Autism Spectrum Disorders: genetic causes and the prospects for therapies

OLLI
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Autism topics in the News

• Definition ...autism spectrum (new DSM)
• Autism and vaccines
• Autism and mitochondria
• Discovery of autism genes
  – Genome-wide associated genes (GWAS)
  – “missing heritability”....SNPs not useful
  – re-sequencing.....rare functional variants are key
  – structural changes of chromosomes (rare CNVs)
• Temple Grandin...gifted brain functions
Autism: *a complex multi-genic disease*

- Affects ~ 2% children (newest CDC)
- Prevalence increasing worldwide over past decade
- Current costs ~$130 B/yr in USA alone
- New DSM-5 groups entire spectrum: diagnosis is ASD
- Causes deficits in:
  - Social skills & behavior
  - Language & communication skills
  - Restricted & repetitive behaviors
  - Also…cognitive impairments; seizures
- Diagnosis is strictly clinical
  - No diagnostic “tests”
- CAUSE is largely UNKNOWN
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‘Callous, unethical and dishonest’: Dr Andrew Wakefield

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
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Why is mitochondrial disease important in ASD
“Vaccine court” settlement
Polling case of ASD

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

• Child had “known pathogenic” mutation
  – mtDNA T2387C mutation: 16s rRNA gene
Mitochondrial DNA mutations cause brain diseases

- mtDNA diseases discovered in 1988 by Doug Wallace, prior UCI faculty

- Now dozens of classical diseases
  - Nuclear and mtDNA mutations
  - Mitochondrial Defects Cause EXTREMELY HETEROGENEOUS CLINICAL PRESENTATION

- Classic phenotypes all overlap
  - Seizures
  - Ataxia
  - Migraines
  - Stroke-like episodes
  - Retinal degeneration
  - Lactic acidosis / Carnitine deficiency
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

- ‘04 Autism Speaks seeded my work on this
- ‘05 National Institutes of Health began support
- ‘08 Autism Speaks seed our UCI autism center
- ‘09 National Institutes of Health initiated UCI center
Clues point to **Energy-Deficiency** as an important “candidate” to evaluate

- **Direct Energy-Deficit Diseases**
  - Mitochondrial respiratory complex defects
    - mitochondrial DNA mutations
    - nuclear chromosomal loci
  - Mitochondrial fatty acid oxidation defects
    - carnitine deficiency
    - nuclear-encoded mitochondrial enzymes (e.g., MCAD)

- **Indirect Energy-Deficit Diseases**
  - Neurotransmitter disease
    - SSADH/ 4-hydroxybutyric aciduria
  - Ion channel / Calcium signaling diseases
    - LQT8 / CACNA1C / Timothy Syndrome *autism*
    - Seizure / Ataxia / Migraine / ASD channelopathies
Empirical treatments in ASD
(not comprehensive)

- Neuropsychiatric medications...symptom control
  - Anti-depressants
  - Anti-psychotics
  - Anxiolytics
  - Anti-seizure medications

- Anti-microbial medications...gut flora

- Chelation...heavy metals

- Metabolite therapy...diet

- Digestive enzymes

- Hyperbaric oxygen

- Stem cell transplants

- Mind-body therapies

- Massage therapies

- ABA has demonstrated efficacy

WHY should these be helpful?
WHAT is the mechanism?
! Clinical trials never done
2 FDA approved drugs in ASD

- Risperidone and Aripiprazole
  - atypical antipsychotics
  - Risperidol and Abilify
  - treat irritability
  - tantrums, self-injurious behaviors

- None treat ASD’s 3 core characteristics
  - huge unmet need

- Common off-label usage
  - ADHD medications
  - SSRIs for anxiety
  - Opiate antagonists for self-injurious behaviors
Behavioral therapies are important, but are symptom-directed & not sufficient
...epidemic increase continues unabated

There is no pharmacological therapy for CORE deficits in ASD

We need new medicines for ASD

Sufficient genomic pathway information exists to begin a FUNCTION BASED approach to CORE deficits in ASD
ASD is the most highly heritable neurodevelopmental disorder

Shows a High Heritability
  • Genes involved

Inheritance pattern…complex
  • Many “at risk” (susceptibility) gene variants (mutations) work together
  • ASD is a COMPLEX MULTI-GENIC DISEASE

ALSO a SIGNIFICANT “non-gene” component
  • “Environment”
Autism: a multi-genic disease
1/88 children with deficits in language, social behavior, cognition

- Common complex multigenic diseases afflict everyone!
- Finding these genes is the medical frontier
- 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
- 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”
Genes are on human chromosomes & they come in PAIRS one from mom & dad
Parents each transmit ONLY ONE example: dominant inheritance

**Brown / blue eyes**

Vertical inheritance

Punnett square
Dominant pedigree
Our bodies GENE-ENCODED proteins form this complex web of BIOCHEMICAL metabolic pathways.

