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Zilkha Neurogenetic Institute

Addressing Heterogeneity of the Autisms – The Brain-Gut Connection

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Topics to Frame the Talk

- **Utility of genetic risk for informing pathogenesis or pathophysiology?**
- **Basic and clinical studies inform each other for understanding disorder heterogeneity?**

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Kanner's/Asperger's Views



- **Emphasis on the social problems**
- **Emphasis on the bizarre language & interests**
- **Emphasis on defective common sense**
- **Emphasis on the uneven cognitive skills, especially superior memory and remarkable ability in limited domains**
- **Neither clear on causation, both inclined in the direction of 'innate' problem**

The Autisms – Heritability of Risk ~.9

“The eleven children (eight boys and three girls) whose histories have been briefly presented offer, as is to be expected, individual differences in the degree of their disturbance, the manifestation of specific features, the family constellation, and the step-by-step development in the course of years.” ----- Kanner, *Nerv. Child* 2:217-250 (1943)

Variety of Co-Occurring Medical Conditions

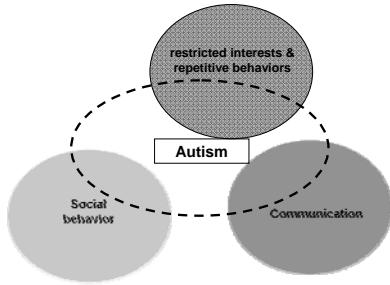
Table 1 Domains of impairment in autistic spectrum disorder (ASD)^a

Domain	Autism	Asperger	PDD-NOS	ASD
social communication	required	required	required	
language	required	-	variable	
repetitive, restrictive behaviors	required	required	variable	
sensory abnormalities	>90%	80%	variable	94%
developmental regression ^b	15%–40%	?	?	15%–40%
motor signs ^c	60%–80%	60%	60%	60%–80%
gross motor delay	10%	?	?	5%–10%
sleep disturbance	55%	5%–10%	40%	50%
gastrointestinal disturbance ^d	45%	4%	50%	4%–50%
epilepsy ^e	10%–60%	0%–5%	5–40%	6%–60%
comorbid psychiatric diagnosis ^f	70%	60%	>25%	25–70%

Geschwind, *Ann Rev Med* (2009)

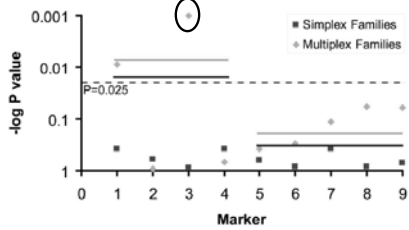
How will we sort through the well-recognized disorder heterogeneity to implement ‘individualized’ best practices?

Strategies for Addressing the Heterogeneity of the Autisms



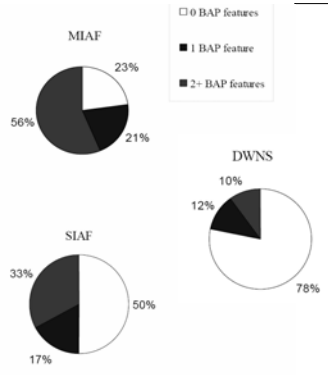
What is the genetic contribution to disorder heterogeneity?

MET – A Common Variant – Distinguishes Risk in Families With More Than One Child With ASD



Campbell et al, PNAS 103:16834-39 (2006)

Multiplex-Simplex Distinctions with Broader Phenotypes



Losh et al Am J Med Gen (2008)

Can We Use Genetic Risk, Combined With Functional Measures That Will Help Stratify Subgroups?

