Autism Spectrum Disorders: genetic causes and the prospects for therapies

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Autism Topics

• Definition ...autism spectrum (new DSM 5)
• Autism as genetic “inborn error” disease
• Autism and (not) vaccines
• Autism and organelle disease
• UCI Center for Autism Research and Translation
• Discovery of functional biomarkers
• Temple Grandin...gifted brain functions
Common Findings

- Repetitive Behaviors
- Narrow Range of Interest

- Impaired Social Relationships

- Impaired Language/Communication

30% have Regression

25% Develop Seizures
Included Within ASD

Gastrointestinal disturbances

Hyper/hypo-sensitivity (~90%)

Intellectual disabilities (~35%)

Sleep abnormalities

Motor deficits
Hypotonia, Apraxia or Motor Delay
1 in 68 children have autism spectrum disorder (ASD)

almost 5x more common in boys than girls

SOURCE: CENTERS FOR DISEASE CONTROL AND PREVENTION
Research Funding NOT Keeping Pace

Autism on the Rise
Estimated Autism Prevalence and NIH Funding for Autism Research

* CDC prevalence estimates are for 4 years prior to the report date (e.g. 2014 figures are from 2010)
Financial Impact on Society

The “No cure” option cannot be ignored!

[Bar chart showing defense spending and associated costs, with the United States having the highest defense spending and the highest associated cost for Autism 2025.]
What do we need in ASD?

**Illness**
- Mechanisms
- Biomarkers
- Interventions

**Injury**
- Triggers
- Predictors
- Preventions

**Services**
- Inclusion
- Civil Rights

**Identity**

After Insel
Current ASD Treatment

Diagnosis clinical only

Therapeutics behavioral interventions only

Drugs do not address root cause
“We need new ASD medicines”

Sufficient genomic pathway information exists to begin a FUNCTION BASED approach to CORE deficits in ASD

Behavioral therapies are important, but are symptom-directed & not sufficient

...epidemic increase continues unabated

Animal models have failed Pharma

There is no pharmacological therapy for CORE deficits in ASD
Key Milestones to Cure Autism

- Define **PATHOGENESIS**
  - how disease arises
  - Best start is genes....
  - not just *statistically associated*...
    - ....but with *roles proven* with *functional analysis*
  - Functional genes are *target* to screen for *environment*

- Genes give **TARGETS** for **DIAGNOSTIC TESTS**

- Genes give **TARGETS** for **DRUG DISCOVERY**

- Genes give **MODEL ORGANISMS** for **DRUG** discovery and **ENVIRONMENTAL** impact

- Genes **REFINE DIAGNOSIS**: **TRANSLATE** into **CLINICAL INVESTIGATIONS** model and drug/environment effects
Autism: a multi-genic disease

- Common complex multigenic diseases afflict everyone!
- Well recognized syndromes in at least 60 million in USA
  - Diabetes/Metabolic Syndrome
  - Atherosclerosis / Hypertension / Asthma
  - Cancers
  - Seizures / Ataxia / Migraine
  - Autism / Schizophrenia / Bipolar / ADHD / Alzheimers
- All show a High Heritability
  - Genes involved….many genes, most with small effect
  - Too many genes …NOT generally useful as a DIAGNOSTIC
- Inheritance pattern…complex
  - Identical twins are MUCH MORE alike than fraternal twins or sibs
  - BUT identical twins can be DISCORDANT
- SO there is ALSO a SIGNIFICANT “non-gene” component
  - “Environment”
- “Functionalizing” these genes makes them “Actionable”
  - Current Frontier for ALL of these diseases
  - Enables …..Diagnostics, Therapeutics
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Genes are on human chromosomes & they come in PAIRS one from mom & dad
Parents each transmit ONLY ONE of each pair
example: dominant inheritance

Brown / blue eyes

Vertical inheritance

Punnett square
Dominant pedigree shows how easy it is to follow ONE gene
Following just two genes is very complicated. Try to imagine following four ... or ten.

But...... ASD is MULTI-GENIC.

MENDEL showed that:

Following just two genes is very complicated.