They 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”
PKU is prototype “genetic inborn error”
...cured...not with a gene....
...but by a change in environment....
newborn screening and diet
successful standard treatment 40 years

Untreated PKU
Profound disability

Treated PKU
Healthy outcome
Extended to several single gene diseases…

Life-threatening disease vs High Dose B12

Methylmalonic Acidemia

30 years successful treatments

Hemodialysis support

Normal Schoolchild

Vitamin B12-Treated
Including Recent Breakthroughs Cystic Fibrosis Mechanism and Cure
CF Breakthrough
new paradigm for personalized medicine:
genomics → cell-based screening → treatment
(NO whole-animal model used)

- Cahalan Lab UCI alum, Paul Negulescu
- Leading discoveries at Vertex
- Novel drug for cystic fibrosis

“the end of the beginning”
and.......NOVEL MEDICINE DISCOVERY for genetic diseases goes on TODAY at UCI

**Liver Disease**
- Fatty liver
- Fibrosis & Cirrhosis
- Liver failure
- Liver transplant
- Premature Death

**Intestinal Tract Disease**
- Persistent vomiting
- Diarrhea
- Severe malabsorption
- Growth failure
- Death

**Cardiovascular Disease**
- MI
- CVA
- Premature Death

**Impaired QOL**
- Reduced physical functioning
- Fatigue

**Wolmans Cholesterol Ester Storage**

4 weeks
- Normal

8 weeks
- Normalizes liver
- SBC-102 Treated
- Untreated
- LAL Deficient
Early Onset LAL Deficiency treatment
...UCI is the USA site......P.I. Gargus

Phase I/II Open-Label
- Growth failure in children due to LAL Deficiency
- Open label dose escalation
- 4 month dosing
- Multicenter
- N= 8
- Active

Open-Label Long-term Extension
- Patients from Phase I/II study
- Continuation with dose regimen from Phase I/II
- Dosing for > 6 months
- Multicenter

Early Onset Natural History Study On-going

Clinical Development

SO...new cures being developed NOW...
....for even lethal diseases...caused by single genes...
...taking advantage of genetic information on the cause.

Transgenic Chicken Lays Golden Egg
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.
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RARE form of autism
15q inverted duplication

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
Molecular chromosomal studies in ASD
Chromosome 15 duplications and deletions
5 unrelated ASD..... M.Smith et al, 2009
Mitochondria and Calcium Signaling complexes....disease targets

Ankyrin B
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

• TRANSLATE into CLINICAL INVESTIGATIONS model and drug/environment effects
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
Approach DOES work: in ASD
Treatment rare **ASD** energy-deficient syndrome
Taurine reverses MRI-documented
**Globus Pallidus lesion in SSADH**
Saronwala, Tournay, Gargus (2008)
ACMG platform presentation

- Mouse Knock-out model leads to 1st human therapy
SSADH Deficiency causes secondary energy deficiency & oxidative stress

Symptoms:
Ataxia, Hypotonia
Autism, PDD
Seizures, Dev delays
Can extend monogenic model to complex disease

- **Monogenic, Mendelian**
  - Mutation in major effect gene MAKES disease

- **Pharmacogenetic syndromes**
  - Mutation dictates response to drug
    - Seizures, heart rhythm disorders
    - Variable response to therapeutic drugs

- **Acquired / stress induced**
  - Transcriptional disease
    - Pattern of NORMAL gene expression
      - is altered by stress...seizures, heart failure

- **Complex polygenic**

- **Potent environmental toxins**
  - Worlds most potent toxins target channels
  - NO MUTATION REQUIRED
CART speeds up the drug discovery process in autism across the “valley of death” through a public-private partnership seeded by visionary entrepreneurial philanthropy.

“So, we have this paradox: we have a great opportunity to develop truly new therapeutic approaches, but are undergoing a real constriction of the pipeline. One solution is to come up with a non-traditional way of fostering drug development.”

NIH Director F. Collins

CART keeps the partnership sustainable with grants, patents, licensing, commercial partnerships, and community philanthropy.

Target Discovery  Candidate Identification  Preclinical Development  Phase I-III  Approval

The valley of death
There is a window of opportunity to discover new medicines for ASD

Neurobiology and genetics are making groundbreaking advances in ASD

There is a renewed interest of the pharmaceutical industry in various ASD

There is strong support of the NIH for translational drug discovery research
The CART proposal

The valley of death

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 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
- Framework structure of CART is now built and functional…Sept 2013
- Initial External Board Review meeting / UCI Foundation meeting…Nov 4, 2013
- 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 1/26-28
Concept of UCI CART

- Center for Autism Research and Translation (UCI CART) is a unique public-private partnership seeded by generous philanthropic support from the William & Nancy Thompson Family Foundation for Autism.
- ... to carry out a comprehensive research and translation effort to develop novel, effective diagnostics and treatments for autism.
- Our goal is to abolish current and future cases of autism through discovery, translation and implementation.
- With the escalating costs of autism care, and fewer Medicare and MediCal funds to cover them, new drug treatments are the ONLY effective approach to stem this tide.
UCI CART is distinctive from other autism research centers

- Real breakthroughs must start with research that is *directed* toward novel treatment approaches, and the CART research team is distinctively organized to do this.

