Coupling sequencing and functional studies in neonates

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Duke University Medical Center
Why babies (apart from the obvious!)

1. Birth defects: 21% of infant deaths
2. Mortality rates plateaued
3. 5-10% of repeat admissions
Why (part II)

1. Early diagnosis/prognosis
2. High-value information content (families)
3. Focused clinical investment
4. Maximum therapeutic window
1. Communication across disciplines

(See Genesis 11:1-9)

Unfortunately, the Babel Roof Crew was unaware that "Send the ladders" now meant "you goat sandal" to the ladder crew.
2. Information management
3. Ethical transmission of information

What i’m about to tell you is gonna change your life forever. Are you really sure you want to know it?
A bold new activity

Duke University Task Force for Neonatal Genomics
synthesis of disciplines around patient needs

clinics

patient

genomics

ethics

function
The process

1. Trio-based recruitment
2. WES/WGS
3. Data filtering
4. Systematic functionalization of ALL candidate alleles
Heavily reliant on inferred data and predictions...

Cartoon:

1. Use the CRS database to size the market.
2. That data is wrong.
3. Then use the SIBS database.
4. That data is also wrong.
5. Can you average them?
6. Sure. I can multiply them too.
Interpretation
**In vivo models in human disease modeling**

**Neuroanatomical**


**Craniofacial anomalies**


**Vascular integrity**


**Cardiac malformations**

**Muscular dystrophy**

In vivo functional testing of missense variants

Loss of function

Dominant negative

Gain of function

Morpholino (MO) injection into 1-4 cell stage zebrafish embryos

Phenotype (1-7 days post-fertilization)

Functional null

Hypomorph

Benign

Dominant negative

Gain of function
to date:
~350 genes modeled
~1,200 human non-synonymous variants

98% sensitivity
84% specificity

suite of models for testing
collapse into recurrent pathways
tools for lead compound discovery
Vignette 1: An opportunity to learn new biology – SCN2A and congenital seizures

**Clinical features**
- intractable seizures
- severe developmental delay
- irritability
- cortical visual impairment
- gastroesophageal reflux

**Clinical testing**
- normal female karyotype
- normal female microarray
- metabolic testing – negative
- genetic testing – negative

**Referred for research studies**
Marie McDonald (Med Genet)

**Consented for research studies**
proband age 21 months
The candidacy of *SCN2A* p.S229P

**Arguments for causality:**
- Novel, *de novo*, protein-altering variant
- *SCN2A* is a known epilepsy gene

**Arguments against causality:**
- Patient phenotype more severe than most other documented *SCN2A* cases
SCN2A S229P: gain-of-function

H. sapiens SCN2A
chr2:166,152,283-166,248,820

p.S229P

209D
Fetal
209N
Adult

Haidun Yan
Geoff Pitt

hyper polarizing shift of voltage-dependent activation
Vignette 2: Surprising diagnoses

Clinical features
severe developmental delay
hypotonia
optic nerve disorder/nystagmus
auditory neuropathy spectrum disorder
demyelination on brain MRI

Clinical testing
normal female microarray
metabolic testing – negative
extensive genetic testing – negative
deafness panel
mitochondrial disease panel
PLP1 and GJC2 sequencing

IKBKAP: Elongator protein complex 1. Causes familial dysautonomia

MGAM: Maltase-glucoamylase

RGS22: Regulator of G-protein signaling
A variant form of familial dysautonomia

Disorganization of vascular networks rescuable by WT human mRNA
Vignette 3: Gene identification when genetic resolution reaches an impasse

Clinical features
Global developmental delay
microcephaly
feeding issues
failure to thrive
abnormal muscle tone
low immunoglobulins
frequent respiratory infections

Clinical testing
normal female microarray
metabolic testing – negative
extensive genetic testing – negative

**BTG2:** Involved in the G1/S transition of the cell cycle

**NOS2:** Nitric oxide synthase 2, inducible

**TTN:** Titin
**BTG2** is responsible for the microcephaly phenotype

**BTG2**: de novo

**NOS2**: de novo
Vignette 4: The case for unbiased analysis of exomes
In vivo testing of epistasis

Chan et al, N Eng J Med in press
Acknowledgements

**Duke Center for Human Disease Modeling:**
- Erica Davis
- Jason Willer
- Natalie Mola
- Azita Sadeghpour
- Christelle Golzio
- Christine Oien
- Dustin Dowless
- Jeremiah Savage
- Nicholas Katsanis

**Duke Neonatal Perinatal Research Institute:**
- C. Michael Cotten
- Kimberley Fisher
- Rebecca Jones
- Cynthia Ross
- Laura Stern
- Mandy Marion
- Margarita Bidegain
- Ronald Goldberg