Try to imagine following four ... or ten.
Autism associated with Extra or missing **blocks of chromosomes..** CNVs

*Can be associated with biochemical signatures*

**Energy deficient signature**

- Autism
- Hypotonia
- Lactic acidosis
- Low carnitine
- High ammonia
- Mitochondria hyper-proliferate
- Defect in Mitochondrial Complex III
- Normal mtDNA sequence

Chromosome 15 +mar
15q Inv dup

Most common chromosomal abnormality in autism
We now study chromosomes with microarrays and score many CNVs in ASD. Most are de novo, increase with dad’s age.

Chromosome 15 duplications and deletions.

5 unrelated ASD..... M. Smith et al, 2009
15q duplications

Dup. 15q11.1-15q13.3 B1 & S1 Autism (maternal origin)

Dup. 15q11.2-15q13.1 AU 210 Asperger (paternal origin)
Single genes build this complex web of BIOCHEMICAL PATHWAYS from the proteins they encode. They work together in teams, PATHWAYS, to provide the energy we need to run complex synthetic and degradative processes that keep us ALIVE and HEALTHY.

Metabolic geneticists specialize in the DISEASES caused by MUTATIONS that disturb these PATHWAYS.

These diseases are called “Inborn Errors of Metabolism”
Inborn errors show perfectly how genes and environment interact
Untreated vs Treated PKU

Reduce phenylalanine in diet CURES the disease phenotype….for past 40 years
Untreated vs $B_{12}$-Treated Methylmalonic Acidemia

30 years successful treatments

Hemodialysis support

Normal Schoolchild
Even applies to ASD
Rare single-gene disease causing ASD
SSADH Deficiency

Symptoms:
Ataxia, Hypotonia
Autism, PDD
Seizures, Dev delays

“date rape drug”
Treatment rare ASD energy-deficient syndrome
Taurine reverses MRI-documented Globus Pallidus lesion in SSADH

Saronwala, Tournay, Gargus (2008)
ACMG platform presentation

Before therapy

1 yr taurine therapy

Mouse Knock-out model leads to 1st human therapy
Inborn Errors approach:

“RARE genetic errors will illuminate the pathway” that causes common disease

• Approach DOES work: MIGRAINE
  • gene discovery in Familial Hemiplegic Migraine
  • pathogenic mechanism
    Scientific American 2008... Dodick & Gargus

• Discovery of 3 genes underlying rare form of migraine re-framed the common disease
  • Migraine is neuronal, NOT BLOOD VESSEL, disease
  • Migraine similar to seizures
  • Use of seizure drugs in migraine therapy makes sense
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Wakefield had not disclosed commercial conflicts of interests. “Your non-disclosure was contrary to your duties,”

...(his) deal with a lawyer, Richard Barr, who was preparing a case against the manufacturers of the MMR vaccine and revealed that the parents of the 12 Royal Free children were mostly litigants, recruited through anti-vaccine campaign groups.

The Lancet said following the judgment of the General Medical Council (GMC) fitness to practise panel last Thursday it had become clear that several elements of the 1998 paper by Dr Andrew Wakefield and others were incorrect, and the paper RETRACTED.

More than 30 charges were found proven against Wakefield.

…in the wake of Wakefield’s Lancet paper immunisation rates in Britain dropped dramatically and led to a surge in measles cases.

....and many law suits claiming damages because of vaccination causing autism
Mitochondrial disease in ASD

“Vaccine court” settlement
Hanah Poling case of ASD
NEJM 358:2089 2008

• Ruling suggested mitochondrial mutation predisposed to vaccine related decompensation causing autism syndrome

• Child had “known pathogenic” mutation
  – mtDNA T2387C “mutation”: 16s rRNA gene variant
  – >1.5M$ award

IN THE UNITED STATES COURT OF FEDERAL CLAIMS
OFFICE OF SPECIAL MASTERS
CHILD, a minor, by her Parents and Natural Guardians, Petitioners,

SECRETARY OF HEALTH AND HUMAN SERVICES, Respondent.
RESPONDENT'S RULE 4(c) REPORT

...and thousands of similar cases were immediately filed

Consequences: Preventable infectious disease outbreaks in affluent communities
A decade before Polling case clues pointed to mito energy-deficiency in ASD

