Clinical Genomics Perspective

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Learning Objectives

1. Participants will learn about the current state of genetics, including genome wide association studies (GWAS), Copy Number Variants (CNVs) and next generation sequencing.
2. Some Case Illustrations will be presented.
3. A review of current progress in psychiatric genetics will be presented.

Learning Objective #1 – Current methods: Association testing

- Is a mutation associated more with people with a disease vs. people without the disease?
- This is Case-Control association.
- If testing only one marker or mutation (or SNP or CNV), then p value of 0.05 is accepted for rejection of null hypothesis with Fisher's exact t-test.
- If one tests, for example, 610,000 mutations or markers, then the significance level must be adjusted for multiple testing, so 0.05 divided by 610,000 = 8e-8.

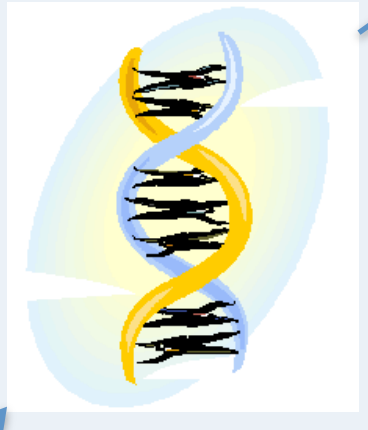
Single Nucleotide Polymorphism (SNP)

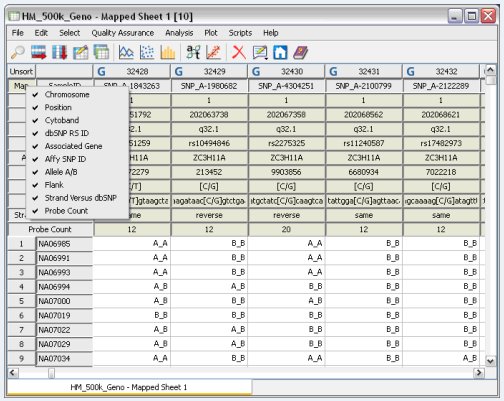
G A T T A C A
C T A A A G T

G A T G A C A
C T A C A G T

- Every 100-200 base pairs
  - ~20,500,000 SNPs currently known
- At least 1% of population
- Labeled with an rs #
  - (i.e. rs1234)

Measuring SNPs





What are SNPs good for?

- Everything?
- i.e. Earwax type →

Genotype	Effect
rs17822931(C;C)	Wet earwax
rs17822931(C;T)	Wet earwax
Rs17822931(T;T)	Dry earwax

nature genetics

Nature Genetics **38**, 324 - 330 (2006)
Published online: 29 January 2006; | doi:10.1038/ng1733

A SNP in the *ABCC11* gene is the determinant of human earwax type

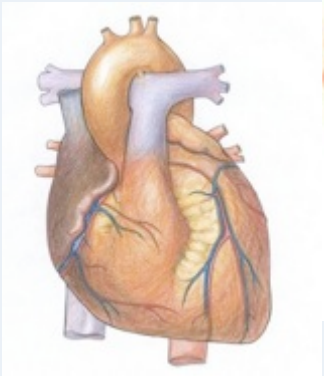
Koh-ichiro Yoshiura[1, 2], Akira Kinoshita[1, 2], Takafumi Ishida[3], Aya Ninokata[3], Toshihisa

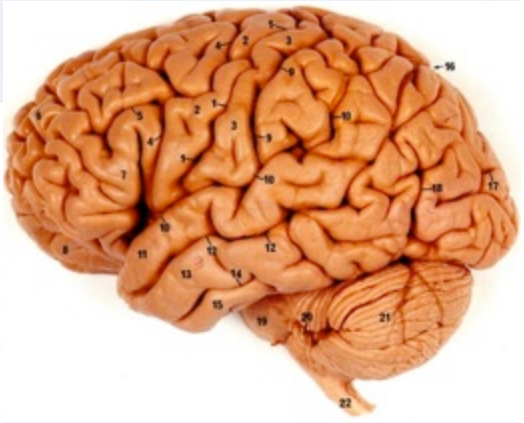
An Example with ApoE4

Alzheimer's – ApoE4

Genotype	Effect
rs429358(C;C)*	>10x increased risk for Alzheimer's 1.4x increased risk for heart disease
rs429358(C;T)	>3x increased risk for Alzheimer's 1.4x increased risk for heart disease
rs429358(T;T)	Common

*~15% of the population carries a C





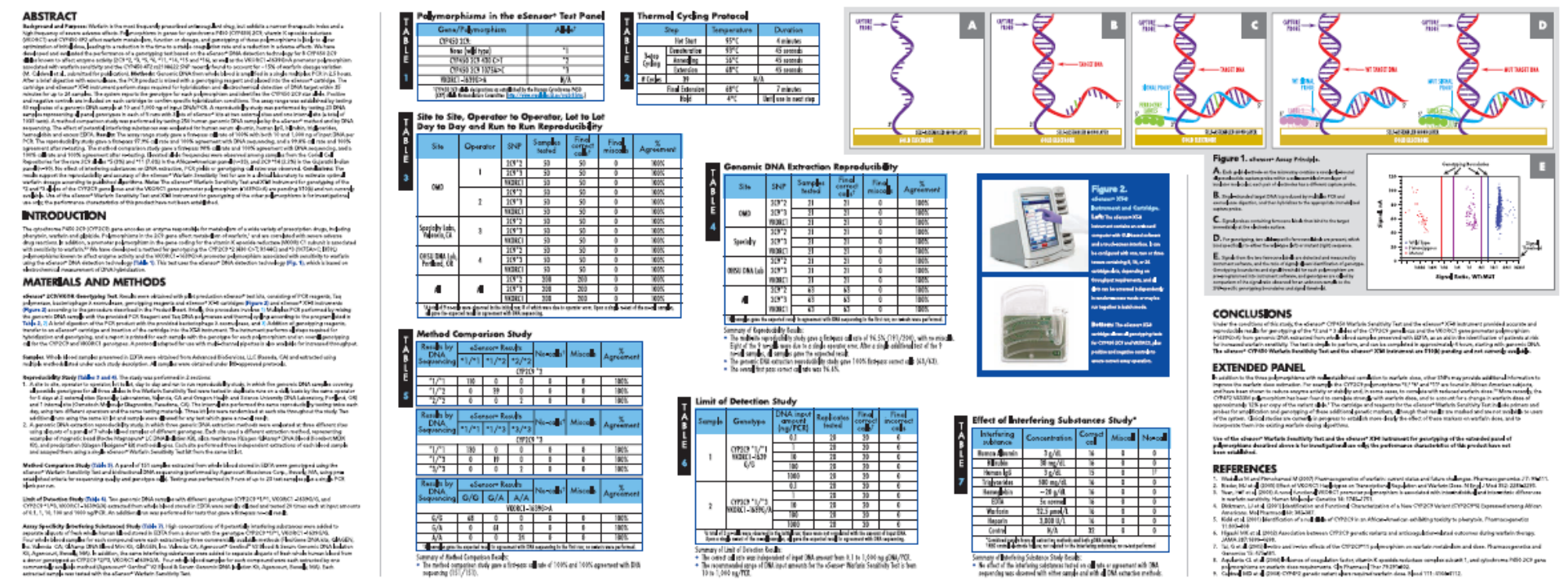
Wafarin Sensitivity

- Polymorphisms in 2C9, VKORC1 & 4F2 to optimize dosage
  - rs2108622 accounts for 15% of dosage variation

DEVELOPMENT AND EVALUATION OF AN eSENSOR® GENOTYPING TEST FOR POLYMORPHISMS ASSOCIATED WITH WARFARIN SENSITIVITY

OSMETECH MOLECULAR DIAGNOSTICS

B. Coty, M. Reed, A. Jacobs, P. Naranatt, R. Hubert, S. Panuganti, Z. Wang, V. Headley, M. Abedi, Y. Liu, D. Cochran, C. Argueta, L. Richter, R. Lopez, H. Wang, G. Gust • Osmetech Molecular Diagnostics, Pasadena, California



ABSTRACT

INTRODUCTION

MATERIALS AND METHODS

TABLE 1 Polymorphisms in the eSensor® Test Panel

TABLE 2 Thermal Cycling Protocol

TABLE 3 Site to Site, Operator to Operator, Lot to Lot Day to Day and Run to Run Reproducibility

TABLE 4 Genomic DNA Extraction Reproducibility

TABLE 5 Method Comparison Study

TABLE 6 Limit of Detection Study

TABLE 7 Effect of Interfering Substances Study*

Figure 1. eSensor® Assay Principle.

Figure 2. eSensor® XT-8 Instrument and Cartridges.

