Abstract book

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The *FMR1* premutation affects millions of people around the globe. Despite the high prevalence of this condition, the extent of its impact on human health is the subject of considerable controversy. A fundamental unanswered question is whether carrying the premutation allele is directly correlated with clinical phenotypes, or whether there are other factors contributing to this observed association. As most past studies were conducted on clinically-ascertained premutation carriers drawn from families including a child with fragile X syndrome, it is possible that ascertainment bias, stressful parenting, or knowledge of one’s own genotype could have influenced the results. Here, we report on the results of analysis of electronic health records that has the potential to address these confounding factors. We created the first population-based *FMR1*-informed biobank to discover the pattern of health characteristics in premutation carriers. Our extensive phenotyping based on the analysis of 40 years of electronic health records shows that premutation carriers experience a significantly higher burden of disease throughout the lifespan compared to those with CGG repeats in the 24-40 range. Using machine learning methods, males and females with the premutation were successfully differentiated from controls with 66% accuracy. For females, the phenotypes that emerged as significantly elevated in premutation carriers vs. controls (p <.01) included mental disorders (agoraphobia, social phobia, panic disorder), genitourinary (infertility, irregular menstrual bleeding, dysmenorrhea), and injuries (fracture of radius and ulna, fracture of upper limb, rotator cuff sprain). For males, the significantly elevated phenotypes (p <.01) were mental disorders (mood disorders, major depressive disorder, other specified nonpsychotic and/or transient mental disorder) and genitourinary (urinary incontinence, other symptoms and disorders of the urinary system). Comprehensive understanding of the clinical risk associated with this genetic variant is critical for premutation carriers, their families and clinicians and has important implications for public health.

**Response Inhibition Skills are Modulated by Age and CGG Repeat Length in Women with the FMR1 Premutation**


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**Introduction.** Limitations in executive function, including deficits in response inhibition, have been documented among carriers of the *FMR1* premutation. However, symptom severity varies widely, particularly in female carriers, and clinical management of this group has been limited by poor understanding of personalized risk factors. The present study investigated age and CGG repeat length as factors that may account for variable presentation of inhibition deficits in female carriers of the *FMR1* premutation. **Methods.** Participants included 134 women with the *FMR1* premutation, aged 39-88 years (mean = 56). All participants were mothers to a child affected by fragile X syndrome. Inhibition skills were measured with both direct-assessment and self-report measures: the Hayling Sentence Completion Test (Burgess & Shallice, 1997) and the Behavior Rating Inventory of Executive Function-Adult Version (BRIEF-A; Roth & Gioia, 2005). Buccal samples were collected and *FMR1* CGG genotyping was conducted. **Results.** Across both inhibition measures, increased symptoms were observed in women who carried mid-range CGG repeat lengths of about 80-100 repeats, relative individuals who carried lower or higher premutation repeat sizes. A second zone of vulnerability was also observed at 120-140 CGG repeats on the Hayling index. Inhibition symptoms became more pronounced with older age, resulting in the greatest symptom presentation among women who were older and carried mid-range CGG repeats. **Conclusion.** Older age and mid-range CGG repeat length may represent personalized risk factors for inhibition deficits among female carriers of the *FMR1* premutation. Inhibitory deficits may contribute to poor outcomes for carriers of the *FMR1* premutation and their families, particularly in midlife and early old age. Understanding of individualized risk factors can facilitate the clinical monitoring and management of executive deficits in women with the *FMR1* premutation.
Clustering of co-morbid conditions among FMR1 premutation carrier women

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About 20% of women who carry an FMR1 premutation (PM) allele develop fragile X-associated primary ovarian insufficiency (FXPOI), defined as hypergonadotropic hypogonadism with irregular or absent menstrual cycles before age 40. A non-linear association between FXPOI and PM repeat size has been documented, where those with mid-range repeats (~70-100 repeats) are at highest risk. Studies also report increased rates of co-morbid mental health and medical conditions by PM women. PM women are also at risk for developing a late-onset fragile X-associated tremor-ataxia syndrome (FXTAS). The risk for FXTAS increases linearly with repeat size, indicating a different molecular mechanism than FXPOI. We collected medical and reproductive histories among 355 PM women, including 87 with FXPOI and 168 without FXPOI. Overall, 22 health conditions were reported by more than 10% of PM women. Notably, anxiety, depression, tension headaches, and migraines were reported by more than 30%. Survival analysis indicated that women with FXPOI compared with women without FXPOI had an earlier age at onset for anxiety, fibromyalgia, and osteoporosis. Cluster analysis identified eight clusters of PM women with similarities in co-morbid conditions. Interestingly, the majority of PM women fell into one of three categories primarily defined by increased rates of only one to a few conditions: “Minimal conditions” (N=123), “Headaches” (N=33), and “FXPOI with minimal other conditions” (N=67). A single cluster defined women with symptoms of FXTAS, and none of these women met criteria for FXPOI. Altogether, there is evidence that some women with a PM experience complex health outcomes, but there is not a global impact of the PM on health with most PM women at risk for only minimal co-morbid conditions. Further, women with symptoms of FXTAS appear to be distinct from women with symptoms of FXPOI, confirming theories that these two PM-associated phenotypes are likely the result of different molecular mechanisms.

Ehlers Danlos Syndrome, Aneurysms and Spontaneous Coronary Artery Dissection (SCAD) in Premutation Carriers

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The lack or deficiency of FMRP leads to significant connective tissue problems in those with the full mutation and also in a subgroup of premutation carriers, especially those a CGG repeat allele in the upper premutation range. Low FMRP leads to elastin abnormalities and MMP9 elevation, the latter leading to extracellular matrix degeneration and negative consequences for connective tissue. Riddle et al (1998) previously reported that 30% of female carriers had prominent ears and hyperextensible finger joints. However, connective tissue problems have been a forgotten phenotype among carriers particularly in the last decade as multiple other medical conditions including FXPOI, FXTAS and FXAND have been documented in carriers. Here we present 2 patients diagnosed with Ehlers Danlos Hyperextensibility syndrome before the diagnosis of the premutation. In addition, we present 3 cases of arterial aneurysms in carriers, problems that have not been reported in those with the full mutation. These cases suggest that factors beyond simply low levels of FMRP may predispose carriers to connective tissue problems including aneurysms and SCAD that have serious medical consequences.
We discuss the relevance of white matter lesions (wmhs) in different brain regions to the broad spectrum of neurological and cognitive changes manifesting in male versus female carriers of Fragile-X Mental Retardation 1 (FMR1) premutation alleles (PM). We present the results of relationships between hemispheric and cerebellar wmhs and motor and cognitive impairments in a total sample of 30 male premutation carriers aged 39-81 years, and separately in a subgroup of 17 of these same carriers affected with Fragile X-Associated Tremor/Ataxia syndrome (FXTAS). Regional and total wmhs on MRI, assessed using semiquantitative scores, were correlated with three motor rating scales (ICARS, UPDRS, Fahn-Tolosa-Marin Tremor), and neuropsychological measures of memory, reasoning and processing speed. Significant relationships of infratentorial wmhs with all motor scales and several cognitive measures occurred across the total sample of carriers. This suggests that progression in infratentorial wmhs may be correlated with progression in motor and cognitive impairments across the categories of clinical status (non-FXTAS carrier versus FXTAS). In contrast, white matter lesions in supratentorial regions were confined to the FXTAS subgroup, where highly significant relationships were recorded between periventricular wmhs and the UPDRS (parkinsonism) score, and between both deep white matter and periventricular burden and the ICARS (ataxia) score. These findings emphasize the relevance of infratentorial white matter changes in male carriers to the extent of tremor/ataxia in female carriers. We discuss the possibility of genetic modifier(s) in carrier females, which might be protective against the development of the cerebellar white matter lesions typically occurring in male PM carriers across the spectrum of neurological involvement.
Establishment of genetic counseling guidelines for risk of FXPOI among premutation women
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Fragile X-associated primary ovarian insufficiency (FXPOI) is seen in about 20% of all women who carry a premutation allele (55-200 CGG repeats). These women develop hypergonadotropic hypogonadism with irregular or absent menstrual cycles before the age of 40. A non-linear association with repeat size and risk for FXPOI has been seen in multiple studies of women with a premutation allele: those with a mid-range of CGG repeats are at highest risk (~70-90 CGG repeats). Definition of the risk for FXPOI by repeat size could provide an important resource for genetic counseling and family planning purposes among premutation carriers. As an initial effort, we have defined the risk by repeat size described in the table below from information collected on 1,579 women in our previous research projects. We propose a collaborative effort for combining information from clinical and research datasets to publish this important resource to be shared with premutation carrier women and their health care providers.

<table>
<thead>
<tr>
<th>Repeat size group</th>
<th>Mean Age at Interview ± SD (N*)</th>
<th>Age at Menopause/POI onset ± SD (N*)</th>
<th>% POI (N*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40 45-53= 8= 16= 5= 453= 49= 2= 5= 92= 2= 146= p=</td>
<td>40-50 37.9 ±14.4 (148) 46.1 ±5.7 (8) 5.6% (18)</td>
<td>51-60 43.4 ±1.64 (74) 47.6 ±9.1 (23) 7.0% (43)</td>
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<tr>
<td>61-70 51.9 ±16.4 (107) 46.0 ±5.6 (58) 11.0% (82)</td>
<td>71-80 49.5 ±13.8 (203) 41.4 ±7.2 (130) 32.1% (159)</td>
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<tr>
<td>81-90 45.2 ±12.6 (231) 39.2 ±8.3 (111) 39.5% (148)</td>
<td>91-100 44.8 ±12.8 (189) 40.8 ±7.8 (88) 31.7% (120)</td>
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<tr>
<td>101-110 45.5 ±12.6 (70) 42.0 ±9.0 (38) 28.6% (49)</td>
<td>111-120 45.1 ±12.5 (33) 41.9 ±7.6 (16) 26.9% (26)</td>
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<tr>
<td>121-130 40.8 ±10.3 (41) 42.4 ±9.9 (9) 19.0% (21)</td>
<td>131-199 38.8 ±13.6 (30) 43.7 ±7.5 (10) 18.7% (16)</td>
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</table>

* Sample size varies because not all women have an assigned age at menopause or meet the definition for having POI or not.

Neuropsychological and motor changes in aging FMR1 premutation carriers: Towards characterization of the FXTAS prodrome
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Fragile X premutation carriers are at risk of developing the neurodegenerative disease fragile X-associated tremor/ataxia syndrome (FXTAS). Longitudinal research plays a critical role in identifying which carriers are at the greatest risk, which factors may influence the age of onset and rate of progression of the disease, and which treatment domains are the most important to target in future trials. Here, we present preliminary results from longitudinal NIH-funded project.

The cohort for the present analysis consisted of 77 males, 52 with the FMR1 premutation (55-199 CGG repeats) and 25 non-carrier controls (less than 40 CGG repeats) between 40 and 80 years. At enrollment, none reported having interfering symptoms of tremor or ataxia. Of these participants, 77 had baseline data (52 carriers), 71 had follow up data from a second visit (50 carriers), and 29 had data from a third visit (19 carriers), with visits about 1-3 years apart. Sixteen carriers have developed FXTAS (“converted”) during the study thus far. The assessment battery consisted of measures of spatial memory, episodic memory, visual and auditory working memory, motor speed, dexterity and control, response inhibition, sustained attention, and planning and problem solving. Diagnosis of FXTAS and staging was carried using a standardized movement disorder assessment.

Linear mixed-effects models based on age showed that premutation carriers demonstrate a significantly faster rate of decline in motor dexterity and speed, and visuospatial working memory compared to controls, declines that are linked to worsening of FXTAS stage. Logistic regression models revealed that CGG repeat length is a significant predictor of FXTAS conversion. Rates of change in the other domains of measurement listed above did not differ between carriers and controls. Changes in visual working memory (highlighted by prior fMRI studies) and bradykinesia may among aspects of the FXTAS prodrome to be monitored in presymptomatic carriers.
Identification of genetic modifiers of FXTAS by combing whole genome sequencing with fly genetics
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Fragile X-associated tremor/ataxia syndrome (FXTAS) is an adult-onset neurodegenerative disorder caused by the premutation CGG repeat expansion (55-200 repeats) within the 5'UTR of FMR1. A significant proportion of male premutation carriers develop the FXTAS phenotype later in adulthood, which includes intention tremor, cerebellar ataxia, progressive neurodegeneration, parkinsonism and cognitive decline, while other male carriers do not exhibit disease at all. In an effort to tackle this conundrum, we set out to identify genetic modifiers that modulate CGG toxicity and that may account for the variable phenotype and onset of disease. We performed whole genome sequencing on 110 male premutation carriers (CGG55-200) and prioritized candidate variants to select candidate genetic modifiers. Out of the 106 genes tested, we found that 25 genes genetically modulate CGG associated neurotoxicity in the fly eye, such as Prosbeta5 (PSMB5), pAbp (PABPC1L), e(y)1 (TAF9) and CG14231 (OSGEPL1). Interestingly, knockdown of Prosbeta5 (PSMB5) in the FXTAS fly resulted in significant suppression of CGG-associated neurotoxicity. Moreover, TRAP-seq performed on FXTAS mice cerebella showed an increased level of PSMB5 mRNA compared to wildtype. We further investigated the role of PSMB5 as a genetic modifier of FXTAS and its potential as a therapeutic target. We observed that knockdown of PSMB5 suppressed CGG associated neurodegeneration in the fly as well as in the mammalian N2A cell line, and an eQTL variant in PSMB5, PSMB5rs11543947-A, was found to be associated with decreased expression of PSMB5 and delayed onset of FXTAS in human FMR1 premutation carriers. Finally, we provide evidence that PSMB5 knockdown results in suppression of CGG neurotoxicity via both the RAN translation and RNA-mediated toxicity mechanisms. Taken together, our analyses suggest the presence of multiple genetic modifiers that could modulate the age-of-onset in FXTAS patients and PSMB5 could serve as a novel potential therapeutic target for FXTAS.