**Duke Fetal Diagnostic Center:**
- Kristin Weaver
- Amanda French
- Brita Boyd
- Amy Murtha

**Duke Pediatric Specialty Clinics:**
- Medical Genetics
  - Katie Sheets
  - Courtney Downtain
  - Marie McDonald

- Blood and Marrow Transplant:
  - Jessica Sun
  - Kristin Page
  - Rebecca Lewis
  - Sloan Kojis
  - June Allison Thacker
  - Joanne Kurtzbarg

- Urology/Nephrology:
  - Jennifer Stout
  - Rasheed Gbadegesin
  - John Wiener

- Neurology:
  - William Gallentine

- Cardiology:
  - Jennifer Li

**Duke Clinical Laboratory:**
- Erica Owens
- Catherine Rehder

**Baylor HGSC:**
- Aniko Sabo
- Shannon-Dugan Rocha
- Donna Muzny
- Richard Gibbs

**Duke Center for Human Genetics:**
- Kristin McDonald
- Heidi Cope
- Allison Ashley-Koch
- Michael Hauser
- Elizabeth Hauser

**Duke Institute for Genome Sciences & Policy:**
- Sara Katsanis
- Joyce Kim
- Misha Angrist

**Duke Ion Channel Group:**
- Haidun Yan
- Geoffrey Pitt

**U Lausanne:**
- Alex Raymond
- Jacqui Beckmann

**Mass General/Harvard:**
- Susan Slaugenhapt

**Funding:** NIH-NIDDK P50 DK096415

[www.dukegenes.org]
Can also model copy number variants
16p11.2 CNV: Complex Mirrored Neurodevelopmental Phenotypes

- AUTISM
- HYPERPHAGIA
- OBESITY
- MACROCEPHALY

... deletion
... normal dose
... duplication

SCHIZOPHRENIA AUTISM
ANOREXIA UNDERWEIGHT MICROCEPHALY
Strategy to Identify Which Gene(s) Cause(s) the Complex Mirror Phenotype at the 16p11.2 Locus

Overexpression human mRNAs

- 29 genes in the CNV
- 26 Zebrafish orthologs

Microinjection mRNAs into Zebrafish embryos 1- to 2-cell stage

Screening for microcephaly at 5 dpf

Candidate gene(s) identified

Identification of the causal gene(s)

Screening for reciprocal phenotype: macrocephaly

Microinjection morpholinos into Zebrafish embryos 1- to 2-cell stage

Design Morpholino(s) against Zebrafish ortholog(s)
KCTD13+CDIPT Overexpression leads to Microcephaly

Golzio et al, Nature 2012
KCTD13 Dosage Changes lead to Head Size Defects

Microcephaly

Golzio et al, Nature 2012
Complex Mirror Phenotypes at the 16p11.2 CNV

Recurrent 16p11.2 microdeletions in autism

Ravinesh A. Kumar1, Samer KaraMohamed1, Jyotsna Sudi1, Donald F. Conrad1, Camille Brune5, Judith A. Badner4, T. Conrad Gilliam1, Norma J. Nowak6, Edwin H. Cook Jr5, William B. Dobyns1,2,3 and Susan L. Christian1,7

1Department of Human Genetics, 2Department of Neurology, 3Department of Pediatrics and 4Department of Psychiatry, University of Chicago, Chicago, IL 60637, USA, 5Department of Psychiatry, University of Illinois at Chicago, Chicago, IL 60612, USA and 6Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY 14236, USA

Microduplications of 16p11.2 are associated with schizophrenia

Shane E McCarthy1,4, Vladimir Makarov1, George Kirov2, Anjene M Addington3, Jon McClellan4, Seungtae Yoon1, Diana O Perkins5, Diane E Dicke6, Mary Kusenda1,7, Olga Krasoshevskaia8, Verena Krause8, Ravinesh A Kumar9, Detelina Grozeva2, Dheeraj Malhotra1, Tom Walsh8, Elaine H Zuckai10, Paege Kaplan11, Jaya Ganes11, Ian D Krantz10, Nancy B Spinner10, Patricia Rocanova1, Abhishek Charadari1, Kevin Pavon1, B Lakshmi1,12, Anthony Loetta1, Jude Kendall1, Yoon-ha Loe1, Vladimir Vacic1, Sydney Gary1, Lilia M Iakovacheva13, Timothy J Crow14, Susan L Christian15, Jeffrey A Lieberman15,16, T Scott Stroup15, Terho Lehtimaki17, Kaija Puura18, Chad Haldeman-Engel11, Justin Pearl11, Meredith Goodell20, Virginia L Willour20, Pamela DeRosa21, Jo Steele19, Layla Kassem19, Jessica Wolff19, Nisha Chitkara19, Francis J McMahon19, Anil K Malhotra21, James B Potash20, Thomas G Schulze19,22, Markus M Nothen23,24, Sven Cichon23,24, Marcella Rietschel22,25, Ellen Leibenluft26, Vlad Kustanovich27, Clara M Lajonchere27, James S Sutcliffe28, David Skuse29, Michael Gill30, Louise Gallagher30, Nancy R Mendell11, Welcome Trust Case Control Consortium31, Nick Craddock2, Michael J Owen1, Michael C O’Donovan2, Tamim H Shaiikh19, Ezra Susser15, Lynn E DeLisi31,34, Patrick F Sullivan35, Curtis K Deutsch33,36, Judith Rapoport1, Deborah L Levy3,8,33, Mary-Claire King6 and Jonathan Sebat1

