ASD through the lens of fragile X-associated disorders

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Fragile X Associated Disorders

Fragile X Syndrome (FMR1 gene, full mutation) -
Most common heritable form of cognitive impairment ~1/3,000 (males>females)
Most common single-gene disorder associated with autism
~30% with autism (>60% with ASD) ~2-6% with autism have FXS

FMR1 (Premutation) carrier-associated disorders –
~ 1:130 females and ~1:300 males is a carrier
Most common heritable form of premature menopause; cause of late-onset neurodegenerative syndrome (FXTAS)
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Most common heritable form of premature menopause; cause of late-onset neurodegenerative syndrome (FXTAS), ASD, and developmental disorders

Caused by a large **CGG-repeat** expansion in a non-coding portion of the FMR1 gene

- Premutation expansions - increased mRNA
- Larger (full mutation) expansions – loss FMRP
**Fragile X Associated Disorders**

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**Premutation-associated ASD and developmental delays**
- ASD in 73% of boys with a premutation referred clinically to the UC Davis MIND Institute ([proband] bias towards ASD) Farzin et al., 2006
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- 7% in boys with premutation identified through cascade testing

19% (11/57) males with a premutation had a diagnosis of autism (1200 families with FXS); 5% of control boys.
33% of boys with a premutation had developmental delays; 1.8% of control boys. Bailey et al., 2008
Fragile X Syndrome

• Broad spectrum of involvement
  - lowered IQ
  - shyness/social anxiety $\rightarrow$ autism/ASD
  - mild to severe mood instability
  - seizures in $\sim$20% of children

• Mild physical features
  - large/prominent ears
  - macroorchidism
  - hyperextensible joints
  - high arched palate
Fragile X Syndrome

- Behavior problems
  - tactile defensiveness 80%
  - poor eye contact 90%
  - hand biting/hand flapping
  - ADHD
  - perseverative speech or behavior in almost all-routines
The penetrance and severity of the clinical features increase in succeeding generations as the CGG repeat increases.
FMR1 protein (FMRP)

FMRP is highly expressed in neurons of the CNS

- RNA binding protein
- Reduces the expression of many other proteins involved in development and maintenance of the synapses between neurons.
Two *FMR1* pathogenic mechanisms

Normal

(CGG) \(<\) 45

mRNA

FMRP

Clinical

Typical

Full mutation

(CGG) \(>\) 200

Gene silencing / **No** mRNA

Absence of *FMR1* protein (FMRP)

Fragile X syndrome

Autism/ASD

Seizure disorder
mGluR5 theory of intellectual disability in FXS

An early model for Fragile X syndrome:
- mGluR5 stimulation leads to long-term depression of synapse function (LTD)
- This process requires protein synthesis, which is regulated by FMRP
- In FXS (absence of FMRP), there is dramatically increased LTD

- But the situation is far more complex

Bear et al. 2004
Deregulated mTOR signaling increases the risk of autism in patients with mutations in neurofibromin (NF1), tuberous sclerosis 1 (TSC1), TSC2 or phosphatase and tensin homologue (PTEN), among others.
Main synaptic functions associated with ASD


In the absence of FMRP, there is a net shift toward protein synthesis of a large number of mRNAs that would normally be bound by FMRP.
FMRP binds multiple mRNAs to (down)regulate their protein expression


The authors used two related measures to isolate all of the mRNAs that are bound by FMRP:

- **RIP-C**H**IP**: ribonucleoprotein immunoprecipitation followed by microarray analysis (RIP-chip)
- **P**AR-**C**L**IP**: 4-thiouridine (4SU) photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation

They found **3,593 FMRP mRNA targets**, of which 939 genes were two- to sixfold enriched.

Among the highly enriched FMRP targets were **93 genes** independently implicated in ASD, including mTOR and TSC2.

Ascano et al. found genes involved in Angelman, Prader–Willi, Rett, and Cornelia de Lange syndromes.

- **Molecular link to tie together elements of clinically overlapping disorders, and identify connections between FXS and its associated phenotypes.**
Proteins encoded by ASD-related genes are colored in dark green (detected by PAR-CLIP) or light green (not detected).

Yellow indicates signaling pathway components also identified by PAR-CLIP.

FMRP targets at the synapse implicated in ASD
A second *FMR1* pathogenic mechanism

**Normal**
- (CGG)< 45
- mRNA
- FMRP
- Clinical: Typical

**Premutation**
- (CGG) 55 - 200
- Excess mRNA
- RNA "toxicity"
- Developmental delay
- *Autism/ASD*
- Seizure disorder
- Premature menopause
- Neurodegeneration (FXTAS)

**Full mutation**
- (CGG) > 200
- Gene silencing / No mRNA
- Absence of *FMR1* protein (FMRP)
- Fragile X syndrome
- *Autism/ASD*
- Seizure disorder
Fragile X-associated tremor/ataxia syndrome (FXTAS) – problem of awareness

Carrier mothers concerned with specific problems with their own (carrier) fathers

- Frequent falls/ balance problems
- Difficulty writing, eating
- Memory loss / dementia
- Loss of feeling / paresthesias
- Parkinsonism
- Incontinence

Prior diagnoses in FXTAS patients

Hall et al. (2005) Neurol 65:299
FXTAS

**Case DR**: Premutation carrier with 89 CGG repeats, identified through two grandchildren with fragile X syndrome. *First identified case of a carrier grandfather with progressive neurological dysfunction*

**Core features**

**Tremor** – Onset in right hand at age 54, left hand within two years; writing illegible at 58 yr; retired early as an electrician at 58 yr.