CONCLUSIONS

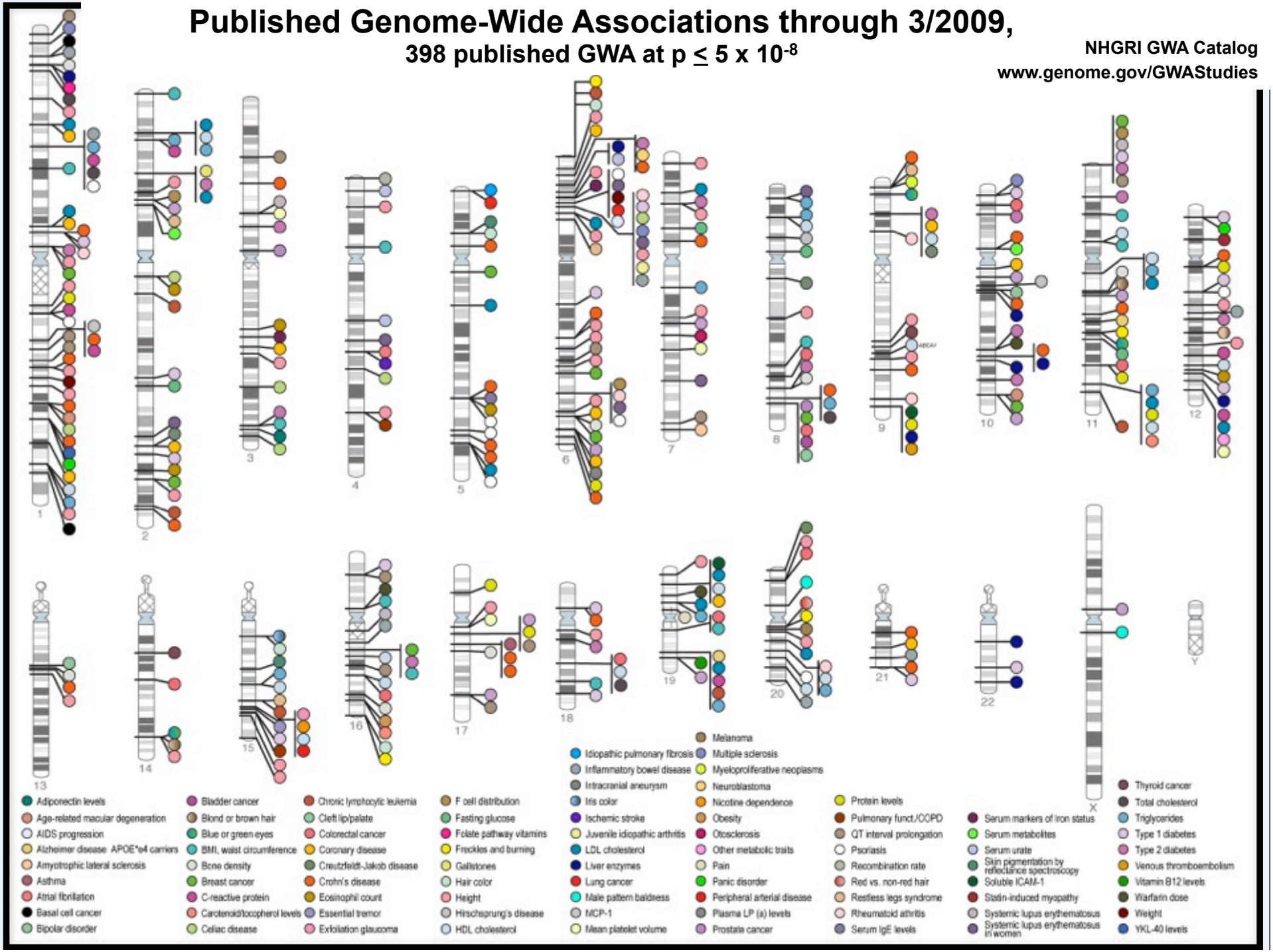
EXTENDED PANEL

REFERENCES

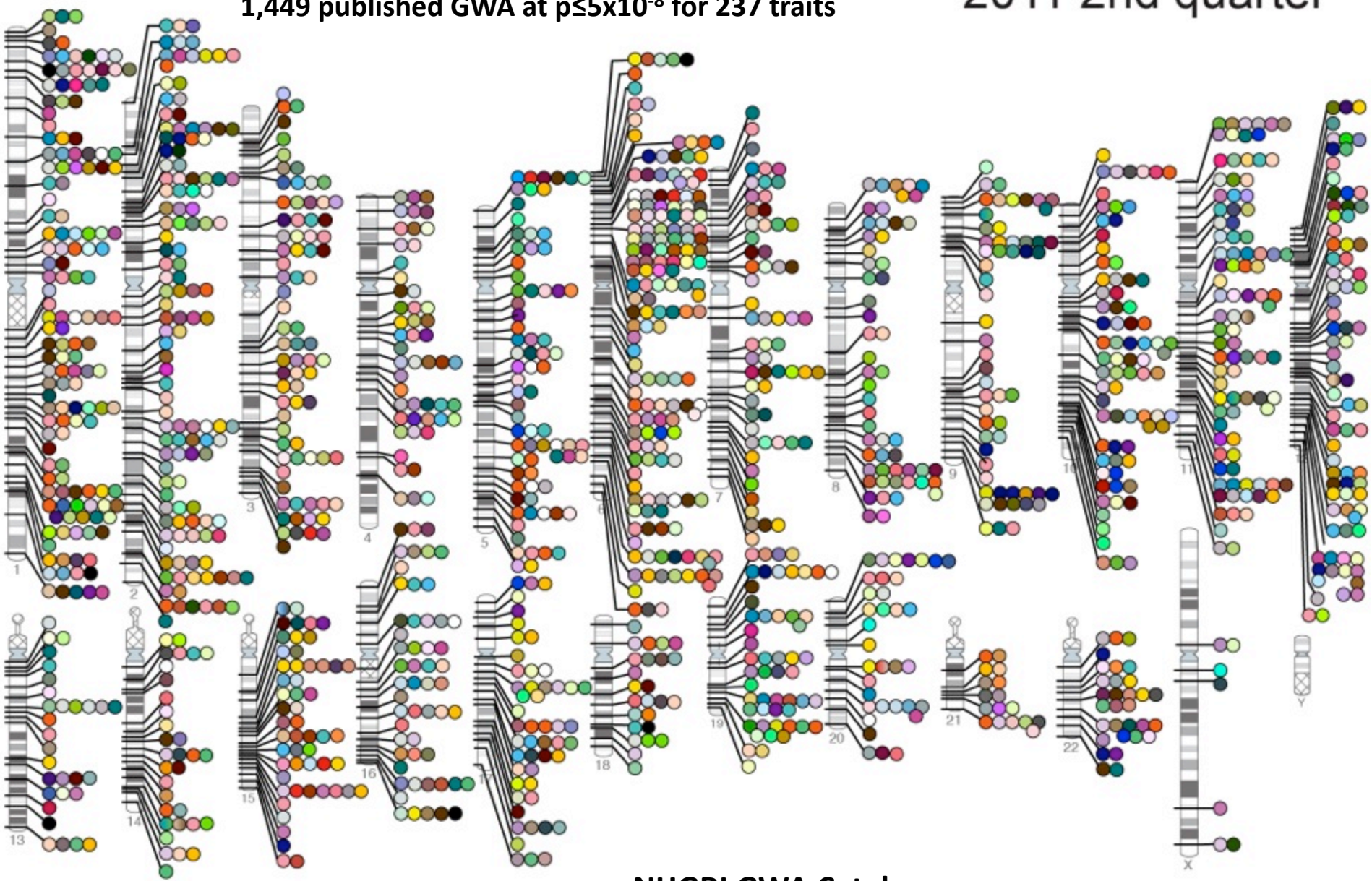
Published Genome-Wide Associations through 3/2009,

398 published GWA at $p \leq 5 \times 10^{-8}$

NHGRI GWA Catalog
www.genome.gov/GWAStudies



Published Genome-Wide Associations through 06/2011,
1,449 published GWA at $p \leq 5 \times 10^{-8}$ for 237 traits



NHGRI GWA Catalog
www.genome.gov/GWAStudies


Abdominal aortic aneurysm
Acute lymphoblastic leukemia
Adhesion molecules
Adiponectin levels
Age-related macular degeneration
AIDS progression
Alcohol dependence
Alopecia areata
Alzheimer disease
Amyloid A levels
Amyotrophic lateral sclerosis
Angiotensin-converting enzyme activity
Ankylosing spondylitis
Arterial stiffness
Asparagus anosmia
Asthma
Atherosclerosis in HIV
Atrial fibrillation
Attention deficit hyperactivity disorder
Autism
Basal cell cancer
Behcet's disease
Bipolar disorder
Biliary atresia
Bilirubin
Bitter taste response
Birth weight
Bladder cancer
Bleomycin sensitivity
Blond or brown hair
Blood pressure
Blue or green eyes
BMI, waist circumference
Bone density
Breast cancer
C-reactive protein
Calcium levels
Cardiac structure/function
Cardiovascular risk factors
Carnitine levels
Carotenoid/tocopherol levels
Celiac disease
Celiac disease and rheumatoid arthritis
Cerebral atrophy measures
Chronic lymphocytic leukemia
Chronic myeloid leukemia
Cleft lip/palate
Coffee consumption
Cognitive function
Conduct disorder
Colorectal cancer
Corneal thickness
Coronary disease
Creutzfeldt-Jakob disease
Crohn's disease
Crohn's disease and celiac disease
Cutaneous nevi
Cystic fibrosis severity
Dermatitis
DHEA-s levels
Diabetic retinopathy
Dilated cardiomyopathy
Drug-induced liver injury
Drug-induced liver injury (amoxicillin-clavulanate)
Endometrial cancer
Endometriosis
Eosinophil count
Eosinophilic esophagitis
Erectile dysfunction and prostate cancer treatment
Erythrocyte parameters
Esophageal cancer
Essential tremor
Exfoliation glaucoma
Eye color traits
F cell distribution
Fibrinogen levels
Folate pathway vitamins
Follicular lymphoma
Fuch's corneal dystrophy
Freckles and burning
Gallstones
Gastric cancer
Glioma
Glycemic traits
Hair color
Hair morphology
Handedness in dyslexia
HDL cholesterol
Heart failure
Heart rate
Height
Hemostasis parameters
Hepatic steatosis
Hepatitis
Hepatocellular carcinoma
Hirschsprung's disease
HIV-1 control
Hodgkin's lymphoma
Homocysteine levels
Hypospadias
Idiopathic pulmonary fibrosis
IFN-related cytopeni
IgA levels
IgE levels
Inflammatory bowel disease
Insulin-like growth factors
Intracranial aneurysm
Iris color
Iron status markers
Ischemic stroke
Juvenile idiopathic arthritis
Keloid
Kidney stones
LDL cholesterol
Leprosy
Leptin receptor levels
Liver enzymes
Longevity
LP (a) levels
LpPLA(2) activity and mass
Lung cancer
Magnesium levels
Major mood disorders
Malaria
Male pattern baldness
Mammographic density
Matrix metalloproteinase levels
MCP-1
Melanoma
Menarche & menopause
Meningococcal disease
Metabolic syndrome
Migraine
Moyamoya disease
Multiple sclerosis
Myeloproliferative neoplasms
Myopia (pathological)
N-glycan levels
Narcolepsy
Nasopharyngeal cancer
Natriuretic peptide levels
Neuroblastoma
Nicotine dependence
Obesity
Open angle glaucoma
Open personality
Optic disc parameters
Osteoarthritis
Osteoporosis
Otosclerosis
Other metabolic traits
Ovarian cancer
Pancreatic cancer
Pain
Paget's disease
Panic disorder
Parkinson's disease
Periodontitis
Peripheral arterial disease
Personality dimensions
Phosphatidylcholine levels
Phosphorus levels
Photic sneeze
Phytosterol levels
Platelet count
Polycystic ovary syndrome
Primary biliary cirrhosis
Primary sclerosing cholangitis
PR interval
Progranulin levels
Progressive supranuclear palsy
Prostate cancer
Protein levels
PSA levels
Psoriasis
Psoriatic arthritis
Pulmonary funct. COPD
QRS interval
QT interval
Quantitative traits
Recombination rate
Red vs.non-red hair
Refractive error
Renal cell carcinoma
Renal function
Response to antidepressants
Response to antipsychotic therapy
Response to carbamazepine
Response to clopidogrel therapy
Response to hepatitis C treat
Response to interferon beta therapy
Response to metaformin
Response to statin therapy
Restless legs syndrome
Retinal vascular caliber
Rheumatoid arthritis
Ribavirin-induced anemia
Schizophrenia
Serum metabolites
Skin pigmentation
Smoking behavior
Speech perception
Sphingolipid levels
Statin-induced myopathy
Stroke
Sudden cardiac arrest
Suicide attempts
Systemic lupus erythematosus
Systemic sclerosis
T-tau levels
Tau AB1-42 levels
Telomere length
Testicular germ cell tumor
Thyroid cancer
Thyroid volume
Tooth development
Total cholesterol
Triglycerides
Tuberculosis
Type 1 diabetes
Type 2 diabetes
Ulcerative colitis
Urate
Urinary albumin excretion
Urinary metabolites
Uterine fibroids
Venous thromboembolism
Ventricular conduction
Vertical cup-disc ratio
Vitamin B12 levels
Vitamin D insuffiency
Vitiligo
Warfarin dose
Weight
White cell count
White matter hyperintensity
YKL-40 levels