- World-class research has been taking place at UC Irvine in Genomics and the Neurosciences.

- CART takes existing UCI Science that wasn’t autism-related, re-directs it and tunes it...... to immediately apply to studies key to autism.

CART has launched an innovative drug discovery effort uniting multidisciplinary campus scientists in a common purpose: to develop an effective pharmaceutical therapy for the core deficits of autism, not symptoms.
Mitochondria and Calcium Signaling complexes

Ankyrin B

ATP

Mito

"Bulk" Cytosol

ECF

S/ER

NCX

Na pump

PMCA

Agonist

Agonist

IP$_3$

IP$_3$R

SERCA

RYR
Calcium Channel INTERACTOME
1- Genomics… whole genome sequence, computers, ASD pt database w/ DNA/cells/arrays
2- Cell signaling… super-resolution STORM signaling, monogenic ASD model cell lines
3- Synapse… deconvolution microscopy, behavioral assays, monogenic murine lines
4- iPSC… rare diseases iPSC and neurons & produce genuine WGSed ASD iPSC/neurons
5- Brain clinical trials outcomes… fancy & portable EEGs, sleep study, ASD phenotyping
7- Electrophysiology & Optogenetics… fancy control of channel activity in cells & animals

PHARMA
Lynch… ampakines
Gee… "duallys"… combination 15q located GABA/α7
Piomelli… endocannabinoids

* = year 2 funding
Distinguished External Advisory Board of UCI CART

Margaret Bauman, MD
- Pediatric neurologist and research investigator who has been a pioneer in the study and treatment of autism
- Associate Professor of Neurology, Harvard Medical School
- Founding Director, LADDERS Program
- Mass General Distinguished Scholar, Lurie Family Autism Center for Children

Uta Francke, MD
- Leader in the field of model mechanisms of autism such as Rett and Prader-Willi syndromes
- Past President, American Society of Human Genetics
- Senior Medical Director, 23andMe
- Professor Emeritus of Genetics and Pediatrics, Stanford University School of Medicine
- Recipient of the Allan Award

Gregg Mashberg, Esq
- Partner and co-head of the Securities Litigation & Enforcement Group and former Chair of the Litigation Department of Proskauer, an international corporate law practice

Paul Negulescu, PhD
- Biotech expert who led recent successful cystic fibrosis drug discovery
- Vice President of Research at Vertex

Jonathan Shestack
- Co-founder, Cure Autism Now
- Member, NIH Internal Autism Coordinating Committee

Gabriel Vargas, MD, PhD
- Executive Medical Director & Neuroscience Therapeutic Area Head, Early Development at Amgen
NextGen DNA Sequencing

Sample Preparation

Sequencing

SNPs, and structural variants

Data Analysis
Sophisticated Microscopy
Mapping Neuron Spine Complexes

Map proteins and mRNAs with the CART super-resolution microscope to:

- Define novel molecular machines at the spine base.
- Determine if autism-implicated proteins participate.
- Assess whether these machines are disrupted in mouse models of ASD.
Stem Cell derived Neurons
Sophisticated measures of Brain & Behavior for clinical trials outcomes

Aim 1: Characterize Sensory deficits in responses to simple amplitude modulated sounds: (1) tones (2) noise (3) speech

Aim 2: Characterize deficits in speech processing and audio-visual integration

Horton et al., 2013 J Neurophysiology
High-density EEG

Capabilities

- 128 EEG electrodes
- Saline interface (no gel or prep)
- 10-15 minutes preparation time
- Adult and children (down to about 4 years)
- Resting state + sensory responses.
Electrophysiology & Optogenetics

Drug development

Electrophysiology

Optogenetics

Behavior
Deficit in Regulation
Endocannabinoids
2-AG, Anandamide
Endocannabinoid System as a Therapeutic Target for Autism
Deficit in Regulation
Endocannabinoids
2-AG, Anandamide

Pharma lead compounds
Targeting $\alpha_7$ nACh and $GABA_A$ receptors simultaneously ASD

Endocannabinoid System as a Therapeutic Target for Autism
Deficit in Regulation
Endocannabinoids
2-AG, Anandamide

Ampakine
Rescue Synaptic Plasticity In ASD
Functional **infrastructure is in place** to drive discoveries for Autism

but there is a gap between the basic science research entrepreneurial philanthropy launched

and the final stages of drug research

and this would be an important gap community support could help close
Three year goals for CART

• Achieve **recognition** placing CART among leading USA ASD translational research centers
  • Largely happening ALREADY !

• Negotiate major **corporate partnership** leveraged from CART Discovery Platform
  • Promising leads should allow by 2015

• Launch one **novel compound clinical trial**
  • Before 2017