PGC Worldwide Lab Call Details

DATE: Friday, December 12th, 2014

PRESENTER: Robert Yolken, M.D.
Stanley Division of Developmental Neurovirology, The Johns Hopkins University School of Medicine

TITLE: "Gene-Environmental Interactions in Human Psychiatric Disorders and Cognition"

START: We will begin promptly on the hour.
1000 EST - US East Coast
0700 PST - US West Coast
1500 GMT - UK
1600 CET - Central Europe
0200 AEDT – Australia (Saturday, December 13th, 2014)

DURATION: 1 hour

TELEPHONE:
- US Toll free: 1 866 515.2912
- International direct: +1 617 399.5126
- Toll-free number? See http://www.btconferencing.com/globalaccess/?bid=75_public
- Operators will be on standby to assist with technical issues. "*0" will get you assistance.
- This conference line can handle up to 300 participants.

PASSCODE: 188 641 29 (then #)

Lines are Muted **NOW**

Lines have been automatically muted by operators as it is possible for just one person to ruin the call for everyone due to background noise, electronic feedback, crying children, wind, typing, etc.

Operators announce callers one at a time during question and answer sessions.

Dial *1 if you would like to ask a question of the presenter. Presenter will respond to calls as time allows.

Dial *0 if you need operator assistance at any time during the duration of the call.

UPCOMING PGC Worldwide Lab

DATE: Friday, January 9th, 2015
PRESENTER: Patrick F. Sullivan, MD, FRANZCP
TITLE: **“**Planning the PGC3 NIMH Grant”
START: We will begin promptly on the hour.
1000 EST - US East Coast
0700 PST - US West Coast
1500 GMT - UK
1600 CET - Central Europe
0200 AEDT – Australia (Saturday, January 10th, 2015)
DURATION: 1 hour

TELEPHONE:
- US Toll free: 1 866 515.2912
- International direct: +1 617 399.5126
- Toll-free number? See http://www.btconferencing.com/globalaccess/?bid=75_public
- Operators will be on standby to assist with technical issues. “*0” will get you assistance.
- This conference line can handle up to 300 participants.

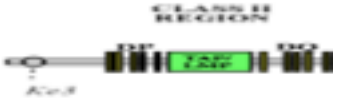
PASSCODE: 188 641 29 (then #)

Gene-Environmental Interactions in Human Psychiatric Disorders and Cognition-Outline

- Introduction to Inflammation and Psychiatric Disorders
- Description of the Sheppard Pratt Study Population
- C Reactive Protein and the Polygene Score (Pilot)
- Metagenomic Analysis of the Microbiome
- Characterization of Novel Agents Associated with Cognition
- Role of Defined Microbial Agents in Psychiatric Disorders and Cognition (Toxoplasma, Herpesviruses)
- Multi-analyte analyses of inflammation
- Clinical Trials

Inflammation and Schizophrenia-Converging Evidence

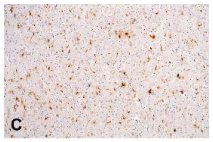
Genetic



Genome-Wide Association Study Implicates HLA-C*01:02 a Risk Factor at the Major Histocompatibility Complex Locus i Schizophrenia

Irish Schizophrenia Genomics Consortium and the Wellcome Trust Case Control Consortium 2

Neuropathologic

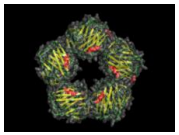


Molecular Psychiatry (2013) **18,** 133; doi:10.1038/mp.2012.199

Markers of inflammation in the prefrontal cortex of individuals with schizophrenia

S G Fillman[1,2,3], N Cloonan[4], L C Miller[5] and C S Weickert[1,2,3]

Systemic

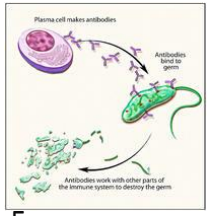


Schizophr Res. 2013 Jan;143(1):198-202. doi: 10.1016/j.schres.2012.10.041. Epub 2012 Dec 4

C-reactive protein is elevated in schizophrenia.

Dickerson F, Stallings C, Origoni A, Vaughan C, Khushalani S, Yang S, Yolken R.

Pharmacologic



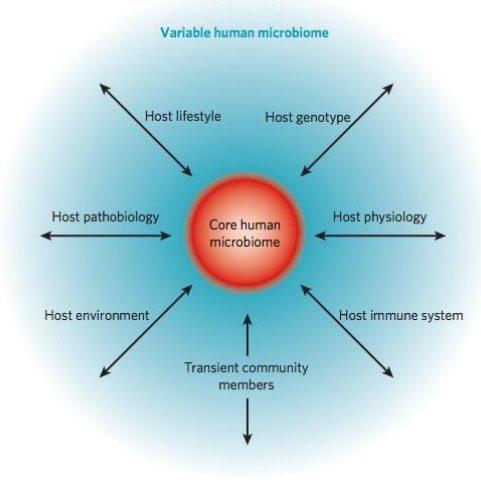
The Lancet Psychiatry, Early Online Publication, 10 December 2014
doi:10.1016/S2215-0366(14)00122-9 Cite or Link Using DOI

Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment

Dr Golam M Khandaker PhD [a b c †], Lesley Cousins DPhil [a b c †], Julia Deakin PhD [a b c], Belinda R Lennox DM [d], Prof Robert Yolken PhD [e], Prof Peter B Jones PhD [a b c]

Why study the Microbiome in Serious Psychiatric Disorders

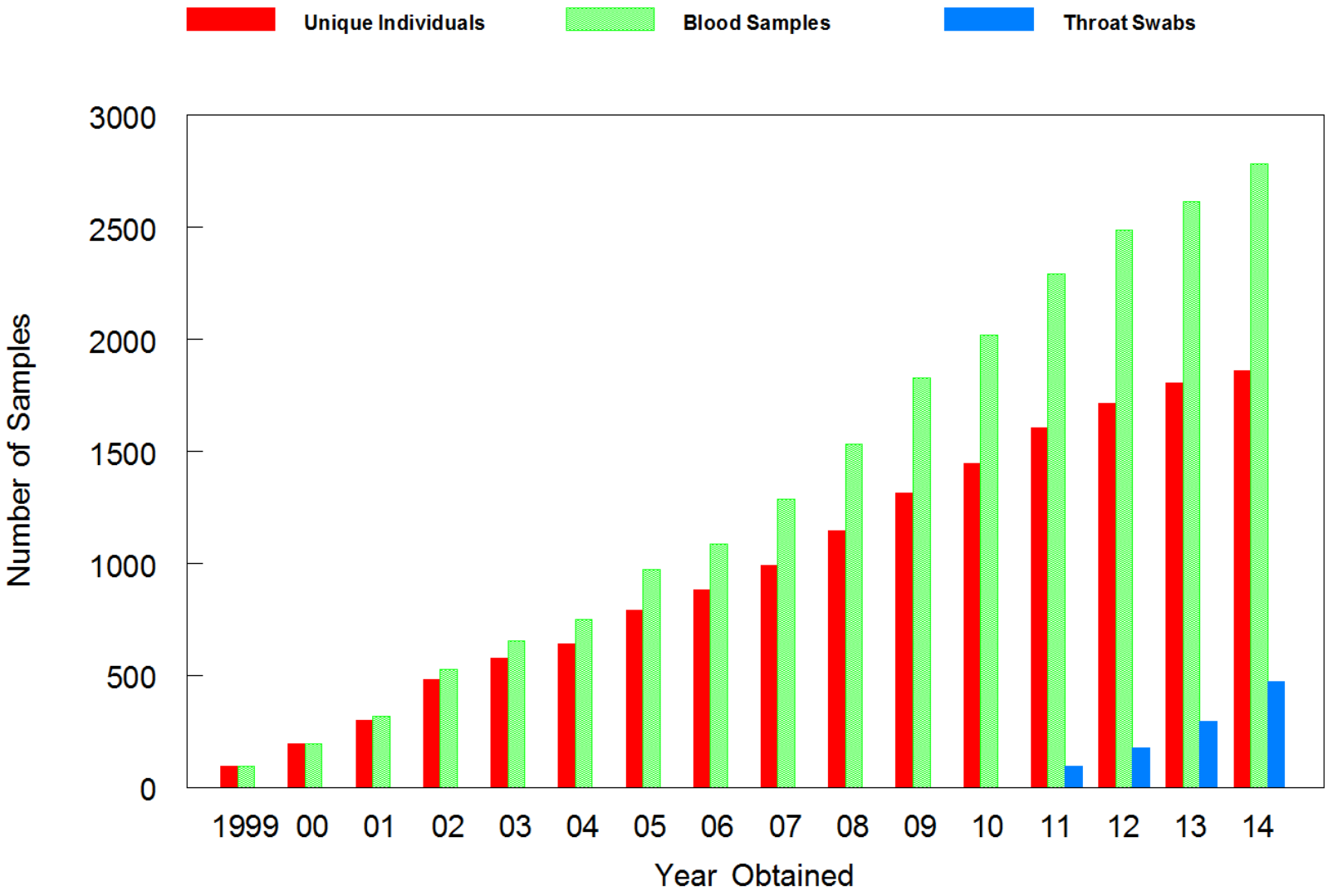
- Source of inflammation not identified in most individuals
- Evolutionary effects of of multiple microbes
- Interaction with host genes
- Effect on immune function and behavior
- Association with fetal development
- Potential for prevention and treatment
  - Antimicrobials
  - Probiotic organisms (Bifidobacteria)
  - Anti-inflammatory agents
  - Diet



Sheppard Pratt Study Population

- Participants drawn from the same geographic area over a 12 year period
- Different psychiatric disorders and controls enrolled and evaluated using similar protocols
- Biological samples processed at the same facility and analyzed in an unbiased manner
  - Antibodies to Infectious Agents
  - Markers of Inflammation
  - DNA from whole blood
  - Mucosal samples for microbiome characterization
  - Samples saved for additional analyses
- Same cognitive tests performed in entire population
  - Repeatable Battery for the Assessment of Neurological Status (RBANS)
  - Trail Making Test Part A (Trails A)
  - Wisconsin Card Sorting Test
- Longitudinal studies
  - Mood disorders
  - Mortality

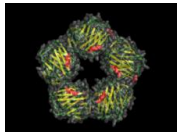
Sheppard Pratt Stanley Research Program: Individuals and Samples in Studies by Year



Sheppard Pratt Research Program

Clinical Study Populations

Study Population	Number 12/01/2012	Number 12/01/2013	Number 12/01/2014
Schizophrenia	714	738	756
Bipolar disorder	373	435	438
Recent onset of psychosis	199	206	213
Mania	60	71	90
Bipolar depression	38	46	46
Major depression	---	9	31
Non-psychiatric controls	482	530	542
Total participants*	**1766**	**1803**	**1883**
Total number of samples	**2489**	**2636**	**2784**



C Reactive Protein(CRP)

- Pentameric protein in the pentraxin family synthesized in the liver in response to macrophage factors
- Component of several arms of the immune system including complement activation and innate immunity
- Meta-stable in most individuals with basal levels increasing following immune stimulation
- Increased levels found in schizophrenia
  - Differences with controls independent of co-factors such as smoking, BMI, and medications
  - Associated with cognitive impairments
  - Elevations in some mood disorders
  - Elevations prior to disease onset
  - Most reliable marker of inflammation in schizophrenia
  - Effect size ~.45; 28% of individuals with elevated levels
- Levels based on genetic and environmental factors

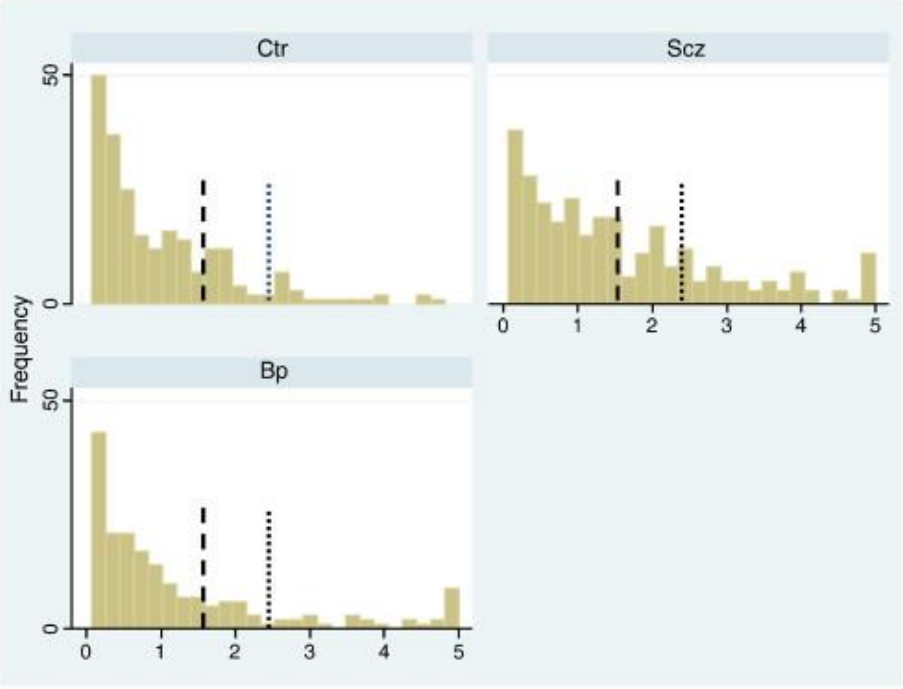
Am J Psychiatry. 2014 Sep;17

Schizophr Res. 2013 Jan;143(1):198-202. d

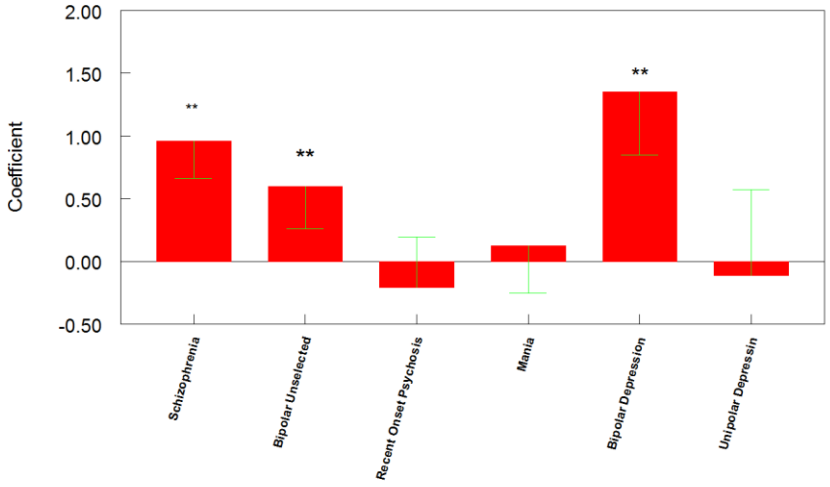
Clin Schizophr Relat Psychoses. 2014 Jan;7(4):223-30

CRP and Psychiatric Disorders

Distribution of Values



Coefficients adjusted for demographic variables



Polygene Score and Immune Activation Pilot Study

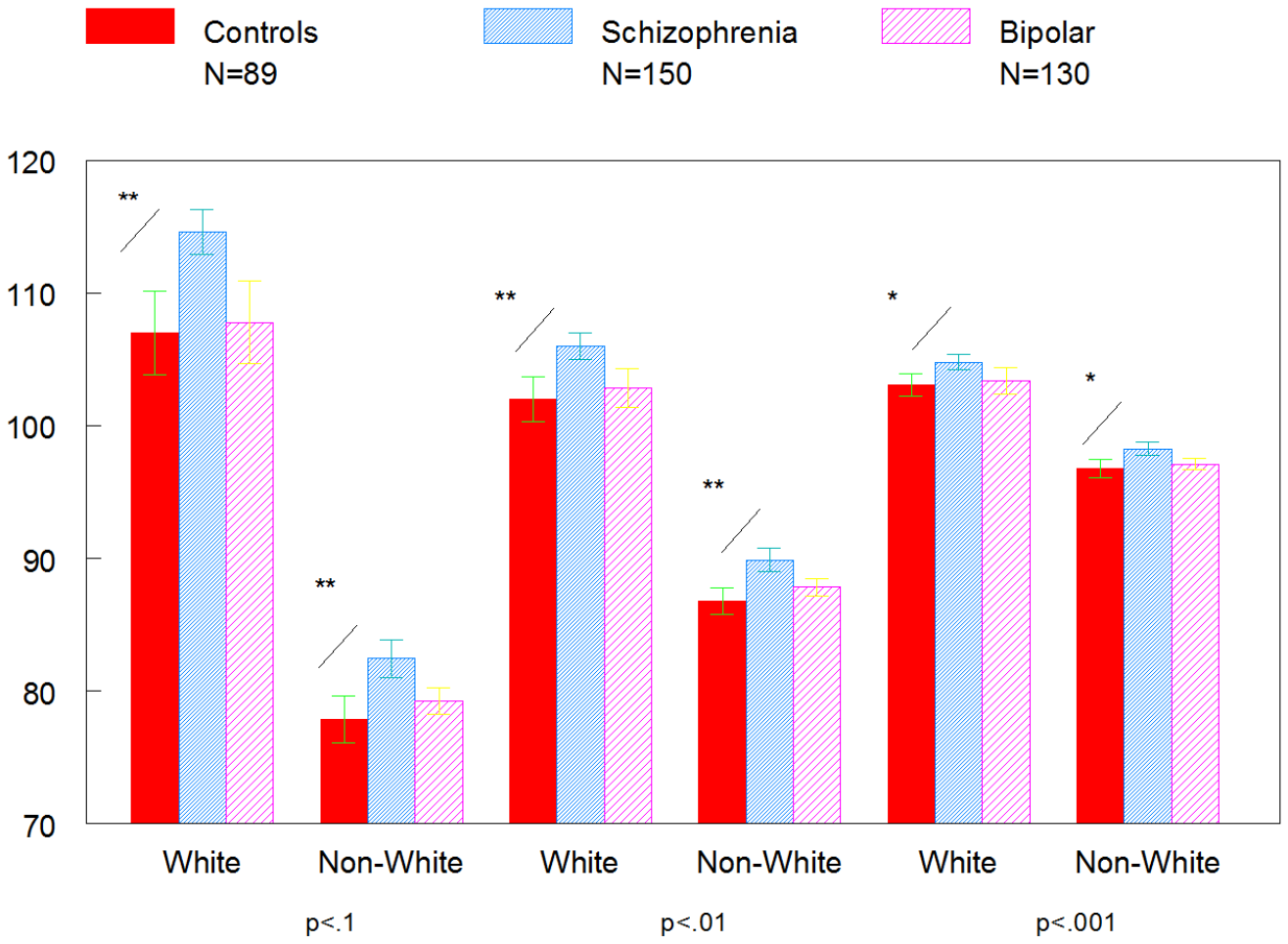
Participants

- N=396 individuals enrolled since 2008
  - Diagnosis: Schizophrenia, N=161; Bipolar disorder, N=146; Non-psychiatric controls, N=89
  - Race: Caucasian, N=232 (59%)
  - Gender: Females, N=214 (54%)