One approach to sort through complexity - test associations of a second variable (i.e. genetic variation) with individual phenotype scores (ADI-R, ADOS) and quantitative trait measures (SRS), or other phenotypic measures (e.g. language, mental health, medical)

Two Examples

•**SLC6A4** – **ASD & Obsessive Compulsive Disorder** (Mulder et al, 2005; Sutcliffe et al, 2005)

•**CNTNAP2** – **ASD & Language Delay** (Alcarcon et al, 2008)

AGRE Families - Stratification Analyses of *MET*

TABLE II. Description of Phenotypes Available for the AGRE Sample

	All families genotyped	Families with co-occurring GI	Families without co-occurring GI
Number of families	367	118	96
Number of individuals phenotyped			
Social Responsiveness Scale [SRS]—Teacher Report	294	108	71
Social Responsiveness Scale [SRS]—Parent Report	363	123	101
Autism Diagnostic Interview—Revised [ADI-R]	742	242	181
Autism Diagnostic Observation Schedule [ADOS]	528	220	160

Campbell et al, Am J Med Gen B 153 (2010)

MET Risk Variant – Associated with all SRS Subscales

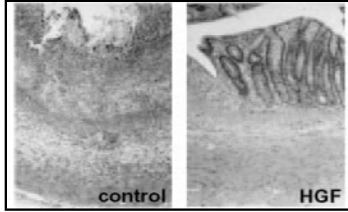
TABLE III. Association of *MET* rs1858830 C Allele With SRS Teacher Report Phenotypes (P-Values)

Trait	All families genotyped	
	FBAT additive model	FBAT dominant model
SRS total	0.033	0.001
Social awareness	0.036	0.001
Social cognition	0.032	0.002
Social communication	0.034	0.001
Social motivation	0.029	0.001
Autistic mannerisms	0.030	0.001

P-values <0.05 are shown in bold.

Campbell et al, Am J Med Gen B 153 (2010)

In Experimental Models, MET Can Help Repair the Damaged Gastrointestinal Tract

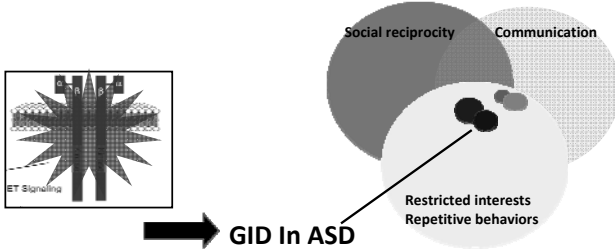


Tahara et al. J Pharm Exp Ther 2003

In many neurodevelopmental disorders, increased (or reduced) risk for other medical conditions is common – because each gene that impacts risk also happens to be important in more than one developmental process.



Working Hypothesis: Disrupting MET Function Contributes to the Disorder Pathogenesis Uniquely in Children with ASD & GID



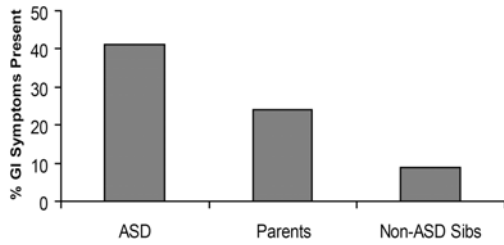
MET — ASD — GID

Tummy Troubles



Phil Gorrindo, MD/PhD student
Levitt Lab

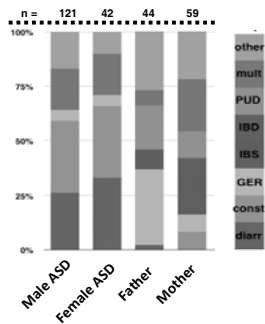
Presence of GI symptoms in AGRE sample –
Parent reports available on 214 families



Chi-Square P=0.0000000000015

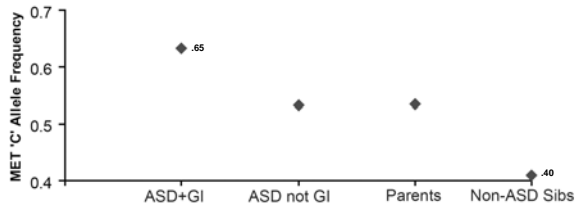
Campbell et al, Pediatrics 123 2009

Majority of Individuals With ASDs In the Report
Have Constipation and Diarrhea



After Campbell et al, Pediatrics 123, 2009

METrisk variant is further enriched in co-occurring ASD + GI in AGRE Families with discordant ASD and GI



Campbell et al, Pediatrics123 (2009)