Copy Number Variants (CNVs) or INDELS (insertions or deletions)

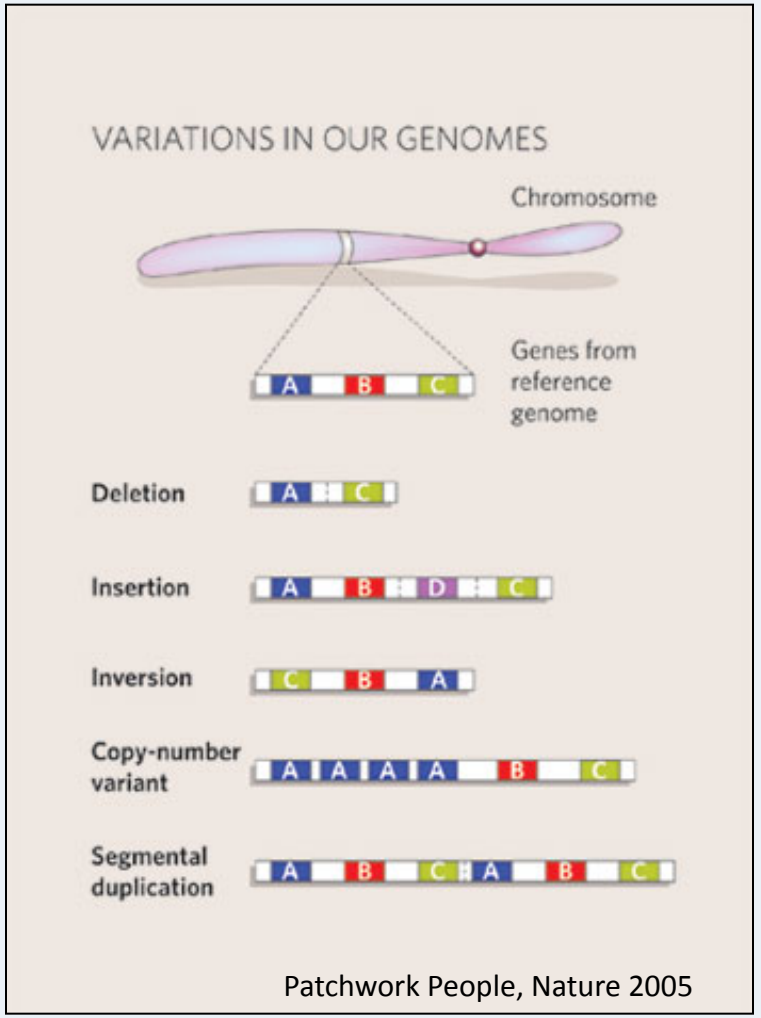
A microindel:

G A T T A C A

C T A A A G T

G A C A

C T A A A G T



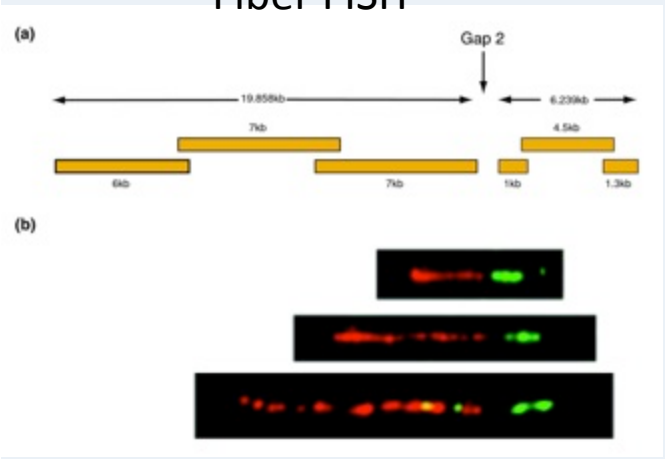
Patchwork People, Nature 2005

Some facts about 

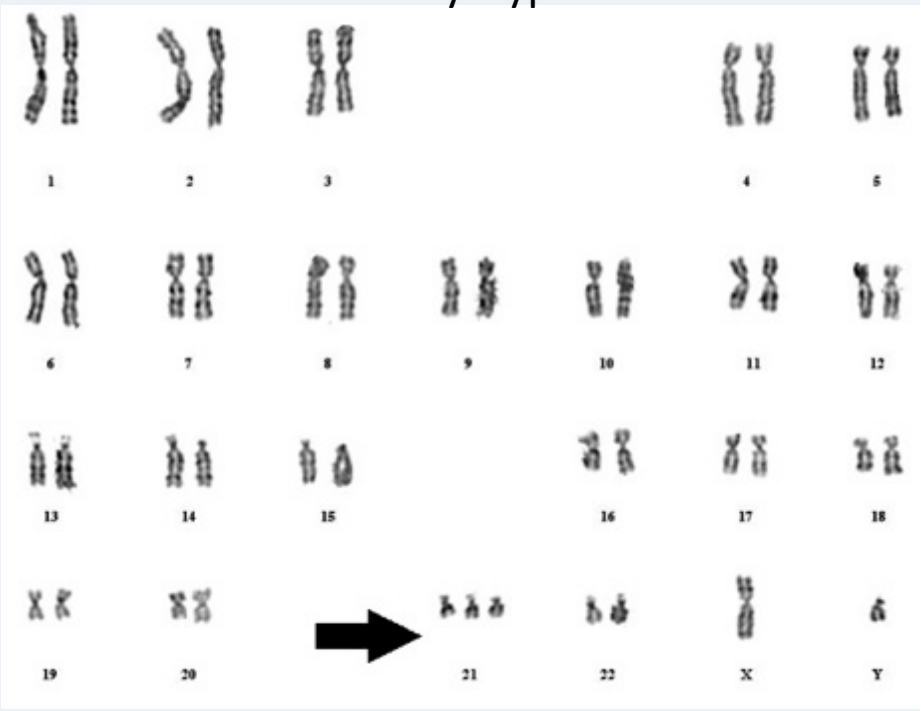
- Insertions & Deletions (1000 base pairs or more)
- >1% of our genomes differ in copy number
  - Compared to ~0.1% for SNPs
- New mutation rate 3-4x higher than SNPs
  - But only 1% of CNVs are new mutations (most are inherited)
- Can influence gene expression even 1 megabase away
- Types of CNVs
  - ~40% are deletions
  - ~40% duplications
  - ~20% other (i.e. both, complex, multi-allelic)

Measuring CNVs

Fiber FISH



Karyotype



Microarray

qPCR

CGH Array

Q: Do I have any CNVs?

A: Yes. Lots.

Q: How many?

A: It varies per person but in the range of Hundreds.

CNV Detection: An Imperfect Science

Venter DNA

Platform	Software	CNVs	Unique	Median Size (kb)
Affy 6.0	Partek	12	0	38
	Affy GTC	29	9	46
	iPattern	77	24	9
	Birdsuite	126	75	10
Illumina 1M	quantiSNP	36	9	38
	iPattern	53	28	28
	PennCNV	63	41	23
Nimblegen 42M	GADA	1095	974	3

CNVs & Disease

- Autism
  - dup 15q11-13 (maternal) (1994)
- Schizophrenia
  - del 22q11 (VCFS) (1999)
- Parkinsons
  - dup SNCA (2003)
- HIV/AIDS risk
  - dup CCL3L1 (2003)
- Autoimmunity
  - del FCGR3B & SLE (2006)
- Crohn's disease
  - ≤ 3 copies of HBD-2 = higher risk (2006)
- Age-related macular degeneration (2006)
  - del CFHR1 and CFHR3 = lower risk
- Alzheimer's disease
  - dup APP (2006)
- Pancreatitis
  - dup PRSS1 (2006)
- Prostate cancer
  - del UGT2B17 ? (2006)
- ITP
  - variation in FCGR2 and FCGR3 (2008)

1990 1995 2005 **2010**

Clinical Management and Genetics

Genet Med. 2011 Sep;13(9):770-6.

Chromosomal microarray testing influences medical management.

Coulter ME, Miller DT, Harris DJ, Hawley P, Picker J, Roberts AE, Sobeih MM, Irons M.

PURPOSE:

Chromosomal microarray (CMA) testing provides the highest diagnostic yield for clinical testing of patients with developmental delay (DD), intellectual disability (ID), multiple congenital anomalies (MCA), and autism spectrum disorders (ASD). Despite improved diagnostic yield and studies to support cost-effectiveness, concerns regarding the cost and reimbursement for CMA have been raised because it is perceived that CMA results do not influence medical management.

METHODS:

We conducted a retrospective chart review of CMA testing performed during a 12-month period on patients with DD/ID, ASD, and congenital anomalies to determine the proportion of cases where abnormal CMA results impacted recommendations for clinical action.

RESULTS:

Among 1792 patients, 13.1% had clinically relevant results, either abnormal (n = 131; 7.3%) or variants of possible significance (VPS; n = 104; 5.8%). Abnormal variants generated a higher rate of recommendation for clinical action (54%) compared with VPS (34%; Fisher exact test, P = 0.01). CMA results influenced medical care by precipitating medical referrals, diagnostic imaging, or specific laboratory testing.

CONCLUSIONS:

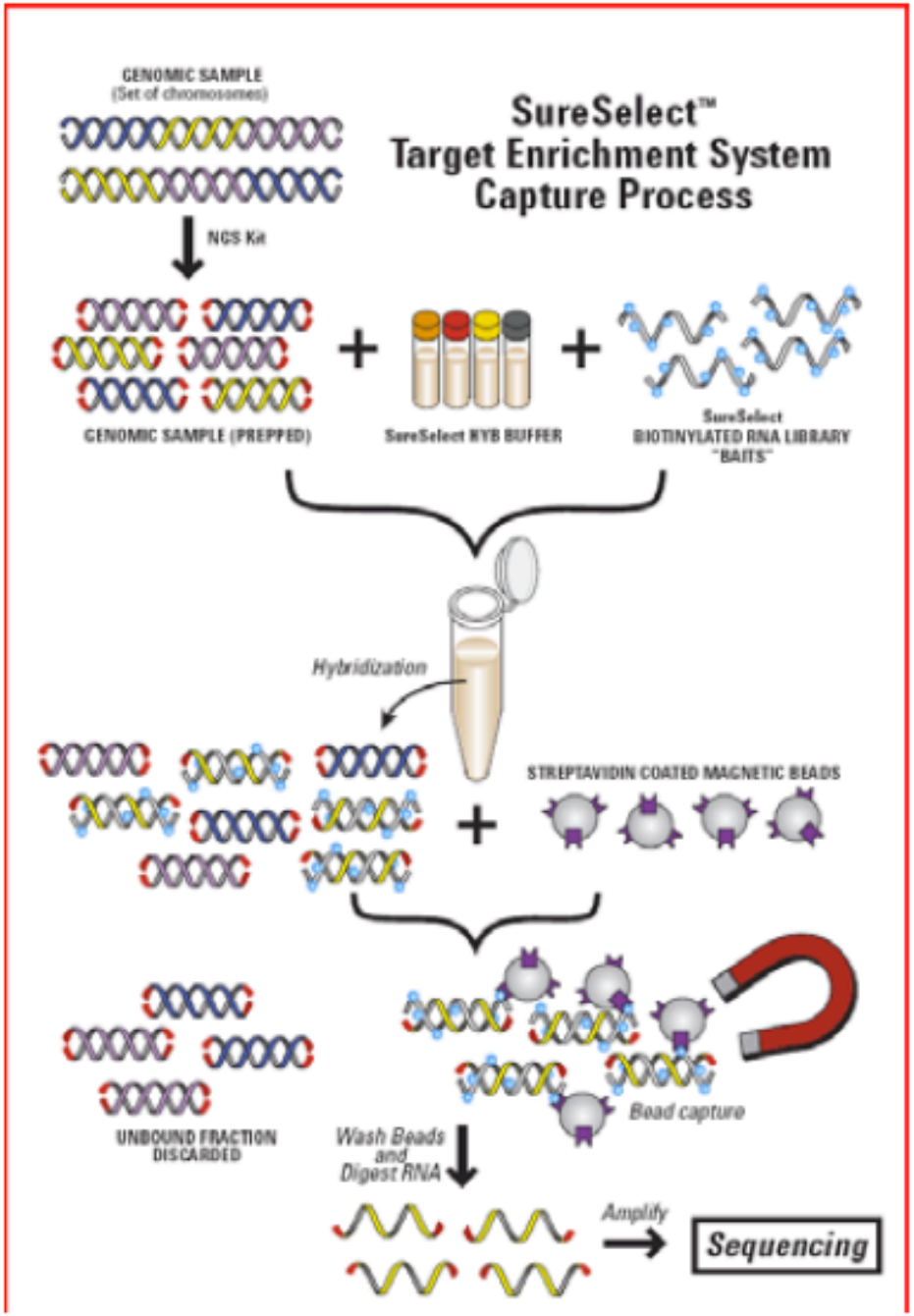
For all test indications, CMA results influenced medical management in a majority of patients with abnormal variants and a substantial proportion of those with VPS. These results support the use of CMA as a clinical diagnostic test that influences medical management for this patient population.

Learning Objective #1: Massively Parallel and Next Generation Sequencing

Exome Sequencing is a Revolutionary Technology!

Agilent Technologies SureSelect method

Whole-exome kit 38Mb and 50Mb



http://cp.literature.agilent.com/litweb/pdf/5990-3532EN.pdf

Learning Objective #2:
Some Case Illustrations

The Biology of MENTAL DEFECT

BY

LIONEL S. PENROSE, M.A., M.D.

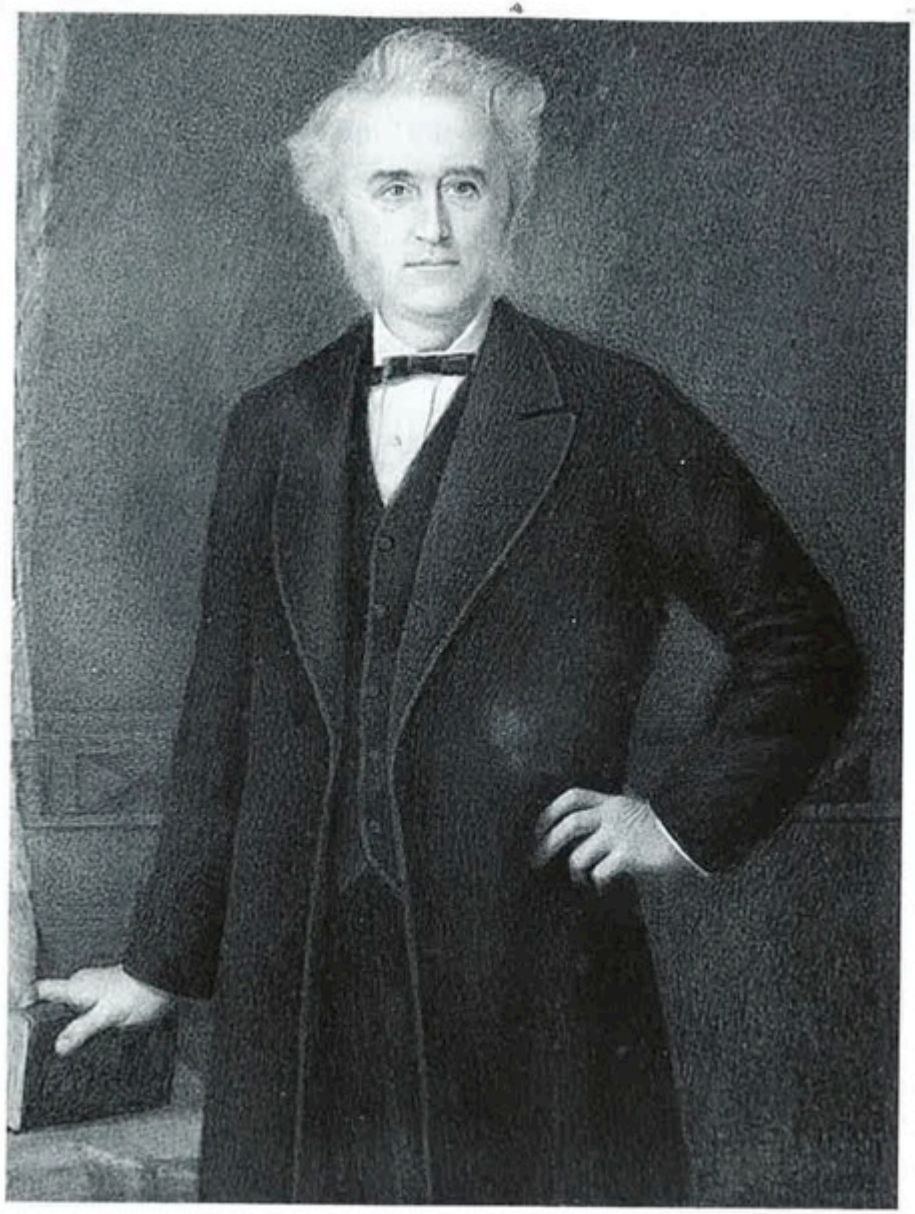
WITH A PREFACE BY

PROFESSOR J. B. S. HALDANE, F.R.S.

GRUNE & STRATTON

New York

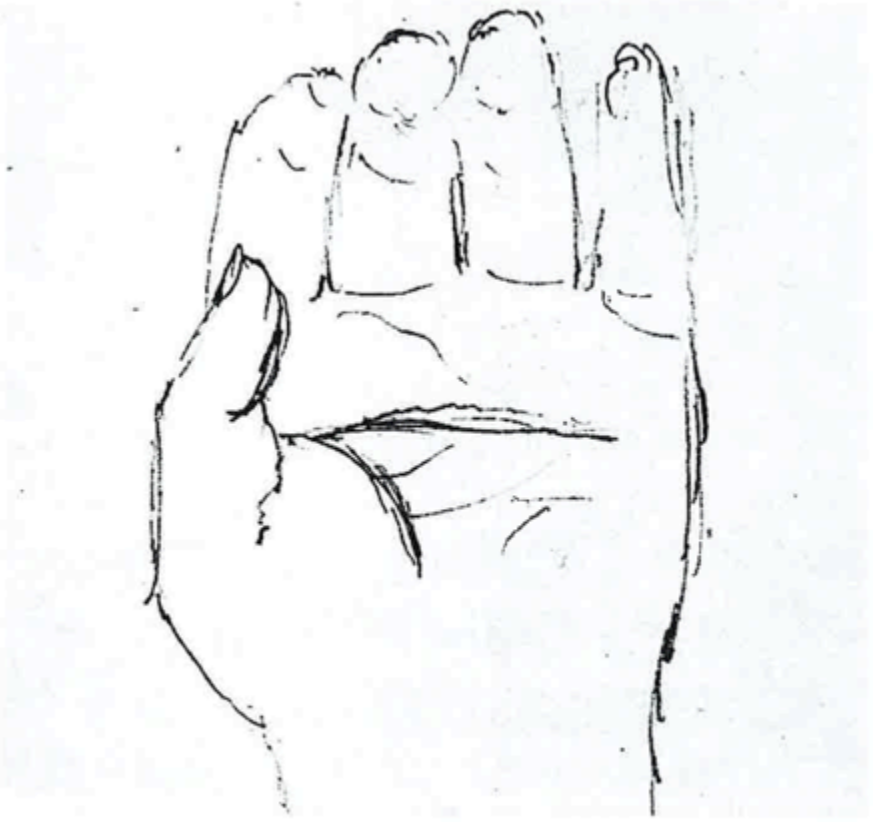
1949



Portrait of Langdon Down, painted by Sydney Hodges in 1883.

Plate VII—Mongolism in two imbecile brothers aged 10 (Colchester Survey, 1938, Case No. 750) and 5 years, with a normal child aged 2½ years.

As compared with the normal child, the younger mongoloid is seen to have a small head, decreased stature and dysplastic features. The characteristic fold of skin covering the inner canthus of each eye (epicanthic fold) was clearly marked in this case.



Reginald Langdon Down was the first to describe the pattern of creases in the palm in Down's syndrome patients. He drew this sketch in 1908.

Published in “Biology of Mental Defect”, by Lionel Penrose, 1949
And “John Langdon Down: A Caring Pioneer”, by O Conor Ward, 1998.

Mary A, the first Down's syndrome patient admitted to Normansfield, photographed when she was 19 and again when she was 55. She lived to the age of 58.

Florence T, a Down's syndrome patient at Normansfield. Photographed in 1886 when she was seven and again in 1899 aged 20.

Published in “John Langdon Down: A Caring Pioneer”, by O Conor Ward, 1998.

Langdon Down began to take clinical photographs in 1862. His first photograph of an Earlswood resident with Down's syndrome was this unnamed girl in the 1865 series. She was probably the first ever Down's syndrome patient to be photographed.

Published in "John Langdon Down: A Caring Pioneer", by O Conor Ward, 1998.

Four Down's syndrome patients. Part of the Earlswood series, photographed in 1865.

Dr Reginald Langdon Down with his daughters Stella and Elspie. Stella married Russell Brain and became Lady Brain. Elspie was an artist. The only son was John, who had Down's syndrome.

Dr Percival Langdon Down with his wife and children. His son Norman, was to be the last Langdon Down superintendent of Normansfield, ending a family connection that had lasted for 102 years. The elder daughter, Molly, was also a doctor and worked in Normansfield.