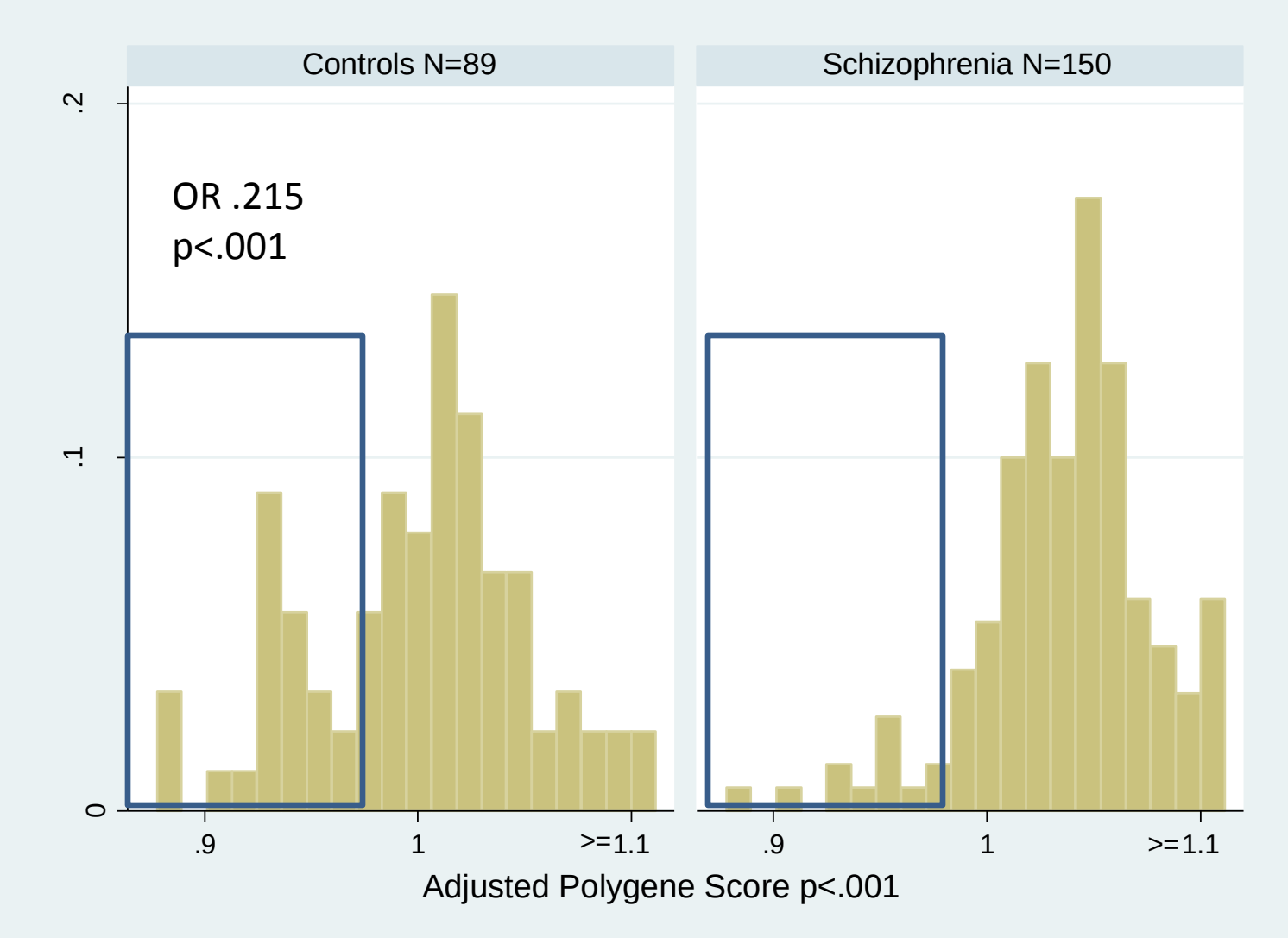
Methods

- GWAS performed at Stanley Center, Broad Institute using the Psych Chip
- Polygene score calculated from PGC2 (Schizophrenia) and PGC1 (Bipolar disorder) and analyzed at different cutoffs
- Interactions with C-Reactive Protein analyzed using regression models including age, gender, race, maternal education, smoking, bmi score

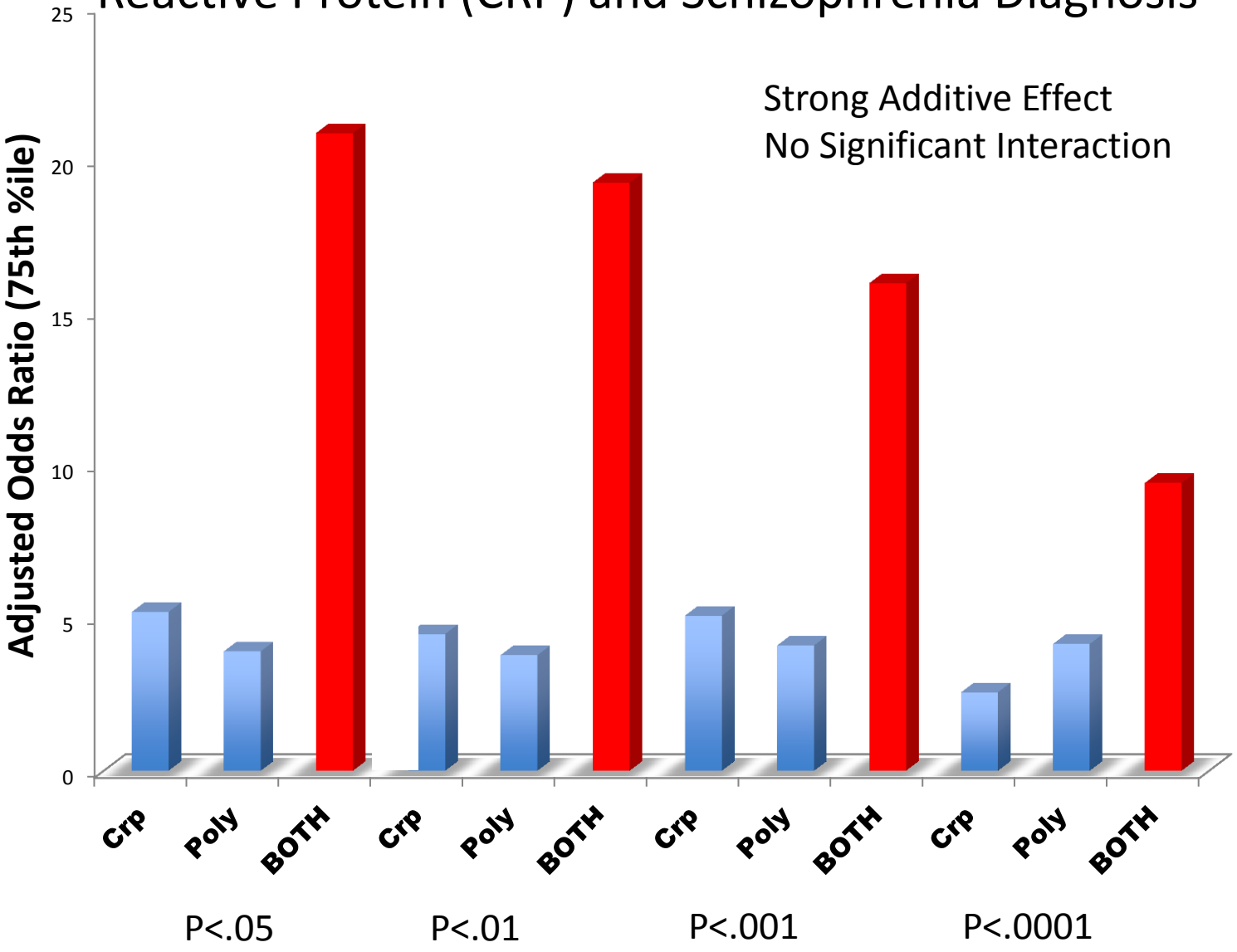
Schizophrenia Polygene Score by Diagnosis and Race



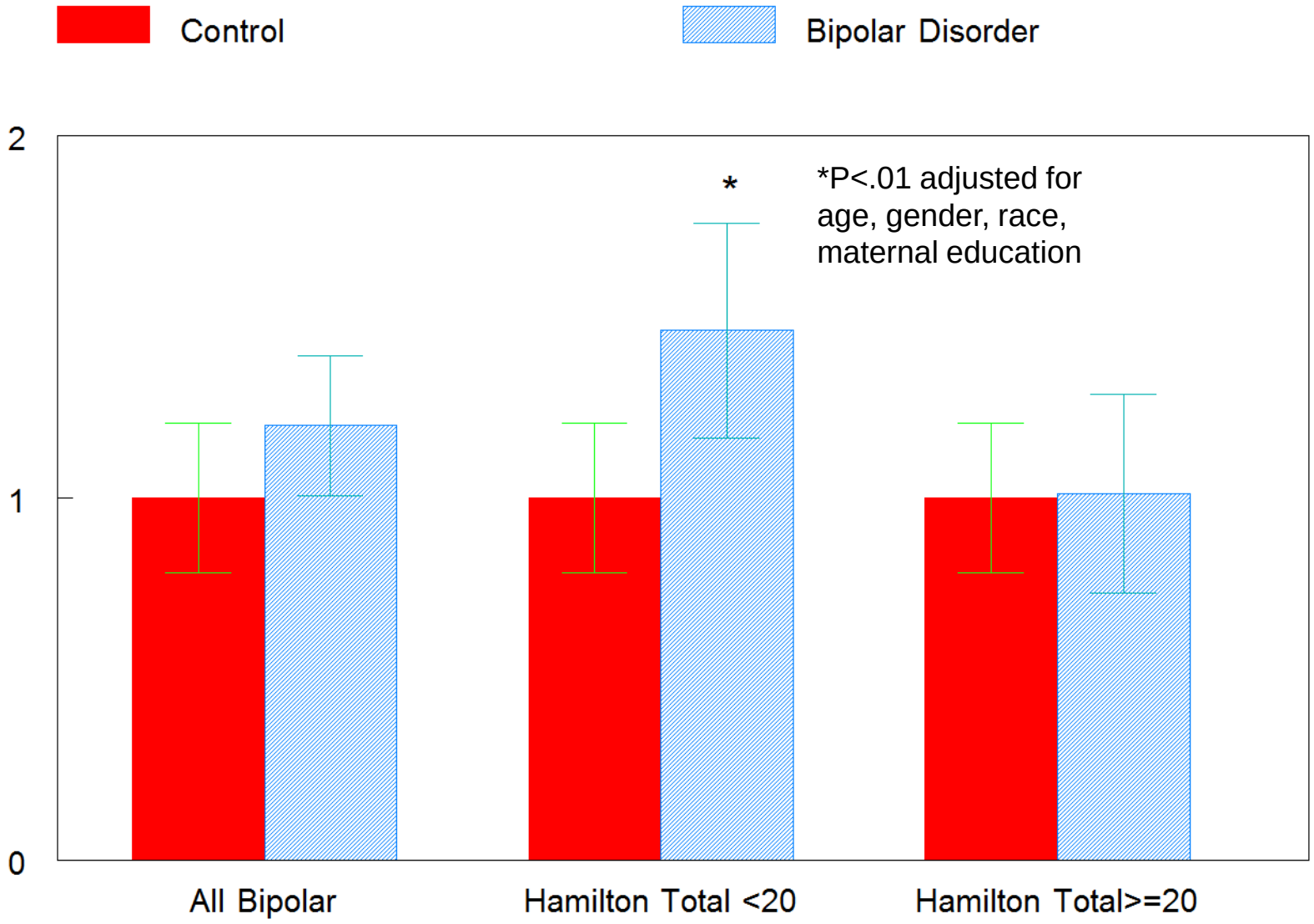
Adjusted Schizophrenia Polygene Scores in Control and Schizophrenia Groups



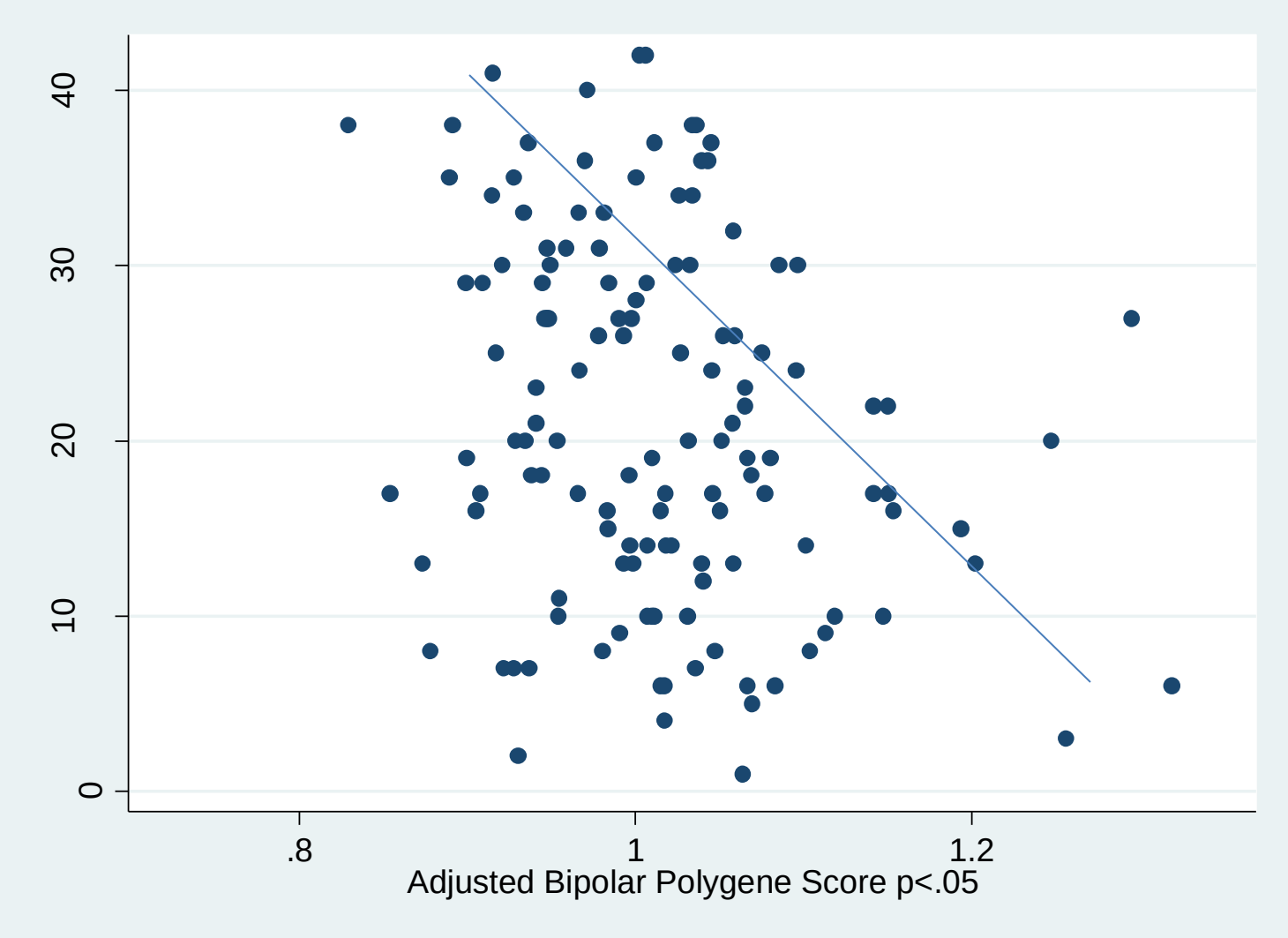
Association between Adjusted Schizophrenia Polygene Score, C-Reactive Protein (CRP) and Schizophrenia Diagnosis

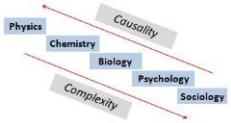


Bipolar Polygene Score in Bipolar Disorder by Hamilton Depression Score (N=130)



Association between Hamilton Depression Score and Polygene Score in Bipolar Disorder (N= 130)

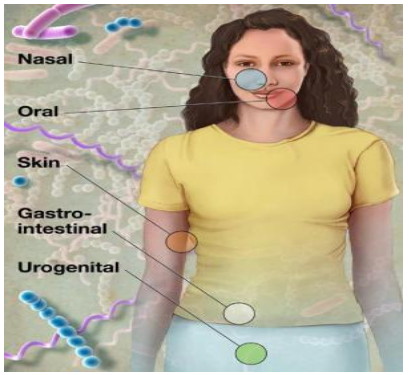




The Microbiome

The Other Human Genome

- Every individual has a unique set of micro-organisms at mucosal sites determined by genetics and environmental exposures
  - Skin
  - Oral pharynx
  - Intestinal Tract
  - Genital tract
  - Amniotic fluid
- Micro-organisms include
  - Bacteria
  - Viruses
  - Fungi
  - Protozoa



- The microbiome is both inherited from the mother and acquired environmentally.
- The genome of the microbiome is larger and more complex than the human genome (at least 10X) and includes some RNA genomes.