Crespi et al. (2010) PNAS 107:1736-1741
Etiology: Gene(s) Within the 16p11.2 CNV but Which One(s)?

<table>
<thead>
<tr>
<th>Gene name</th>
<th>CDS start</th>
<th>CDS end</th>
<th>Strand</th>
<th>Change in Expr</th>
<th>Protein function</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOLA2</td>
<td>29365833</td>
<td>29373786</td>
<td>-</td>
<td>0.6 (0.8)</td>
<td>Possibly involved in cell proliferation or cell-cycle regulation</td>
</tr>
<tr>
<td>BOLA2B</td>
<td>30111739</td>
<td>30112815</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GIYD1</td>
<td>29373376</td>
<td>29377041</td>
<td>+</td>
<td>1.0 (1.5)</td>
<td>GIY-YIG domain containing</td>
</tr>
<tr>
<td>GIYD2</td>
<td>30112906</td>
<td>30116288</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SULT1A4</td>
<td>29373902</td>
<td>29383801</td>
<td>+</td>
<td>1.1 (1.1)</td>
<td>Induced in response to fasting or as a result of a defect in leptin signalling</td>
</tr>
<tr>
<td>SULT1A3</td>
<td>30119550</td>
<td>30122742</td>
<td>+</td>
<td></td>
<td>Catalyzes the sulfate conjugation of phenolic monoamine neurotransmitters</td>
</tr>
<tr>
<td>SPN</td>
<td>29582550</td>
<td>29583753</td>
<td>+</td>
<td>1.2 (1.3)</td>
<td>Sialophorin, CD43, Activator of JNK1 and MAPK3 signalling</td>
</tr>
<tr>
<td>QPRT</td>
<td>29598019</td>
<td>29616233</td>
<td>+</td>
<td></td>
<td>Catabolism of quinolinic acid, a neural excitotoxin and NMDA receptor agonist</td>
</tr>
<tr>
<td>C16orf54</td>
<td>29663098</td>
<td>29663773</td>
<td>-</td>
<td>0.5 (0.8)</td>
<td></td>
</tr>
<tr>
<td>MAZ</td>
<td>29725523</td>
<td>29728564</td>
<td>+</td>
<td>0.7 (0.8)</td>
<td>Interacts with SPI in regulating transcription of serotonin receptor gene HTRIA</td>
</tr>
<tr>
<td>PRRT2</td>
<td>29731876</td>
<td>29733460</td>
<td>+</td>
<td></td>
<td>Proline-rich transmembrane protein</td>
</tr>
<tr>
<td>C16orf53</td>
<td>29733347</td>
<td>29738576</td>
<td>+</td>
<td>0.8 (0.8)</td>
<td></td>
</tr>
<tr>
<td>MVP</td>
<td>29749371</td>
<td>29766811</td>
<td>+</td>
<td>0.5 (0.8)</td>
<td>Regulates cytoplasmic localisation of PTEN</td>
</tr>
<tr>
<td>CDIPT</td>
<td>29778010</td>
<td>29781679</td>
<td>-</td>
<td>0.4 (0.5)</td>
<td>Phosphatidylinositol synthesis</td>
</tr>
<tr>
<td>SEZ6L2</td>
<td>29790520</td>
<td>29817841</td>
<td>-</td>
<td>0.9 (0.9)</td>
<td>Seizure-related. May contribute to specialized ER function in neurons</td>
</tr>
<tr>
<td>ASPHD1</td>
<td>29819793</td>
<td>29824719</td>
<td>+</td>
<td>1.0 (1.1)</td>
<td>Aspartate beta-hydroxylase domain containing</td>
</tr>
<tr>
<td>KCTD13</td>
<td>29825693</td>
<td>29844855</td>
<td>-</td>
<td>0.6 (0.6)</td>
<td>Similar to TNFAIP1, a mediator of insulin resistance in rodent obesity models</td>
</tr>
<tr>
<td>TMEM219</td>
<td>29881965</td>
<td>29890367</td>
<td>+</td>
<td>0.6 (0.7)</td>
<td>Transmembrane protein</td>
</tr>
<tr>
<td>TAOK2</td>
<td>29896594</td>
<td>29906802</td>
<td>+</td>
<td>0.8 (0.8)</td>