New discoveries in the pathology of FXTAS
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Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder associated with premutation alleles of the FMR1 gene. Expansions of more than 200 CGG repeats give rise to fragile X syndrome. FXTAS is characterized by cerebellar tremor and ataxia. The major pathology of FXTAS is the presence of intranuclear inclusions in neurons and astrocytes throughout the brain. In addition, FXTAS pathology presents as a broad white matter disease within cerebrum and cerebellum by cortical atrophy, ventriculomegaly, and brain stem atrophy. Abnormal areas of white matter show spongiosis, axonal degeneration and myelin loss. We have discovered that inclusions are also contained in endothelial cells and that microhemorrhages are common in the FXTAS brain. In addition, we discovered that FXTAS present with iron accumulation and with modified levels of iron-binding and iron-regulating proteins in the brain, indicating an alteration of iron transport and metabolism. We also found the presence of senescent microglial cells in half of the postmortem cases with FXTAS analyzed, and microglial activation in the remaining cases. Our findings indicate that inclusion-bearing endothelial cells in white matter fail to maintain capillary integrity, resulting in the release of erythrocytes that, upon breakdown, release iron. Iron accumulates in the capillaries and white matter tissue inducing activation of microglia and astrocytes. We here propose that FXTAS should be classified as a small vessel disease and that iron accumulation, microglial senescence and activation, and microhemorrhages, should be used as additional pathology signs for postmortem diagnosis of FXTAS.
Identifying modifying genes to explain the variation in severity of fragile X-associated primary ovarian insufficiency
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Fragile X-associated primary ovarian insufficiency (FXPOI) is a disorder among women with a premutation (PM), characterized by oligo/amenorrhea and hypergonadotropic hypogonadism before age 40. Co-morbid conditions include depression and anxiety related to hormonal changes and infertility, and reduced bone mineral density leading to osteoporosis. The risk for FXPOI is ~20% among carriers and depends on repeat length, the highest risk occurring among those with ~80-100 repeats. The presence and variability of onset is not completely explained by repeat length and we hypothesize that modifying genes are involved. To test this hypothesis, we have conducted a whole genome sequencing, case/control study among women who carry a PM. Cases are defined as those diagnosed with FXPOI before age 35 and controls as those with natural menopause after age 50. For the first 65 cases and 55 controls, we used a gene-based associate test (SKAT-O) to obtain evidence for genes being identified as potentially associated with case/control status, adjusting for repeat size. No gene was genome-wide significant, as expected for this sample size. To define possible candidate genes for further investigation, we ranked gene results by: 1) association test p-value <0.001, 2) presence of a fly ortholog, 3) published literature supporting a possible function in the ovary, and 4) gene expression in the ovary as indicated in GTEx. We chose 12 genes meeting these criteria to screen for altered ovarian function using the Drosophila model. The level of fecundity was compared among four genotypes: wild type, 90-repeat PM, candidate gene knockdown (KD), and double mutant PM x KD. We will present the evidence for these 12 candidate genes. Whole genome sequencing data from a second case/control sample of comparable size have been recently obtained and these will be analyzed to gain further evidence of the candidate FXPOI-modifying genes.

Autophagic alterations in skin fibroblasts from Fragile X-associated tremor/ataxia syndrome patients
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Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder with reduced penetrance that appears in adult FMR1 premutation carriers (55-200 CGGs). The neuropathological hallmark of FXTAS consists of presence of ubiquitin-positive nuclear inclusions that are broadly distributed throughout the brain. Additionally, bioenergetic deficits and mitochondrial dysfunction have been also reported. Since autophagy promotes cell survival by elimination of damaged organelles and proteins aggregates, we aimed to study its potential role in the pathogenesis of FXTAS. Skin fibroblasts cultures from FXTAS patients (n=4) were used to quantify autophagic flux (time-course bafilomycin a1-mediated autophagic inhibition) by measuring by western blot autophagic substrates (p62 levels), autophagosome formation (LC3-BII content) and mitochondrial amount (VDAC1 expression). The analysis of autophagic flux in skin fibroblasts cultures evidenced enhanced autophagic pathway activation and increased mitochondrial amount in FXTAS patients compared to controls. Increased autophagosome number was also confirmed by confocal microscopy. These findings suggest that the decrease in autophagic flux, abnormal cell waste may contribute to the pathology of FXTAS fibroblasts by causing the accumulation of incomplete degraded mitochondria, among other substracts. As a result, we postulate that FXTAS fibroblasts suffer from a bioenergetic collapse, leading to cell stress that triggers cell death. Acknowledgements: This work was supported by the Instituto de Salud Carlos III (PI17/01067 and PI18/00498), co-financed by Fondo Europeo de Desarrollo Regional (FEDER) “una manera de hacer Europa” and AGAUR from the Autonomic Catalan Government (2017 SGR1134). The CIBER de Enfermedades Raras is an initiative of the Instituto de Salud Carlos III.
OS03 - Oral session III
Oral presentation

O12

Antisense Oligonucleotides block RAN translation, enhance FMRP and reduce toxicity in CGG repeat expansion patient neurons.
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Large transcribed CGG repeats located in the 5’UTR of the FMR1 gene are potentially toxic as RNA or by triggering Repeat associated non-AUG initiated translation (CGG RAN). Moreover, large repeats can impede translation of FMRP even when transcription is sufficient. Therefore, effective therapies targeting large transcribed CGG repeats must simultaneously block CGG RAN and enhance production of FMRP while potentially also eliminating repeat RNA mediated toxicity. To this end, we developed a series of antisense oligonucleotides that selectively target RAN initiation sites (RAN ASOs) on the FMR1 transcript. In neurons derived from patients with expanded CGG repeats, RAN ASOs effectively reduced accumulation and toxicity of CGG RAN products and enhanced neuronal FMRP expression, suggesting that CGG RAN acts normally to inhibit FMRP synthesis. Blocking endogenous CGG RAN also altered activity dependent FMRP synthesis in response to mGluR5 stimulation, a critical regulatory component of long-term depression. Together, these data suggest a native function for CGG RAN in regulating FMRP synthesis and demonstrate that targeting CGG RAN has the potential to correct multiple disease relevant features in Fragile X-associated disorders.

OS04 - Oral session IV
Oral presentation

O13

Secondary structural choice of DNA and RNA associated with CGG/CCG trinucleotide repeat overexpansion leads to the RNA misprocessing in FXTAS
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Trinucleotide repeats belong to the family of microsatellites (a tract of 1 to 6 repetitive nucleotides) that are commonly observed in eukaryotes and exhibit repeat length polymorphism. The inherent ability of trinucleotide repeats to undergo abnormal expansion (viz. increase in repeat length) leads to many incurable genetic disorders that are mainly neurodegenerative. For instance, CGG repeat overexpansion in the 5’ un-translated region (UTR) of fragile mental retardation (fmr1) gene form unusual nucleic acid conformations and causes genetic instabilities. This results in fragile X syndrome (FXS) and fragile X tremor/ataxia syndrome (FXTAS). We have shown here that the number of G...G/C...C mismatches dictate the secondary structural choice of the sense and antisense strands of fmr1 gene and the corresponding transcripts. Circular dichroism (CD) spectra & electrophoretic mobility shift assay (EMSA) spectra reveal that CGG sense strand and its transcript favour quadruplex structure due to the intolerance for periodic G...G mismatch in a hairpin/duplex. Further, CD and molecular dynamics (MD) simulations show that more than four C...C mismatches cannot be accommodated in a RNA duplex consisting of CCG repeat (antisense transcript), instead, i-motif structure is favored. In contrast, CCG can form hairpin/duplex structure at the DNA (antisense strand) level irrespective of the number of C...C mismatches. Such unusual structures may be responsible for the increased R-loop stability, bidirectional transcription, RNA foci formation and repeat associated non-AUG translation for monoplypeptide aggregates in FXTAS, a mechanism similar to C9ORF72 GGGGCC repeat expansion that causes amyotrophic lateral sclerosis.
FMRpolyG may play a role in the pathogenesis of FXPOI by altering mitochondrial function.
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Sheba Medical Center, RAMAT GAN, Israel

Background:
It was recently demonstrated that the cytosolic FMRpolyG aggregates interact with mitochondria in Fragile X-associated tremor/ataxia syndrome (FXTAS) cells, animal model and neurons of FXTAS patients. Functional mitochondria are essential for oogenesis and ovarian follicle development and survival. Therefore, mitochondrial abnormalities may point to the mechanism of FXPOI.

Aim:
To investigate the effect of FMRpolyG on mitochondrial genes expression in COV434 FXPOI cell model and granulosa cells (GC) from FMR1 premutation carriers.

Methods:
Cov434 were transfected with pcDNA3CGG20x-(CGG)20x, 5'UTRGLY99xFMRPeGFPFMRpolyG-(CGG)99x-FMRpolyG and GLY99xeGFPCTL-(CGG)99x-CTL plasmids [Chantal Sellier, et al 2010]. Total RNA was isolated, quantified and cDNA was synthesized from FSH stimulated cells. qPCR was performed to assay the levels of mitochondrial DNA encoded transcripts. Human mural GC were isolated from follicular aspiration of a FMR1 premutation carrier (90 CGG repeats) and from five healthy, fertile controls (<55 CGG repeats) undergoing IVF-PGD treatments. β-actin was used as a reference gene. Relative expression levels were calculated using the $2^{\Delta\Delta CT}$ method.

Results:
Expression of functional mitochondrial genes (ND1, ND2, COX1, COX3 and ATP8) was significantly decreased in (CGG)99x-FMRpolyG compared to (CGG)20x repeats transfected GC. Moreover, 48h post transfection expression levels of mitochondrial structural genes (MFF, MFF1 and DRP1) were significantly reduced in (CGG)99x-FMRpolyG compared to (CGG)20x repeats transfected GC. Mitochondrial DNA content decreased gradually starting at 24h post transfection (p≤0.05). GC from a FMR1 premutation carrier demonstrated decreased functional and structural mitochondrial genes expression compared to healthy individuals. Furthermore, the pathogenic FMRpolyG protein was detected specifically in (CGG)99x-FMRpolyG transfected COV434 cells and in FMR1 premutation carrier GC.

Conclusions:
Our preliminary results suggest association between FMRpolyG expression and aberrant mitochondrial genes expression in GC. This may lead to defect in mitochondrial functions and structure. Further studies are essential for understanding the role of mitochondria in FXPOI pathophysiology.
OS04 - Oral session IV

Oral presentation

O15

Identification of common modifying genes related to age of onset of premutation-associated disorders.


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The molecular etiology involved in premutation (PM)-associated disorders, fragile X-associated tremor/ataxia syndrome (FXTAS) and fragile X-associated primary ovarian insufficiency (FXPOI), involves the consequence of the long CGG-repeat track in the mRNA of an FMR1 PM allele. However, the variability in the age of onset and severity of both disorders indicate other factors that interact with or are additive to the consequences of carrying a PM allele. Such factors may be common among the two PM-associated disorders and can provide insight into the associated molecular mechanism(s). To identify modifying genetic variants, we have obtained whole genome sequencing data from PM carriers: 89 men and women with onset of FXTAS before age 65; 58 men with no symptoms of FXTAS by age 70; 90 women with onset of FXPOI before age 35; and 90 women with natural age at menopause after age 50. We implemented our rigorous analytical pipeline to ensure high quality genotyping results. We conducted principal component analyses to identify any population substructure and adjusted subsequent analyses accordingly. We combined early onset FXTAS and FXPOI cases and compared them to late onset FXTAS and FXPOI controls using logistic regression or Fisher Exact test for common variants and gene-set burden analyses for rare variants. Initial findings revealed no common or rare variation reaching genome-wide significance, as expected for this limited sample size. We ranked gene-set burden p-value signals to identify candidate genes. Top ranked genes include: CACNB4, a gene encoding a calcium gated channel associated with episodic ataxia, and CAPR1, whose protein is reported to interact with FMRP in neuronal ribonucleoprotein complexes. From these initial analyses, we conclude that there are no large-effect modifying genes that explain age of onset common to both PM-associated disorders; however, we will conduct additional analyses to rank possible candidate genes for further investigation.

OS04 - Oral session IV

Oral presentation

O16

Monitoring and Modulating RAN translation in Fragile X-associated disorders

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Expansion of unmethylated and transcribed CGG repeats in the 5'UTR causes Fragile X-associated Tremor/Ataxia Syndrome and other Fragile X premutation associated disorders. This repeat supports translation in the absence of an AUG codon to produce multiple potentially toxic proteins through a process known as Repeat associated non-AUG initiated (RAN) translation. Our group has explored how this repeat allows aberrant translation and whether the products of RAN translation contribute to toxicity in model systems. Using both candidate based approaches and a genome-wide siRNA screen, we identified selective modifying factors for RAN translation at CGG repeats. These modifiers suggest that failures in start codon fidelity and RNA helicase activity are critical drivers of these RAN translation. In parallel, we are developing tools to quantitatively monitor RAN translation from the endogenous locus. These tools will allow us to accurately define the relationship between RAN translation and human neuronal phenotypes while also serving as a potential disease biomarker. Our long term goal is to develop tools that will identify novel targets for therapeutic development and aid clinical trial design in these currently untreatable conditions.
O17
Pathogenesis of FXTAS: an inclusion-centric view
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Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is a late adult-onset neurodegenerative disorder that affects a portion of carriers of premutation alleles (55-200 CGG repeats) of the FMR1 gene. The principal features of the syndrome; progressive gait ataxia and intention tremor, with ancillary features of cognitive impairment, peripheral neuropathy and dystautonmia, all occur in mid-late adulthood. However, growing evidence from animal models suggests that the underlying pathogenic mechanism(s) may be operating at or even before birth. Recent studies of mouse and human brain tissues suggest that a central feature of the pathogenesis of FXTAS involves calcium dysregulation and oxidative stress, although the initial triggering events remain incompletely defined; evidence and models for such triggers will discussed. Moreover, a systematic, data-independent acquisition (DIA) mass spectrometric analysis of purified inclusions, the central pathologic feature of FXTAS, has identified more than one-hundred proteins. These proteins fall into several classes that point to possible mechanisms for inclusion formation. The hope is that through an understanding of the events leading to FXTAS, we will be better able to develop targeted treatments for the earlier onset clinical presentations (e.g., ADHD, ASD, FXPOI) among carriers of premutation FMR1 alleles.