Proc Natl Acad Sci U S A. 2011 Feb 15;108(7):3047-52. doi: 10.1073/pnas.1010529108. Epub 2011 Jan 31.

Normal gut microbiota modulates brain development and behavior.

Diaz Heijtz R, Wang S, Anuar F, Qian Y, Björkholm B, Samuelsson A, Hibberd ML, Forssberg H, Pettersson S.

Department of Neuroscience, Karolinska Institutet, 171 77 Stockholm, Sweden.

Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve

Javier A. Bravo[a,1], Paul Forsythe[b,c,1], Marianne V. Chew[b], Emily Escaravage[b], Hélène M. Savignac[a,d], Timothy G. Dinan[a,e], John Bienenstock[b,f,2], and John F. Cryan[a,d,g,2]

[a]Laboratory of NeuroGastroenterology, Alimentary Pharmabiotic Centre, [d]School of Pharmacy, and Departments of [e]Psychiatry and [g]Anatomy, University College Cork, Cork, Ireland; [b]The McMaster Brain–Body Institute, St. Joseph's Healthcare, Hamilton, ON, Canada L8N 4A6; and Departments of [c]Medicine and [f]Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada L8S 4L8

Edited by Todd R. Klaenhammer, North Carolina State University, Raleigh, NC, and approved July 27, 2011 (received for review February 27, 2011)

Original Article

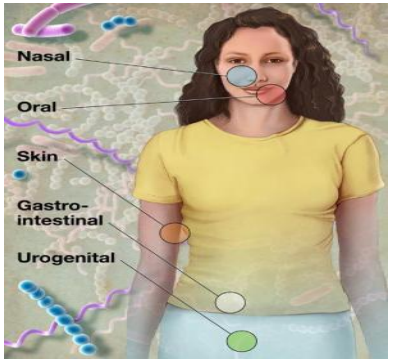
Molecular Psychiatry advance online publication 12 June 2012; doi: 10.1038/mp.2012.77

The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner

G Clarke[1,2], S Grenham[1], P Scully[1], P Fitzgerald[1], R D Moloney[1], F Shanahan[1,3], T G Dinan[1,2] and J F Cryan[1,4]

Exploring the Microbiome with Metagenomic Sequencing

- Measurement of large numbers of DNA or RNA sequences in biological samples
- Can detect any microbial DNA or RNA in any sample
  - Bacteria
  - Viruses
  - Fungi
  - Protozoa
  - Novel agents



- Mammalian mRNA can be detected in the same run (gene-environmental interactions)
- Main limitations are cost and bioinformatics
  - Cost $1000/sample
  - Hardware and software capable of matching to database many times larger then the human genome
  - Analysts with knowledge of both bioinformatics and microbiology

High Throughput Sequencing Discovery and Verification

Sequencing

- Illumina HiSeq
- 300,000,000 reads per lane
- Indexing up up to 3 samples per lane
- 100 nucleotides per paired end run

Characterization

- Filtering of human and contaminating sequences
- Matching to RefSeq databases
  - Bacteria
  - Protozoa
  - Fungi
  - Viruses
- BLAST Searches
  - DNA (BlastN)
  - Protein (BlastX)

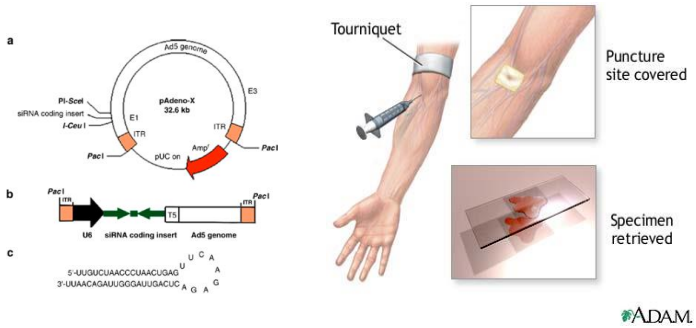
Verification

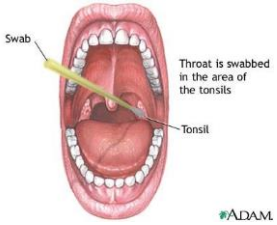
- Gel based PCR
- Real Time PCR (Taqman)
- Antibody Measurement
- Biological Characterization
- Animal models

High Throughput Sequencing
Removal of “Irrelevant” sequences

- Human DNA
  - Assembled Chromosomes
  - Unassembled Fosmids
  - Individual Variants

- Microbial Contaminants
  - E Coli
  - Skin flora (Proprionobacteria)
  - Water born bacteria (Pseudomonas)
  - Saprophytes (Fungi)
  - Vectors

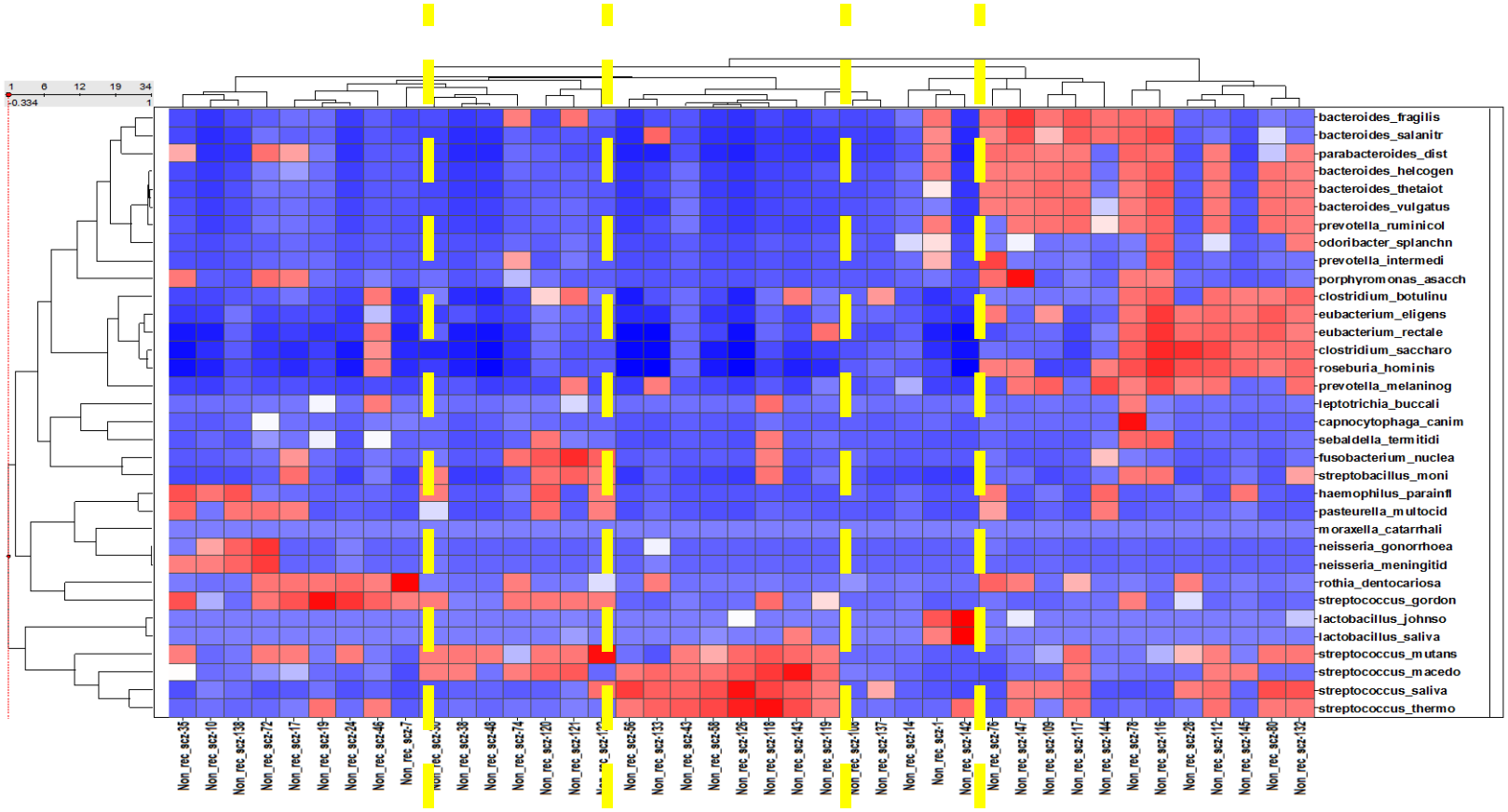




Microbiome and Schizophrenia
Throat swab samples

- Obtained from individuals with schizophrenia other psychiatric disorders and controls without a psychiatric disorder at Sheppard Pratt Hospital
- Samples obtained with standard throat swabs, sent to laboratory by messenger, and stored frozen until DNA extraction
- Samples analyzed
  - High Throughput Sequencing
    - N=41 individuals with established schizophrenia
    - N=32 controls without a psychiatric disorder
    - Discovery Subset of N=16 cases and N=16 controls
  - Verification by Real Time PCR (Taqman)
    - N=72 individuals with schizophrenia (126 samples)
    - N=34 individuals with bipolar disorder (44 samples)
    - N=82 controls (82 samples)
  - Blood samples, demographic, and cognitive data from all individuals

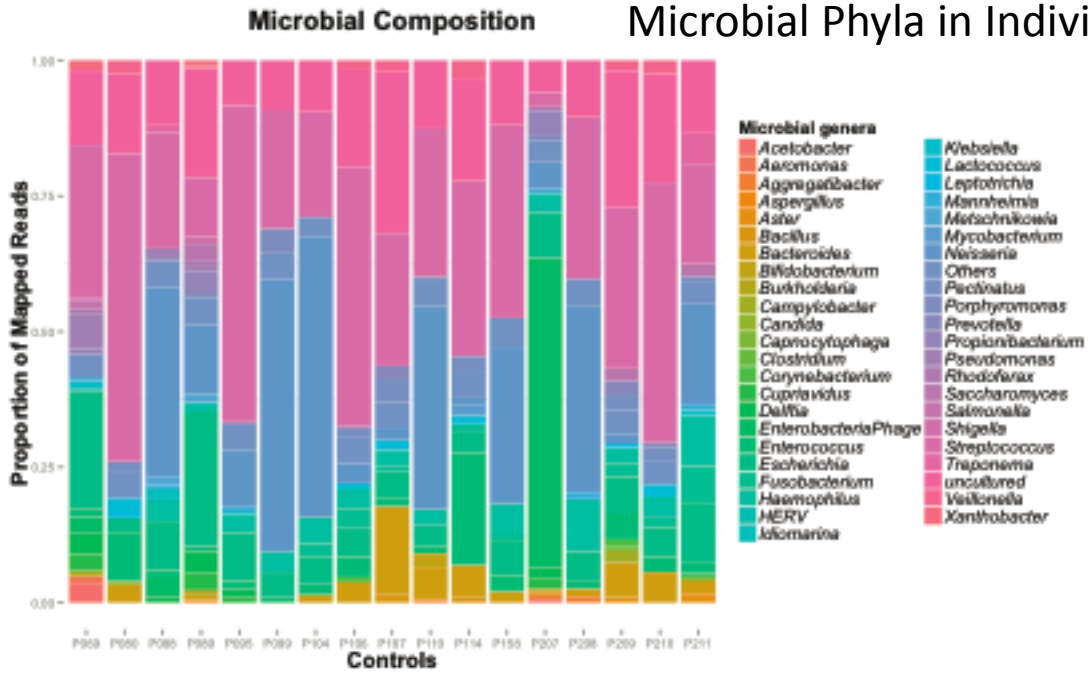
Oral microbiome in Schizophrenia-Grouping



Groups do not differ in age, gender, race, cigarette smoking, oral hygiene, current antibiotics

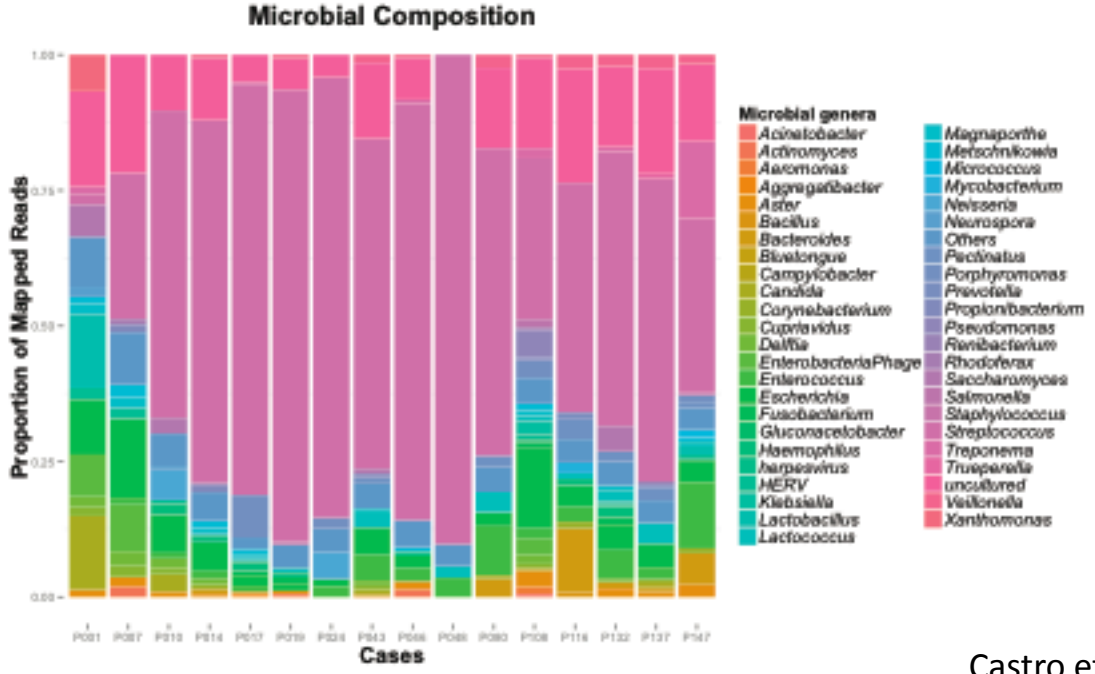
Groups **do** differ in having a higher rate of **deficit syndrome** (negative symptoms) and evidence of **intestinal inflammation**

