successfully conducted in two-year-old infants, and that specific preferential-looking behavior in ASD infants is not always observed, but is dependent on the image. Our findings are useful for future cohort study of high-risk infants for ASD.

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Treatment of Fragile X Syndrome targeting the cAMP pathway

Introduction. Fragile X syndrome (FXS) is the most common cause of single gene intellectual disability (ID) but remains without treatments. In addition, patients may suffer from autism spectrum disorder, sleep disorder and epilepsy. We have developed a FXS fruit fly, Drosophila, model that recapitulates cognitive, sleep and social interaction defects seen in FXS patients. Using a classical conditioning paradigm we showed that excess protein synthesis was key to the memory dysfunction in FXS. In addition, memory in human and Drosophila depends on the cyclic AMP (cAMP)-CREB pathway. We used well-established assays for memory in Drosophila and mice to test if cAMP signaling modulation can rescue memory in FXS.

Methods: We used the classical olfactory memory and the courtship memory assays in Drosophila. We tested the effect of phosphodiesterase inhibitors (PDEi) administered either acutely or chronically on memory performance. Next, we tested the effect on long-term depression. We performed acute and chronic PDEi application on hippocampal tissue from FX mice.

Results: We observed that FX flies presented significant rescue of short and long-term memory performance after administration of PDEi for both olfactory and courtship memory. Also, acute and chronic administration of PDEi were able to rescue the enhanced LTD seen in FX mice.

Conclusion/Significance: FXS is the most common single gene cause of ID but also ASD. It is also associated with epilepsy and circadian rhythm dysfunction, making it a very important for the treatment of neurodevelopmental disorders. These results suggest a new avenue for the treatment of FXS and ID.


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ICNC-0491  **Classifying paroxysmal events in Rett syndrome**

Introduction Rett syndrome (RTT) is a rare neurodevelopmental disorder, which predominantly affects females. Epileptic seizures, breathing- and movement disorders are often present, causing paroxysmal events. The correct diagnosis of these paroxysmal events is mandatory for treatment. Method As part of our assessment protocol a comprehensive clinical and neurophysiological examination is performed at least once in every Rett female. The examination consists of a multimodal registration with recording of electroencephalogram (EEG) with time-locked recording of video and autonomic parameters (heart rate, cardiac vagal tone, blood pressure, breathing patterns, pO2, pCO2, oxygen saturation). The registration is performed during wakefulness and (night) sleep and has a duration of around 20 hours. Results Multimodal video-EEG polygraphy was performed in over 50 females with RTT between 2012 and 2015. Preliminary results show that diagnosis of paroxysmal events was changed from epileptic to non-epileptic and vice-versa in several RTT females, having significant impact on handling and treatment. In the presentation the clinical features of paroxysmal events will be discussed and EEG- and video examples will be given to highlight the pitfalls in clinical judgment. Conclusion In RTT EEG-video polygraphy is essential to characterize paroxysmal events, differentiating autonomic dysfunctions from epileptic seizures and stereotyped motor phenomena in order to determine the optimal treatment policy.

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Glatiramer Acetate clinical trial in RTT syndrome – hopes and real life copaxone

BDNF deficiency role in the pathogenesis of the phenotype in RTT mouse models is well established and there is accumulating evidence for positive effects for its replenishment. There are also hints for its role in the syndrome severity in RTT girls. Because there is no practical way to supply BDNF directly researchers in the field of RTT looked for indirect ways to increase its CNS levels.

Use of re-purposing drugs for clinical trials and planning for future treatment is considered much less cumbersome in comparison to introducing novel drugs which were never studied in human overcoming much more easily safety related issues.

Glatiramer acetate (GA) is an immune modulator which is used for more than 20 years for chronic treatment of multiple sclerosis and it is safely used in both adults and children with this disorder although not formally approved yet for the pediatric age. In addition to immunomodulation it was found to increase CNS BDNF levels in several disease models in both direct and indirect modes. After showing in a proof of concept approach that GA treatment increases BDNF cortical levels in KO RTT model and receiving data on its safe use in an MS regimen in a RTT mice model we decided to go into clinical trial.

Our plan was to recruit 10 MECP2 mutation proven females with classical RTT syndrome from 6-16 years of age for a 6 months open label exploratory trial with GA in the approved MS dosage. Our chosen primary outcome measure was degree of reduction of epileptiform activity as measured by 24 hours Video EEG (for this we had to include only girls with significant epileptiform activity assessed by the same method in the baseline phase). Secondary outcome measures were related to safety and tolerability of its use in RTT girls and also to its effects on breathing abnormality assessed by 24 hours repeated breathing irregularities monitoring. In addition general behavioral, gait, sleep, hand stereotypies and use were assessed by parents questionnaires and by direct and short video recording observation of the investigator physicians.

Ten patients were recruited and entered the trial in 2 weeks intervals. To our disappointment we encountered the first severe adverse event (SAE) 3 months into the trial. The SAE was of immediate post injection sudden prolonged apnea followed by a seizure, diffuse flushing and mild edema in one patient. This was followed by a similar but of differing severity response in 2 more girls soon after leading us to hold the trial. After thorough re-evaluation of the response we concluded that it is paralleling the acute post injection response described in up to 10% of GA MS treated patients. We received IRB re-approval to re-start the trial but after recruiting 4 girls and 2.5 month into the trial another child had the same severe response and we decided to stop the trial.