O18
Expression of CGG repeat-containing mRNA in either oocytes or granulosa cells leads to differential effects on female fertility in mice
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The Fragile X premutation represents the most common genetic etiology of primary ovarian insufficiency in women with a normal karyotype, but our current understanding of the molecular underpinnings of FXPOI is limited. Determining the consequences of CGG repeat expression in reproductive-aged women is hindered by lack of access to ovarian tissue for direct analysis. In this study, we use a mouse model to describe effects of premutation-length CGG repeat expression in specific follicular cell types. Two conditional alleles producing either CGG RNA-only (RNA-onlyPG) or CGG RNA that supports translation of the polyglycine product FMRpolyG (FMRpolyG+RNAPG) are used to examine oocyte (Gdf9-Cre) or granulosa-restricted (Cyp19-Cre) expression. We demonstrate that heterozygous females with oocyte-specific expression of FMRpolyG+RNAPG;Gdf9-Cre ovulate fewer oocytes, but heterozygous RNA-onlyPG;Gdf9-Cre females have oocyte counts comparable to controls. Superovulation of females with granulosa-specific expression reveals no change in retrieved oocyte number regardless of FMRpolyG production. We expected that ovarian dysfunction in response to hormonal cues would be exacerbated with age, as we observed in females with ubiquitous expression of CGG alleles. Interestingly, we found that both FMRpolyG+RNAPG;Gdf9-Cre and RNA-onlyPG;Gdf9-Cre females produce a comparable number of cumulative progeny through reproductive lifespan. Assessment of FMRpolyG+RNAPG;Cyp19-Cre and RNA-onlyPG;Cyp19-Cre females demonstrates that FMRpolyG+RNA expression in granulosa cells leads to a reduction in cumulative progeny toward the end of the breeding period studied. Histological evaluation of ovaries with oocyte- or granulosa-specific expression revealed that both recapitulated morphological features identified in females with ubiquitous CGG expression. Molecular analyses to confirm these similarities are underway. In summary, we demonstrate that expanded CGG expression in specific follicular cell populations leads to perturbed ovarian function, and CGG RNA plus the FMRpolyG peptide, but not CGG RNA alone is sufficient to confer these phenotypes.
Women carrying an FMR1 premutation risk developing Fragile X-associated Primary Ovarian Insufficiency (FXPOI). While approximately 20% of carriers will be clinically diagnosed with POI, the molecular underpinnings remain understudied. Ectopic expression of an expanded CGG tract is sufficient to drive ovarian dysfunction and reproductive senescence in mice with the FMR1 premutation. Using heterozygous females from two conditional mouse lines expressing either CGG RNA-only (RNA-only) or CGG RNA and its translated polyglycine product FMRpolyG (FMRpolyG+RNA) we demonstrate in this study that deficits in ovarian function are apparent in young mice and intrinsic to the ovary. Further, we leverage this early impaired ovarian response in CGG-expressing females to determine specific molecular processes that lead to reduced oocyte ovulation. Histological assessment revealed no statistical difference in the number of periovulatory follicles in CGG-expressing ovaries compared to controls. We observe a clear deficit in cumulus expansion and reduced meiotic resumption of oocytes in the periovulatory follicles of CGG-expressing ovaries. These findings suggest that dysfunction within existing follicles may underlie the decreased number of oocytes collected after gonadotropin stimulation. Follicles in FMRpolyG+RNA ovaries appear to have a broader range of abnormal follicular morphologies compared to follicles in RNA-only ovaries, but both CGG-expressing lines have a clear decrease in ovulated oocyte number. Analysis of canonical cumulus expansion-enabling factors revealed some changes in RNA-only but not in FMRpolyG+RNA ovaries. These alterations cannot completely account for our robust histological findings. Ultimately, we utilized RNA-sequencing of unstimulated immature ovaries to identify perturbed signaling pathways in CGG-expressing ovaries. Transcriptomic analyses implicated multiple pathways influencing granulosa cell function, including effectors of EGFR and MAP3K1 signaling. Cumulatively, we have identified cumulus expansion and meiotic resumption as critical follicular processes impaired by CGG expression, and we describe differential effects of FMRpolyG+RNA and CGG RNA alone that ultimately lead to reduced oocyte ovulation.

Establishment of a novel Granulosa cell-line model for the research of FXPOI pathophysiology

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The establishment of a novel granulosa cell-line model offering the opportunity to investigate the molecular mechanisms responsible for Fragile X-associated premature ovarian insufficiency (FXPOI) is not available. Therefore, the development of such model systems is important. In the current study we present a novel human granulosa cell-line model over-expressing pathogenic FMRpolyG protein for FXPOI research.

Aim:
The establishment of a novel granulosa cell-line model mimicking FXPOI conditions in vitro.

Methods:
Cov434, human immortalized granulosa cells (GC) were transfected with pcDNA3_CGG20x ((CGG)20x), 5'UTR-GL99x-FMRPeGFP-FMRpolyG ((CGG)99x-FMRpolyG) and GL99xGFP_CTL ((CGG)99x-CTL) plasmids [Chanté Elizur, et al. 2010]. FMRpolyG expression was detected using western blot analysis. Cells proliferation and viability was quantified by trypan blue staining and cell counting in a hemocytometer chamber. 24h post transfection total RNA was isolated, quantified and cDNA was synthesized from FSH stimulated and unstimulated cells. qPCR was performed to validate transfected cells response to recombinant FSH (rFSH) stimulation. β-actin was used as a reference gene. Relative expression levels were calculated using the \( 2^{-ΔΔCt} \) method. Western blot analysis was performed to assay AKT/mTOR pathway activation levels.

Results:
FMRpolyG expression was detected in (CGG)99x-FMRpolyG transfected GC. An increase in FSH receptor expression levels as well as cell proliferation was observed in response to rFSH induction in all plasmids transfected COV434 cells. However, (CGG)99x-FMRpolyG transfected cells viability was decreased 24h post transfection, compared to (CGG)20x) transfected cells (p=0.026). Moreover, activation of mTOR/AKT pathway was reduced in rFSH induced (CGG)99x-FMRpolyG compared to (CGG)20x transfected cells.

Conclusions:
Our preliminary results suggest that COV434 over-expressing pathogenic FMRpolyG, may serve as a novel human cellular model for investigating FXPOI, since mimicking its conditions in vitro.
**OS06 - Oral session VI**

**Oral presentation**

**O22**

**Breaking bad: The mechanism of repeat expansion in the FMR1-related disorders.**
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Expansion of the CGG repeat tract at the 5’ end of the FMR1 gene is responsible for the FMR1-related disorders. The expansion mutation occurs at high frequency via a mechanism that is still unknown. We have used a combination of patient samples and a mouse model to try to understand the underlying molecular basis of this unusual mutation. The mouse model recapitulates key aspects of expansion in humans including the requirement for transcription or transcriptionally competent chromatin and the fact that expansion involves a mechanism that is independent of cell division. Using this model, we have identified a number of proteins required for expansion, others that contribute to, but are not essential for, expansion and proteins that protect against expansions. Importantly, some of these factors have been shown to affect the Age At Onset or severity of other diseases that are thought to share a common expansion mechanism, suggesting that our data may also have implications for some human FX premutation carriers. One of the protective factors we have identified is Lig4, the DNA ligase essential for non-homologous end-joining (NHEJ), a process involved in the repair of double-strand breaks. This provides the first evidence for expansion proceeding via a double-strand break intermediate and as such, has important implications for the mechanism of repeat expansion. Furthermore, monitoring of the repeats in mice over time and in different cell types also provides interesting insights as to repeat dynamics that may be relevant for disease risk in humans.

**OS07 - Oral session VII**

**Oral presentation**

**O20**

**A Catalogue of the CGG Short-Tandem Repeats in the Human Genome**
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Greater than 30 genetic disorders have been identified resulting from short-tandem repeat (STR) expansions. Highly repetitive genomic regions are a challenge to analyze, especially when GC-rich and this has resulted in limited information on CGG-repeat distribution in the human genome. Nonetheless, there is strong evidence linking expanded CGG-repeats to multiple rare disorders. The CGG-repeat is best known as the genetic aetiology of fragile X syndrome and fragile X-associated tremor/ataxia syndrome in front of the FMR1 gene. We hypothesize that multiple as yet undisclosed CGG-repeat expansions in the genome exist and contribute to human disease. Here, we aimed to catalogue the CGG-repeats within the human genome utilizing genome-wide STR genotyping tools, including LobSTR and GangSTR. A control cohort of ninety human genomes was analyzed to detect and characterize CGG-repeats. 4662 unique uninterrupted CGG-repeats of 2 units or above were detected in our control cohort. 55% of the CGG-repeat loci were polymorphic, while the remaining 45% of the CGG-repeat loci were stable. Different CGG-repeat distributions were observed across the chromosomes. Chromosome 22 showed the highest density of both total repeat and unstable repeat numbers with 4.16 repeats per Mb and 2.33 polymorphic repeats per Mb, respectively. Every individual of our cohort displayed polymorphisms and between 7.86% to 13.22% of the CGG-repeat loci in each individual were polymorphic. Furthermore, it was observed that approximately 43% of the polymorphic repeat loci could undergo a significant expansion event, with several loci reaching a size equivalent to a premutation range as observed in other disorders. In general, the larger the median repeat length, the more polymorphic the CGG-repeat is, with repeats with a median size of 14 or higher always being polymorphic. However, unexpectedly, we observed occasional substantial expansions in CGG-repeat loci which normally exhibited median repeat lengths as short as 3 repeat units or less.
OS07 - Oral session VII

Oral presentation

O23

Ethnicity is an independent Risk Factor for Full Mutation Expansion in FMR1 Premutation Carriers

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Objective: To assess whether ethnicity affects the risk for full mutation expansion among Jewish FMR1 premutation carriers.

Materials and METHODS: All Jewish FMR1 premutation carriers who underwent chorionic villus sampling (CVS) or amniocentesis (AC) at Sheba Medical Center and the Tel Aviv Medical center during the period of 2011-2018 were included in this study. Ethnicity was determined by self-report as appeared in the medical records. Long range PCR for FMR1 (Asuragen, TX, USA) was performed in order to determine the number of CGG repeats and AGG interruptions in all women and fetuses. General distribution and data analysis were calculated using SPSS 18.0 and Microsoft Excel.

RESULTS: Seven hundred sixty six FMR1 premutation carriers underwent CVS or AC. Of them, 592 carriers had parents concordance for ethnic background (361 Ashkenazi and 231 Non-Ashkenazi Jewish). The number of CGG repeats was significantly higher among the Ashkenazi carriers compared to Non-Ashkenazi women (median 69 vs. 64; p<0.0001). Moreover, the number of AGG interruptions was lower in Ashkenazi (35% with no AGG’s) compared to the Non-Ashkenazi carriers (20% with 0 AGG’s); p<0.0001.

In 322/708 (45%) pregnancies the permuted allele was transmitted to the offspring. Multivariate analysis using women’s age, CGG repeats, AGG interruptions and ethnicity, revealed that women’s ethnicity was an independent risk factor for a full mutation expansion with a large Likelihood Ratio of 15.7 for Ashkenazi compared to Non-Ashkenazi population (p<0.03).

CONCLUSIONs: Carrier’s ethnicity may be an independent risk factor for a full mutation expansion among FMR1 premutation carriers, suggesting the existence of further components affecting the mother-to-offspring transmission process.

Further research is needed in order to identify other ethnic groups at risk and to better understand the process of CGG inter-generation expansion and the different variables that affect it. In addition, these findings may contribute for a precise genetic counselling.

OS07 - Oral session VII

Oral presentation

O24

Newborn screening for FMR1 expansions: Uptake rates from the first year of Early Check

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Aims. Early Check, which is funded by the National Center for Advancing Translational Science (NCATS U01TR001792-01) offers voluntary newborn screening for FMR1 expansions to 120,000 birthing parents annually in North Carolina. The goal of the study is to better understand the benefits of pre-symptomatic screening and treatment for rare disorders. This presentation will summarize the uptake rate from our first year of screening for FMR1 expansions, describe participant’s motivations for seeking screening, and present feedback about the screening process.

Methods. Through multiple outreach methods, all birthing parents in the state of North Carolina are offered additional newborn screening for FMR1 expansions, including fragile X syndrome (FXS) and the FMR1 premutation. Although both conditions require permission, receipt of FMR1 premutation results requires a second tier of consent after agreeing to FXS screening. Families of infants who participated in the screening were surveyed to obtain feedback on the screening process.

Results. To date, approximately 4,000 sample have been screened. Of those who agreed to FXS screening, approximately 45% have agreed to the return of FMR1 premutation results. The follow-up survey found that 66% of families remembered being offered the chance to receive FMR1 premutation results, whereas 9% did not and 25% were unsure. Although most families were satisfied with Early Check, some had frustrations with the screening process and interpretation of results (i.e., short sign up period, uncertainty of results).