Microbial Phyla in Individuals with Schizophrenia and Controls



Controls

Veillonella
Neisseria
Haemophilus
Other non-pathogenic organisms

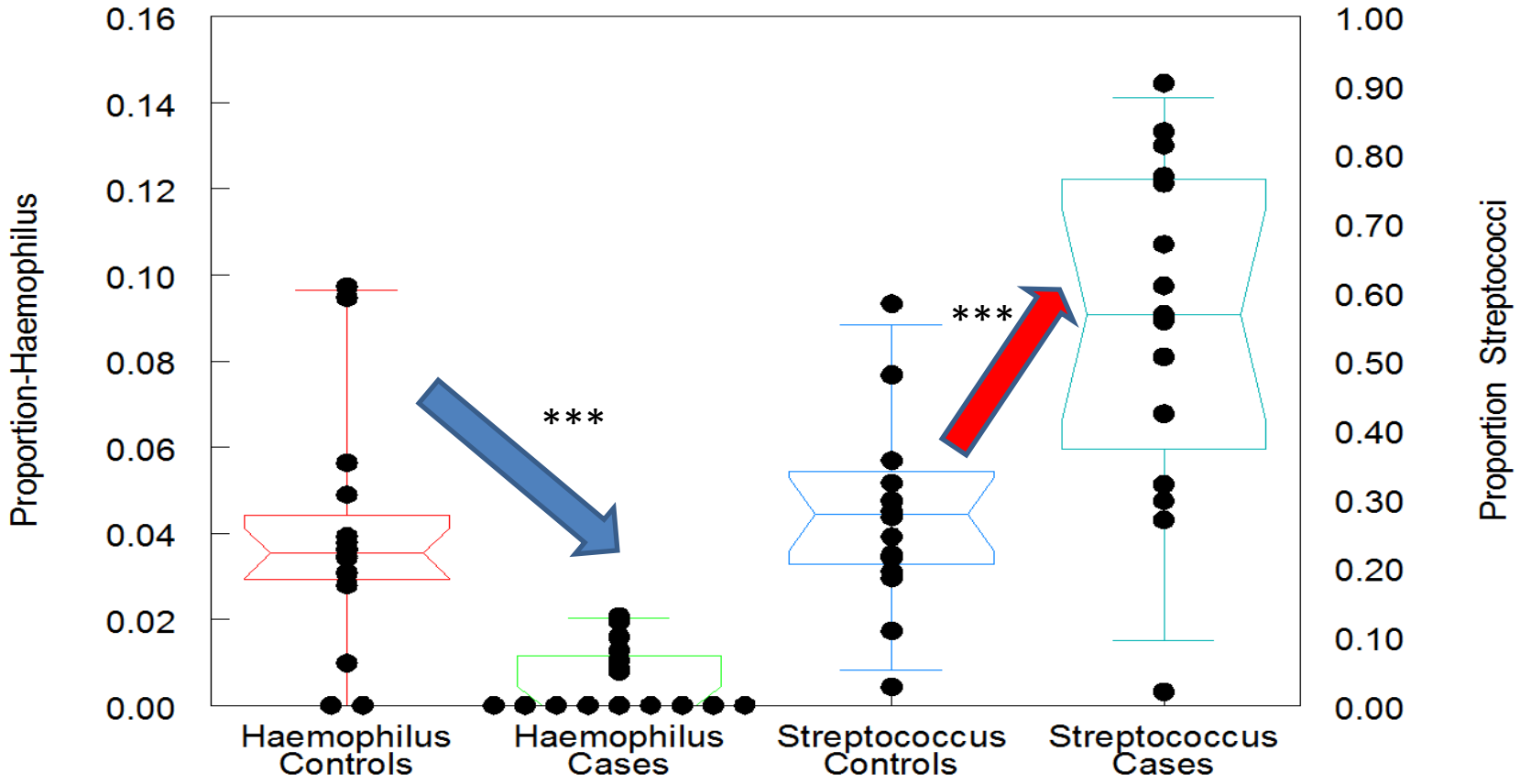


Schizophrenia

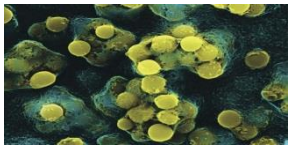
Streptococci
Staphylococci
Mycobacteria
Pseudomonas
Lactobacilli

Castro et al, submitted for publication

Pharyngeal Microbiome-Individuals with Schizophrenia and Controls



*** P<.001 adjusted for age, gender, race, maternal education



Streptococci

- Common inhabitants of the oropharynx
- Range of pathogenicity-some neurotropi
- Associated with abnormal behavior (PANDAS)

1947

Bacteriologic, Etiologic, and Serologic Studies in Epilepsy and Schizophrenia I.

EDWARD C. ROSENOW

LONGVIEW HOSPITAL, CINCINNATI

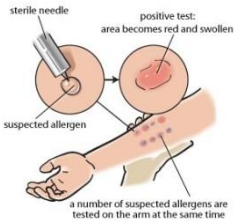
1947

Positive to:	A	B	C	F
Schizophrenics	33/73 (45%)	32/73 (45%)	7/73 (10%)	11/73 (15%)
Idiopathic epileptics	6/14 (43%)	7/14 (50%)	1/14 (7%)	4/14 (28%)
Controls	6/32 (18%)	6/32 (18%)	2/32 (6%)	4/32 (12%)

From our findings we may conclude that (1) thermal antibodies prepared by Dr. Rosenow from streptococci, isolated and cultured from groups of patients with schizophrenia (*A*) and epilepsy (*B*), produce larger reactions when injected into patients suffering from schizophrenia and epilepsy than do antibodies derived from streptococci, isolated and cultured from patients suffering from arthritis (*C*), or well persons (*F*).

Likewise, these same substances *A* and *B* produce

specific types of alpha streptococci which are not harmless or casual invaders but which are specifically antigenic; (2) the streptococci and the "neurotoxins" which they produce have predilection for certain structures in the brain, and (3) they may play an important role in the pathogenesis of epilepsy and schizophrenia. Studies on the nature of the

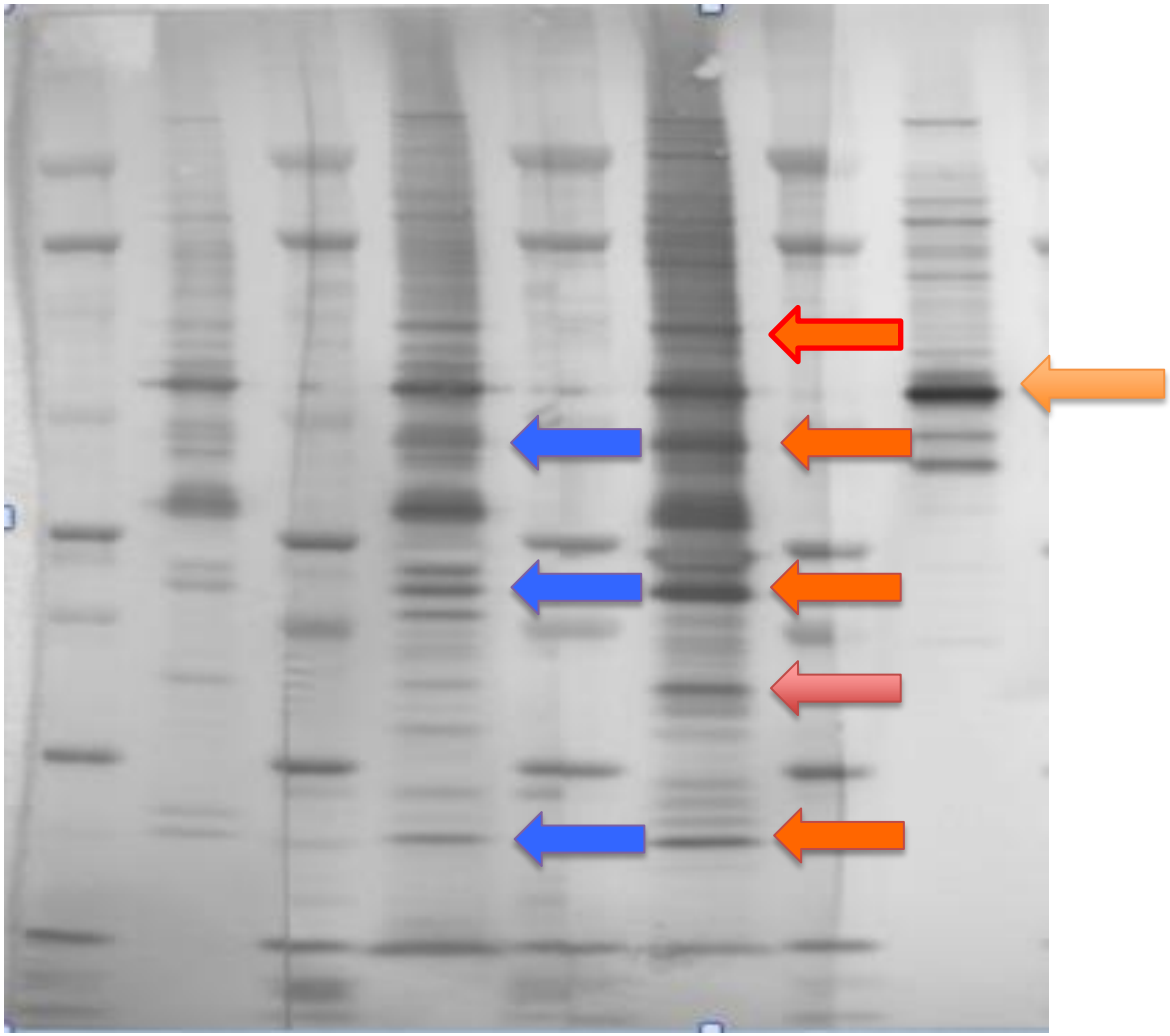


Streptococcal skin test 1955

Treatable with specific antibiotics and probiotics

Antibodies to *Streptococcus pyogenes* *Sheppard Pratt Cases and Control*

M Ctr M Scz M ROP M Rab



Ctr-Non-psychiatric Control

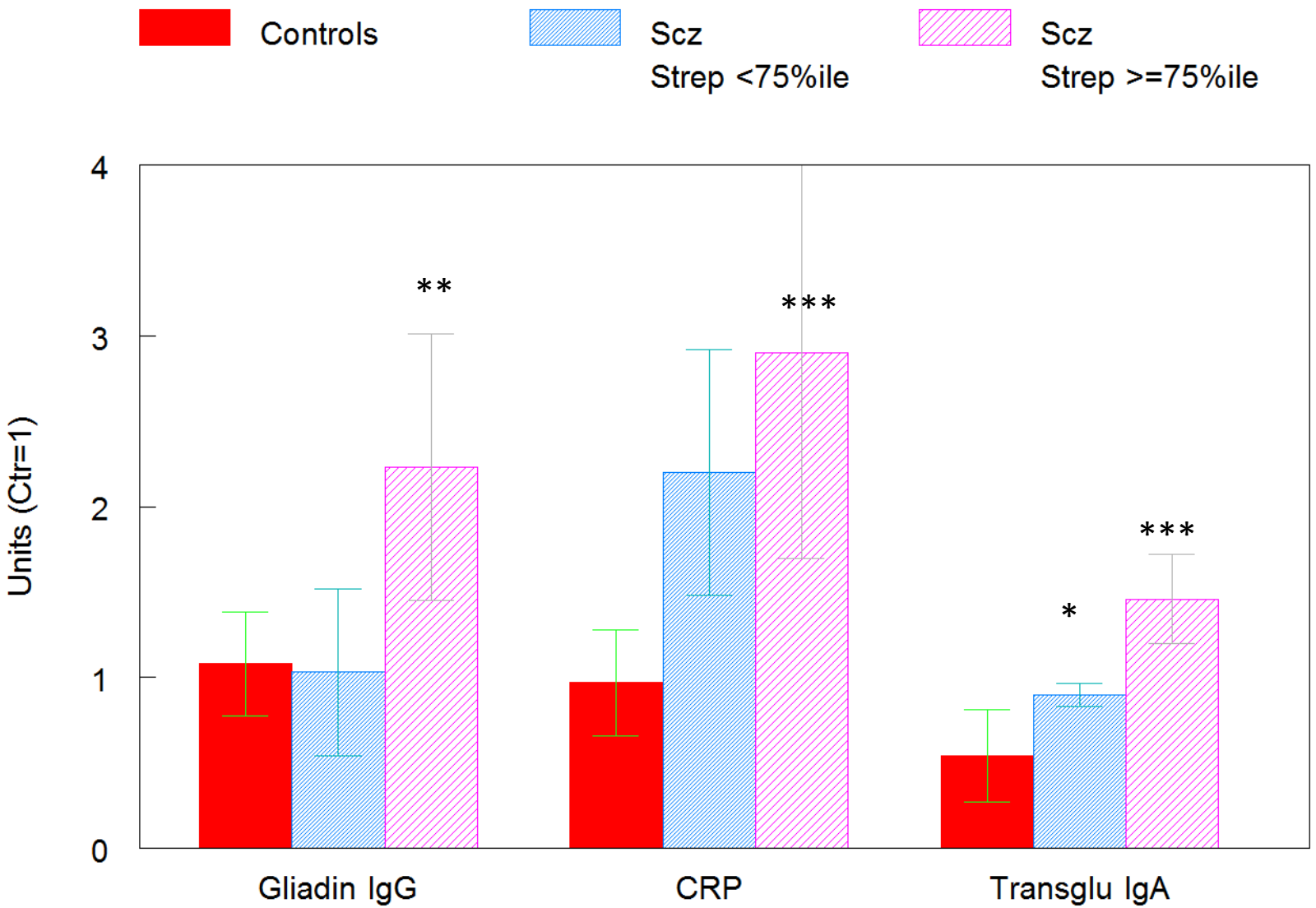
Scz-Established Schizophrenia

ROP-Recent Onset Psychosis

Rab-Rabbit anti-Streptolysin

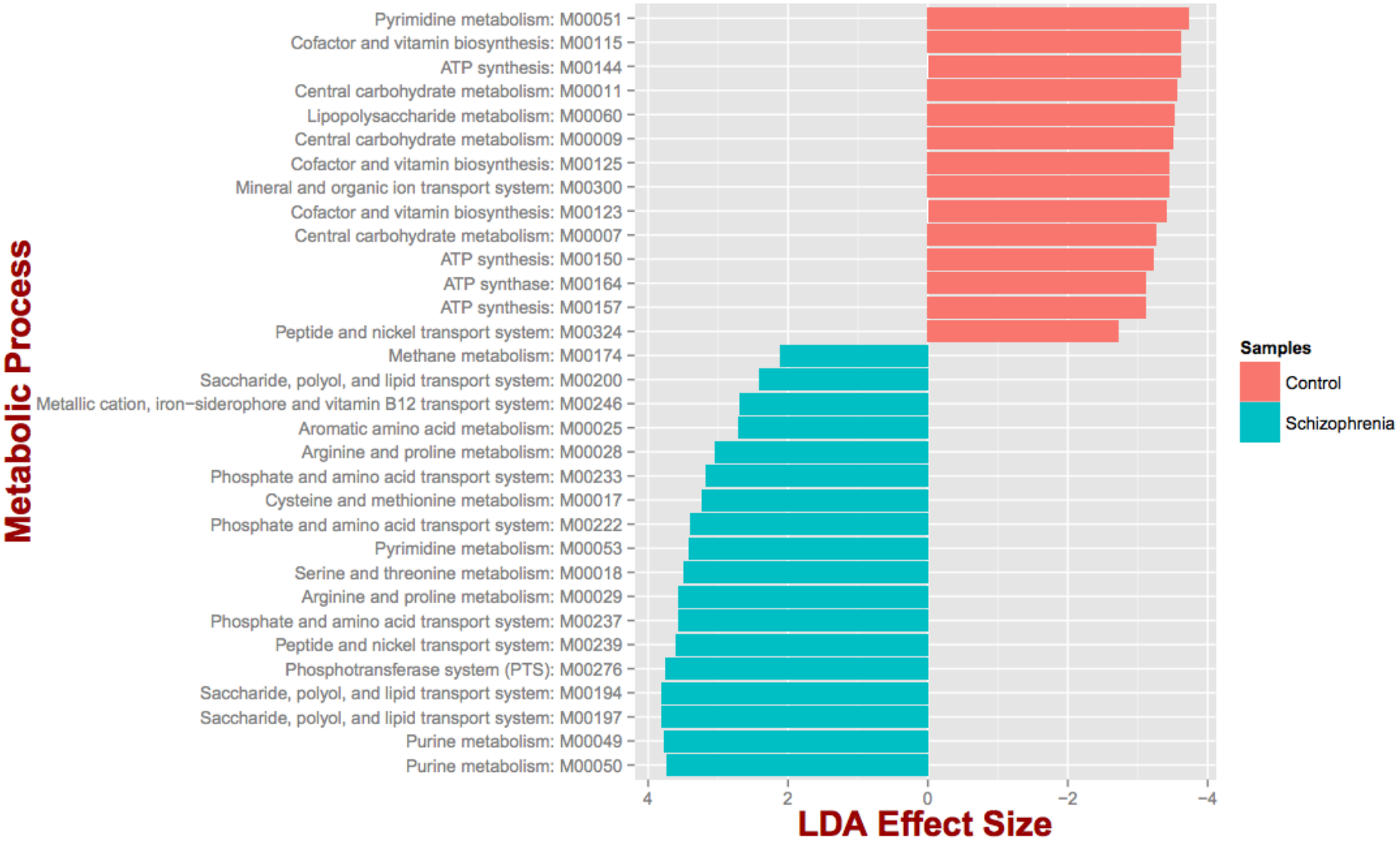
M-Markers

Relationship of Streptococci to Inflammatory Markers



Strep=*S mitis*+ *S oralis*+ *S gordonii*

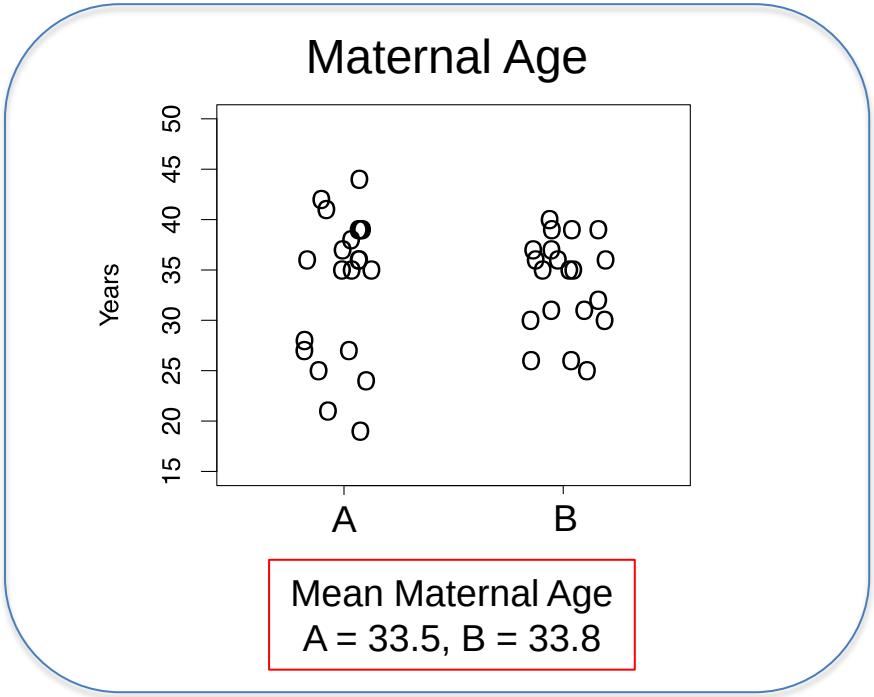
* p<.05, **p<=.01, ***p<=.001 compared to control adjusted for age, gender, race, maternal education, and bmi