Our partial results related to the chosen outcome measures and the procedures of evaluating the SAE will be presented. We will also present additional laboratory related data on the effect of GA in a cellular model of RTT.

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Calcium Channelopathies: Genotype-Phenotype Correlations to Neuropsychiatric Disorders

Introduction: Calcium channelopathies are associated with various neurological phenotypes, but little is known about their association with neuropsychiatric disorders (NPD), such as anxiety disorders, mood disorders, obsessive-compulsive disorder, functional symptoms (fatigue, pain) and/or autonomic phenomena. Methods: We performed a retrospective review of patients undergoing targeted exome DNA sequencing of nuclear mitochondrial genes (Courtagen Life Sciences, Woburn, MA, USA). We identified those with at least one variant of uncertain significance in CACNA1A, CACNA1S, CACNA1H, RYR1, and RYR2. Variants were compared to clinical information to determine genotype-phenotype relationships for NPD with/without neurological disorders, autonomic over-responsivity (AOR), or other organ dysfunction (GI, Cardiac). Results: There were 49 patients with at least one VUS in the genes of interest. 96% had an identifiable underlying neurological disorder, and 98% NPD and/or AOR. 35% had cardiac symptoms (tachycardia, postural hypotension, arrhythmia), 65% gastrointestinal, 63% functional, and 29% frank dysautonomia. 29% of patients experienced worsening of symptoms with stress. 18 patients received medical treatment with calcium channel modifying medications or supplements. 9 (50%) showed clinical improvement, 4 (22%) showed no improvement, and the remaining 5 patients have not yet been reevaluated. Signs of clinical improvement included improved behavior patterns and/or reduction in autonomic symptoms, as well as improved overall functionality. Conclusions: Calcium channelopathies may be causative or contributory to NPD and concomitant symptomatology. Genotype-phenotype correlates are redefining NPD into biological sub-phenotypes that can inform diagnostic and therapeutic interventions. Future studies are planned to compare NPD and neurological phenotypes of patients with and without conserved calcium channel variants.

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Ciliopathies: Clinical and Genetic Spectrum

Introduction:

Ciliopathies comprise a spectrum of multisystemic disorders caused by mutations in genes encoding proteins that are fundamental to the structure and function of primary cilia. Our objective is to demonstrate the diversity of clinical manifestations and genetic heterogeneity of ciliopathies.

Methods:

Seven subjects were ascertained with ciliopathy in a cohort of patients. Molecular studies were performed by next generation sequencing and Sanger sequencing.

Results:

Biallelic mutations were found in the KIAA0586, CEP290, NPHP1 (3 siblings), CSorf42, and B9D1 genes. Brain MRI revealed molar tooth sign in all patients. The organ involvement, degree of intellectual disability and outcome varied. The patient with KIAA0586 mutation had lethal short-rib polydactyly syndrome. Joubert syndrome due to CEP290 mutation was associated with retinal dystrophy and renal involvement. Whole deletion of the NPHP1 gene resulted in chronic kidney disease. The phenotype in CSorf42 resembled classic Joubert syndrome. B9D1 mutation caused mild Joubert syndrome.

Discussion:

The lethal phenotype due to an early Sonic hedgehog signalling defect in the KIAA0586-affected patient represents the severe end of the spectrum. The proteins encoded by CEP290, NPHP1 and CSorf42 are parts of interacting proteomic modules. Defects in multiple interlinked cellular mechanisms may lead to tissue organization defects, such as renal cysts, or retinal degeneration, whereas altered Sonic hedgehog signalling appears responsible for cerebellar malformation with molar tooth sign. The mild end of the spectrum is represented by the B9D1 mutation, previously found only in Meckel syndrome. Studies on ciliary protein networks might elucidate further the genotype-phenotype correlations.

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Introduction: In vitro and in vivo modeling of neurodevelopmental disorders is often labor-intensive and time-consuming. Here, we aimed to apply a newly developed method, clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 genome editing, to create a cellular model for a microcephaly syndrome, asparagine synthetase (ASNS) deficiency. Methods: We studied two families (Omani and Saudi) with congenital microcephaly, seizures and infantile death by whole-exome and Sanger sequencing. We generated ASNS cDNA with mutations by standard methods. We generated ASNS-deficient HEK293FT cell lines by CRISPR-Cas9 genome editing. We measured cell proliferation and apoptosis using PrestoBlue and Apo-ONE assays, respectively. Results: We identified homozygous mutations in ASNS in both families: p.Leu190* (Omani) and p.Arg404His (Saudi). Mutant cDNAs showed reduced protein abundance compared to the wild-type when transfected to HEK293FT cells, suggesting that these mutants represent a loss of function. Analysis of ASNS-deficient HEK293FT cell lines revealed an almost complete lack of cell proliferation as well as increased apoptosis. However, when the culture medium was supplemented with asparagine, cell proliferation and apoptosis rate returned close to that of the wild-type cells. Conclusions: Our results show that CRISPR-Cas9 genome editing can be an effective method for creating cellular models of neurodevelopmental disorders. We show that ASNS deficiency leads to reduced proliferation and increased apoptosis, and suggest that these are likely pathogenetic mechanisms of microcephaly in this condition. We also show that asparagine supplementation alleviates the cellular phenotype, implicating a path toward potential treatment of this devastating condition.

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