- Direct Energy-Deficit Diseases
  - Mitochondrial respiratory complexes
    - mitochondrial DNA mutations
    - nuclear chromosomal loci
  - Mitochondrial fatty acid oxidation
    - nuclear chromosomal loci

- 2008- UCI receives Autism Speaks then NIH support for ASD-mitochondria connection studies
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Cell Organelles and CNS disease

1. Mitochondria
2. Lysosomes
3. Peroxisomes
4. Endoplasmic Reticulum
   NEW MECHANISM
Organelle dysfunction causes energy deficiency / unregulated calcium

• Clear for mitochondria
• Clear for peroxisomes

• NEW findings in lysosomal diseases
  – Gaucher GBA locus major risk factor in Parkinsons
  – Carriers, without storage, have increased risk
• NEW finding in endoplasmic reticulum
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Cure Relies on Entrepreneurial Philanthropy

Entrepreneurial Philanthropy: An Important Model for Autism Research & Treatment in the 21st Century
June 26th 2013; 10:30 AM to 12:30 PM
Sacramento, CA State Capitol Building
CART and Center for Autism And Neurodevelopmental Disorders…launched with $28M Thompson Family Foundation Gift and Children & Family Commission Matching funds

• Center Launch event…..December 13, 2012
• Senate Hearing…..Nov 13, 2013
• Invited keynote presentation of CART at La Jolla Biotech symposium…Dec 11, 2013
• Invited platform presentation of CART at Personalized Medicine World Conference Jan 26, 2014
The Center for Autism Research and Translation (CART)
Launched December 2012
$28 million Thompson Family Foundation gift and Children & Family Commission matching funds.
The valley of death

The CART proposal

Target Discovery | Candidate Identification | Preclinical Development | Phase I-III | Approval

Speed up the drug discovery process in ASD through a public-private partnership catalyzed by CART

Keep the partnership sustainable by spinning off commercial opportunities (start-ups, licensing)
CART Integrated Research Platform

Current Projects
- Calcium signals
- Sensory stimulation
- Oxytocin / MCH pathway
- Rare disease clinical trials
- FraX synapses
- ASD electrophysiology
- Enriched learning
- Sodium channel ASD
- Biomarkers in ASD
- Zinc signals
- Dendritic spine complexes
- Optogenetics
- GABA neurons
- MEG prototype
Cellular Imaging

Core 2
Cell Signaling

Galina Schmunk

Center for Autism Research and Translation
University of California, Irvine – School of Medicine
Synapse
“UCI is at the forefront of scientific research because of the interdisciplinary collaboration among its faculty, staff and departments,” Bill says. “This cross-functional approach has enabled the campus to become a hub of stem cell research in Southern California.”
Brain & Behavior / Clinical Trials Outcomes

Core 5
Brain Function

Irregular periods of sleep

WASI-II

Ramesh Srinivasan
Wendy Goldberg

Center for Autism Research and Translation
University of California, Irvine - School of Medicine
Optogenetics/Electrophysiology

Transplantation allows for *in vivo* studies

Circuit Mapping

Optogenetics

Electrophysiology

Behavior

Human transplanted neurons

Core 7
Optogenetics/Electrophysiology

Robert Hunt

Center for Autism Research and Translation
University of California, Irvine - School of Medicine
Clinical Trial Expertise

Natural History Study

Record of NIH, Autism Speaks and CPEA Center funding.

Current NIH supported rare diseases ASD clinical trial site.
Deficit in Regulation of Endocannabinoids

Endocannabinoid System as a Therapeutic Target for Autism

Ampakine Rescue Synaptic Plasticity In ASD

3 Pharma leads show published efficacy in mouse models

Targeting $\alpha_7$ nACh and $\text{GABA}_A$ receptors simultaneously ASD
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CART Two Pronged Approach

Diagnostics

Therapeutics
Diagnosis

Subjective

Early Intervention

The longer a child with autism goes without help, the harder it is to reach.
With multiple therapeutic targets...