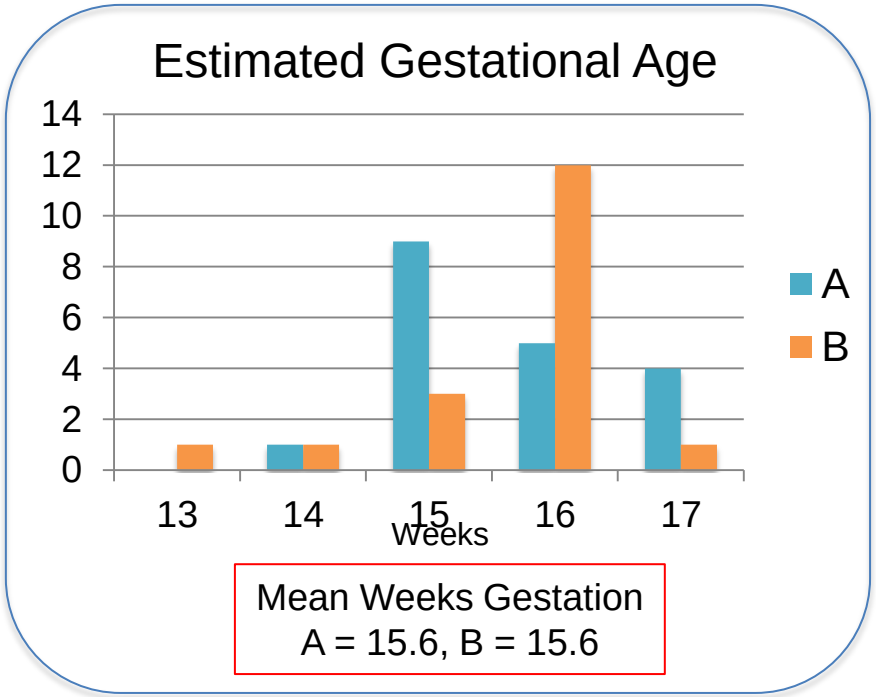
 Castro et al, under review

Microbial and Human Gene Expression in Amniotic Fluid

- Amniotic fluid obtained from pregnant women in Denmark for chromosomal analyses
- Schizophrenia cases in offspring identified by case registry and matched to controls.
- RNA extracted and subjected to high throughput sequencing. Analyzed in coded groups

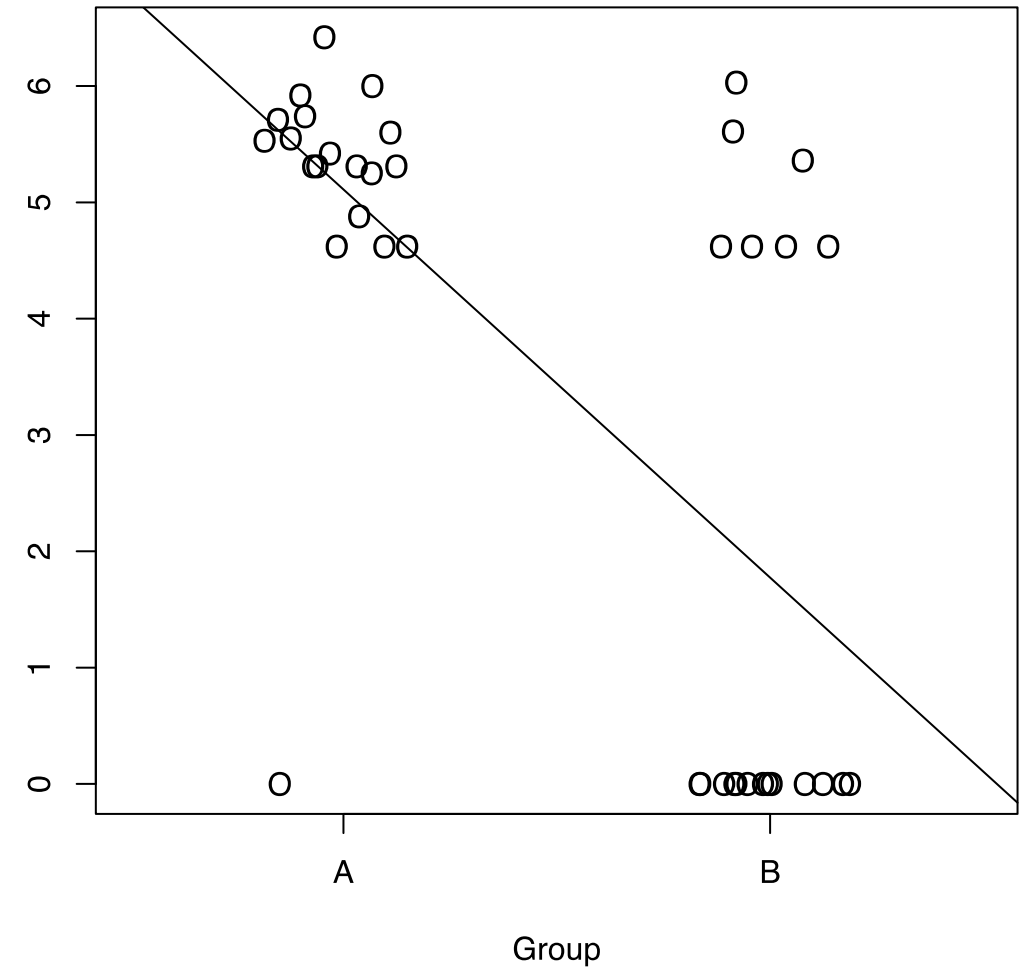


Mean Maternal Age
A = 33.5, B = 33.8

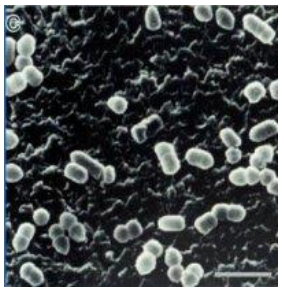


Mean Weeks Gestation
A = 15.6, B = 15.6

Differential Presence of Prevotella in Amniotic Fluid

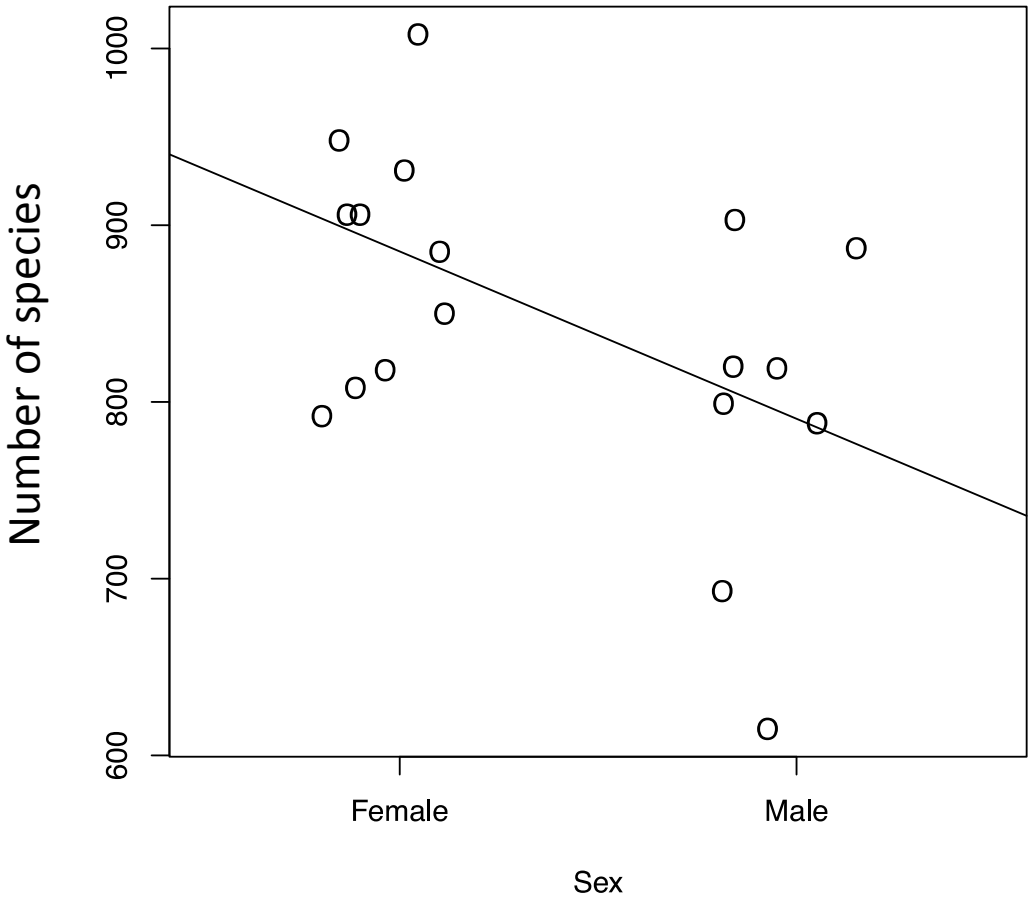


p–value: 8.532718648665 72e–06 R^2: 0.402984771994875

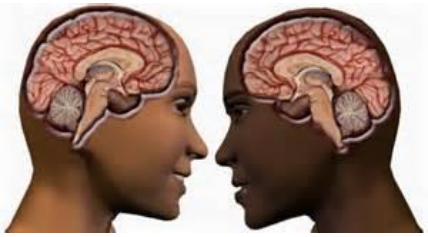


- Gram negative anaerobic organism
- Part of the genital microflora
- Alters phospholipid metabolism in amniotic fluid through phospholipase A2 activity

Amniotic fluid microbiome has more species diversity when fetus is female.

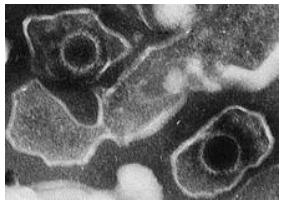


p–value: 0.0262811733718627 R^2: 0.227005053412896

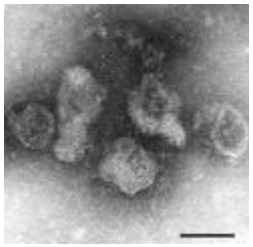


The Pharyngeal Viral Microbiome Revealed by Metagenomic Sequencing

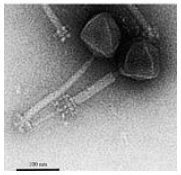
- Herpesviruses
  - Human Herpesvirus 1 (HSV-1)
  - Human Herpesvirus 4 (EBV)
  - Human Herpesvirus 6
  - Human Herpesvirus 7
  - Bovine Herpesvirus

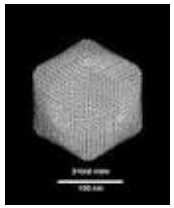


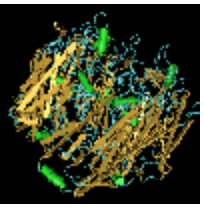
- Retroviruses
  - Endogenous Retroviruses
  - Primate Retroviruses
  - Lentiviruses



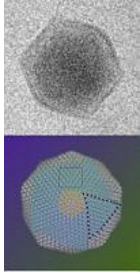
- Other DNA Viruses
  - Human Papilloma Viruses
  - Bacteriophage (Bacteria)
  - Circoviruses (Pigs)
  - Baculoviruses (Insects)
  - Mottle viruses (Plants)
  - Chlorella Viruses (Algae)



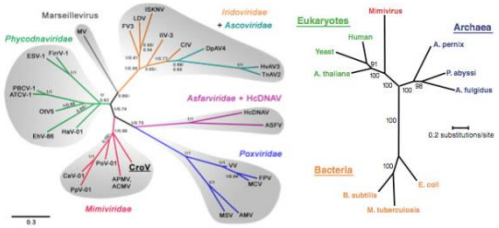


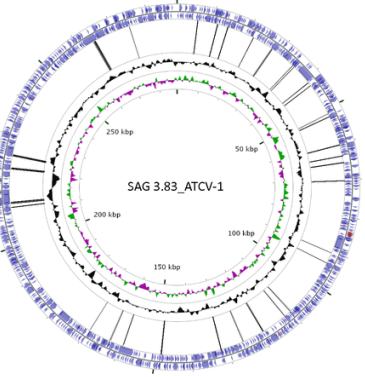


Chlorella Viruses

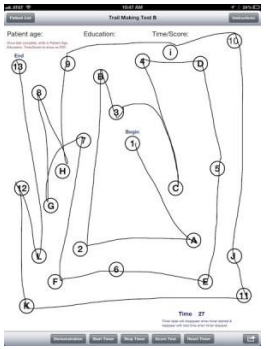


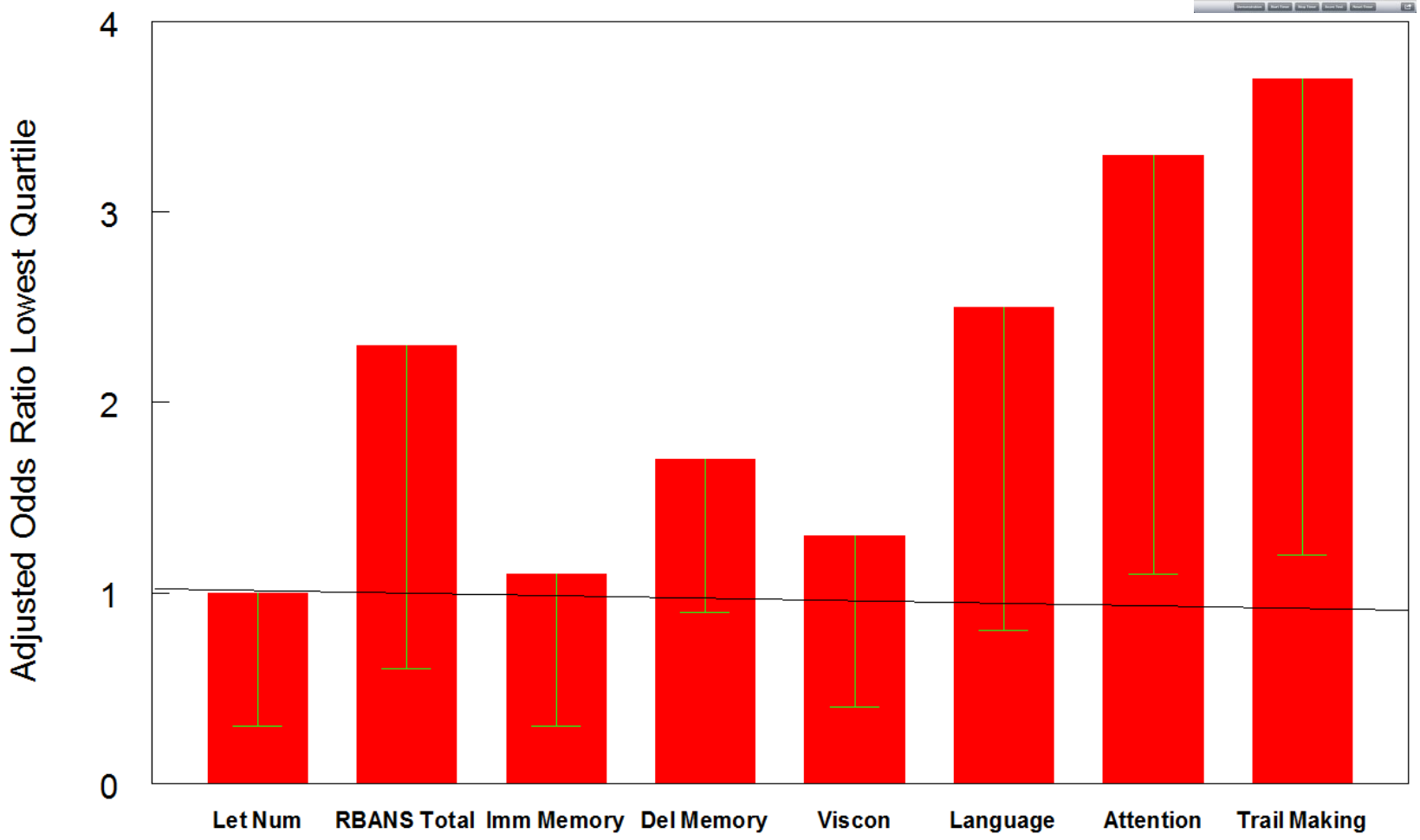
- Large dsDNA genomes - 290 to 370 kb
- Common evolutionary ancestor with the Irido, Pox, Asfar, Asco & Mimiviruses; collectively referred to as Nucleocytoplasmic Large DNA viruses (NCLDVs)
- First isolated from sea water and algae.
- Related to African Swine Fever Virus
- Biological effects on infected cells
  - Generation of ion channels (CACNA1C)
  - Epigenetic effects on host cells (HDAC)
- Prototype strain Acanthocystis turfacea Chlorella Virus (ATCV-1) replicates in algae Chlorella SAG 3.83
- Other viruses replicate in different algae