Langdon Down's personal patients with his syndrome[2]

Name	Age Admitted	Date Admitted	Outcome	Comment
Mary A	19	12.5.68	Died 1907, age 58	Cardiac failure, Alzheimer's
Cecelia GA	10	7.6.68	Died 31.1.70, age 12	Fatal scarlet fever
Herbert H	8	15.7.68	Discharged 10.10.68	Improved
Edward GP	11	1.5.69	Died 1908, age 50	
Laura M	7	5.4.69	Died 5.4.77, age 15	Tuberculosis: Query
Walter AP	4	4.11.75	Discharged 27.1.77	Masturbation cured
Margaret DE	11	14.4.74	Died 15.5.74, age 11	Fatal scarlet fever
Norah MT	12	23.4.74	Died 26.6.74, age 12	Acute Bronchitis
James DKW	5	10.1.77	Died 30.12.77, age 12	Bronchitis and Pneumonia
Norman MB	10	14.2.77	Died 12.1.12, age 45	Alzheimer's?
Thomas N	6	13.11.77	Died 1896, age 25	Cardiac failure
Margaret AW	4	11.3.80	Died 1885, age 9	Sudden death on holiday
George HW	6	27.3.80	Died 27.11.80, age 7	Laryngo bronchitis, croup
Cathy MS	9	28.3.82	Died 20.8.82, age 9	Bronchitis and pneumonia
Lucy EN	11	22.8.82	Died 3.11.85, age 14	Broncho-pneumonia, cardiac failure
Ada FH	15	2.12.82	Alive 1895	
Elizabeth G	5	27.10.83	Discharged 16.2.87	Improved
Florence ET	7	8.3.86	Alive 1895	
David AH	6	5.4.72	Died 1915. age 49	Late onset of blindness and deafness
Constance AW	13	31.7.86	Discharged 12.5.88	Improved
Ann MR	17	18.11.86	Discharged 26.5.91	Improved
John GT	15	6.7.74	Died 4.6.18, age 59	Alzheimer's?

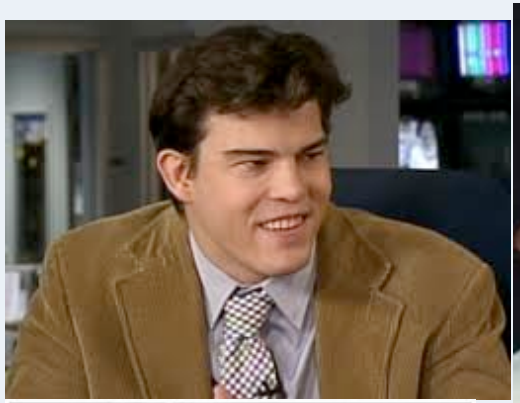
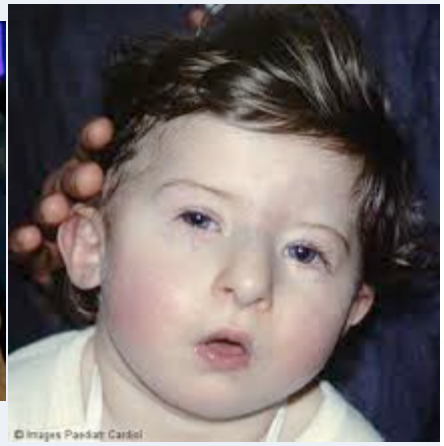
Down Syndrome

Down Syndrome

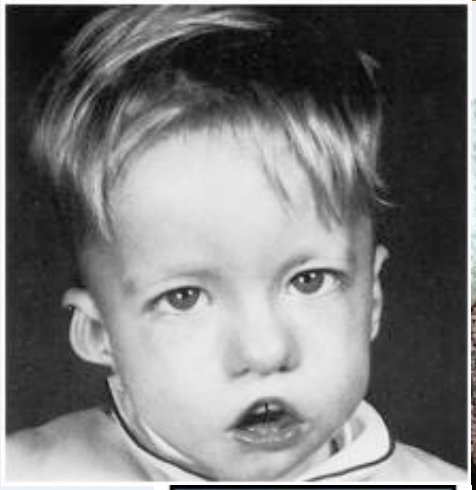
Christopher Joseph "Chris" Burke (born August 26, 1965) is an American actor and folk singer, who lives with Down syndrome, who has become best known for his character Charles "Corky" Thacher on the television series Life Goes On.

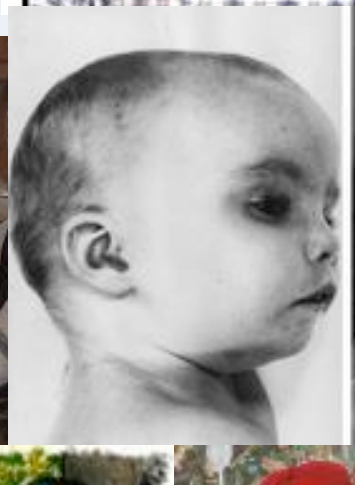
And there are people with Mosaic Down Syndrome, who are much less affected.

Velocardiofacial (22q11.2) Syndrome

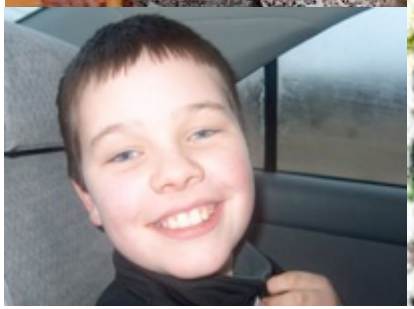





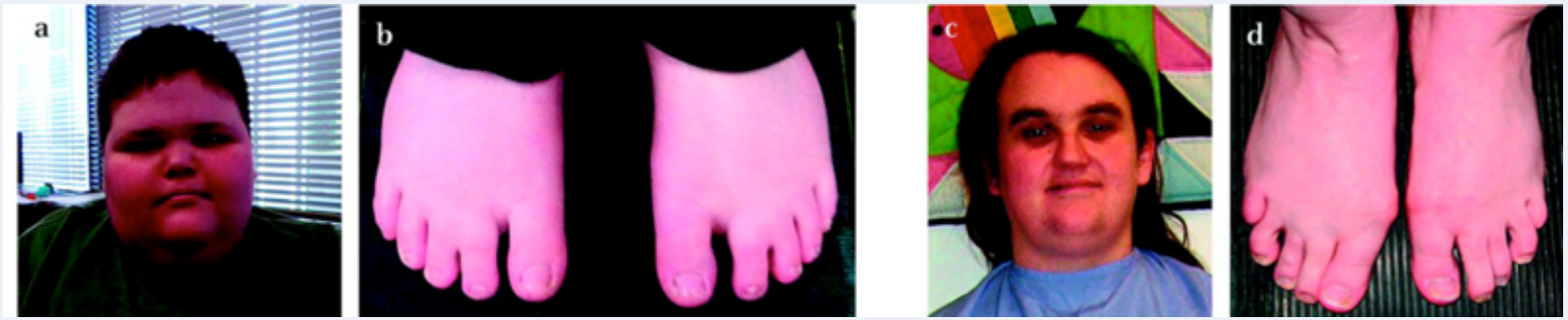








16p11.2 deletion



Clinical photographs. (a and b) Proband 2 (de novo deletion 16p11.2). Note long narrow palpebral fissures, short delicate nose, short neck and brachydactyly with 2–3 cutaneous toe syndactyly. (c and d) Mother of proband 3 (both with deletions). Note her large ears, smooth philtrum and short fifth toes.

Fernandez B A et al. J Med Genet 2010;47:195-203

JMG

16p11.2 duplication

Clinical photographs. (e) Proband 5 who has a maternally inherited duplication. (f) Proband 5 (note smooth philtrum) and her healthy duplication positive sister. (g) Duplication positive mother of proband 5, who also has a smooth philtrum. (h) Proband 6 (inherited duplication and oliogohydramnios sequence). Note her frontal bossing, receding hairline, hypoplastic supraorbital ridges and smooth philtrum. (i) Proband 6's right hand showing fifth finger clinodactyly.

Fernandez B A et al. J Med Genet 2010;47:195-203

16p11.2 deletion, not in mother or father, only in child.

5 years old, but developmental age of 2 year old.
Speaks a few words, almost unintelligible.
Very hyperactive.
Can be withdrawn and has at times been diagnosed with “autism”.

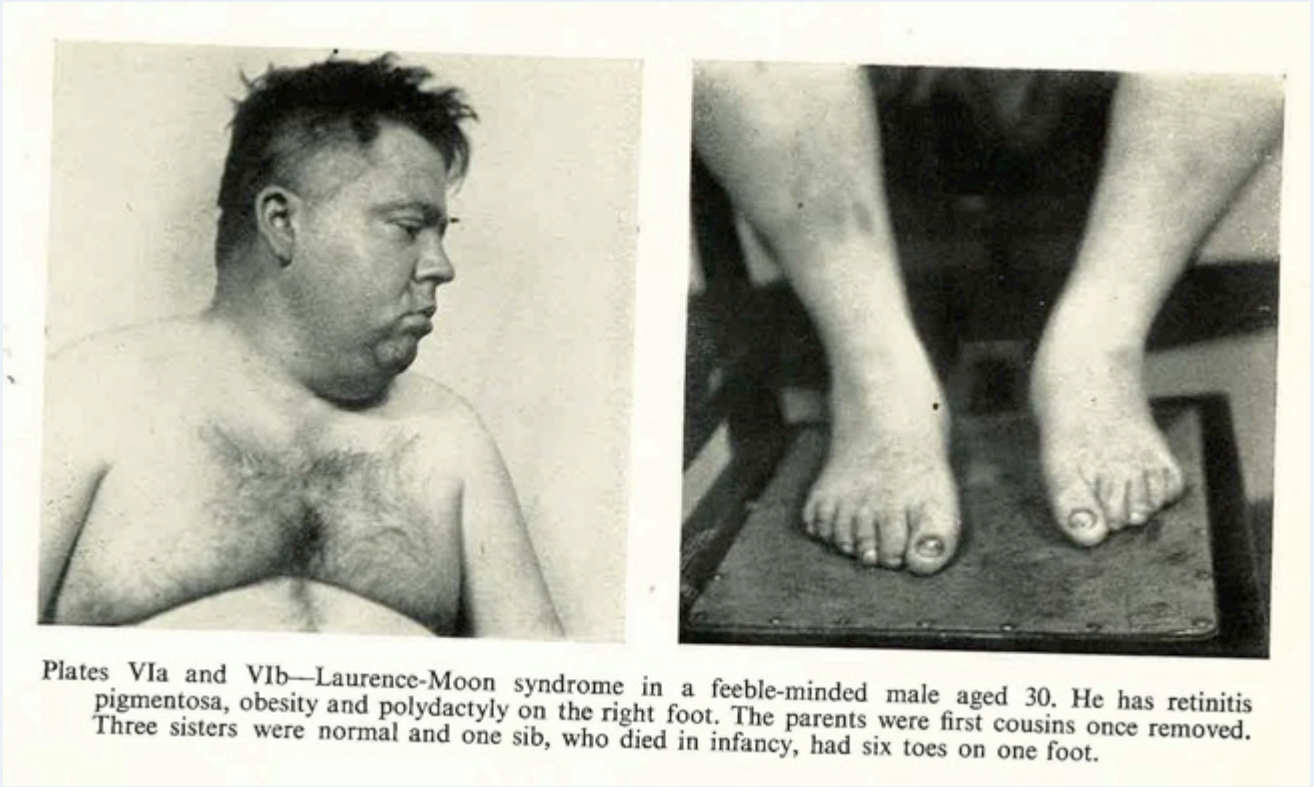
Current Diagnoses under Evaluation (DSM IV-TR)

AXIS I	299.00	Autism Disorder
	314.01	Attention-Deficit-Hyperactivity Disorder, Combined Type
AXIS II	V71.09	No Diagnosis
AXIS III	16p11.2	Microdeletion
AXIS IV		Psychosocial Stressors: Moderate(Adaptive/Behavioral and Educational/Learning Problems)
AXIS V		Current GAF: 60

Assessment Procedures:

Wechsler Preschool and Primary Scale of Intelligence (WPPSI)
Wide Range Achievement Test 4rd Edition (WRAT-4)
Test of Memory and Learning 2 (TOMAL, 2)
Beery VMI 6th Edition (Beery-Buktenica Developmental Test of Visual-Motor Integration, 6th Edition; Visual Perception, 6th Edition; Motor Coordination, 6th Ed)
Wide Range Assessment of Visual Motor Abilities (WRAVMA)
Conners' Comprehensive Behavior Rating Scales (CBRS) (Parent Report)
The Social Responsiveness Scale
Autism Diagnostic Interview Revised (ADI-R)
Mental Status Examination
Steinmann Neuropsychiatric Developmental Questionnaire
CNS Vital Signs Neuropsychological Screening
Clinical Interview with Patient
Clinical Interview with Parent
Clinical Observations
Review of Medical, Psychiatric, and Scholastic Records

Laurence-Moon Syndrome, now known as Bardet-Biedl Syndrome



Plates VIa and VIb—Laurence-Moon syndrome in a feeble-minded male aged 30. He has retinitis pigmentosa, obesity and polydactyly on the right foot. The parents were first cousins once removed. Three sisters were normal and one sib, who died in infancy, had six toes on one foot.