Conclusion. Feedback from families about the Early Check screening process will help to refine consent procedures. In addition, planned in-person recruitment will lead to higher participation rates and earlier identification of babies with an FMR1 expansion.

** This presentation is complimentary to Anne Wheeler’s presentation which provides a summary of the short term follow-up of infants identified with an FMR1 premutation through Early Check screening.**
Early Developmental Profiles of Infants with a Premutation: Updates from the first year of Early Check
RTI International, DURHAM, United States of America

Aims. Evidence suggesting a genetic vulnerability to cumulating environmental stressors for individuals with a premutation highlight a need to understand the trajectory of symptom onset and severity. We now have an unprecedented opportunity to study very early development in infants with FXS and the premutation through our voluntary newborn screening program, Early Check. We are conducting a natural history study to explore the range and nature of disease expression and progression of the full spectrum of \( FMR1 \) expansions. This presentation will summarize our findings from the first year of screening.

Methods. All birthing parents in the state of North Carolina are offered expanded newborn screening for FXS, with the option of also receiving information about carrier status in their newborn. Families of infants who screen positive for an \( FMR1 \) expansion receive confirmatory testing, genetic counseling, and developmental surveillance through in-person and telemedicine modalities.

Results. As of this submission, 2 infants (1 male, 1 female) have been identified with a full mutation, and 7 (2 males, 5 females) have been identified with a premutation. All families are participating in follow-up. This presentation will provide a case series overview of the infants identified through Early Check, with an emphasis on family history, family reaction to the diagnosis, and developmental and behavioral findings. We will also describe our protocol for providing ongoing support, surveillance and intervention for the families involved in the Early Check program.

Conclusion. This presentation will summarize findings from the first large scale newborn screening study focused on identifying \( FMR1 \) expansions. Early Check provides an unprecedented opportunity to understand symptom onset and potential co-morbidities related to the premutation in infants and young children.

**This presentation is complimentary to Melissa Raspa’s presentation which provides an overview of Early Check and the procedures for recruiting and consenting families for premutation screening.**

FMR1 alleles and ovarian reserve: is the AGG pattern a neglected biomarker?
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The impact of the Fragile Mental Retardation-1 (\( FMR1 \)) gene in the female reproductive function is well established as the premutation carriers (55<CGG<200) are prone to develop primary ovarian insufficiency (FXPOI). This ovarian dysfunction is believed to be caused by a toxic effect due to the accumulation of \( FMR1 \) mRNA in ovarian cells, leading to follicular atresia and impairing follicle development, by a mechanism that remains to be elucidated. Additionally, recent studies suggest a higher predisposition to ovarian impairment in carriers of “normal” \( FMR1 \) alleles, sized under 26 or over 34 CGG triplet repeats. As these results are controversial further investigation is needed. It is particularly important to clarify if this new normal-low \( FMR1 \) sub-genotype (CGG<26) predisposes to an increased risk of ovarian dysfunction. Herein, statistical analyses were performed using data from 131 young and healthy females. A mathematical formula was developed taking into account total \( FMR1 \) CGG allele size, and AGG interspersions number and pattern, scoring the allelic complexity (allelic score). Plotting the score of both alleles showed distinct clusters and linear regression allowed categorization in two distinct groups (R\(^2\)=0.9631 and R\(^2\)=0.9751). Although no association with hormonal markers was found, one of these groups has an enrichment of the sub-genotype normal-low (CGG<26) alleles, suggesting that in addition to the allele size also the AGG pattern should be considered. Overall, our results suggest the use of this additional parameter to study \( FMR1 \) associated ovarian dysfunction.
Presymptomatic identification of FMR1 premutations in infants via population screening presents a myriad of challenges for genetic counseling: the effects are uncertain in children and unpredictable throughout the lifespan, the complex genetic mechanism is difficult to understand yet has far-reaching implications for unsuspecting family members, and low health literacy and income can limit access to information and support for many. The Early Check research study—which offers free, voluntary, expanded newborn screening (NBS) for all babies who have standard NBS in North Carolina–tests for the FMR1 full mutation with the option for parents to also receive premutation results. Approximately 450 babies with the FMR1 premutation are born each year in North Carolina, and due to the sensitivity of the screening assay virtually all are potentially identifiable through Early Check. Helping parents of children identified with the premutation understand and begin to adapt to the potential implications of these results demands a robust yet scalable model for return of results, education and ongoing support accessible to the general population. Here we present a multidimensional, patient-centered approach that capitalizes on web-based platforms using low literacy concepts. It layers a password-protected participant portal, direct phone access to a genetic counselor, secure telegenetic counseling, and scheduled follow-up outreach. Early Check fragile X premutation educational resources for parents and families reiterate and augment standardized core genetic counseling content and provide an enduring resource for families.

** This is complimentary to Melissa Raspa's and Anne Wheeler's presentations, which provide overviews of Early Check, the procedures for recruiting and consenting families for premutation screening, and early developmental profiles of infants with a premutation.

**OS08 - Oral presentation**

O27

Expanded newborn screening for fragile X premutation: Challenges in genetic counseling

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Objective: To determine the association between FMR1 gray zone expansions and the presence of parkinsonism, motor, and cognitive function in an elderly community-based population.

Background: FMR1 gray zone alleles (41-54 CGG repeats) have been associated with neurological signs that are seen in fragile X-associated tremor ataxia syndrome in a small number of cases. Neuropathological findings in a large population of gray zone carriers has not been reported in the past.

Design/Methods: Automated FMR1 PCR was performed on existing samples from two longitudinal aging studies at the Rush Alzheimer’s Disease Center, whose subjects agree to brain donation. A detailed clinical evaluation included a modified Unified Parkinson Disease Rating Scale score, a composite score of global motor function, and 17 cognitive tests summarized as a global measure of cognition. Neuropathological examination was performed to investigate for the presence of neuronal and glial intranuclear inclusions and other age-related pathologies.

Results: The average age of the population (n=2362) was 85.9±7.3 and average age at death was 88.6±6.4 (n=2362), with 72% women. The prevalence of FMR1 gray zone alleles was 5.2% (122/2362). There was no significant difference between gray zone participants and normal (≤40 CGG) in global cognition, global motor function, nor clinical diagnosis; after controlling for age, sex, education, race, and ethnicity. Gray zone alleles were associated with parkinsonism in men (p=0.01) and were more likely to die, dying approximately 3 years earlier than normal controls (p<0.001). There were no intranuclear neuronal nor glial inclusions seen in gray zone allele carriers (n=36).

Conclusions/Relevance: This is the largest study to investigate gray zone alleles in an older community population. The key outcome is that in men, the gray zone allele is associated with parkinsonism and an earlier age at death. This study is also the first to report neuropathology in FMR1 gray zone carriers.

**OS08 - Oral presentation**

O38

Fragile X Gray Zone Alleles in Men are associated with Parkinsonism and Earlier Death in an Elderly Community Population

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²United States of America

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Conclusions/Relevance: This is the largest study to investigate gray zone alleles in an older community population. The key outcome is that in men, the gray zone allele is associated with parkinsonism and an earlier age at death. This study is also the first to report neuropathology in FMR1 gray zone carriers.
Characterization of Antisense FMR1 (ASFMR1) Gene and Identification of Novel Splice Variants in Premutation Carriers

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Fragile X Associated Tremor/Ataxia Syndrome (FXTAS) is a late adult-onset neurodegenerative disorder that affects movement and cognition mainly in male carriers of an allele of 55-200 CGG repeats (premutation) in FMR1 gene. Comprehensive analysis of human FMR1 locus identified a novel Antisense gene (ASFMR1) that similar to the FMR1 gene is upregulated in premutation alleles and not expressed in full mutations, suggesting a potential role of bidirectional transcription to the clinical phenotype of FMR1 related disorders. The structure of ASFMR1 has not been well characterized, and multiple studies have only reported a single differentially expressed isoform between premutation carriers and controls. The primary objective of this study was to determine the structure of the ASFMR1 gene and to investigate the alternative splicing transcriptional landscape in premutation carriers as compared to healthy individuals.

For this purpose, we isolated total RNA from post-mortem cerebellar tissues derived from three male premutation carriers and three age-matched normal male controls. We generated multiplexed, full-length cDNA prepared using the Clontech® SMARTer® kit followed by target enrichment of the ASFMR1 gene using IDT probes. The library was sequenced on the PacBio Sequel system using one SMRT Cell. After sequencing, we obtained full-length, high-quality transcript sequences using the PacBio Iso-Seq bioinformatics analysis pipeline.

Preliminary results show the presence of 45 novel ASFMR1 isoforms spanning approximately 60 kb of the genomic region, 19 of which are present only in premutation carriers, 17 only in normal controls and remaining in common. Our initial findings indicate that altered alternative splicing of the ASFMR1 gene is present in premutation carriers. Further, the characterization of the expression levels of these unique ASFMR1 isoforms will help us to elucidate the mechanism(s) by which “toxic gain of function” of the ASFMR1 mRNA may play role in the pathogenesis of FXTAS or other FMR1 associated disorders.

OS09 - Oral session IX

Oral presentation

O28

Reversal of multiple manifestations of Fragile X-associated tremor/ataxia syndrome by short antisense oligonucleotides

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The Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is a neurodegenerative disorder caused by expansion of CGG repeats (CGGₙ₎ in 5’ untranslated region of FMR1 gene encoding FMRP protein. The CGGₙ₎-induced sequestration of the important component of microprocessor (DGCR8) and alternative splicing regulator (SAM68) as well as biosynthesis and accumulation of the aggregated form of polyglycine protein (FMRpolyG), which is a product of Repeat-Associated Non-AUG translation of long CGG repeats are considered to be main factors triggering neurodegenerative processes in FXTAS patients. Since the causative molecular targets are well defined, FXTAS is highly amenable to the development of RNA targeting therapy. We showed high efficiency of antisense oligonucleotides (ASOs) as potential therapeutic agent. Short ASOs composed of locked nucleic acid residues bind along the CGGₙ₎ RNA (rCGGₙ₎ with very high affinity both in vitro and in vivo. These ASOs were delivered to FXTAS cell models or directly into cerebrospinal fluid in brain of mouse model expressing the transgene with 90 CGG repeats exclusively in neural cells. ASOs reduced the overall burden of the toxic RNA. As DGCR8 and SAM68 were released from sequestration, the total level of miRNAs increased significantly and defect of alternative splicing was corrected. The treatment decreased biosynthesis of soluble form of FMRpolyG and accumulation of its insoluble form within nuclei, but also interfered translation of native FMRP protein. Importantly, reduction of FMRpolyG intranuclear inclusions in the cerebellum positively correlates with behavioral features of FXTAS in treated animals.

Moreover, gene expression analysis and gene ontology classification performed basing on RNAseq results showed significant correction of ASOs treated FXTAS mice towards control mice. Our data demonstrate that short ASOs rescue both nuclear and cytoplasmic effects of toxic rCGGₙ₎ and can be considered as therapeutic strategy in FXTAS.

This work was supported by the Foundation for Polish Science, TEAM [POIR.04.04.00-00-5C0C/17-00] and POWR.03.02.00-00-1006/17.

OS09 - Oral session IX

Oral presentation

O29
OS09 - Oral session IX

Oral presentation

O30

**Compound 1a reduces toxic FMRpolyG levels in in vitro and in vivo models for Fragile X-associated Tremor and Ataxia Syndrome.**

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**Introduction**

Fragile X-Associated Tremor/Ataxia Syndrome (FXTAS) is a neurodegenerative disorder caused by a limited expansion of CGG repeats in the 5'-untranslated region of the FMR1 gene. Two non-exclusive pathogenic mechanisms have been proposed for the disease, the RNA gain-of-function model and the repeat-associated non-ATG (RAN) translation mechanism which causes pathogenicity through translation of the toxic FMRpolyG protein. Ubiquitin positive intranuclear inclusions together with the accumulation of the FMRpolyG protein form a major pathological hallmark. To study both mechanisms the inducible mouse model with ubiquitous expression of an expanded CGG repeat was used. Small chemical compound 1a seems to be a promising candidate in preventing the formation of these inclusions and reducing FMRpolyG levels. The aim of this study is to decrease overall production of FMRpolyG in vitro and in vivo through targeted therapeutic intervention.

**Results**

IF staining showed that mouse neurons stain positive for ubiquitin as well as FMRpolyG, and that both co-localize in the vast majority of neurons. After quantification of the inclusion bodies in GFP-positive cells, we could show that number of ubiquitin-positive intranuclear inclusions significantly increased over time. Compound 1a was able to significantly reduce the amount of intranuclear inclusions. Compound 1a concentrations in the livers were measured and quantified using LC-MS/MS. Histological analysis showed formation of FMRpolyG inclusions that contain Rad23B. After treatment with compound 1a FMRpolyG and Rad23B levels could be reduced but there was no significant effect on the number of FMRpolyG inclusions in the liver. We could show a significant decrease in Rad23B inclusions as well.

**Conclusion**

In summary this model allows us to quickly study the effects of potential therapeutics on pathologies induced by the expression of an expanded CGG repeat. Compound 1a seems to be a promising therapeutic to reduce FMRpolyG levels and its possibly its effects in mice.