In fact, ASD + GI is driving the signal in association with ASD phenotypes using SRS, ADI-R, ADOS

TABLE III. Association of MET rs185830 C Allele With SRS Teacher Report Phenotypes (P-Values)

Trait	All families genotyped		Families with co-occurring GI		Families without co-occurring GI	
	FBAT additive model	FBAT dominant model	FBAT additive model	FBAT dominant model	FBAT additive model	FBAT dominant model
SRS total	0.033	0.001	0.003	0.007	0.279	0.611
Social awareness	0.036	0.001	0.050	0.005	0.511	0.612
Social cognition	0.032	0.002	0.054	0.008	0.549	0.660
Social communication	0.034	0.001	0.061	0.007	0.636	0.693
Social motivation	0.029	0.001	0.096	0.007	0.606	0.670
Autistic mannerisms	0.030	0.001	0.056	0.007	0.562	0.613

P-values < 0.05 are shown in bold.

TABLE VI. Association of the MET rs185830 C Allele With Phenotypes on the ADOS (P-Values)

Variable	Trait description	All genotyped families		Families with co-occurring GI		Families without co-occurring GI	
		FBAT additive model	FBAT dominant model	FBAT additive model	FBAT dominant model	FBAT additive model	FBAT dominant model
CSACommunication	Communication Autism Cutoff	0.159	0.008	0.038	0.029	0.460	0.288
CSACommunication	Communication Autism Spectrum Cutoff	0.048	0.001	0.032	0.010	0.332	0.199
CSA Social	Social Autism Cutoff	0.145	0.002	0.073	0.011	0.455	0.187
CSASocial	Social Autism Spectrum Cutoff	0.095	0.001	0.034	0.009	0.598	0.217
CSACom+Social	Communication + Social Autism Cutoff	0.096	0.005	0.058	0.018	0.333	0.216
CSACom+Social	Communication + Social Autism Spectrum Cutoff	0.113	0.002	0.026	0.019	0.555	0.273

P-values < 0.05 are shown in bold.

Campbell et al, Am J Med Gen B 153 (2010)

Genetics, Metabolomics and Microbiomics – Children with ASD and Co-Occurring GID are Biologically Distinct

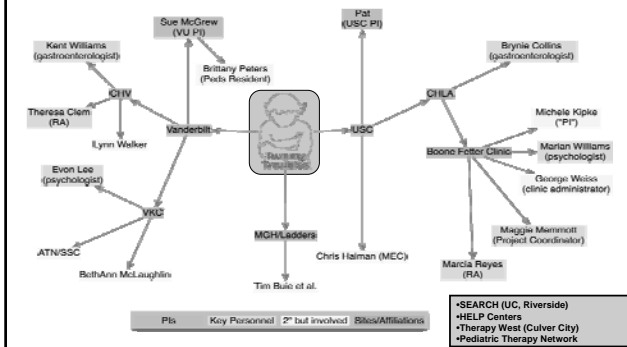
J. Nicholson and colleagues – Kock et al J. Proteom Res (2010)
 A. McCartney and colleagues - Parracho et al J. Med. Microbiol (2005)

Tummy Troubles: The Logical Next Study



- prospectively collected
- rich characterizations by pediatric gastroenterologists
- a standardized instrument for functional GI disorders
- in the context of nutritional status
- combined with biomarker analyses

The Tummy Troubles *Dream Team*

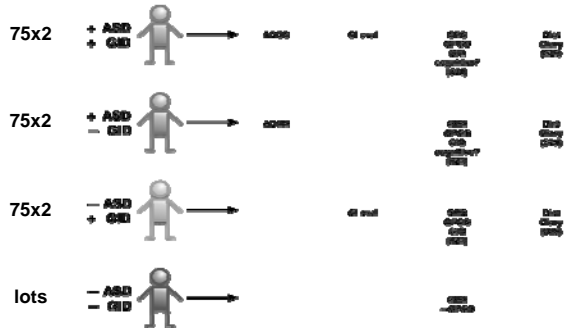


The Two Aims of Tummy Troubles

•To richly characterize the GID present in children with ASD – PROSPECTIVELY!!!