Langdon Down's patient Elizabeth C. She has the short stature, severe obesity and characteristic facial appearance of Prader–Willi syndrome.

Published in "John Langdon Down: A Caring Pioneer", by O Conor Ward, 1998.

James Henry Pullen, the idiot savant *who designed the prize winning exhibit for the Paris exhibition in 1867, dressed in the admiral's uniform which he accepted in return for not pursuing his plan to marry. He also designed a realistic model of the* Great Eastern, *a famous transatlantic vessel built by Brunel.*

Master Craftsman
Most famously designed The Great Eastern, a 10 foot long model ship with incredible detail.

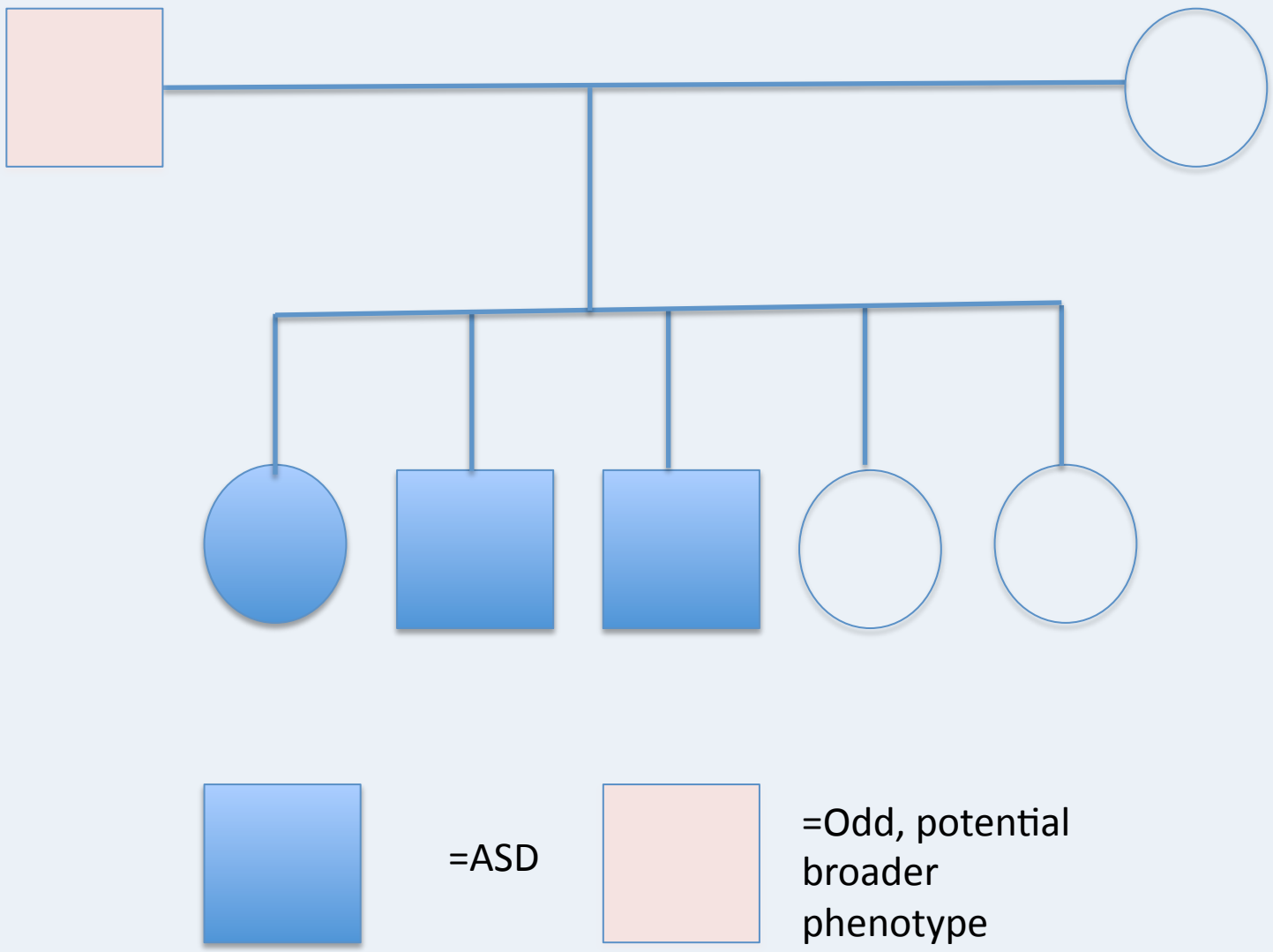
Deaf and nearly mute – Nonverbal,
Obsessed with one topic of building things.
Thought to be mentally retarded.
Usually quiet and reserved, but sometimes was intolerant of advice, suspicious of strangers, and ill-tempered and violent.

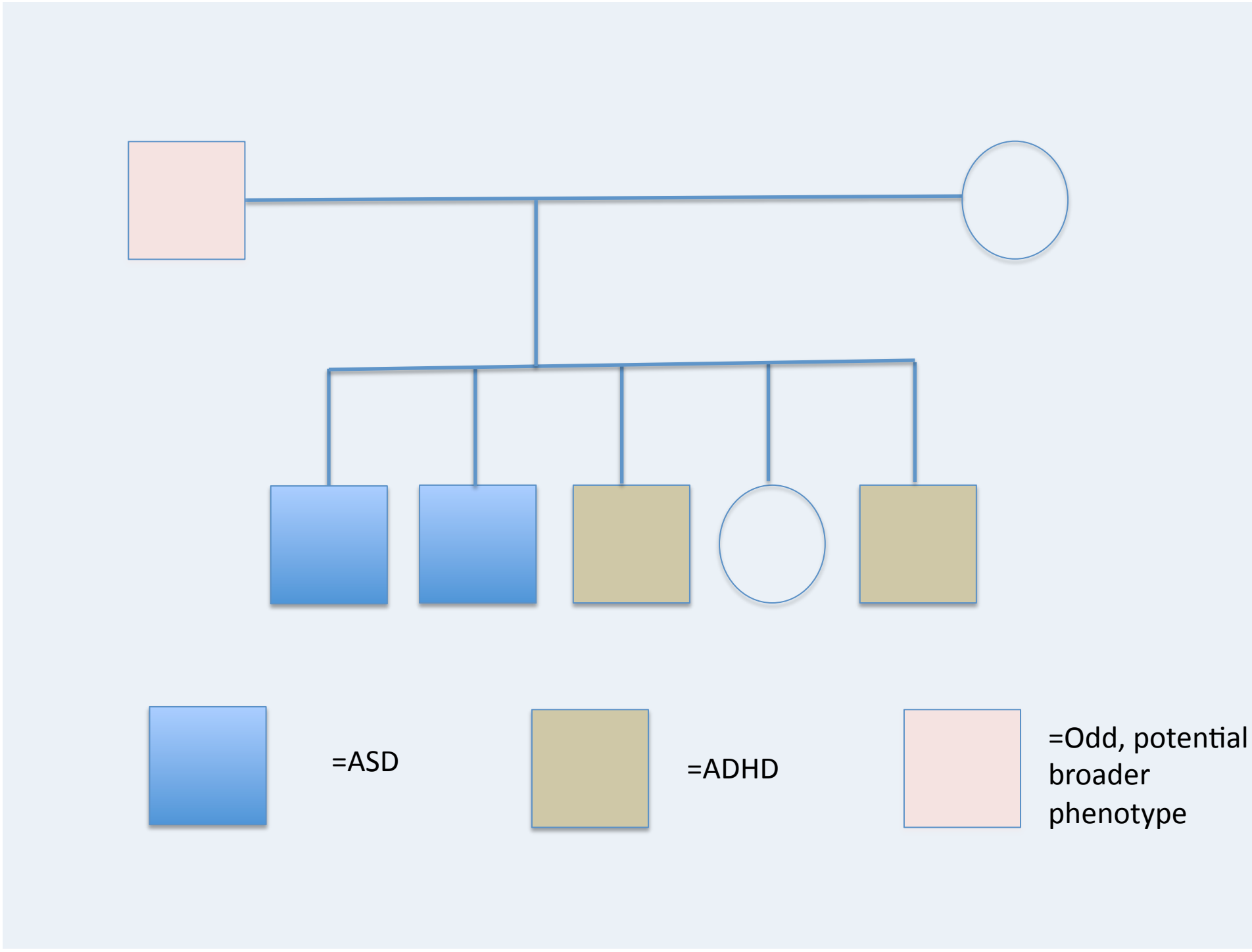
"The clinical and pathological evidence of a pervasive developmental disorder points to a retrospective diagnosis of autism."