<td>Activates JNK1 and MAPK3 pathways via the upstream MKK3 and MKK6 kinases</td>
</tr>
<tr>
<td>HIRIP3</td>
<td>29912028</td>
<td>29914427</td>
<td>-</td>
<td>0.6 (0.5)</td>
<td>Possibly functions in some aspects of chromatin and histone metabolism</td>
</tr>
<tr>
<td>INO80E</td>
<td>29915132</td>
<td>29924264</td>
<td>+</td>
<td>0.5 (0.5)</td>
<td>INO80 complex subunit F</td>
</tr>
<tr>
<td>DOC2A</td>
<td>29925007</td>
<td>29929044</td>
<td>-</td>
<td>1.0 (1.0)</td>
<td>Possibly involved in Ca^{2+}-dependent neurotransmitter release</td>
</tr>
<tr>
<td>C16orf92</td>
<td>29942176</td>
<td>29943049</td>
<td>+</td>
<td>1.1 (1.1)</td>
<td></td>
</tr>
<tr>
<td>FAM57B</td>
<td>29944004</td>
<td>29949349</td>
<td>-</td>
<td>0.9 (0.8)</td>
<td></td>
</tr>
<tr>
<td>ALDOA</td>
<td>29986076</td>
<td>29989034</td>
<td>+</td>
<td>0.5 (0.6)</td>
<td>Fructose-bisphosphate aldolase A</td>
</tr>
<tr>
<td>PPP4C</td>
<td>29995199</td>
<td>30003884</td>
<td>+</td>
<td>0.7 (0.8)</td>
<td>Regulates JNK1 signalling</td>
</tr>
<tr>
<td>TBX6</td>
<td>30005046</td>
<td>30010015</td>
<td>-</td>
<td>1.0 (1.0)</td>
<td>Transcription factor involved in regulation of early developmental processes</td>
</tr>
<tr>
<td>YPEL3</td>
<td>30011531</td>
<td>30014190</td>
<td>-</td>
<td>0.6 (0.6)</td>
<td>Possibly involved in proliferation and apoptosis in myeloid precursor cells</td>
</tr>
<tr>
<td>GDPD3</td>
<td>30023693</td>
<td>30032300</td>
<td>-</td>
<td>0.8 (0.9)</td>
<td>Glycerophosphodiesterase domain</td>
</tr>
<tr>
<td>MAPK3</td>
<td>30035658</td>
<td>30042031</td>
<td>-</td>
<td>0.7 (0.7)</td>
<td>ERK1. Multiple roles in proliferation and differentiation of preadipocytes</td>
</tr>
<tr>
<td>CORO1A</td>
<td>30104031</td>
<td>30107786</td>
<td>+</td>
<td>0.3 (0.5)</td>
<td>Coronin. Actin binding protein</td>
</tr>
</tbody>
</table>
Strategy to Identify Which Gene(s) Cause(s) the Complex Mirror Phenotype at the 16p11.2 Locus

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Golizio et al, Nature 2012
KCTD13 Dosage Changes lead to Head Size Defects

Golzio et al, Nature 2012
**KCTD13** Dosage Changes lead to Proliferation and Apoptosis Defects at 2-3 dpf

C

<table>
<thead>
<tr>
<th>kctd13 MO</th>
<th>Control</th>
<th>KCTD13 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histone H3</td>
<td><img src="histone_h3.png" alt="Image" /></td>
<td><img src="histone_h3.png" alt="Image" /></td>
</tr>
<tr>
<td>TUNEL</td>
<td><img src="tunel.png" alt="Image" /></td>
<td><img src="tunel.png" alt="Image" /></td>
</tr>
</tbody>
</table>

D

![Graph](graph.png)

Golzio *et al*, Nature 2012
De Novo deletion of exon 4 of *KCTD13* in a Patient with Autism

Golzio et al., Nature 2012
Summary

✓ Accelerated molecular diagnosis, early diagnosis

✓ Complementation of WES data with functional assays aid interpretation significantly

✓ Identify genetic phenomena such as synergistic effects, modifiers in trans, etc

✓ can be used to model CNVs with anatomical surrogates