OS09 - Oral session IX

Oral presentation

O31

**Assessing CRISPR mediated deletion of CGG repeats in the brains of Fmr1 CGG knock-in mice**

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RNA mediated gene editing, using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), can result in short indels or large deletions following a targeted double stranded break. The expanded CGG repeat in the Fragile X Mental Retardation 1 (FMR1) gene is a potential target for gene correction using CRISPR. Such gene-editing strategies have been shown in vitro, but has not yet been reported in vivo. We evaluated Cas9 for its ability to correct expanded FMR1 trinucleotide repeats in human cells and the Fmr1 CGG knock-in mouse. We tested multiple guideRNAs for cutting efficiency in HEK 293 cells and patient derived fibroblasts. Effective editing resulted in complete or partial deletion of the CGG repeats as well as nucleotides proximal to the repeats. Two guideRNAs were further evaluated in CGG knock-in mice. AAV-guideRNAs and AAV-SpCas9 were injected into mice striatum. Three weeks later, tissues were harvested and isolated DNA was subjected to PCR amplification and Sanger sequencing to map the CRISPR-induced deletions. We found complete or partial deletion of the CGG repeats and deletion of 3-48 nucleotides upstream and downstream of the target site. In all cases the transcriptional start site and the start codon were intact, elements required for functional gene expression. In RNA isolates assessed for Fmr1 mRNA levels by qRT-PCR, we found that transcripts were normalized from 3-fold elevation in control treated animals to indistinguishable from normal after gene editing. Additionally, FMRP typically has lower translational efficiency in Fmr1 CGG knock-in mice. Treated mice showed levels similar to those seen in WT mice. These results are the first in vivo report of editing the Fmr1 trinucleotide repeat with CRISPR. Our data demonstrate that CRISPR mediated deletion of the CGG repeats in Fmr1 rescues transcript upregulation while maintaining FMRP expression, and provides an important path forward for FXTAS treatment.
Mito-miR, hsa-miR-320a modulates mitochondrial functions and cell death in FXTAS
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Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is an X-linked inherited neurodegenerative disorder that affects adult (>50 years) males and females who are carriers of an expansion of 55 to 200 CGG repeats at the 5’-untranslated region of the Fragile X Mental Retardation 1 (FMR1) gene (Hagerman et al., 2001). The clinical features of FXTAS include progressive development of tremor, ataxia and neuropsychological problems, including parkinsonism, dementia and cognitive decline. Importantly, in our lab and others have recently found that the expanded CGG repeats can sequester RNA binding proteins and translated to toxic FMRpolyG. Recent studies on FXTAS have revealed the mitochondrial dysfunctions are associated with it (Gohel D et al., 2019). miRNAs are noncoding RNAs involved in posttranscriptional regulation of mRNAs. The emerging role of translation of miRNA to mitochondria suggests its possible involvement in the regulation of mitochondrial functions. Therefore, we hypothesize that sequestration of RNA binding proteins and the presence of FMRpolyG can dysregulate the transport of nuclear encoded mRNA/miRNA to the mitochondria. We isolated miRNAs from mitochondria of HEK293 cells transfected with expanded CGG repeats and performed NGS analysis. We observed altered miRNA association at mitochondria in FXTAS condition as compared to control. We characterized the role of mito-miR, hsa-miR-320a in the modulation of mitochondrial functions and cell death under premutation condition. Our results suggest the important role miRNA transport to mitochondria and its alteration in premutation condition. miRNA based therapy should be the focus of future studies to alleviate the mitochondrial functions and neuronal cell death in FXTAS and related neurodegenerative disorders.

Precision visuomotor behavior and cortical-cerebellar function in aging FMR1 premutation carriers
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Significant progress has been made to define molecular mechanisms and clinical symptoms associated with Fragile X tremor/ataxia syndrome (FXTAS). Objective, quantifiable measures of neuromotor degeneration associated with FXTAS are needed to identify disease risk markers, monitor disease progression, and define brain-behavior linkages that may be useful for determining neurobiological pathways. In the present study, 26 FMR1 premutation carriers ages 44 to 77 years and 34 healthy controls matched on age, sex, and handedness completed a test of visuomotor control. Participants pressed with their thumb and forefinger against precision load cells while viewing two horizontal bars. They were instructed to press so that the FORCE bar moved upwards to the level of the TARGET bar, and then to maintain this force level as steadily as possible. We predicted that FMR1 premutation carriers would show increased motor variability and regularity relative to controls. Sixteen of these FMR1 premutation carriers and 20 of the healthy controls completed a similar visuomotor test during functional magnetic resonance imaging (fMRI). We expected atypical cortical-cerebellar activation during visuomotor behavior in FMR1 premutation carriers compared to controls. During visuomotor behavior, FMR1 premutation carriers showed increased motor regularity relative to controls associated with greater CGG repeat length. Increased motor variability in premutation carriers was correlated with more severe clinically rated FXTAS symptoms. All fMRI data has been collected and preprocessed, and analyses of imaging data are ongoing and will be presented. Our behavioral results suggest cortical-cerebellar networks involved in dynamically adjusting motor output in response to sensory feedback are compromised during aging in FMR1 premutation carriers. Precision visuomotor issues also appear to be associated with both disease risk (increased CGG repeat length) and progression (clinical symptoms). Our results suggest quantitative measures of precision visuomotor control may serve as objective biomarkers useful for tracking neurodegenerative processes associated with FXTAS.
Thickness of Motor Cortex as a Biomarker of FXTAS
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Individuals with premutation alleles of the FMR1 gene are at risk for developing a neurodegenerative disease, fragile X-associated tremor/ataxia syndrome (FXTAS). The disease has a variable penetrance but there are a lack of valid biomarkers for the disease. We examined whether thickness of a key region related to FXTAS symptomology, primary motor cortex, is related to development of FXTAS. We collected T1-weighted structural MRI from 51 adult male premutation carriers and 25 IQ-matched controls. Thickness estimations for the region of interest (precentral) and a control region containing primary visual cortex (pericalcarine) were extracted and averaged across hemispheres. FMR1 mRNA expression levels and CGG repeat size were also collected. Three groups were identified: controls (n=25), carriers without FXTAS (n=40; FXTAS stage 0 or 1) and carriers with FXTAS (n=11; stage ≥2). Analyses revealed a main effect of group, with both groups of carriers having significantly decreased thickness compared to controls. FXTAS stage and mRNA expression, but not CGG repeat size, were significant predictors of cortical thickness in carriers. We also conducted longitudinal analyses with a subset of participants (25 carriers, 16 controls) who returned for two additional visits. Carriers were grouped into those with FXTAS at initial visit (n=6), those who developed FXTAS at a follow-up visit ("converters"; n=10) and those who showed no signs of FXTAS at any visit ("non-converters"; n=9). Results showed that those with FXTAS at their initial visit had decreased motor cortex thickness compared to both controls and non-converters. A main effect of visit indicated thinning over time in all groups. There was no main effect of group or significant associations with genetic measures detected in the control region. These findings suggest that thickness of the motor cortex may be a useful biomarker of the development of FXTAS.

A unique visual attention profile associated with the FMR1 premutation
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Atypical visual attention patterns have been observed among carriers of the FMR1 premutation, including differences in efficient gaze-language coordination during language processing tasks (Nayar et al., 2019) and altered sensitivity to averted gaze (Klusek et al., 2017). Observed differences in gaze show some resemblance to visual attention patterns observed in autism spectrum disorder (ASD), and have also been shown to correlate with ASD-related clinical-behavioral phenotypes. Such patterns of visual attention could therefore constitute important biomarkers spanning diagnostic boundaries, and help to inform the neurocognitive profile of the FMR1 premutation. This study examined patterns of eye movement across an array of fixation measurements from three distinct eye-tracking tasks to investigate profiles of visual attention among premutation carriers, ASD parents, and parent controls using a structural equation modeling framework, as well as clinical-behavioral correlates. Participants included 80 premutation carrier, 271 ASD parents, and 56 parent controls. Analyses of fixations across the eye-tracking tasks, and their corresponding areas of interest, revealed a distinct "Premutation Fixation Profile" characterized by increased fixations on inanimate objects, the mouth, and the setting components of scenes. An "ASD Fixation Profile" also emerged that was comprised of decreased fixations on the face, eyes, and animate objects. Stronger expression of the Premutation Fixation Profile was associated with lower activation ratios (r = -.39, p < .01) in the premutation group. Further, individuals in the premutation group who showed evidence of the ASD Fixation Profile exhibited increased subclinical ASD-related features (r = -.24, p < .05). In sum, results demonstrate distinct visual attention profiles in premutation carriers and ASD parents that were associated with FMR1 variation and ASD-related clinical-behavioral features, suggesting that visual attentional profiles may constitute meaningful biomarkers reflective of underlying genetics and broader clinical-behavioral characteristics.
Prenatal biomarkers of the FMR1 premutation
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Importance: No studies have examined whether differences in molecular biomarkers are present in early development of individuals carrying the FMR1 premutation.

Objective: To evaluate biological pathways in amniotic fluid from fetuses with and without premutation alleles.

Participants: Nineteen pregnant female carriers of premutation alleles who had undergone amniocentesis for fragile X prenatal diagnosis at gestational age of (average) 17 weeks. Consent was obtained to use residual amniotic fluid from the clinical studies for research.

Main Outcomes and Measures: The analysis included proteomics and metabolomics of 9 amniotic fluids from non-carriers and carriers as well as determination of maternal and fetal CGG repeat size.

Results: Untargeted metabolomic and proteomic data of amniotic fluid from pregnancies of mothers with premutation alleles showed significant differences in pathways related to antioxidant capacity, neurotransmission, intermediary metabolism, and neurodevelopment in samples from carriers compared to those of non-carrier fetuses matched for sex and gestational age.

Conclusions and Relevance: Neurodevelopment in amniotic fluid suggest those with premutation exhibit early differences that may be related to the development of emotional or behavioral issues observed in some children with the premutation.

Metabolic changes associated with development and prediction of fragile X-associated tremor/ataxia syndrome
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FMR1 premutation carriers are at increased risk for developing FXTAS, a late-onset neurodegenerative disorder. Although FXTAS represents a significant health risk for many individuals, to date no treatment or interventions to prevent or slow the disease progression are available.

Leveraging on an ongoing longitudinal study of male carriers and controls and utilizing at least two longitudinal time points, we examined the global metabolic profiles in three subgroups, matched by age and CGG number: those who entered the study symptom-free and later developed symptoms of FXTAS at subsequent visits (converters, n=10), those who remained symptom free, (non-converters n=10) and normal age matched controls (n=10).

Our preliminary findings indicate that several metabolites were altered in the premutation groups compared to controls. Importantly, several pathways involved in lipid metabolism were altered only in the converter group. Specifically, levels of Free fatty acids, Acyl-Carnitines, Diacylglycerides, Ceramides and Phosphatidylethanolamine were altered at visit 2 in the converter group in comparison to visit 1 and, importantly, compared to visit 2 in the non-converters group. Further, several metabolites, particularly in the lipid metabolism, were significantly different at visit 1 between converters and non-converters and remained statistically different at visit 2. These differences may implicate changes in liver or pancreas metabolism as contributing to the FXTAS phenotype, which agree with altered liver and mitochondrial function reported in the premutation mouse model.

Thus, our preliminary findings, demonstrate metabolic changes in premutation carriers that developed FXTAS symptoms with time serving as indicators of early sign of disease. Importantly, we also identified metabolites, which levels were significantly different at the time that participants were symptom-free (visit 1) and that remained different later on (visit 2) and therefore able to predict which carrier will and will not go on to develop FXTAS. Our results may provide us insight regarding disease pathogenesis.
Healthcare Needs of FMR1 Premutation Carriers: A Semi-Structured Interview Study
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Background Although the FMR1 premutation is quite common, with recent estimates of 1:291 for women and 1:855 for men, relatively little attention has been paid to its associated disorders. Premutation carriers are at increased risk of several associated disorders, including fragile X-associated primary ovarian failure (FXPOI) and fragile X-associated tremor/ataxia syndrome (FXTAS). Pre-clinical research continues to focus on understanding the underlying molecular processes and the development of a therapeutic intervention. While our knowledge is growing, it remains largely unknown whether the current state of knowledge regarding the premutation and its associated disorder has found its way to the doctor’s consultation room.

Aims This study aims to chart the obstacles premutation carriers experience in the healthcare they receive. The results of this study will be translated to suggestions to ensure an optimal healthcare organization for FMR1 premutation carriers.

Methods A qualitative study was performed using semi-structured interviews with FMR1 premutation carriers. Up until now, 27 interviews were conducted and 16 interviews have been transcribed verbatim.

Results Results of at least 27 interviews will be presented at the FMR1 conference. So far, the first 16 interviews indicate a variety of healthcare needs. Premutation carriers reported the following needs: more easily accessible information about complaints that are associated with the FMR1 premutation, more awareness of the FMR1 premutation among healthcare professionals, to be taken seriously by healthcare professionals, access to FMR1 premutation experts, and more follow-up after the initial premutation diagnosis. Premutation carriers expressed that they often need to be very assertive in their contact with healthcare professionals, and that they often need to explain the (consequences of a) FMR1 premutation to a healthcare professional.

Conclusion The first interview results indicate the need for a healthcare system that is organized around the premutation carrier, instead of by the premutation carrier.
Fragile X-associated tremor and ataxia syndrome (FXTAS): A small vessel disease
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We herein report the presence of cerebral microbleeds (CMBs) in grey and white matter in the postmortem FXTAS brain. We have examined 10 FXTAS and 10 control brains for the presence of CMBs and are in the process of analyzing 10 additional FXTAS cases. Areas of interest included cerebral cortex, hippocampus, putamen, amygdala, substantia nigra, and cerebellum. Tissue was sectioned into 10 micrometers slices and stained with H&E and with an antibody against ubiquitin. We read 3 slides per area per case for the presence of extravasated erythrocytes that stained bright pink in H&E. We found that all the FXTAS cases presented with CMBs, being more abundant in the white matter of both cortical cortex and cerebellum. Control cases presented with a sporadic presence of CMBs. We also quantified the number of inclusions within endothelial cells in ubiquitin stained slides. We did not find a direct correlation between the percentage of vessels with intranuclear inclusions in endothelial cells and the number of CMBs, but more cases should be analyzed to account for heterogeneity. History of stroke and treatment with aspirin do not seem to be associated with a more severe presentation of CMBs. However, major anticoagulants such as warfarin may be correlated with a higher number of CMBs. We hypothesize that the concomitant presence of white matter disease and CMBs suggest a correlation between endothelium abnormalities and a more severe presentation of a small vessel disease, contributing to the progression of cognitive impairment in FXTAS. We also propose the use of CMBs as a further postmortem finding for the diagnosis of FXTAS.