Ir J Psych Med 2005; 22(4): 151-155

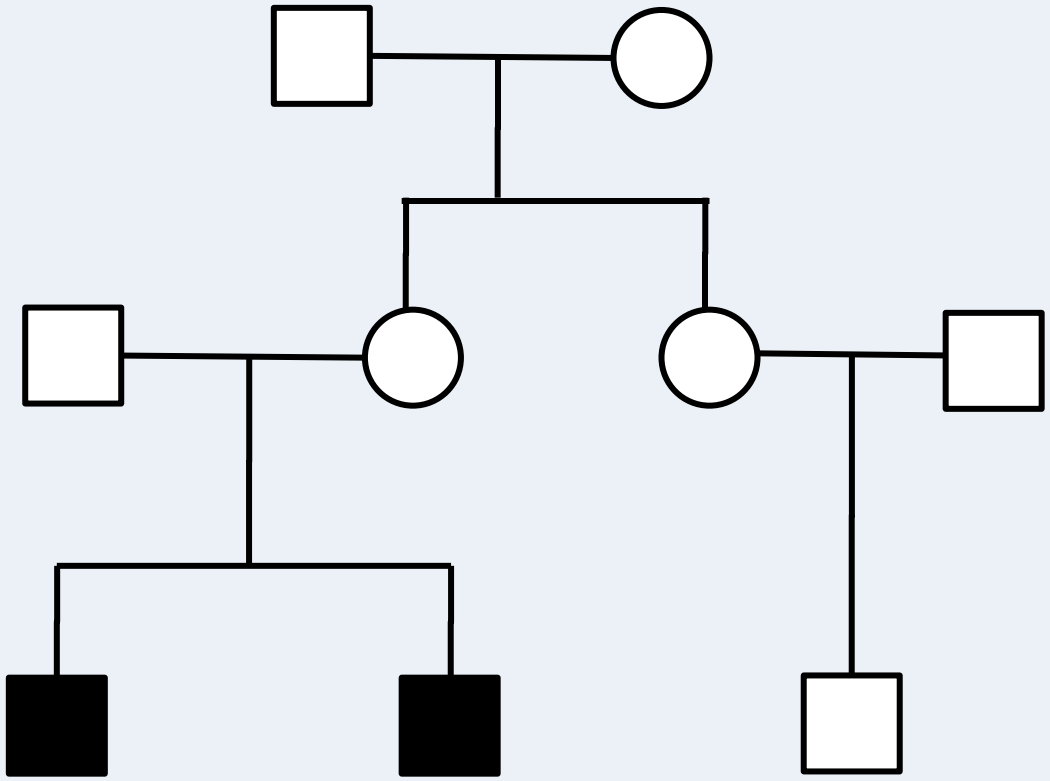
Sibling Defense Theory

- Defense or modifier Genes? – mutations that somehow protect against or modify the effects of a primary mutation.
- Or, can female gender also somehow be protective with certain mutations?
- Henry Pullen was one of 13 children, but only 3 lived to adult life. His brother, William, was also institutionalized and had exceptional artistic skills. Their parents were first cousins.


=ASD
=Odd, potential broader phenotype


=ASD
=ADHD
=Odd, potential broader phenotype

New Syndrome with Mental Retardation, “Autism”, “ADHD”



Likely X-linked or Autosomal Recessive, with X-linked being supported by extreme X-skewing in the mother

Workup Ongoing for past 10 years

- Numerous genetic tests negative, including negative for Fragile X and MANY candidate genes.
- Found one missense mutation in a known mental retardation gene, but the mutation is a very conservative nonsynonymous Asp to Glu. Is it relevant or not? What about the whole rest of the genome?

Sequenced whole genomes of Mother, Father and Two Boys, using Complete Genomics

- Sequenced “whole” genomes to obtain noncoding and other non-exonic regions.
- No obvious pathogenic CNVs – microarrays normal.
- ~6 million variants total in the 4 people different from Hg19 reference genome.
- No homozygous autosomal recessive mutations found.
- No Nonsense/Frameshift mutations in both boys.
- 2 mutations present in mother and two boys, on X-chromosome, not in father, not in dbSNP135, not in 1000Genomes April 2012 release, and not in NHLBI 6500 Exomes

2 mutations present in mother and two boys, on X-chromosome, not in father, not in dbSNP135, not in 1000Genomes April 2012 release, and not in NHLBI 6500 Exomes

- Nonsyn SNV ZNF41 c.1191C>A p.Asp397Glu
- Nonsyn SNV TAF1 c.4010T>C p.Ile1337Thr

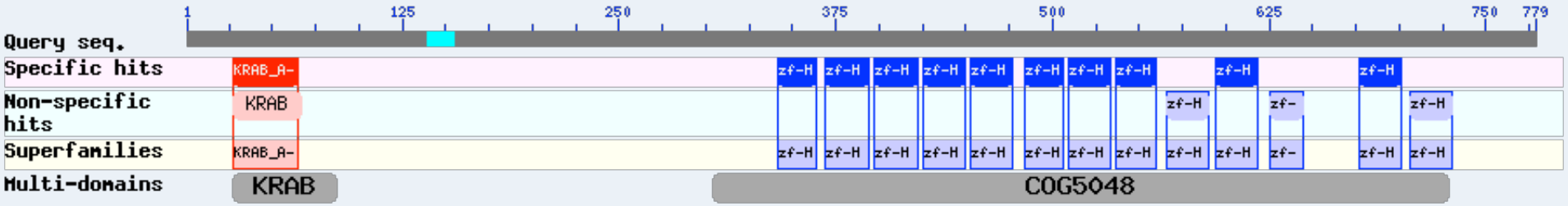
TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 250kDa

Mutations in the *ZNF41* Gene Are Associated with Cognitive Deficits: Identification of a New Candidate for X-Linked Mental Retardation

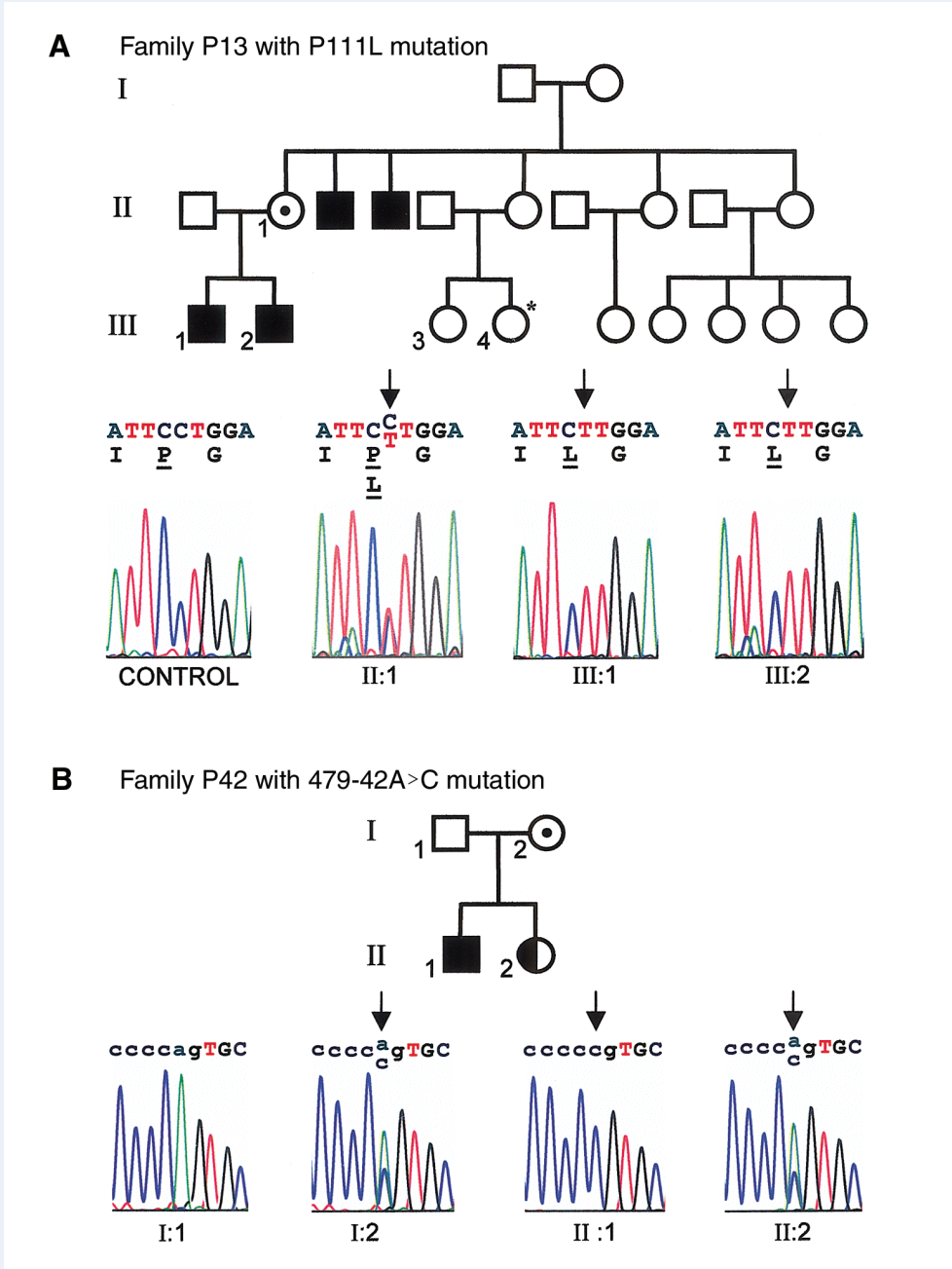
Sarah A. Shoichet,[1] Kirsten Hoffmann,[1] Corinna Menzel,[1] Udo Trautmann,[2] Bettina Moser,[1] Maria Hoeltzenbein,[1] Bernard Echenne,[3] Michael Partington,[4] Hans van Bokhoven,[5] Claude Moraine,[6] Jean-Pierre Fryns,[7] Jamel Chelly,[8] Hans-Dieter Rott,[2] Hans-Hilger Ropers,[1] and Vera M. Kalscheuer[1]

[1]Max-Planck-Institute for Molecular Genetics, Berlin; [2]Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen-Nuremberg; [3]Centre Hospitalier Universitaire de Montpellier, Hôpital Saint-Eloi, Montpellier, France, [4]Hunter Genetics and University of Newcastle, Waratah, Australia; [5]Department of Human Genetics, University Medical Centre, Nijmegen, The Netherlands; [6]Services de Génétique–INSERM U316, CHU Bretonneau, Tours, France; [7]Center for Human Genetics, Clinical Genetics Unit, Leuven, Belgium; and [8]Institut Cochin de Génétique Moleculaire, Centre National de la Recherche Scientifique/INSERM, CHU Cochin, Paris

Am. J. Hum. Genet. 73:1341–1354, 2003



- KRAB (Kruppel-associated box) domain -A box.
- The KRAB domain is a transcription repression module, found in a subgroup of the zinc finger proteins (ZFPs) of the C2H2 family, KRAB-ZFPs. KRAB-ZFPs comprise the largest group of transcriptional regulators in mammals, and are only found in tetrapods.
- The KRAB domain is a protein-protein interaction module which represses transcription through recruiting corepressors. The KAP1/ KRAB-AFP complex in turn recruits the heterochromatin protein 1 (HP1) family, and other chromatin modulating proteins, leading to transcriptional repression through heterochromatin formation.