...how do we know where to shoot?
CART Solution

Rapid Discovery Platform

1. Non-Invasive High Throughput Diagnostic Screen
2. Stratify Trial Cohort Based on Screen/Phenotype
3. Choose Candidate Therapeutics Based on Phenotype/Pathway
4. Generate Cohort Specific Investigator IND Application Data
5. Initiate Clinical Trial
Diagnostic Screening Development

Pre-Clinical Studies
High Resolution Phenotype Collection

Unique Discriminator Discovery

Evidence based diagnosis

Establish unique group discriminators

Findings

Phenotype Group 1

Phenotype Group 2

Diagnosis and treatment based on unique group discriminators
CART Rapid Discovery Platform

60+ ASD Families Screened

Biorepository 100+ ASD Cell Lines Established
Ca$^{2+}$ signaling as a biomarker for autism

- Diagnosis is strictly behavioral, only after the age of 2
- Mean age of ASD diagnosis is 5 years
- Early intervention is important, especially for high risk children
- No current biomarkers for autism
What we found...

Unique Discriminator
Calcium Signal
Genes associated with calcium signaling
Examine how to “functionalize” the mitochondria / calcium signaling complexes
From global to local $\text{Ca}^{2+}$ events
Visualizing local to global Ca$^{2+}$ events
Local Ca²⁺ events in FXS and TS

QUALITATIVELY altered in rate constant

Unchanged: amplitude, latency, frequency

Fast flicker closure in ASD

15 ms both FXS and TS

32 ms control
Global IP$_3$-induced Ca$^{2+}$ response is decreased in FXS and TS.
ATP-mediated response in sporadic ASD

Schmunk et al, 2017
FLIPR – from subcellular events to high-throughput screening

*FLIPR High Throughput Cellular Screening* – reads fluorescence values from 96 well plate in response to activation with an agonist.
Ca$^{2+}$ signaling is suppressed in sporadic ASD

ATP response normalized to IM

N=12  N=4  N=6  N=3  N=2  N=23

Schmunk et al, 2017
ROC curve

- Receiver operating characteristic
- True positive rate (TPR) against the false positive rate (FPR)
- Trade-off between true positive and false positive
Sensitivity vs Specificity

- A negative result in a test with high **sensitivity** is good for ruling out the disease
- A positive result in a test with high **specificity** is useful for ruling in the disease.
Ca$^{2+}$ signaling in syndromic ASD

AUC=0.86

Schmunk et al, 2017
Ca^{2+} signaling in sporadic ASD

AUC=0.83

Schmunk et al, 2017
Ca$^{2+}$ signaling as a diagnostic tool

Sporadic and syndromic ASD combined

AUC=0.84

Schmunk et al, 2017
### a)

<table>
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<th>Identity</th>
<th>hiPSC</th>
<th>EB</th>
<th>NE</th>
<th>Neuronal progenitors</th>
<th>Mature neurons</th>
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<td>Day 0</td>
<td>7</td>
<td>10</td>
<td>26</td>
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</tr>
</tbody>
</table>

### b)

- **hiPSC**
- **EB**
- **Rosette**
- **Progenitor**
- **Neurosphere**
- **Neuron**
IP₃-mediated Ca²⁺ signaling is decreased in neuronal progenitors from a FXS patient, similar to fibroblasts.

Mean amplitudes and latencies to peak of Ca²⁺ fluorescence signals in FXS progenitors (red) and matched controls (black)
UV uncaging of t-ACPD induces Ca\textsuperscript{2+} release in proximal dendrites of murine cortical neurons

Mean peak amplitude and latency of somatic Ca\textsuperscript{2+} response in murine Neurons does NOT differ statistically between FXS and WT neurons (p-value>0.05)

GS thesis and GS and JJG unpublished results
IP$_3$–induced intracellular Ca$^{2+}$ signals are decreased in human fibroblasts and neural progenitor cells with FXS, but not in murine cells with FXS.