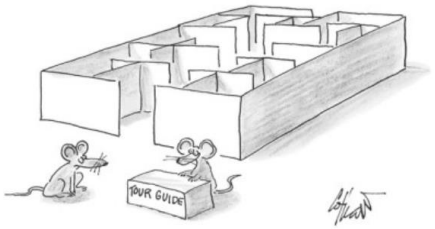


ATCV-1 in throat swabs and Cognitive Functioning in Controls (40/92)

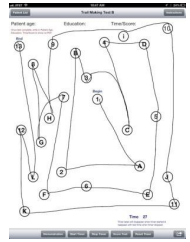


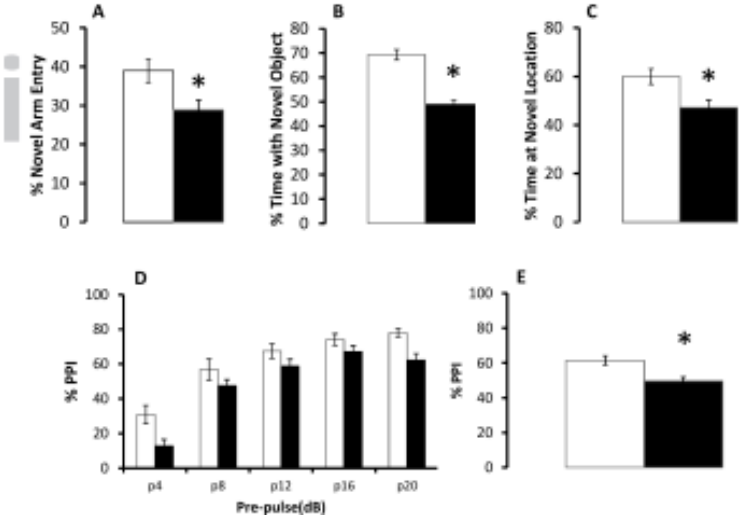


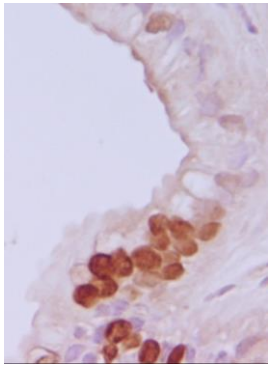
Yolken et al, PNAS 2014



Confirmation of Chlorella Virus Infection in Mice



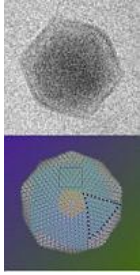


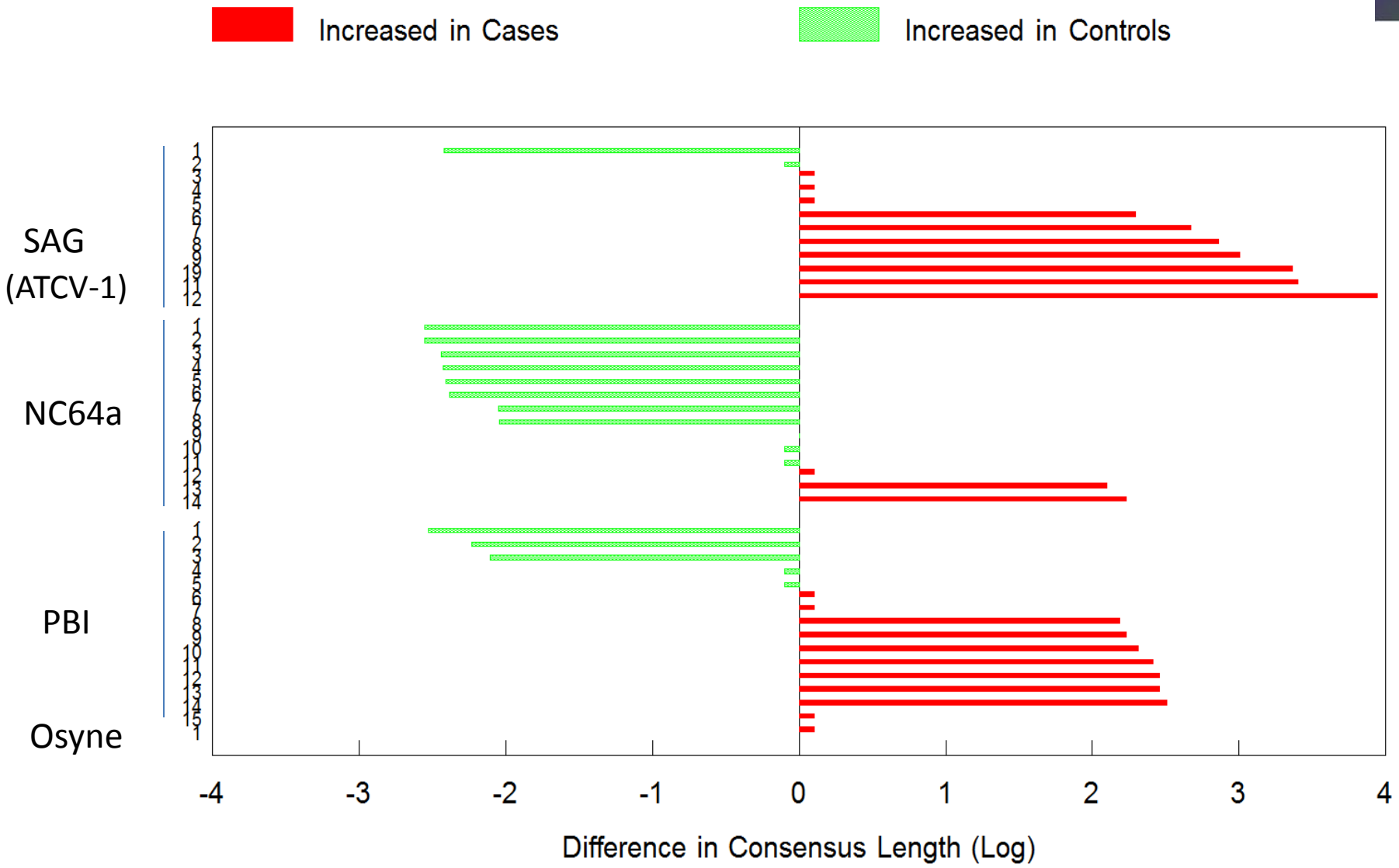


Significantly altered canonical pathways from Ingenuity Pathways Analysis based on differences in gene expression in the hippocampus of ATCV-1 exposed vs. control mice

Canonical pathway	p-value[1]	Ratio[2]	Genes	Function(s)	Reference[3]
EIF2 Signaling	3.47E-04	9.45E-02	Rpl11,Ppp1CC,Rpl4, Rpl37A,Rps18,Ppp1CB,Rps8, Rps13,Rps21,Ins1,Rpl6, Rps27, Rpl39, Eif2S3, Rplp2, Rps15A, Eif2Ak2,Rps3, Rpl13	Regulation of protein synthesis. Enhanced eIF2 phosphorylation leads to deficits in synaptic plasticity and spatial memory	Ma,T.et al., Nat NeuroSci, 2013 Rathore AP, Virol J. 2013
Agranulocyte Adhesion and Diapedesiss	4.79E-04	9.9E-02	Cxcl9,Tnfrsf1A,Itga2,Itga6,Mmp2, Ccl5,Actg1,Ccl9,Cxcl10,Glycam1, Podxl2,Ccl7, Cxcl13, Cldn1, Ccl2, Ccl3l1/Ccl3l3,Actg2,Itga4,Msn	Activated monocytes and lymphocytes leaving the blood vessels in respond of infection, allergen, inflammation, coagulation.	Dörries R., et al. Curr. Top. Microbiol. Immunol, 2001
LXR/RXR Activation	1.00E-03	1.01E-01	Kng1,Scd,Ttr,C3,Tnfrsf1a, Serpinf1,Serpinf2,C4a/C4b,Ly96, Ldlr,Ccl7,Ptgs2,Lbp, Pon3	Heterodimers of LX (retionid) and RX (liver) nuclear receptor initiate transcription of target genes involved in lipid metabolism, inflammation, and cholesterol to bile acid catabolism	Burgener A, J Virol.2013
Acute Phase Response Signaling	1.00E-03	9.94E-02	Socs3,Ttr,Ftl,Itih3,C3, Tnfrsf1A,Socs2,Serpinf1, Serpina3,Mapk12,Rbp1,Serpinf2, Hnrnpk,C1r,C4a/C4b, Osmr,Lbp,A2m	Orchestrated response to tissue injury, infection or inflammation. Limit activity of cytokines by increasing the levels of acute-phase proteins made by the liver	Koj A., Biochem Biophys Acta., 1996 Nonnecke BJ, J Dairy Sci. 2014
Neuroprotective Role of THOP1 in Alzheimer's Disease	1.70E-03	1.27E-01	Kng1,Hla-a,Hla-b, Prkaca,Serpina3,Ace,Hla-e	Metallopeptidase that catalyzes the thiol-dependent cleavage of many neuropeptides.It also participates intracellularly in the antigen presentation of peptides generated by the proteasome.	N/A
Antigen Presentation Pathway	2.19E-03	1.43E-01	Hla-a,Hla-b,Hla-dmb, Psmb8,Cd74,Hla-e	Presentation of viral peptides on MHC class I molecules, taken up from extracellular sources.	Rosendahl Huber, SR, Front. Immunol, 2014
Caveolar-mediated Endocytosis Signaling	2.29E-03	1.18E-01	Itgad,Flna,Hla-a, Flnc, Itga2,Hla-b, Itga6, Actg2,Actg1,Itga4	Endocytic internalization of membrane components, extracellular ligands, molecules, viruses and bacteria toxins	Bernacchi S, Biochem Soc Trans. 2004
CDK5 Signaling	2.63E-03	1.13E-01	Fosb,Ppp1cc,Drd1,Ppp2ca, Cables1,Ppp1r3c,egr1,Itga2,Itga6, Prkaca,Ppp1cb	Involved in postsynaptic transmission, modulates dopaminergic signaling through dopamine receptors (DRD)	Bibb J. A, et al. Nature, 1999 Patrick C, Am J Pathol. 2011
Dopamine Receptor Signaling	2.24E-02	8.33E-02	Ppp1cc,Drd1,Ppp2ca,Ppp1r3c, Ddc ,Prkaca,Ppp1cb,Slc18a2	Dopamine is a neurotransmitter that is important to vital brain functions like motor control, short term memory, rewarding	Klanker M, Front Neurosci. 2013 Furuyashiki T., J Pharmacol Sci. 2012 Smith JL, J Virol. 2014.
Complement System	4.79E-02	1.14E-01	C1r,C4a/C4b,C3,C1qb	Complement activation and recruitment of inflammatory leukocytes is an important	Stoermer KA., Virology. 2011

Chlorella Virus Sequences in the Oropharynx of Individuals with Schizophrenia (N=16) and Controls (N=16)





Proc Natl Acad Sci U S A. 2014 Oct 27. pii: 201418895. [Epub ahead of print]

Chlorovirus ATCV-1 is part of the human oropharyngeal virome and is associated with changes in cognitive functions in humans and mice.

Yolken RH[1], Jones-Brando L[2], Dunigan DD[3], Kannan G[4], Dickerson F[5], Severance E[2], Sabunciyan S[2] Talbot CC Jr[6], Prandovszky E[2], Gurnon JR[3], Agarkova IV[3], Leister F[2], Gressitt KL[2], Chen O[2], Deuber Ma F[3], Pletnikov MV[4], Van Etten JL[7].

New 'Stupidity Virus' Discovered, Scientists Say

Nov 11, 2014, 12:12 PM ET

By LIZ NEPORENT via **GOOD MORNING AMERICA**

The next time you lose your keys or bomb a test, try blaming it on a virus. Researchers from Johns Hopkins University and the University of Nebraska have discovered a virus that makes you just a little bit dumber.

Brain Behav Immun. 2008 Oct;22(7):1103-7. doi: 10.1016/j.bbi.2008.04.156. Epub 2008 Jun 20.

Association between cognitive functioning, exposure to Herpes Simplex Virus type 1, and the COMT Val158Met genetic polymorphism in adults without a psychiatric disorder.

Dickerson F[1], Stallings C, Sullens A, Origoni A, Leister F, Krivogorsky B, Yolken R.

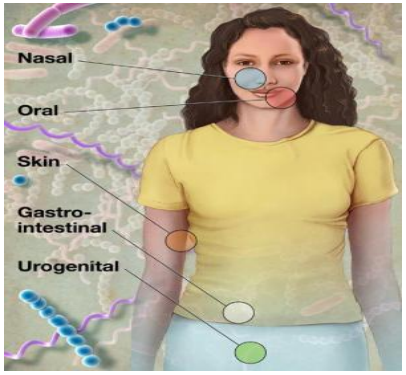
J Infect Dis. 2014 Aug 8. pii: jiu437. [Epub ahead of print]

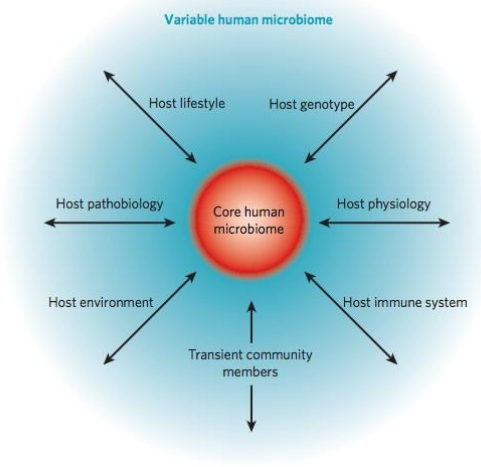
Cytomegalovirus Infection and Risk of Alzheimer Disease in Older Black and White Individuals.

Barnes LL[1], Capuano AW[2], Aiello AE[3], Turner AD[4], Yolken RH[5], Torrey EF[5], Bennett DA[2].

The Metagenomic Microbiome and Psychiatric Disorders Ongoing and Planned Studies

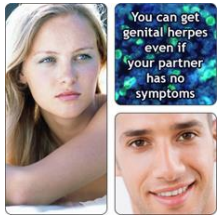
- Additional body sites
  - Intestinal Fluids
  - Skin
  - Urine
- Blood
  - Individuals with psychiatric disorders
  - Discordant Twins
  - Neonatal samples
- CSF
- Amniotic Fluid
- Clinical trials
  - Probiotic microorganisms
  - Antimicrobials/Anti-viral agents
  - Prebiotics and other dietary interventions





Infectious Agents and Animal Behavior
Mechanisms that increase transmission

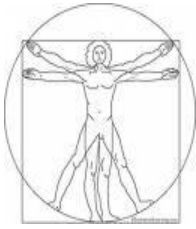
- Toxoplasma gondii
  - Altered risk assessment
  - Predation
  - Accidents/Suicide
- Malaria/Leishmania
  - Listlessness
  - Increased insect exposure
- Guinea Worm
  - Itching
  - Extrusion through blisters
- Rabies Virus
  - Increased aggression
  - Transmission by biting
- Respiratory viruses
  - Coughing
  - Droplet transmission
- Herpesviruses/Retroviruses
  - Cognitive impairment
  - Sexual transmission



Many agents which have not yet been characterized in humans

The Microbiome and Human Psychiatric Diseases-*Implications*

- Human psychiatric disorders are diseases involving genomes.
- Genomes involved include:
  - Human genome
  - The Microbiome
  - Persistent microbial pathogens
- The modulation of the microbiome might result in a new approach to the treatment of psychiatric disorders and a dramatic decrease in the massive personal, social and economic impact of these disorders.