Disruption in the hippocampal network function in an inducible mouse model of FMR1 premutation
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PrP-rtTA/TRE-90CGG-eGFP is a Tet-ON bigenic mouse model of fragile X-associated tremor/ataxia syndrome (FXTAS) that expresses a premutation (PM) range CGG-repeat expansion tract outside of the context of FMR1 gene under the control of doxycycline (DOX). Our previous work with this model has shown that motor as well as emotional alterations manifest in these transgenic mice after 12 weeks of induction with DOX starting at postnatal day 28. In order to study cognitive and emotional aspects of the PM without any interference from motor deficits, in this study we have adopted an early induction schedule starting from embryonic development that does not result in the manifestation of a motor phenotype. We have identified that an anxiety-like phenotype was already present albeit no motor changes. A rescue was possible after a period without transgene expression (washout phase) that was also reflected in the decreased ubiquitin-positive intranuclear inclusion load in the basolateral amygdala and DG of the hippocampus but not in CA3. Whole electrophysiological read-outs have shown disruptions in the DG and lateral amygdala upon DOX administration, these also tend to normalize after transgene shutdown. In line with the persistent inclusion load in CA3, we have observed a worsening physiology as recorded from Schaffer collaterals despite the cessation of transgene expression. We have further identified that deteriorating CA3-CA1 physiology is accompanied by changes in the protein markers that reflect an altered excitation level in the hippocampus. Lastly, we were able to describe a cognitive phenotype that only manifests after the washout phase. These findings suggest that emotional disturbances are present despite the lack of the motor deficits seen in FXTAS when adopting an early induction schedule. Although these are amenable to rescue after a washout period, the overall network integrity of the hippocampus is lost and a total rescue is not possible.
PS01 - Poster session
Poster presentation

P04

Optimizing Pregnancy Potential in Young Women with Fragile X Associated Primary Ovarian Insufficiency (FXPOI)
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Fragile X Associated Primary Ovarian Insufficiency (FXPOI) is a chronic disorder characterized by oligo/amenorrhea and hypergonadotropic hypogonadism before age 40 years. These women may experience significant emotional distress due to the associated infertility and the related increased prevalence of depression and anxiety. Many young women with FXPOI desire to conceive. However, presently there is: 1) insufficient evidence regarding how to optimize this possibility, and 2) no centralized and professionally managed process through which these women can work with professionals to investigate and manage this issue in an integrated manner. Previously we reported on the case of a young woman with FXPOI who conceived three times after diagnosis. She delivered two healthy children while taking a standardized approach to Physiologic Hormone Replacement Therapy (PHRT). Despite published evidence to the contrary, clinicians and patients alike frequently consider POI equivalent to an early menopause, meaning a state of complete ovarian follicle depletion. In fact, published evidence has demonstrated in most cases POI is a state of follicle dysfunction. Importantly, the evidence has shown the major mechanism of follicle dysfunction in POI is inappropriate follicle luteinization due to high serum Luteinizing Hormone (LH) levels. A standardized PHRT approach has been demonstrated to reduce serum LH levels to normal in women with POI. Evidence has shown women with FXPOI are frequently undertreated with regard to HRT. To address the issue of maximizing fertility in young women with FXPOI Conover Foundation has initiated a collaboration with 1) the https://endo-ern.eu/ and 2) https://www.rareconnect.org/en, an organization promoting global conversation and collaboration to improve the lives of those dealing with a rare disease. Many women with FXPOI and their clinicians do not realize that it is possible to conceive without medical intervention and do not understand the need for appropriate hormone replacement.

PS01 - Poster session
Poster presentation

P05

Increased severity of fragile X spectrum disorders in the agricultural community of Ricaurte, Colombia
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Premutation carriers of the FMR1 gene (CGG repeats between 55 and 200) usually have normal intellectual abilities but approximately 20% are diagnosed with developmental problems or autism spectrum disorder. Additionally, close to 50% have psychiatric problems such as anxiety, ADHD and/or depression. The spectrum of fragile X disorders also includes Fragile-X-associated primary ovarian insufficiency (FXPOI) in female carriers and Fragile-X-associated tremor/ataxia syndrome (FXTAS) in older male and female carriers. We evaluated 25 premutation carriers in the rural community of Ricaurte Colombia and documented all behavioral problems, social deficits and clinical signs of FXPOI and FXTAS as well as reviewed the medical and obstetric history. We found an increased frequency and severity of symptoms of fragile X spectrum disorders, which might be related to the vulnerability of FMR1 premutation carriers to higher exposure to neurotoxic pesticides in this rural community.
Parent report suggests increased neurobehavioral problems among boys with a premutation measured in an unbiased cohort of children with premutation alleles

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To assess risks for neurodevelopmental problems in children with premutation (PM) alleles, we recruited a cohort of children aged 3-10 years identified prenatally as PM carriers and control children who were non-carriers (NC). Four surveys were completed online by parents: a survey to capture child clinical diagnoses and special services received, Child Behavior Checklist (CBCL), Social Responsiveness Scale (SRS-2), and Behavior Rating Inventory of Executive Function (BRIEF). To date, 288 children have enrolled and 148 have completed all surveys. Per parent report, more PM boys (8/72) were enrolled in special ed compared to NC boys (1/79; p=0.01), though the number of boys receiving ≥1 clinical diagnoses or special services was similar. On standardized questionnaires, data were dichotomized into those with a T score ≥60, distinguishing those at increased risk for clinical concerns. Between-group comparisons were run. On the CBCL, PM boys were more likely to score in the “at risk” range for social withdrawal (8/51 PM vs 1/48 NC; p=0.03) and anxiety (10/51 PM vs 2/48 NC; p=0.03), but there were no between group differences on other behavior problem indices. For the SRS-2, 7/50 PM boys scored in the “at risk” range for social problems (5 mild, 1 moderate, 1 severe) compared to none of the NC boys. For the BRIEF, 10/43 PM boys experienced difficulties in planning and organizing compared to 2/35 NC boys (p=0.06); however, other components of executive dysfunction were not indicated. As hypothesized for an X-linked trait, PM girls had fewer indications of behavioral problems than boys and no striking differences were identified between PM and NC girls. The only significant finding was that 6/54 PM girls were enrolled in special ed compared to 2/81 NC girls (p=0.02). These preliminary results suggest a possible excess of neurodevelopmental problems in PM boys compared to NC boys.

Higher base line expression levels of folliculogenesis related genes in mural granulosa cells from females FMR1 premutation carriers

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Introduction: FMR1 has a premutation category with expansions of 50-200 repeats. Female premutation carriers are at an increased risk of Fragile X-Associated Primary Ovarian Insufficiency (FXPOI). (1) Various genes have been previously correlated with folliculogenesis including stem cell factor (SCF), (2) growth and differentiation factor 9 (GDF9), (3) Follicle-Stimulating Hormone (FSH) and Anti-Müllerian hormone (AMH), (4, 5) FSH stimulates granulosa cells (GCs) of early antral follicles and drives the formation of a large pre-ovulatory follicle. (6) AMH, secreted by granulosa cells, might be involved in the recruitment of dormant primordial follicles with other regulators of follicles growth initiation. (2) AMH’s inhibitory character is suspected to assist in dominant follicle selection, possibly reducing the follicle’s sensitivity to FSH stimulation. (7, 8) AMH serum levels are assumed to be a reliable biomarker of ovarian reserve. (9) During the menstrual-cycle AMH serum levels remain constant, while GCs of growing follicles might reflect a different pattern. Salmon et al. (10) showed that AMH expression was first detected in GCs of early primary follicles. The highest expression was found in GCs of preantral and small antral follicles.

Aim: To investigate the expression levels of folliculogenesis related genes in mural granulosa cells (MGCs) obtained from female FMR1 premutation as compared with non-carriers undergoing in vitro fertilization treatments.

Methods: Isolated MGCs from follicular fluid following oocyte retrieval were cultured for 4 days to achieve a state resembling early non-luteinized follicles. Gene expression levels were analyzed using RT-qPCR. Fixed MGCs were immunostained with AMH antibody.

Results: MGCs demonstrated higher base line expression levels of both FSHR and AMH in FMR1 premutation carriers compared to non-carriers. (FSHR; 9.4 ±8.2 and 3.7±3.6, p=0.005; AMH: 4.2±3.9 and 1.2±1.4, p=0.003 respectively). Immunostaining revealed higher AMH expression in FMR1 premutation carriers than non-carriers.

Conclusions: FMR1 premutation carriers demonstrated dysregulation in folliculogenesis related genes expression levels, possibly due to a compensatory mechanism.
PS01 - Poster session
Poster presentation

P08

Fertility Preservation for FMR1 Premutation Carriers - Results of the First Year of the New Health Insurance Policy in Israel
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Introduction:
In Israel, all citizens are entitled to a mandatory health insurance coverage. Every year a professional committee of the health ministry decides which medications or medical procedures will be added to this mandatory health coverage. In 2018 the committee has decided to include fertility preservation treatments for FMR1 premutation carriers who are at increased risk of FXPOI (elevated basal FSH serum levels, low serum AMH level or low antral follicle count). Each carrier is entitled to undergo up to 6 IVF cycles or cryopreserve up to 40 oocytes or embryos.

Material & Methods:
We present the results of the first year of this novel policy from the six largest IVF centers in Israel.

Results:
In 2018 eight FMR1 premutation carriers underwent 13 fertility preservation treatment cycles. Median women age was 33 (range 18-38). Number of CGG repeats ranged from 60-135. All women had regular menstrual cycles. Mean oocyte retrieved and cryopreserved were 8.1±4.5 and 5.6±4.4 per cycle, respectively. The total dose of gonadotrophins (mean 5233 IU±1692 vs. 1803±1062) used during the treatment for FMR1 premutation carriers was much higher and the number of oocyte retrieved (8.1±4.5 vs. 13.6±6.5) was much lower compared to age-matched women with normal CGG repeat undergoing IVF cycle.

Discussion:
In Israel, every year approximately 360 new FMR1 premutation carriers are diagnosed. Estimating a 20% risk of FXPOI, we expect that about 70 FMR1 premutation carriers will be suitable for fertility preservation treatments every year.

Conclusion:
In the first year of the novel fertility preservation health policy in Israel, only about 10% of the expected FMR1 premutation carriers underwent fertility preservation treatments. A national public campaign is suggested in order to increase public awareness for the risk of FXPOI.

PS01 - Poster session
Poster presentation

P09

Does the presence of AGG interruptions within the CGG repeat tract has a protective effect on the fertility phenotype of female FMR1 premutation carriers?
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Introduction: Fragile X-associated primary ovarian insufficiency (FXPOI) is caused by the expansion of a CGG repeat sequence in the 5’ untranslated region of the X-linked Fragile-X Mental Retardation gene (FMR1). The size of the repeat expansion affects the severity of the different genetic phenotypes. AGG interruptions may contribute to different secondary structures of the FMR1 mRNA and are found in the CGG repeat tract with 0–2 AGG interruptions in premutation alleles (55–200 CGG repeats). AGG interruptions may have a protective effect on the pathogenesis of premutation carriers. Yrigollen et al. looked at FMR1 mRNA blood levels and Peprah et al. investigated the transcriptional and translational activity of FMR1. Both reported a lack of association to the number of AGG interruptions. Nolin et al., predicted that AGG interruptions might increase the stability of the repeat tract during maternal transmission reducing the risk of a full mutation expansion. Recently Lekovich et al. reported that AGG interruptions correlates with improved ovarian reserve in accordance with higher AMH serum levels and antral follicles counts.

Aim: To examine a correlation between the number of AGG interruptions and the in-vitro fertilization (IVF) outcome of FMR1 premutation carriers.

Methods: Our cohort included 58 premutation carriers who underwent IVF treatments. A statistical analysis examined the effect of the number of AGG interruptions on various IVF outcome markers; The number of oocytes retrieved; the number of top-quality embryos; the ratio between top-quality embryos and oocytes number and the peak level of estradiol at the day of human chorionic gonadotropin (hCG).

Results: All four IVF parameters examined, did not show statistical significances. Further analysis of FSH stimulation duration and dose, age and CGG repeats revealed no correlation to AGG interruptions.

Conclusions: The number of AGG interruption may not correlate with the IVF outcome of FMR1 premutation carriers.
C. elegans as a model to study FMRpolyG related toxicity in FXTAS

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Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is a late-onset neurodegenerative disorder caused by expansion of the CGG repeat in the 5’ untranslated region (5’UTR) of the Fragile X Mental Retardation (FMR1) gene. This expanded repeat can be translated into a toxic FMRpolyG protein through a mechanism called Repeat-associated Non-AUG (RAN) translation. Previously we have shown that FMRpolyG in mice is needed for development of pathology.

Our main goal is to use the model-organism C. elegans to study the underlying molecular mechanisms of FXTAS and perform unbiased screens for modifiers of FMRpolyG related toxicity. We have made transgenic worms expressing FMRpolyG from a canonical ATG codon in frame with eGFP under control of the panneuronal rab-3 promoter. Several concentrations of the construct were injected. As a control a similar construct missing the FMRpolyG coding sequence was used.