•To explore association of altered *MET* signaling system abnormalities with ASD and GID

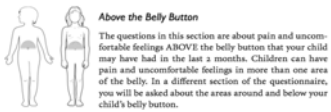
Aim 1: To richly characterize the GID present in children with ASDs



SRS

- 1 - NOT TRUE 2 - SOMETIMES TRUE 3 - OFTEN TRUE 4 - ALMOST ALWAYS TRUE
1. Seems much more fidgety in social situations than when alone. 1 2 3 4
 2. Expressions on his or her face don't match what he or she is saying. 1 2 3 4
 3. Seems self-confident when interacting with others. 1 2 3 4
 4. When under stress, he or she shows rigid or inflexible patterns of behavior that seem odd. 1 2 3 4
 5. Doesn't recognize when others are trying to take advantage of him or her. 1 2 3 4
 6. Would rather be alone than with others. 1 2 3 4
 7. Is aware of what others are thinking or feeling. 1 2 3 4
 8. Behaves in ways that seem strange or bizarre. 1 2 3 4
 9. Clings to adults, seems too dependent on them. 1 2 3 4
 10. Takes things too literally and doesn't get the real meaning of a conversation. 1 2 3 4
 11. Has good self-confidence. 1 2 3 4
 12. Is able to communicate his or her feelings to others. 1 2 3 4
 13. Is awkward in turn-taking interactions with peers (e.g., doesn't seem to understand the give-and-take of conversations). 1 2 3 4
 14. Is not well coordinated. 1 2 3 4

QPGS



1. In the last 2 months, how often did your child have pain or an uncomfortable feeling in the upper abdomen above the belly button?
 - 0 ... Never
 - 1 ... 1 to 3 times a month
 - 2 ... Once a week
 - 3 ... Several times a week
 - 4 ... Every day
- If your child has not had ANY pain or uncomfortable feelings above the belly button in the past 2 months, please go to Section B.*
2. Which of the following feelings did your child have above the belly button? (You may check one or more than one.)

a. Pain	<input type="checkbox"/> No	<input type="checkbox"/> Yes
b. Nausea	<input type="checkbox"/> No	<input type="checkbox"/> Yes
c. Bloating	<input type="checkbox"/> No	<input type="checkbox"/> Yes
d. Feeling of fullness	<input type="checkbox"/> No	<input type="checkbox"/> Yes
e. Not being hungry after eating very little	<input type="checkbox"/> No	<input type="checkbox"/> Yes

QPGS

Circle a number for your answer to each question below.	0% of the time	25% of the time	50% of the time	75% of the time	100% of the time	I don't know
In the last 2 months, how often	Never	Once in a while	Sometimes	Most of the time	Always	(check box)
4. Did your child have to rush to the bathroom to poop?	0	1	2	3	4	<input type="checkbox"/>
5. Did your child have to strain (push hard) to make a poop come out?	0	1	2	3	4	<input type="checkbox"/>
6. Did your child pass mucus or phlegm (white, yellowish, stringy, or slimy material) during a poop?	0	1	2	3	4	<input type="checkbox"/>
7. Did your child have a feeling of not being finished after a poop (like there was more that wouldn't come out)?	0	1	2	3	4	<input type="checkbox"/>

QPGS: FGID diagnoses

- functional dyspepsia
- irritable bowel syndrome
- abdominal migraine
- functional abdominal pain
- functional abdominal pain syndrome
- functional constipation
- nonretentive fecal incontinence
- aerophagia
- cyclic vomiting syndrome
- adolescent rumination syndrome
- operational definition of diarrhea (you know it when you see it)

GIQ

- based on ATN
- includes questions regarding:
 - special diets
 - food selectivity
 - food allergies
 - breastfeeding
 - medications

Progress.....

- Complete recruitment in Nashville by December, 2010
- Analyze full complement of GI biopsies from MGH site
- Emphasize recruiting in Los Angeles for completion by Spring, 2011
- Apply for NIH funding to expand Tummy Troubles Study
- DoD grant to explore treatments

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Research Informs The Way That We Intervene



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