Stanley Laboratory
Johns Hopkins

Stanley Research Center
Sheppard Pratt Hospital

Danish Amniotic Fluid Collection

- Preben Bo Mortensen
- Marianne Giørtz Pedersen
- Lasse Stenberg Jønsson
- Bent Nørgaard-Pedersen

Stanley Center , Broad Institute

- Colm O'Dushlaine
- Steve McCarroll
- Jennifer Moran
- Kimberley Chambert

Department of Virology, U Nebraska

- James VanEtten
- David Dunnigan

PGC

- Patrick Sullivan

Stanley Medical Research Institute

- E Fuller Torrey
- Maree Webster
- Julie Friese

Complex Human Diseases
Beyond Koch and Mendel

Mendel-Human traits are determined by individual genes which function independently of other genes and of environmental influences

Koch-Many human diseases are caused by individual microbes which exert their effect independently of other microbes, environmental factors and genes

Complex Human Diseases
Beyond Koch and Mendel

Mendel-Human traits are determined by individual genes which function independently of other genes and of environmental influences

Koch-Many human diseases are caused by individual microbes which exert their effect independently of other microbes, environmental factors and genes

Methods

- Study populations: Individuals with schizophrenia, bipolar disorder, or non-psychiatric controls
  - Assessed by Structured Clinical Interview for Diagnosis (SCID)
- Cognitive Functioning Measures:
  - Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)
  - Wisconsin Card Sorting Test
  - Trail Making Test
  - CATIE neurocognitive battery
- Symptom Measure:
  - Positive and Negative Syndrome Scale (PANSS)
  - Mood scales for bipolar disorder participants
- Blood sample drawn for all participants
  - Immunoassay for test of C-reactive protein, antibodies to HSV-1 and other infectious agents

Sheppard Pratt Stanley Research Program
Measures

Demographics

- Age, race, gender, educational level, maternal education, place of birth

Clinical Data

- Psychiatric symptoms: Positive and Negative Syndrome Scale (PANSS), YMRS, Ham-D
- Cognitive functioning: Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), Wisconsin Card Sort, Letter Number Sequencing, Trail Making Test
- Exposures: Medications, smoking, substances of abuse, pets, dietary exposures
- Health conditions: Diabetes and other co-occurring illnesses, body mass index, mortality

Laboratory Evaluations

Antibodies to infectious agents
- Herpes viruses HSV1, HSV2, CMV, VZV, EBV, HHV6
- Retroviruses
- Coronavirus
- Measles
- Influenza virus
- Chlorella viruses
- Toxoplasma gondii

Genetic polymorphisms
- Polygene score

Microbiome
- Viruses
- Bacteria

DNA Methylation

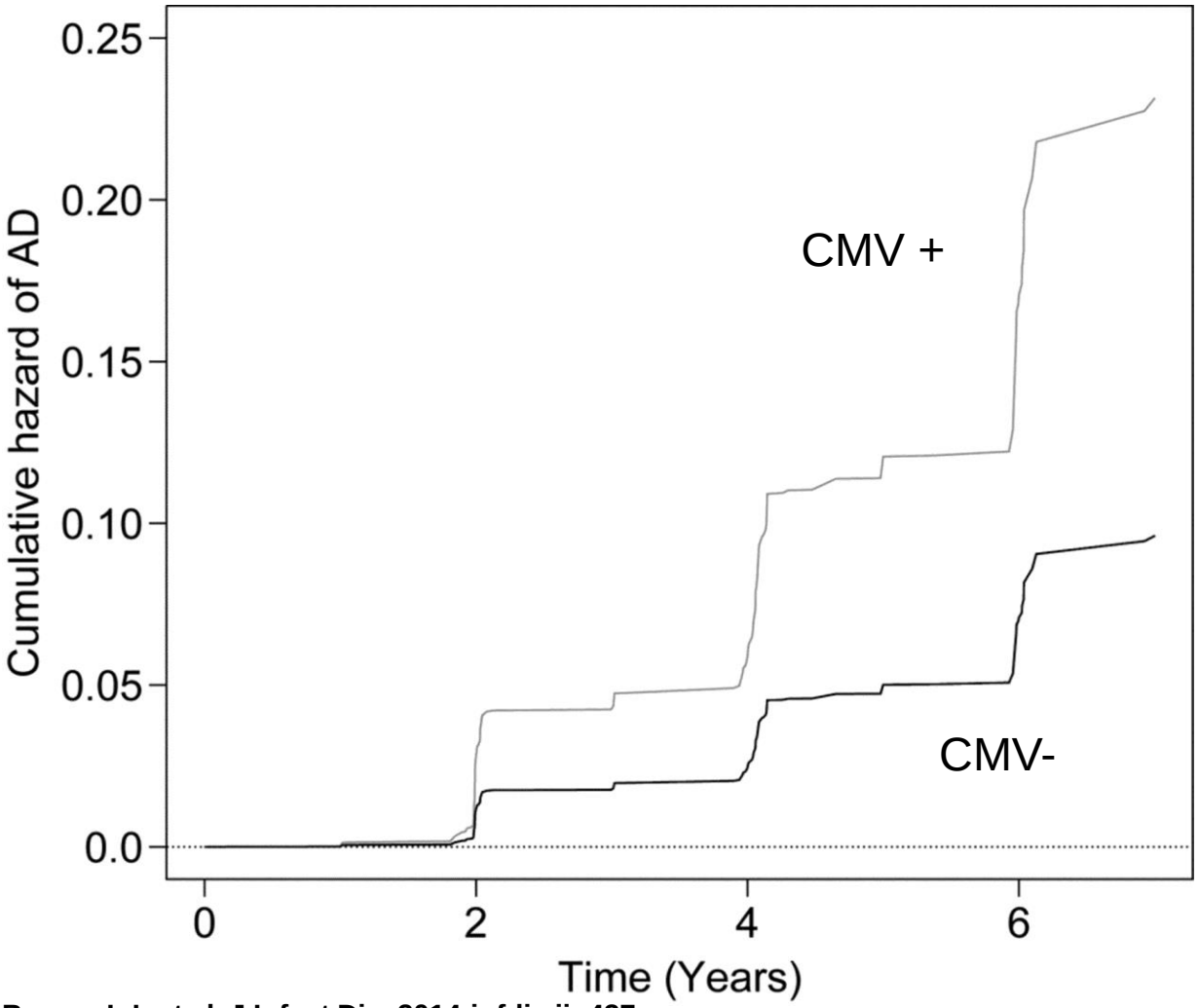
Antibodies to food antigens
- Gliadin and casein
- Saccharomyces cerevisiae (ASCA)

Auto-antibodies
- NMDA receptor
- Tissue transglutaminase

Markers of inflammation
- C-Reactive protein
- Pentraxin 3
- Cytokines, other markers
- Multi-analyte markers

All samples saved for future analyses

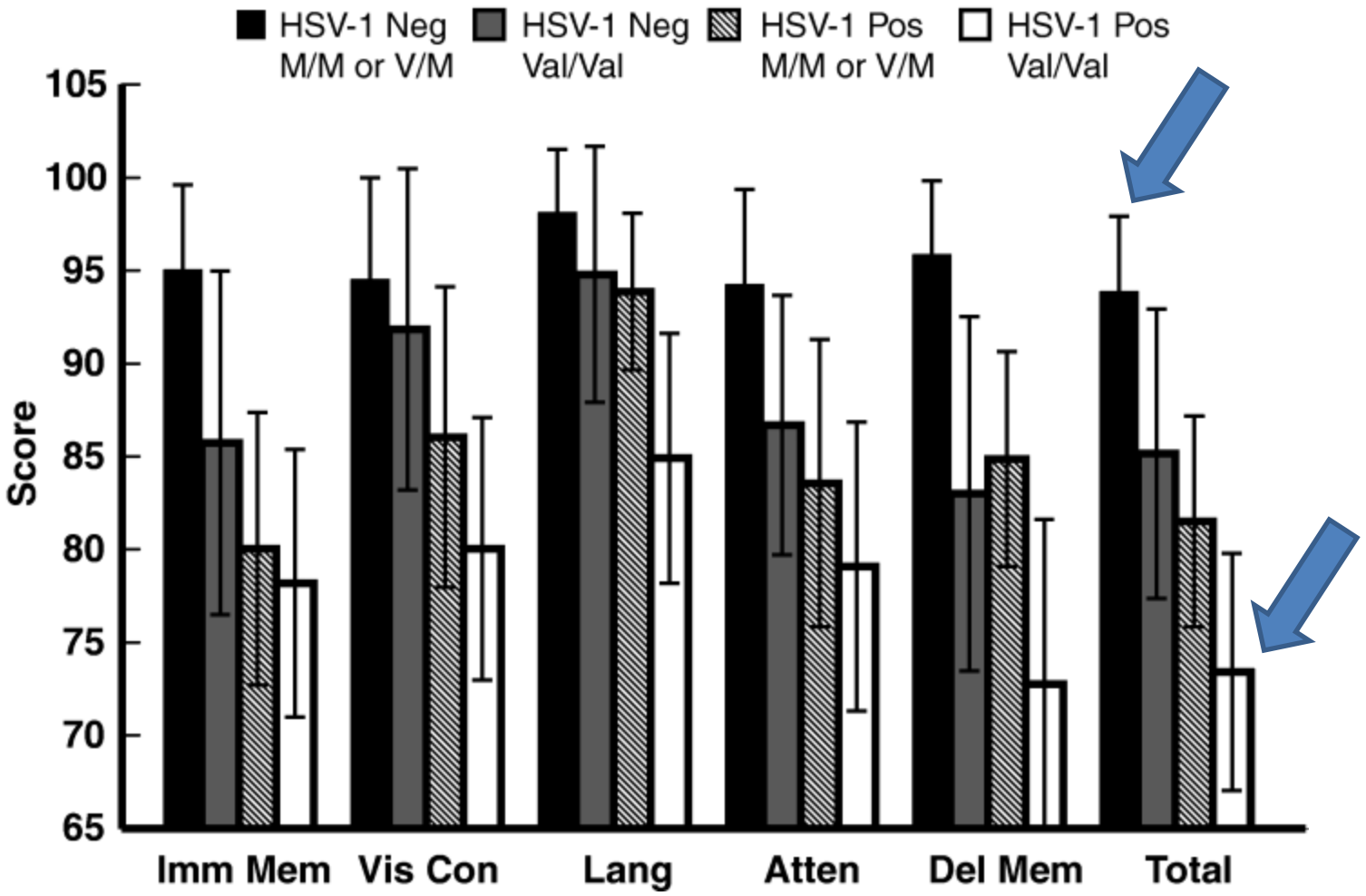
Risk of Alzheimer disease (AD) in persons infected with cytomegalovirus (CMV; top line) relative to those without CMV infection (bottom line), adjusted for age, sex, education duration, and race.



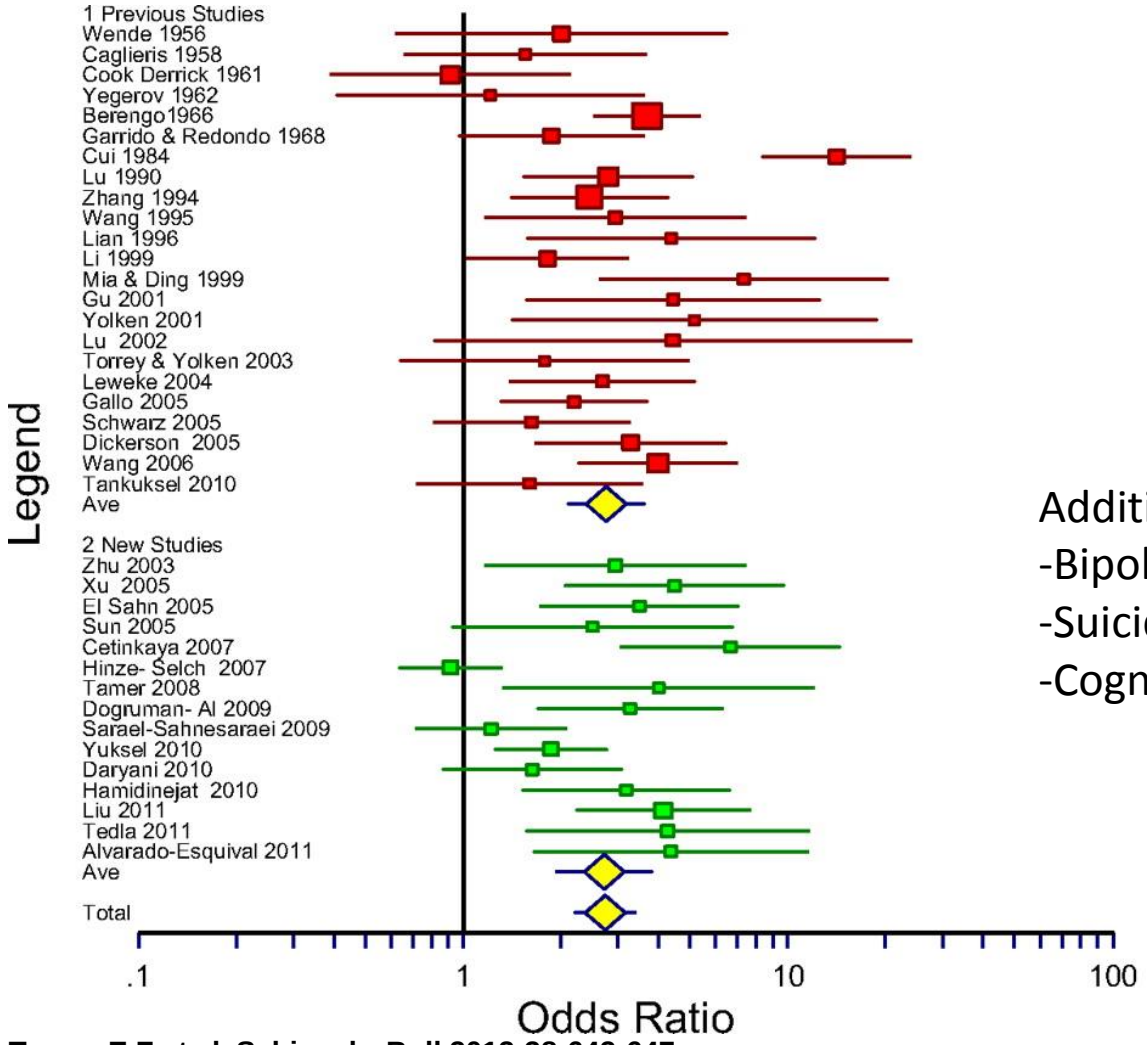
Barnes L L et al. J Infect Dis. 2014;infdis.jiu437

The Journal of Infectious Diseases

HSV-1, COMT, and Cognitive Functioning in Bipolar Disorder



Toxoplasma gondii
Meta-analysis of Association with Schizophrenia

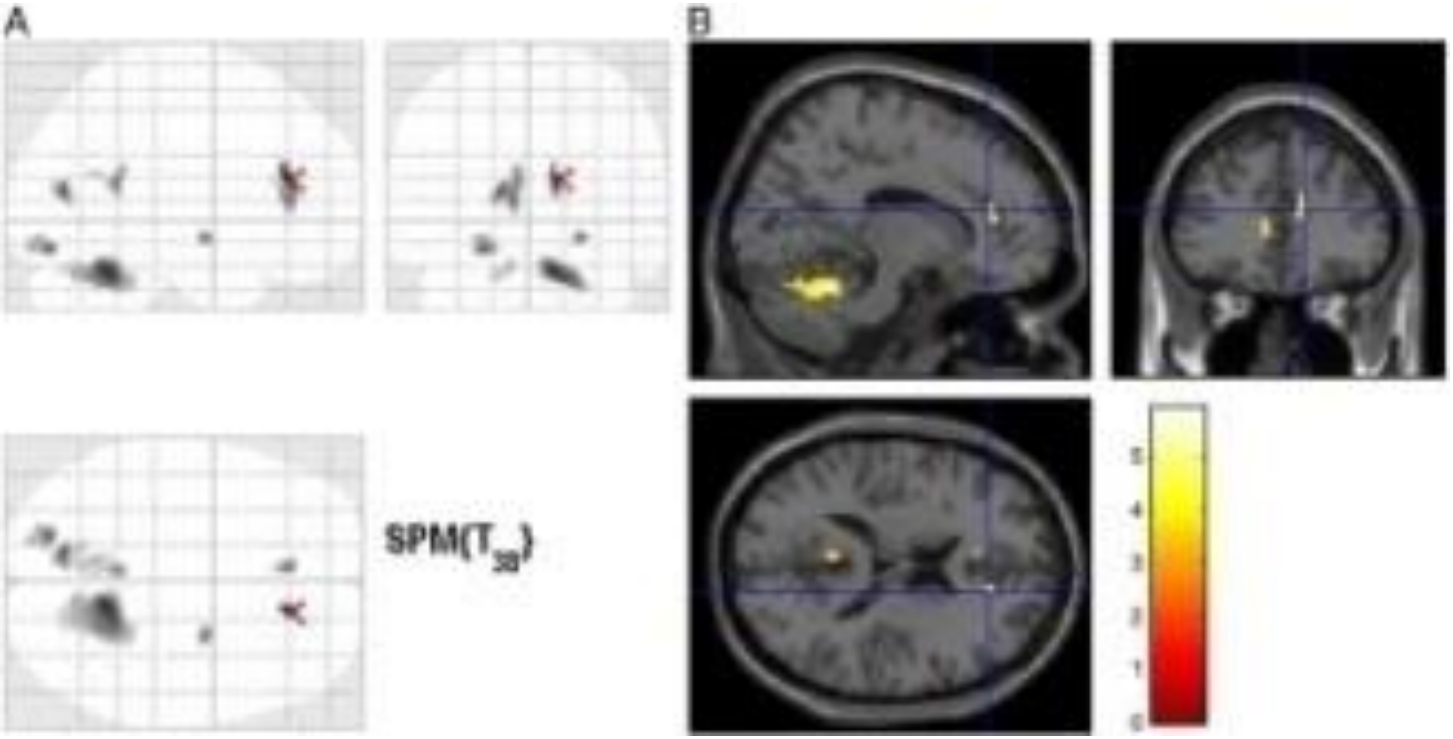


Additional associations with
-Bipolar disorder
-Suicide
-Cognitive impairment

Torrey E F et al. Schizophr Bull 2012;38:642-647

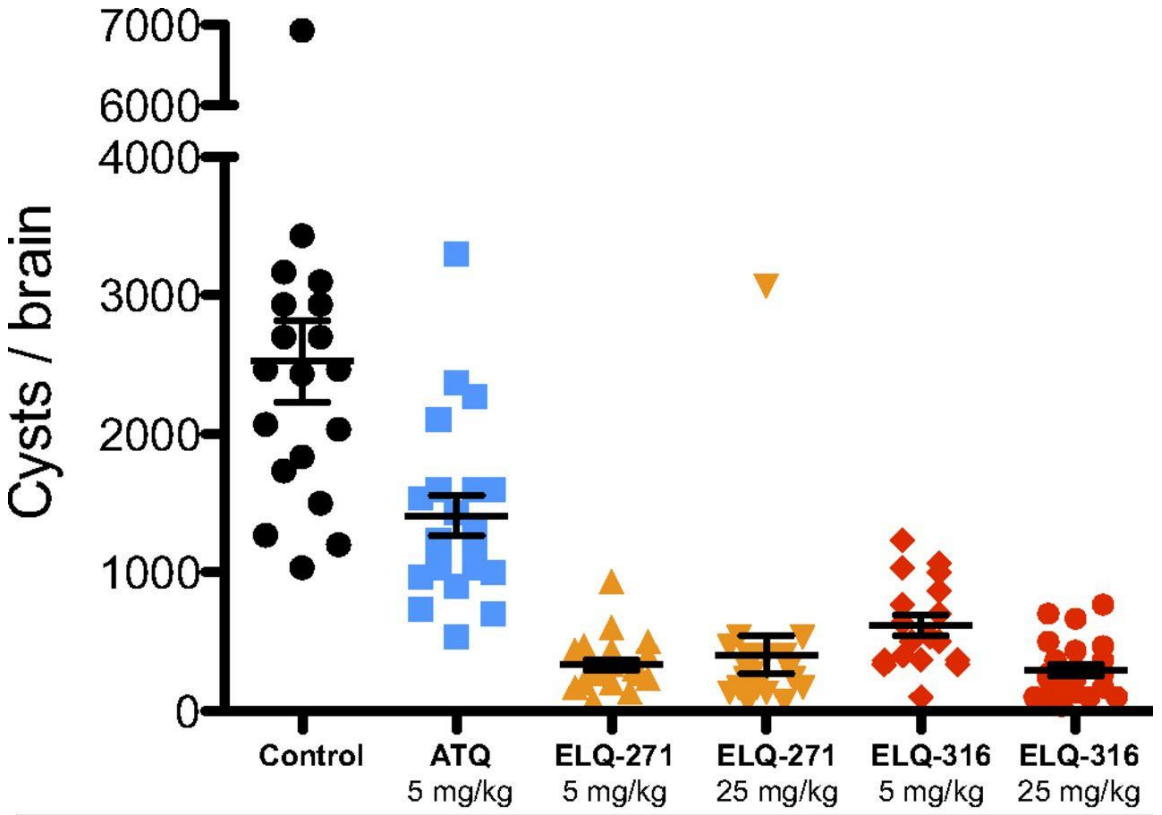
Schizophrenia Bulletin

Neuroanatomic abnormalities related to herpes simplex virus type 1 in schizophrenia