**Human fibroblasts**

**Human NPCs**

**Mouse fibroblasts**

YM & JJG unpublished

GS thesis and unpublished GS & JJG
Mitochondrial energy-deficient phenotype

Ca\(^{2+}\) signaling in neuronal cells

impact of MEMs on mitochondrial energetics
Therapeutic Target Testing

CART Discovery Platform

DNA, RNA, Metabolite, Clinical Data, Protein, Literature

ASD Subtype 2

Healthy Network

Toxicity Network

ASD Gene Reporter Set

Screening Assays

HIPSC-derived Neurons

ASD Patients and Controls

hiPSCs, NPCs

Neurons

Dopaminergic, GABAergic, Glutamatergic

Targeted pharmacotherapies

CENTER FOR AUTISM RESEARCH AND TRANSLATION
UNIVERSITY OF CALIFORNIA, IRRVINE - SCHOOL OF MEDICINE
CF Breakthrough

new paradigm for personalized medicine:
genomics → cell-based screening → treatment
(NO whole-animal model used)

“the end of the beginning”
Conclusions

• ASD has no defined biomarkers for diagnostics or novel drug discovery

• In rare forms of monogenic ASD syndromes, a molecular defect in IP$_3$ channel gating is resolved showing all forms have a short flicker open time.
  • Must be post-translational....covalent, protein complex, membrane domain, ?

• A high-throughput screen was developed to capture this defect in the monogenic ASD and typical, sporadic ASD samples

• iPSC- derived neuronal precursors from patient fibroblasts share this signaling defect....mature neurons being studies in vivo and implanted in murine brain

• Therefore, IP$_3$R signaling appears to be at a node in a signaling pathway at which many forms of ASD are unified into a shared defective output.

• ROC curves can distinguish, with high sensitivity and specificity, between syndromic and sporadic ASD samples, which signal similarly in this assay, and neurotypical controls....captures 75 – 85% of ASD

• This biomarker may come to be useful as an adjunct diagnostic and potentially in a screen for novel therapeutics for ASD and environmental stress risks for ASD.

• In the future, it may help guide clinical trials based on the Ca$^{2+}$ signaling phenotype

.....ps...Murine FXS cells DO NOT share this phenotype....they signal like WT
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Derek P. performs at UCI Gala
World Class Facilities and Professionals
CART Integrated Research Platform

CART

Core 1 Genomics
Core 2 Cell Signaling
Core 3 Synapse
Core 4 Stem Cells
Core 5 Brain function/
Clinical trials
Core 7 Optogenetics/
Electrophysiology

NIH Rare Clinical
ASD diseases

PH Drug Discovery

Autism Treatment Network
Affiliated Site

The Center for Autism
& Neurodevelopmental Disorders
Deeply phenotyped and genotyped autism cohort with demographic and clinical data.

Recruit additional participants

Fundraise & Philanthropy

Strengthen Research Platform

Research Grants

Drug discovery platform

Autism Research Kit (ARK)

Funding Opportunities

More Participants = More Discoveries

CENTER FOR AUTISM RESEARCH AND TRANSLATION
UNIVERSITY OF CALIFORNIA, IRVINE – SCHOOL OF MEDICINE
Our Vision...

“We are very excited for the UCI College of Health Sciences to become a national model for integrative health. We believe this model will eventually become the standard approach for promoting health and well-being in our society.” — Henry Samueli

“One of the root causes of our country’s healthcare challenges is our failure to focus on prevention and well-being. We need to do better at preventing diseases rather than just treating them. UCI is making much-needed investments in this area, and I congratulate them on this very generous donation.” — Sen. Dianne Feinstein
So please join us to recruit new participants…

Thank you!

Center for Autism Research and Translation
University of California, Irvine - School of Medicine

...and hence discover new diagnostics, therapeutics and ultimately a CURE!
Thank you

Acknowledgements

- Galina Schmunk, Ian Parker, Ian Smith
  - Publication..Translational Psychiatry this year.
- Dave Ferguson, Sam Reiter, Rachel Nguyen
  - CART staff
- 50 member faculty scientists of UCI CART
  - Please see web ....autismresearch.uci.edu
- Members of the Center for Autism and Neurodevelopmental Disorders (CAND)
- Thompson Family Foundation
- Prior center funding from NIH, Autism Speaks, Doris Duke Foundation