Live imaging and FRAP analysis showed that FMRpolyG is synthesized and is aggregated in the nuclei of neurons. Moreover, transgenic worms will be analysed in motility and chemotaxis assays to study the effect of FMRpolyG expression on neuron function. Our first data suggest that C. elegans is a good model to investigate FXTAS.
Although on average, low zone CGG alleles are not associated with compromised functioning, this average masks those with low zone CGGs who had 26-40 CGGs on both alleles (n = 286). We investigated gene x environment interactions by comparing how mothers in these two categories responded to stress exposure. More than one-third of the mothers in both groups (38.7%) had children with disabilities - the measure of stress exposure used in this study. We found significant gene x environment interactions for a range of phenotypes - executive functioning, mental health, and motor functioning. Mothers with low zone CGG repeats had greater phenotypic limitations when they had children with disabilities as compared with mothers with normal-range CGGs who had children with disabilities. In contrast, women with low zone CGG repeats had better functioning when their children were non-disabled than women in the normal-range whose children were similarly non-disabled. This pattern is reflective of “differential susceptibility” or the “flip-flop phenomenon” whereby people with certain genotypes are more reactive to the environment than those with other genotypes. In this analysis, those with low zone CGG repeats were more vulnerable to parenting stress than those who had normal range CGGs. Conversely, those with low zone CGGs fared better than those in the normal range when their children were not disabled. Although on average, low zone CGG alleles are not associated with compromised functioning, this average masks differential response to the environmental context.
PS01 - Poster session

Poster presentation

P15

**Genotype-phenotype associations in male and female FMR1 premutation cases**


**Background:** FMR1 genetic markers are a hallmark of fragile X syndrome, which has co-morbidities in autism and psychiatric symptoms. The FMR1 premutation (PM, 55-200 CGGs) length is linked to psychopathology in some PM females with mid-size expansions, though additional studies are needed. Here, we explore genotype-phenotype links in clinically-affected PM patients.

**Methods:** Five PM individuals at Kennedy Krieger Institute were examined for FMR1 genetic, epigenetic and protein (FMRP) blood-based indices using AmpliDex® PCR/CE FMR1 and mPCR (Asuragen), and FMRP liquid bead assays, respectively. Clinical assessments included DSM-5, clinician’s impression and parental measures. Standard cognitive skills measures were used. SPSS software (v25; IBM) was used for statistical analyses. The JHH’s Institutional Review Board approved the study.

**Results:** Five White PMs were assessed (2 males, 56 and 72 CGGs, aged 4-13 years) and (3 females, 58-133 CGGs, 8-39 years). FMR1 alleles were unmethylated (males) or partially methylated (females). The mean FSIQ score was 87±21.9 (females) and 89.5±4.9 (males). The FMRP level [pg/ng] was slightly higher in females (17.1±13.2 vs. 13.1±10.0 in males), but skewed by a teenage female with 113 CGGs (32) who had major depression and social anxiety. She required a SSRI and individual therapy. Males had slightly more clinical diagnoses of ADHD whereas females had slightly more anxiety, including a 39 yo subject with FXPOI (75 CGGs, FMRP 6.7). While a higher mean ABC-CFX score was found in females (30.7±27.2 vs. 25.5±21.2 in males), it did not reflect accurately on overall impairment as CGI-S scores were comparable in both gender (3.7±1.1 vs. 3.5±0.7 in males). Overall, SSRIs were the most frequently used drugs (80%); no antipsychotics were prescribed.

**Conclusion:** These case studies underscore the complexity of PM clinical presentations relative to measured molecular markers. Well-powered, multi-analyte studies with deep clinical annotation are required to characterize PM genotype-phenotype relationships.

PS01 - Poster session

Poster presentation

P16

**FMR1 gene premutation: A case-series study in pediatric population with Fragile X-associated Neuropsychiatric Disorders (FXAND)**

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**Background:** The FMR1 gene premutation (PM, 55-200 CGGs) leads to higher frequency of both medical/neurological and psychiatric disorders that were recently labelled by Hagerman and colleagues as Fragile X-associated Neuropsychiatric Disorder (FXAND). As gaps in knowledge exist among clinicians in terms of these psychiatric disorders in the PM pediatric population, we present FXAND-related manifestations in children with PM.

**Method:** This is a descriptive case-series study of 7 pediatric patients (57% males) from the Fragile X Clinic, Kennedy Krieger Institute aimed to illustrate their spectrum of FXAND and medications that were prescribed. The study was approved by JHH’s IRB.

**Results:** All of these patients had the FMR1 PM (56-113 CGGs, mostly unmethylated). Cases 1-4 are white males, aged 10-15-yo unmethylated CGGs 57-71. Case 1, a 10yo who displayed autistic features. Case 2, a 11yo with generalized anxiety and oppositionality. Neither Case 1 nor Case 2 received pharmacological therapy. Case 3, a 13yo with ASD who was treated with antipsychotic, stimulant and ABA-therapy. Case 4, a 15yo with social anxiety (SA), ADHD, and learning disabilities who received alpha-agonist, stimulant and SSRI. Cases 5-7 are white females, 12-15-yo with 57 (42% methylated), 82 and 113 unmethylated CGGs, respectively. Case 5, 12yo with ADHD, mixed receptive-expressive language disorder and learning disability who did not receive pharmacological treatment. Case 6, a 13yo with moderate intellectual disability and problem behaviors who was medicated with alpha-agonist and an anticonvulsant. Case 7, a 15yo with SA and major depression who received SSRI and an individual psychotherapy.

**Conclusion:** We described a case-series study of pediatric patients with PM aged 10-15-years both genders, who displayed a spectrum of FXAND such as ASD, depression, anxiety, ADHD, learning disability, intellectual disability-“second hit”. Majority of them (4/7, 57%) received medications classes of stimulants, SSRI, antipsychotics, alpha-2 agonists. Non-pharmacological interventions were also common.
Cortical Gyrification and Its Relationships with FMR1 Molecular Measures and Cognition in Children with the FMR1 Premutation

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Cognitive and psychiatric problems may occur early in children carrying a premutation allele (55-200 CGG repeats) of the fragile X mental retardation 1 (FMR1) gene. However, the neurobiological basis for these issues has not been explored. Knock-in mouse models of the premutation revealed defects in embryonic cortical development that may affect cortical folding.

We therefore conducted a retrospective analysis of gyrification in 62 children (age 8-12 years, 20/14 male/female carriers, 15/15 male/female controls). Vertex-wise analysis of local gyrification index (LGI) that quantified folding complexity was performed for group comparisons and for correlations with FMR1 molecular measures and IQ. Individuals with aberrant gyrification in 68 cortical areas were identified using Z scores of LGI relative to controls controlling for age, sex, and total cranial volume.

Significant three-way group-sex-age interaction in LGI was detected in right inferior temporal and fusiform cortices (p=0.027), which displayed a negative correlation with CGG repeat length in male carriers (p=0.015) but no significant correlation in female carriers. Using a cutoff of extreme 1% of Z scores, 17 male carriers (hyper/hypo: 8/9) and 10 female carriers (hyper/hypo: 2/5, 3 with both) displayed hyper- and/or hypo-gyrification in 1-17 regions per person in contrast to 2 control boys (hyper/hypo: 0/2) and 2 control girls (hyper/hypo: 1/1) who met the criteria in only one region per person. In addition, LGI correlated positively with perceptual reasoning, working memory, and processing speed in both male controls and male carriers (p=0.0002-0.003) while negatively in female carriers (p=0.006) but no significant correlation in female controls. Regions showing significant correlation were relevant for the functions, including the precuneus, cingulate, and various frontal regions. These findings demonstrate aberrant gyration in children with the FMR1 premutation, which may underlie cognitive deficits. Further study of differential effects of hyper- and hypo-gyrification on cognition is warranted.

Probing functional neural correlates of postural instability in FMR1 premutation carriers: an EEG study.

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Fragile X Associated Tremor/Ataxia Syndrome (FXTAS) affects up to 40% of FMR1 premutation carriers over 50 years and is characterized by tremor and balance impairments. While subtle changes in balance have been observed in younger carriers prior to a diagnosis of FXTAS, the neural mechanisms underlying these changes have not yet been explored. Increases in frontal and parietal theta spectral power are believed to reflect an error signal caused by unstable balance. This study investigated cortical theta activity during continuous balance and its relationship to balance performance in Fragile X premutation carriers.

Ten premutation carriers and six controls each stood on a forceplate with their eyes open and eyes closed (EO/EC) and also while conducting two cognitive tasks (N-back and SART tasks). Postural sway and EEG data were simultaneously recorded to measure changes in theta power in response to each balance task. Postural sway parameters included sway area, path length, and velocity. Cortical areas for analysis included frontal, central, and parietal regions.

Sway parameters between groups were comparable across tasks. Carriers showed an increase in path length during EC, N-back and SART tasks, as well as sway area during the N-back task, compared to EO condition (p=0.005 for all). However, theta power in frontal and central regions was inversely correlated with sway velocity for the EC condition for the control group (r=−1.0, p=0.001), while no such correlation was found for the carriers. Higher theta power was linked with greater stability in the control group, reflecting an increase in error signalling caused by reduced visual input and a greater discrepancy between expected and actual balance state. Carriers’ theta power did not change with task difficulty, possibly indicating disruptions in error detection and processing. Such results provide new insight into the neural correlates of balance control in Fragile X premutation carriers.
PS01 - Poster session
Poster presentation

P21

Prenatal studies suggest mothers with more than 75 CGG repeats transmit the normal allele in 53% of pregnancies
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We have performed fragile X prenatal studies for 2999 pregnancies of women with intermediate, premutation or full mutation alleles during 1991-2019. The study included chorionic villus and amniotic fluid samples (77% and 23% respectively). Expanded allele was transmitted in 1497 (49.9%) pregnancies with expansion to a full mutation in 460 (30.7%). Mothers with large premutation (75 CGG repeats and above) or full mutation alleles transmitted the normal allele in 53% (595/1118) of pregnancies while mothers with smaller alleles (45-74 repeats) transmitted the normal allele in 48% (902/1881) of pregnancies (p=.005, Chi square 1 df). These surprising results suggest the presence of intermediate and small premutation alleles may provide some early developmental advantage. For larger alleles, some full mutation embryos may be non-viable from loss of the X chromosome carrying the full mutation. Alternatively, double crossover events in meiotic recombination may result in transmission of the normal allele. Such events may lead to skewed distributions of the normal versus the fragile X chromosome. Additionally, maternal transmission outcomes were examined for possible correlation with FMRP levels for more than 480 cases.

PS01 - Poster session
Poster presentation

P22

R-loops in FXTAS locus: helpful or harmful?
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FXTAS is caused by the expansion of CGG repeats in the 5’UTR of FMR1 gene. One of the potential pathomechanism of FXTAS is the process of R-loops formation in the region of expandable CGG repeats. These nucleic acid structures are RNA/DNA hybrids which are formed during transcription when nascent RNA hybridizes to the complementary DNA template strand behind the elongating RNA polymerase II (Pol II). Till now it has been shown that R-loops can be formed at the endogenous human FMR1 locus. However, there are a few regions in the 5’-region of FMR1 gene that are suspected to be responsible for R-loops formation. Nevertheless, it has been presented via in vitro transcription that CGG trinucleotide repeats alone can form RNA/DNA hybrids. R-loops over expanded repeats may form a structural block, directly interfering with Pol II transcription elongation and influence the transcription efficiency. Interestingly, it has been demonstrated that different trinucleotide repeat expansion-associated R-loops can be resolved by over-expressed exogenous RNase H1, which leads to transcription up-regulation of gene expression in vivo. What is more, the knock-down of endogenous RNase H1 by RNA interference tools resulted in a significant increase in the R-loop signal in DIP (DNA immunoprecipitation) experiments. In our studies, we wanted to confirm the possibility of R-loop formation by CGG repeats located in the context of FMR1 5’UTR during in vitro transcription. We also aimed to present that these structures are sensitive to RNase H1 which digest RNA within RNA: DNA heteroduplexes. Moreover, we wanted to correlate the effect of R-loop formation with the transcription efficiency in vitro and in vivo and to establish the effect of antisense oligonucleotides targeting the CGG repeat tract on R-loop formation. This work was supported by the Foundation for Polish Science, TEAM [POIR.04.04.00-00-SC0C/17-00] and POWR.03.02.00-00-I022/16.
Dual task cognitive motor interference exacerbates turn deficits in men with FXTAS

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BACKGROUND: The impact of dual task (DT) cognitive motor interference or fast paced gait which are real life/challenging conditions has never been studied in FXTAS. We hypothesized that these gait “stress tests” would exacerbate gait and turn deficits. METHODS: Thirty individuals with FXTAS and 35 controls performed gait analysis using an inertial sensor-based 25 meter two-minute walk test under a 1) self-selected pace, 2) fast as possible pace and 3) DT condition asking subjects to perform a concurrent verbal memory task while walking at their normal speed. The dual task cost (DTC) for gait and turn parameters was calculated as (ST-DTC)/ST value x 100. RESULTS: FXTAS subjects had significant reductions in stride length and velocity, increased double support and reduced swing phase times, slower turn velocity and greater number of steps to turn compared to controls under all 3 test conditions (0.0001 > p < 0.039). During fast paced gait, FXTAS individuals also displayed increased gait variability (p = 0.025). Men with FXTAS had significantly elevated DTC for turning speed (p = 0.036), indicating cognitive interference for turning, which has greater motor control requirements than straight walking. There were no elevated DTC for spatiotemporal aspects of gait during straight walking, suggesting that men and women with FXTAS prioritized gait over cognition. This was supported by the finding that FXTAS individuals had greater DTC for the cognitive verbal fluency task (p = 0.004). CONCLUSIONS: DT gait paradigms to cognitively challenge subjects significantly exacerbates turn function in men with FXTAS and fast paced gait increases gait variability which is associated with instability and falls. Gait stress testing paradigms and associated gait and cognitive markers may be useful in future studies to help determine fall risk and develop effective treatment interventions for both cognitive and motor deficits in FXTAS.