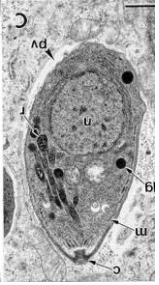
Schretlen et al, 2010

The efficacy of ELQ-271, ELQ-316, and atovaquone against latent T. gondii infection.



	Control	ATQ 5 mg/kg	ELQ-271 5 mg/kg	ELQ-271 25 mg/kg	ELQ-316 5 mg/kg	ELQ-316 25 mg/kg
Mean number of cysts/ brain (SEM)	2523 (±294)	1408 (±143)	333 (±38)	402 (±137)	617 (±74)	296 (±43)
% Cyst reduction from control	0	44%	87%	84%	76%	88%

Doggett J S et al. PNAS 2012;109:15936-15941



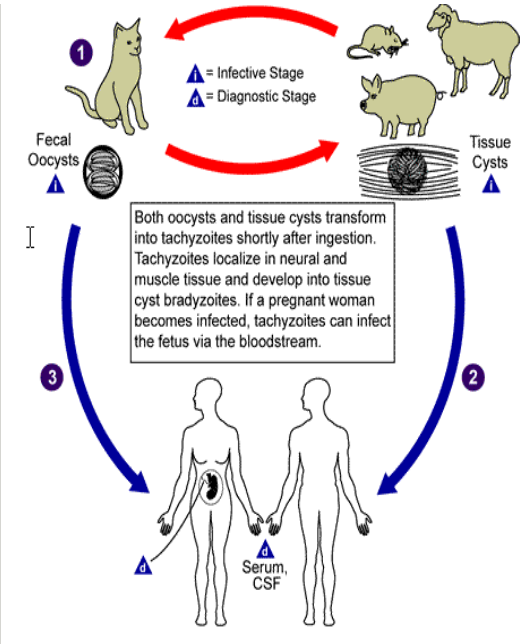
Toxoplasma gondii
Biology and Epidemiology

- Obligate intracellular protozoan of the Family *Apicomplexa*
- Complete life cycle in feline: Definitive hosts
- Abortive life cycle in humans and other animals resulting in lifelong cysts: Intermediate hosts
- 3 major well-defined genotypes I, II, III
- High prevalence in humans
  - 8-25% in developed countries
  - 30%-90% in less developed countries
  - Additional variation by geography and diet.
- Route of infection

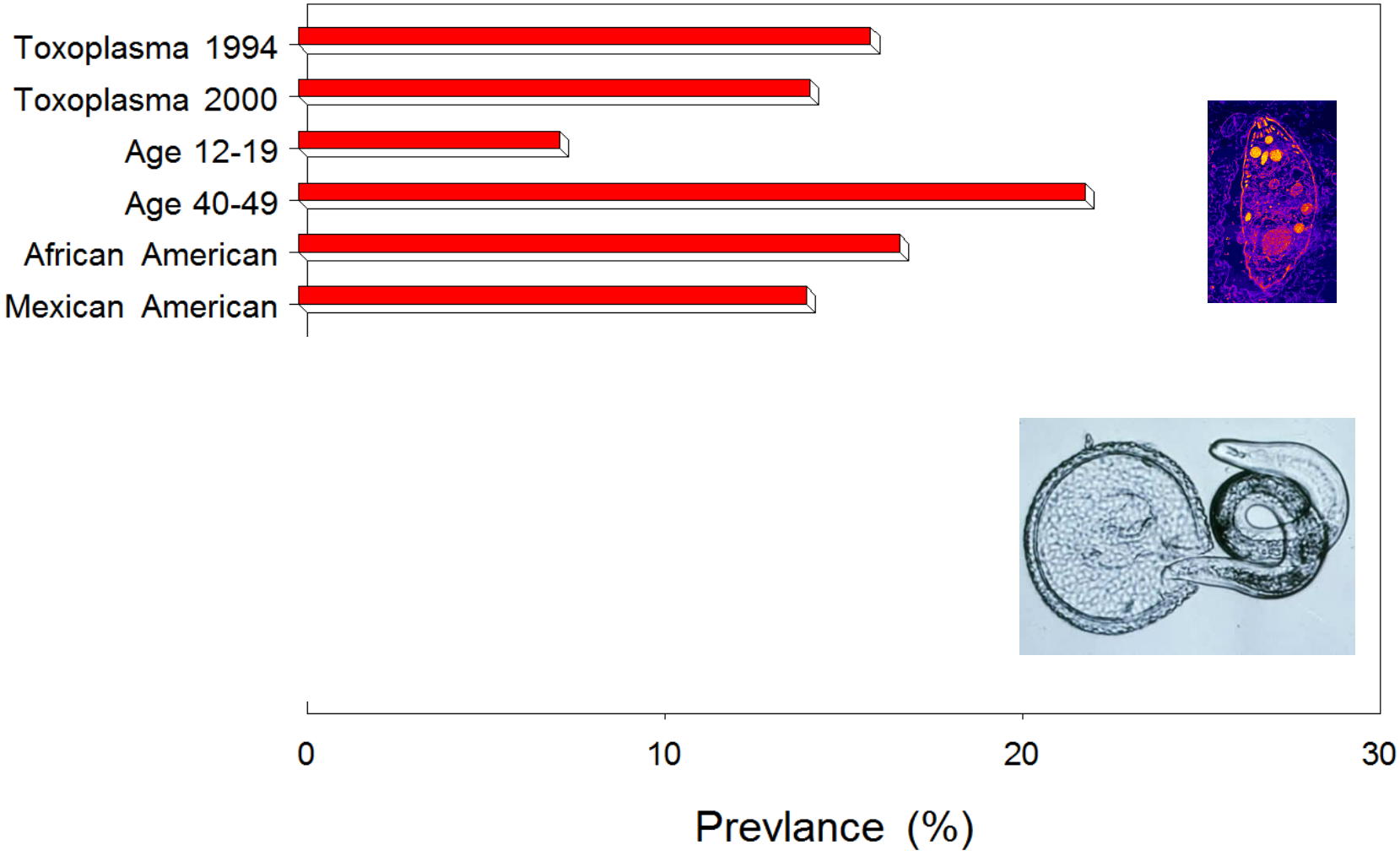
  Ingestion of oocytes from cat feces

  Eating of undercooked meat

  Drinking of contaminated water



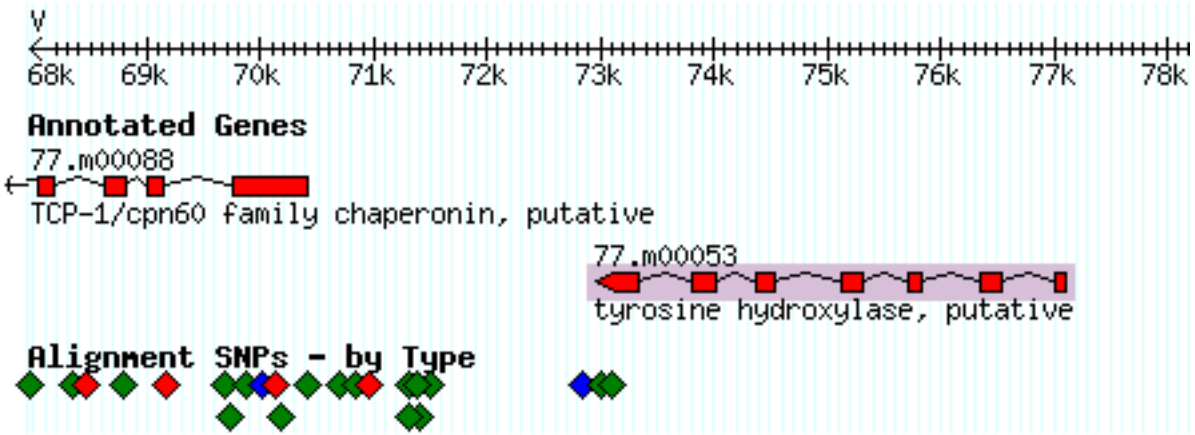
Toxoplasma Infection in the United States (NHANES)

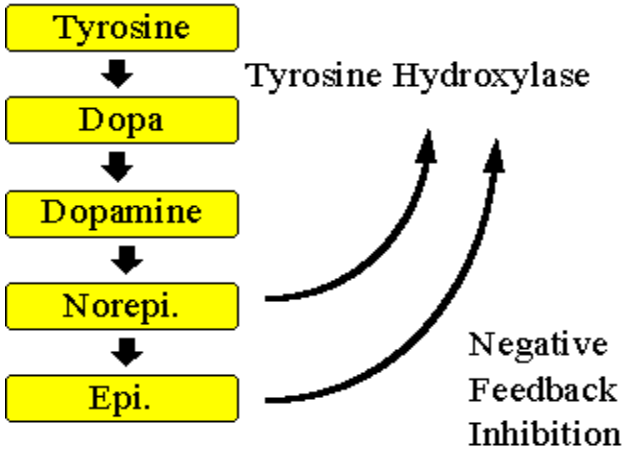


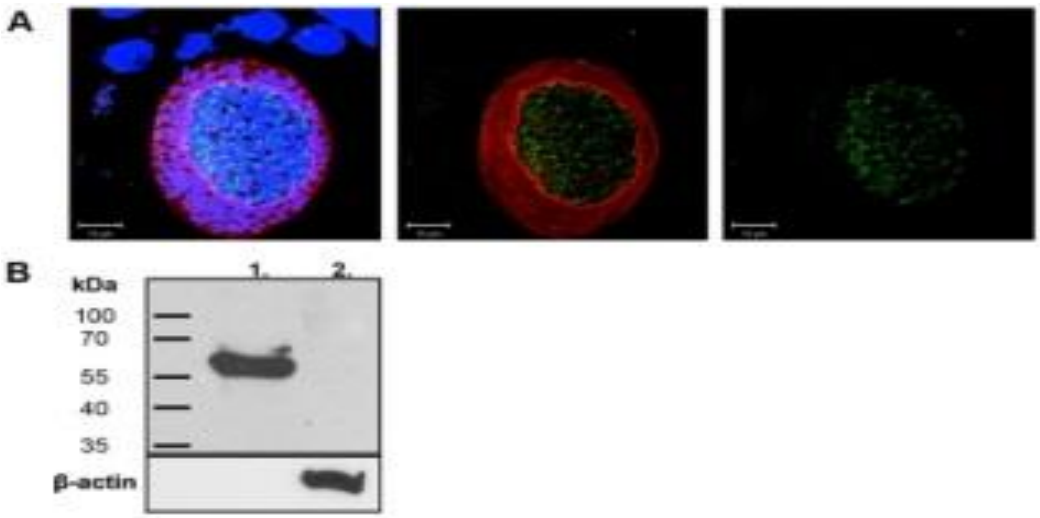
Risk Factors for Toxoplasma Infection

Toxoplasmosis is considered to be a leading cause of death attributed to foodborne illness in the United States. More than 60 million men, women, and children in the U.S. carry the *Toxoplasma* parasite, but very few have symptoms because the immune system usually keeps the parasite from causing illness.

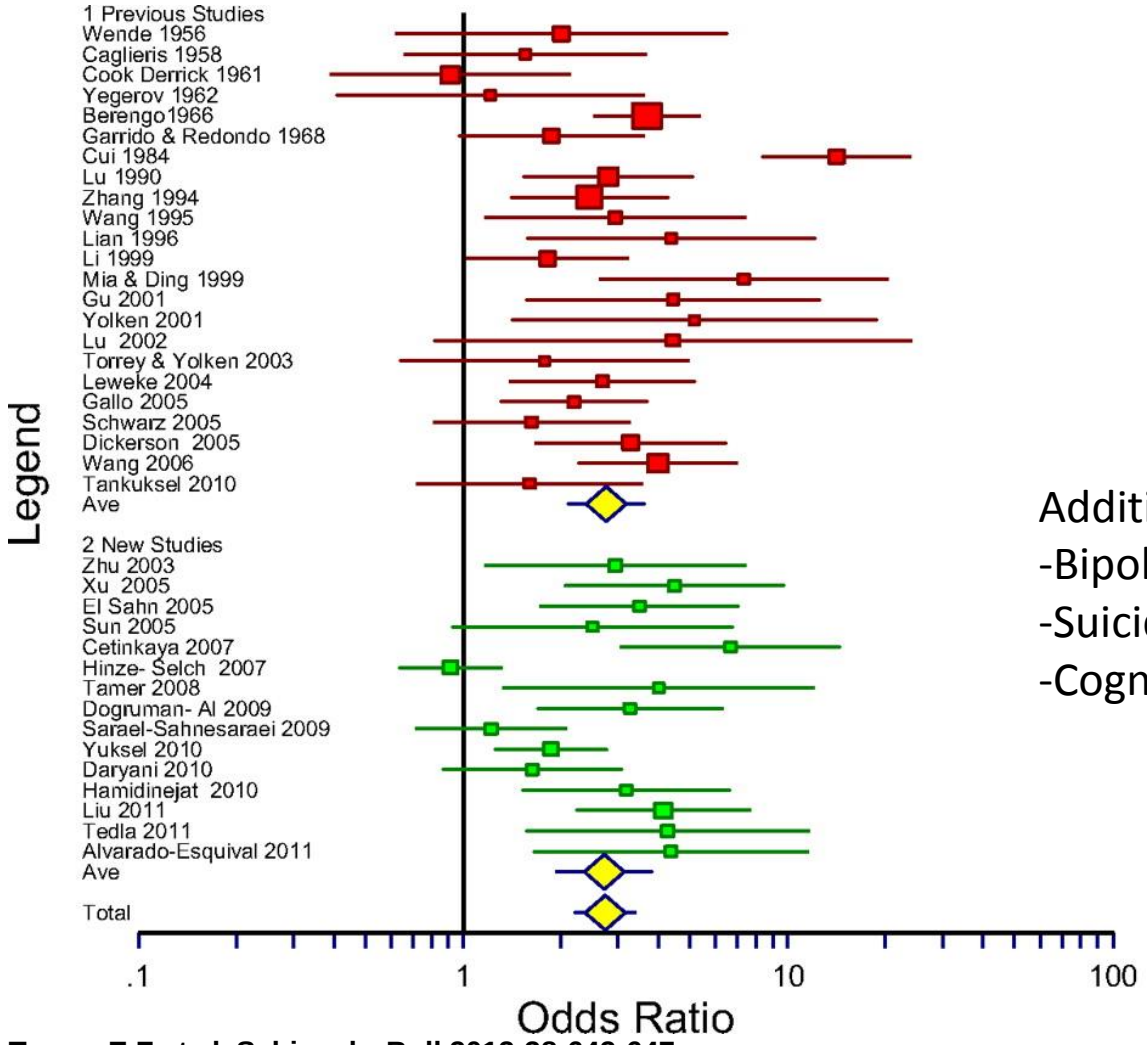
Toxoplasma gondii contains genes which encode Tyrosine Hydroxylase and D4 Receptor







Toxoplasma gondii
Meta-analysis of Association with Schizophrenia



Additional associations with
-Bipolar disorder
-Suicide
-Cognitive impairment

Torrey E F et al. Schizophr Bull 2012;38:642-647

Schizophrenia Bulletin