**Ataxia** – Progressive difficulty with balance and gait; frequent falls
Decreased viability of premutation neurons in culture

- Mouse hippocampal neurons from neonatal mice (normal and premutation) grown in culture.
- Decreased viability suggests greater susceptibility
  - To environmental toxins
  - Trauma, as in seizures
  - Oxidative stress
  - Second genetic hits

Chen et al., 2010 *HMG*
Molecular Etiology of Fragile X Disorders

**Premutation disorders**

*Increased* gene expression

- RNA toxicity

- **Fragile X-associated primary ovarian insufficiency** (FXPOI)
  - Reduced ovarian reserve
  - Early menopause

- **Fragile X-associated tremor/ataxia syndrome** (FXTAS)
  - Late-onset neurodegeneration Syndrome
  - Tremor, gait ataxia, dementia, etc.

**Full mutation disorder**

*Loss* of gene expression

- Absence of FMRP

- **Fragile X syndrome**
  - Neurodevelopmental disorder
  - Altered brain development
  - Seizures

**Autism/ASD**
Molecular etiology of fragile X premutation disorders

Premutation disorders

*Increased* gene expression

RNA toxicity

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Autism/ASD

**DGCR8/DROSHA initiate microRNA production**

- pri-miR
- pre-miR
- mature miRNA
Molecular etiology of fragile X premutation disorders

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*Increased* gene expression

- RNA toxicity

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**Autism/ASD**

**CGG repeat sequesters DGCR8**

- Premutation disorders
- Increased gene expression
- RNA toxicity
- Fragile X-associated primary ovarian insufficiency (FXPOI)
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- Autism/ASD
A return to FMRP interactions pointing beyond fragile X
mRNA encoding bone morphogenetic protein type II receptor (BMPR2) is a target of FMRP, which down-regulates BMPR2 translation (protein expression).

Two earlier observations:
(i) RNA immunoprecipitation studies of Ascano et al. (2012) raised the possibility that FMRP interacts with the mRNA for Bone Morphogenic Protein Receptor 2 (BMPR2)
(ii) BMPR2 signaling is involved in synapse formation

In FXS, BMPR2 protein is over-expressed in the absence of FMRP
Function of BMPR2 in neuronal morphology

BMPR2 subunit extracellular space

BMPR1 subunit cytosol

CTD

LIM1 kinase
Function of BMPR2 in neuronal morphology
Function of BMPR2 in neuronal morphology

Normal balance

F-actin

G-actin

Profilin,

cofilin

Phosphatases (e.g., slingshot)
Consequences of reduced FMRP: 
*increased BMPR2*

Abnormal actin polymerization

F-actin

G-actin
A nexus for altered gene-brain function: the dendritic spine / synapse

Many disorders involving intellectual disability (ID) and autism/ASD involve altered spine shape

Srivastava et al. (2011)

Super-Resolution Dynamic Imaging of Dendritic Spines Izeddin et al. (2011)
A nexus for altered gene-brain function: the dendritic spine / synapse

Many disorders involving intellectual disability (ID) and autism/ASD involve altered spine shape or dynamics.

Dual-channel time-lapse imaging of total (EGFP-actin) and filamentous (Lifeact-mRuby) forms of actin.

Super-Resolution Dynamic Imaging of Dendritic Spines Izeddin et al. (2011)

Time-lapse sequence from the two regions (i and ii) in a neuronal growth cone.
Consequences of reduced FMRP: 

*increased BMPR2*

- Absence of FMR1 activity (FMRP) leads to overexpression of BMPR2 and LIMK1, and abnormal spine development

- Genetically reducing BMPR2 corrects the defect
Consequences of reduced FMRP: *increased BMPR2*

- Absence of FMRP
- Overexpression of BMPR2
- Increased LIMK1
- Inhibition of cofilin
- More immature spines (poor connectivity)

**Augmented noncanonical BMP type II receptor signaling mediates the synaptic abnormality of fragile X syndrome** (2016) Science Signaling 9:ra58

FMR1: \[ \begin{array}{c} 0 \ \ \ \ \ \ + \\ - \ \ \ \ \ \ + \end{array} \]

LIMK inhibitor: \[ \begin{array}{c} - \ \ \ \ \ \ + \\ - \ \ \ \ \ \ + \end{array} \]

- Addition of a LIMK1 inhibitor corrects the defect caused by the absence of FMRP
- The inhibitor does not lead to further alterations of spine morphology even in the presence of normal FMRP / BMPR2 / LIMK levels.
- This experiment suggests a possible treatment of FXS and associated ASD
Roles of LIM kinases in central nervous system function and dysfunction

- Lim kinases integrate signals from Rho GTPase signaling pathways.
- LIMK1 is upregulated by Rac and Cdc42; LIMK2 by Cdc42 and RhoA.
- Both are deactivated by multiple proteins, including NF1
- Both LIMK1 and LIMK2 phosphorylate and inactivate cofilin, which result in actin polymerization.
Synaptic balance

Secondary gene effects link / modify primary disorders
Synaptic balance

Secondary gene effects link / modify primary disorders

ASD + ID

Secondary (background) gene effects

Autism ASD

Primary gene effect

ID
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