Maternal stress and social outcomes in mothers of children with fragile X syndrome and autism spectrum disorders

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Our prior work indicates that women who carry an FMR1 premutation (PM) and have a child with fragile X syndrome (FXS) experience increased social anxiety, an effect not seen in mothers who carry a PM without a child with FXS, indicating an impact of maternal stress. However, it is unclear whether social anxiety manifests due to a genetic vulnerability conferred by the PM in response to elevated maternal stress or also manifests among non-carriers experiencing elevated maternal stress. Thus, we compared social anxiety among 18 mothers of a child with FXS (“FXS mothers”) and 19 mothers of a child with autism spectrum disorder (ASD) (“ASD mothers”). We also explored social outcomes and symptoms of ASD. Social Phobia and Anxiety Inventory (SPAI) scores were not significantly different, with 44% of FXS and 47% of ASD mothers in the probable social anxiety range on the SPAI. For social outcomes, 28% of FXS and 38% of ASD mothers indicated a lack of social support; these rates were higher among mothers with probable social anxiety on the SPAI (38% and 57%, respectively). In addition, 61% of FXS and 81% of ASD mothers indicated that they had a hard time reaching out for help; again, rates were higher in those with probable social anxiety (88% and 100%, respectively). Lastly, similar scores were seen on the Broad Autism Phenotype Questionnaire (BAPO) and the Social Responsiveness Scale (SRS), with 28% of FXS and 39% of ASD mothers scoring in the “high” range of the BAPO and 6% of FXS and 21% of ASD mothers scoring in the “moderate” to “severe” range of the SRS. Overall, social anxiety and social outcomes were not significantly different between FXS and ASD mothers indicating that social anxiety may be a common manifestation among mothers experiencing elevated maternal stress.
**P25**

**Inertial Sensor-Based Tremor and Bradykinesia Quantification and Potential for Early Disease Identification in FXTAS**  
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**BACKGROUND:** Early predictors of FXTAS onset are needed and quantitative measurements of tremor, bradykinesia and coordination may be useful in natural history studies, response to medications and as outcome measures in future clinical trials in FXTAS. **OBJECTIVE:** To quantify the severity of upper extremity (UE) tremor subtypes, bradykinesia and incoordination in FXTAS and potentially identify preclinical symptoms in premutation carriers (PMC) without FXTAS using an inertial sensor system. **METHODS:** 39 PMC with FXTAS, 20 PMC without FXTAS, and 27 healthy controls performed a series of UE motor tasks while wearing an *ETSense™* sensor with the *Kinesia One* system which quantifies several types of tremor, bradykinesia and rapid alternating movements. Regression analyses controlling for age, sex and CGG repeat size with FXTAS diagnosis group as the main predictor was performed to detect potential group differences. The FXTAS Rating scale (FXTAS-RS) was administered to determine whether these clinician-rated subscores correlate with severity scores from the *Kinesia* system. **RESULTS:** PMC with FXTAS had significantly worse postural and kinetic tremor compared to PMC without FXTAS ($p=0.04; 0.03$) and controls ($p=0.001; 0.0001$), respectively, and worse finger tap ($p=0.0009$), hand movement ($p=0.0001$) and rapid alternating movement speed ($p=0.003$) and amplitude ($p=0.04$) than controls. PMC without FXTAS had significantly worse finger tap ($p=0.004$), hand movement ($p=0.01$) and rapid alternating movement speed ($p=0.003$) and amplitude ($p=0.02$) than controls. All quantitative scores were significantly correlated with the same item subscores from the FXTAS-RS ($p=0.02$ to $<0.0001$) except for finger tap speed and amplitude. **CONCLUSIONS:** The *ETSense™* system is a feasible, portable and low-cost method for quantifying UE tremor, bradykinesia and dysdiadochokinesia in FXTAS and may have potential in detecting preclinical symptoms of UE speed and coordination deficits in PMC without FXTAS. Further validation of these measures and confirmation of preclinical disease identification in longitudinal studies is needed.

**P26**

**Feasibility of Dual-Task Treadmill Training to Improve Gait and Balance in Patients with FXTAS: A Pilot Trial**  
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**Background:** Gait ataxia is a major clinical characteristic of FXTAS, and worsening gait and balance along with cognitive decline may be prognostic for future falls, progressive disability and reduced quality of life (QoL). Cardiovascular exercise training with and without cognitive tasks improves both motor and cognitive function in individuals with Parkinson's disease, traumatic brain injury and chronic stroke survivors. A dual-task (DT) intervention simultaneously targeting both gait, balance and cognitive deficits have the potential to improve function and slow disease progression in FXTAS.  

**Objective:** Our primary objective is to determine the feasibility of DT treadmill training (combined aerobic exercise at 65% max HR for 30-45 minutes with an executive function task) in patients with FXTAS. Secondary objectives are to explore the effects of the intervention on gait, balance, cognitive and functional outcome measures compared to a control group.

**Methods:** Ten individuals with possible, probable, or definite FXTAS, who are community ambulators without an assistive device will be recruited for a 6-week DT treadmill training intervention group (3x/week for 50 minute sessions), and ten individuals who are not able to participate in the intervention (due to lack of time/interest/transportation, etc.) will be assigned to the control group. Outcome measures will be collected pre- and post-intervention (immediately after the 6-week intervention, and at 1- and 6-months post-intervention; controls will also be tested at the same timepoints). These include assessments of cardiovascular function (via VO2max), gait, balance, cognitive function, functional outcome measures, and QoL.

**Discussion:** This research will demonstrate the feasibility of a DT treadmill training intervention and potential to improve motor and cognitive function and QoL in FXTAS. The results of the study will provide data to inform the design and conduct of the first exercise-based RCT in FXTAS.
Factors Associated with CGG Repeat Instability in Female Premutation Carriers with Varying Degrees of Mosaicism

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Individuals with a FMR1 premutation CGG expansion are at risk for FXTAS, FXPOI, FXAND and other FMR1-associated disorders. Premutation carriers often present with CGG size instability and mosaicism; however, it is unclear why some premutation carriers present with allelic instability while others do not. We determined the CGG profile and the presence of AGG interruptions in 418 female premutation participants (age ranged from 6 months to 90 years) by triplet primed PCR. The activation ratio (AR) was determined from the Southern blot analysis or HpaII predigestion of the PCR template and the FMR1 mRNA expression levels by real time qRT-PCR.

Of the 418 females analyzed, 243 were mosaic (2 or more CGG size repeat alleles) and 175 were non-mosaic. The mean CGG repeat number was 97.2 ± 17 and 85.3 ± 26 in the mosaic and non-mosaic females. The mean AR was not statistically different and as expected, elevated FMR1 mRNA expression levels were observed in both groups. Using new measures to quantify somatic instability it is apparent that factors that affect intergenerational instability also affect somatic instability, with increased expansion being directly related to increased CGG repeat number and ameliorated by AGG interruptions. Age too is a factor, with older individuals presenting with more instability than younger individuals with the same initial repeat number. Further, we found that there is an inverse relationship between the extent of instability and the AR, consistent with the idea that the alleles on the active X are the ones more prone to expansion and thus that transcription or open chromatin is necessary for instability. Whether variations in DNA repair proteins also contribute to some of the variability in the extent of instability, as they do in other Repeat Expansion Diseases, remains to be seen.
Expression of hnRNP A2/B1 suppresses rCGG repeat-mediated neuronal toxicity associated with Fragile X-associated tremor/ataxia syndrome in a RAN translation-independent manner


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FXTAS (Fragile X-associated tremor/ataxia syndrome) is a neurodegenerative disorder in aged premutation carriers with 55–200 CGG repeats in the 5'UTR of fragile X mental retardation gene (FMR1). Two mechanisms have been proposed for FXTAS: RNA toxicity and non-AUG-initiated (RAN) translation toxicity. Expanded CGG repeat RNA is reported to sequester proteins such as Purα and hnRNP A2/B1 (heterogeneous nuclear ribonucleoprotein A2/B1), thereby blocking them from performing their proper function. Recently, polyglycine-containing protein (FMRpolyG) generated by RAN translation of the expanded CGG repeats was shown to be toxic to cells. Here, we have generated a transgenic mouse line that expresses hnRNP A2/B1 in Purkinje cells, and we have crossed this line with the FXTAS mouse model that we generated previously. We found that the expression of hnRNP A2/B1 can ameliorate the Purkinje cell loss and locomotor deficits induced by the expression of the expanded CGG repeat. Gene expression analyses further confirmed the molecular rescue by hnRNP A2/B1 in the FXTAS mouse model. Surprisingly, pathological analyses revealed that the number of FMRpolyG inclusions were unchanged in the rescued mice. Furthermore, using a FXTAS cell culture model, we observed a similar suppression of rCGG repeat toxicity by hnRNP A2/B1 without altering the number of FMRpolyG inclusions. These results together suggest that hnRNP A2/B1 has an important neuroprotective function by alleviating RNA toxicity, and FMRpolyG may not be essential for the rCGG repeat toxicity associated with FXTAS.

A continuum of learning and attention difficulties in females, extending from FMR1 premutation to full mutation

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Although once thought to be asymptomatic, it is now well documented that both female as well as male premutation carriers might suffer from associated medical comorbidities, beyond FXTAS and FXPOI. Whether fragile-X premutation has a subtle effect on cognition has been under debate for several years. We assume that, there is a continuum of learning and attention deficits that correlate with increased number of repeats. Methods: 98 women were referred to our center due to a related FXS patient, mainly offspring or sibling. Variable data was collected from index cases and relatives. This is an ongoing project. The information included genetic results of CGG repeats, demographic information, structured questionnaires for ADHD, learning disabilities of language and mathematics, and independence. The group carrying FMR1 premutation was analyzed separately and compared to the group carrying full mutation. Females with Fragile X Syndrome, autism and/or intellectual disability were excluded. Results: When analyzed as a continuum, there was a significant increase in reported labor difficulties and C-Section, more FXPOI and less women driving, with higher number of repeats. Significant correlations with higher number of repeats were found for ADHD severity, language difficulties, dyscalculia, inattentiveness, spelling difficulties and executive dysfunction. Conclusions: Our study supports the findings that the premutation FMR1 allele may lead to other disorders in addition to FXTAS and FXPOI. ADHD and learning difficulties correlate with increased number of CGG repeats and are prevalent features of premutation and full mutation in females.
**Saccades in Fragile X Premutation Carriers with and without FXTAS**

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Studies of eye movements in neurodegenerative disorders have provided new understanding and biomarkers for disease progression. While research in eye movements in fragile X-associated tremor/ataxia syndrome (FXTAS) is limited, a previous study demonstrated that eye movements during the antisaccade task was indicative of inhibitory dysfunction in a group of young (30.14 years ± 6.44) asymptomatic fragile X premutation carriers (fXPCs). Here we discuss the results of three oculomotor tasks in a cohort of older fXPCs with and without FXTAS (68.70 years ± 7.90 and 61.06 years ± 8.80, respectively) to assess oculomotor function. Additionally, we examined if the same inhibitory control problems were still present in this older cohort.

We recruited 23 controls, 35 fXPCs with FXTAS and 52 asymptomatic fXPCs to complete three tasks: prosaccade, antisaccade, and smooth pursuit. In the prosaccade task participants were instructed to fixate their eyes on a central crosshair until a peripheral stimulus appeared. In the antisaccade task participants were told to keep their eyes fixated and then look opposite of the peripheral stimulus. Finally, in the smooth pursuit task, participants were asked to maintain gaze on a continuously moving square. Diagnosis of FXTAS was determined by a medical doctor according to standardized neurological examination protocols.

In a non-parametric group (ANOVA) comparison with controls we found that fXPCs with FXTAS had longer saccade latencies on prosaccade (p = .0061) and antisaccade (p = .0001) tasks. There were no differences in latencies between controls and asymptomatic fXPCs. The antisaccade task revealed significant differences between controls and fXPCs with FXTAS regarding the magnitude of saccades (p = .0037) and directional errors (p = .0083). There were no significant differences among the three groups for the speed of saccades, during either the prosaccade or antisaccade task nor the smooth pursuit task.

**Neuropathology of Fragile-X associated neuropsychiatric disorders (FXAND)**


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CGG repeat expansions (55-200) in the FRM1 gene can lead to Fragile-X associated diseases such as FXTAS and FXAND. Clinically, FXTAS is characterized by progressive tremor and ataxia, whereas FXAND presents mainly with neuropsychiatric symptoms. Pathology of FXTAS include ubiquitin-positive FMRpolyG inclusions as a result of CGG-repeat RAN translation. Neuropathology of FXTAS is characterized by white matter changes, astrogliosis and inclusion pathology throughout the CNS. Currently, the pathology of FXAND is unknown.

Here, we describe two cases of FXAND with mild movement disturbances. One donor was diagnosed with vascular dementia and frontotemporal dementia during life. Macroscopically, no white matter lesions were visible but there were vascular infarcts in cortical regions and basal ganglia. On microscopic level, there was a large amount of FMRpolyG inclusions predominantly in cortical regions in neuronal, glial, ependymal, choroid plexus epithelial and endothelial cells. A 107 CGG repeat expansion in the FRM1 gene was identified post-mortem. Donor two was known to carry a repeat expansion of 77 prior to passing. Clinically, he presented with dementia and behavior alterations, and suffered from several infarcts throughout the brain. On MRI, no white matter changes were detected and on microscopic level similar changes were seen as in donor one with a large amount of FMRpolyG inclusions predominantly in cortical regions in neuronal, glial, ependymal, choroid plexus epithelial and endothelial cells.

We conclude that the pathology of FXAND presents different from FXTAS, where few white matter alterations are observed and the inclusions were present in large numbers in cortical regions. Inclusions are prominent also found in endothelial cells, which is likely linked to the vascular insults.
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Thank you!