A
Family P13 with P111L mutation
I
II
III
1
2
3
4
*
ATTCCTGGA
I P G
ATTCCTTGGA
I P L G
ATTCTTGGA
I L G
ATTCTTGGA
I L G
CONTROL
II:1
III:1
III:2
B
Family P42 with 479-42A>C mutation
I
II
1
2
1
2
ccccagTGC
ccccacgTGC
ccccCgTGC
ccccacgTGC
I:1
I:2
II :1
II:2

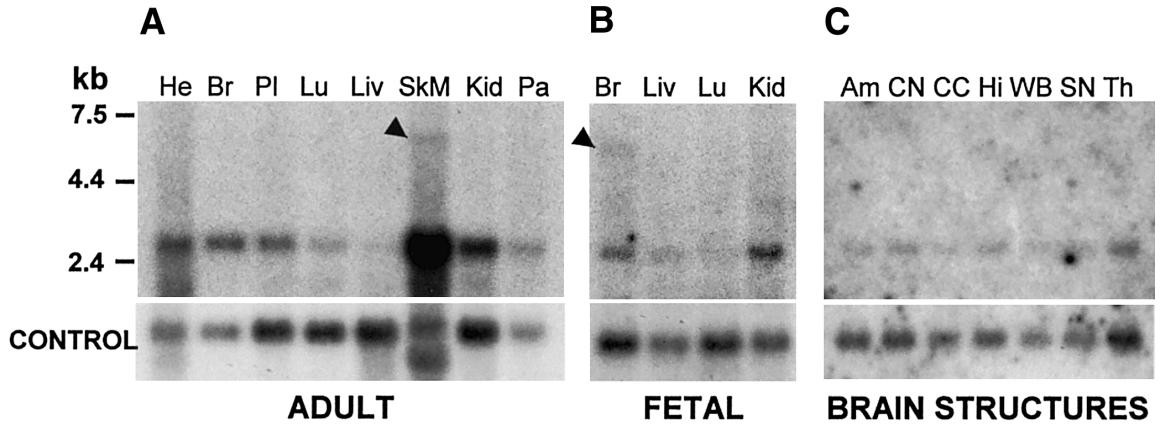


Figure 6 Northern blot hybridization of *ZNF41*, by use of a probe corresponding to nucleotides 621–1099 of *ZNF41* transcript variant 1. *A*, Adult tissues (left to right): heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. *B*, Fetal tissues (left to right): brain, lung, liver, and kidney. *C*, Adult brain structures (left to right): amygdala, caudate nucleus, corpus callosum, hippocampus, whole brain, substantia nigra, and thalamus. Black arrowheads highlight the presence of a novel 6-kb transcript. *Actin* (*A* and *C*) or *GAPDH* (*B*) served as controls for RNA loading.

Proving Causality

- Will need to find a second, unrelated family with same exact mutation and similar phenotype.
- Can also perform in vitro/in vivo studies and structural modeling, and make knock-in mice and/or test in zebrafish, etc… for biological function.

Learning Objective #3: The current state of Psychiatric Genetics

- Very little found with GWAS
- Some CNVs found by association testing, but proving causality is an ongoing issue.

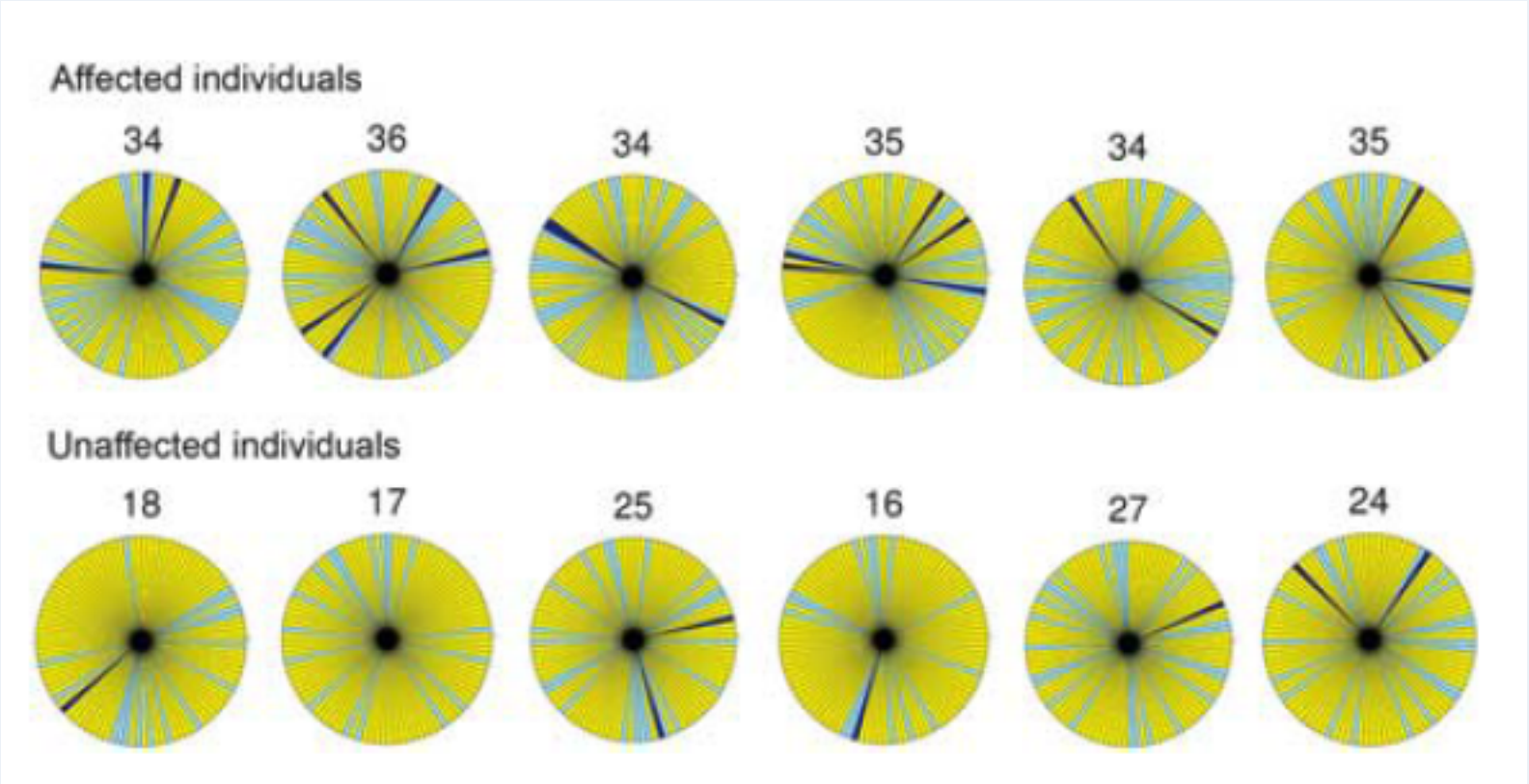
Some Definitions …

- The words “penetrance” and “expressivity”, defined classically as:
- Penetrance: whether someone has ANY symptoms of a disease, i.e. all or none, 0% or 100%. **Nothing in between.**
- Expressivity: how much disease (or how many symptoms) someone with 100% penetrance has.
- This has led to endless confusion!
- Some just use the word “penetrance” to mean the expressivity of disease, i.e. incomplete penetrance, and maybe we should combine the two terms into ONE word with the full expression from 0-100% of phenotypic spectrum.

Definitions. It is unknown what portion of autism will be oligogenic vs. polygenic

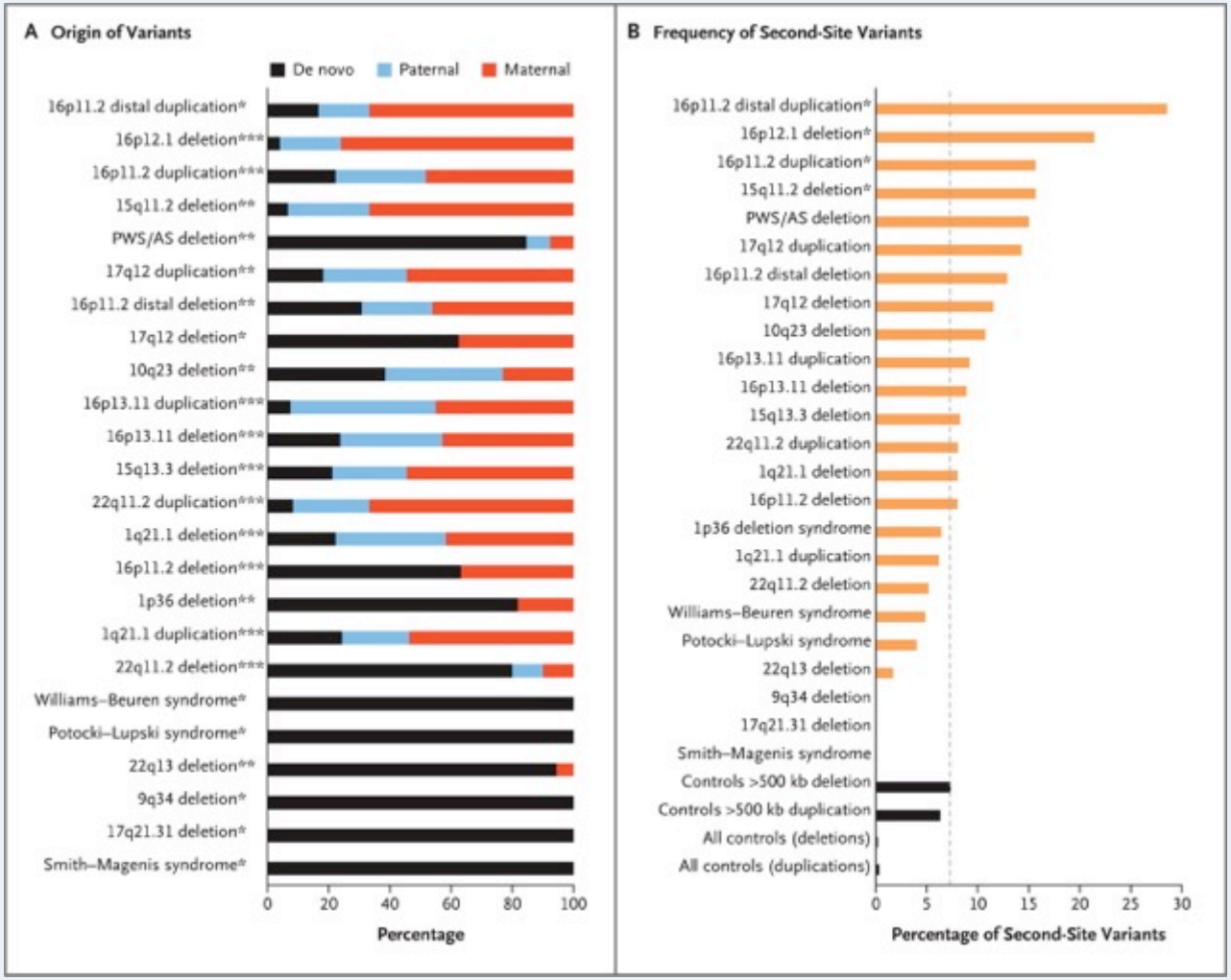
- **Oligogenic** – multiple mutations together contributing to aggregate disease, BUT with only 1 mutation of ~ >10% penetrance (or "effect size) in EACH person.
- **Polygenic** – Dozens to hundreds of mutations in different genes in the SAME person, together contributing to the disease in the SAME person, hence **additive** and/or **epistatic** contribution with ~0.01-1% penetrance for each mutation.

Example of Polygenic Model



Visscher et al. 2011

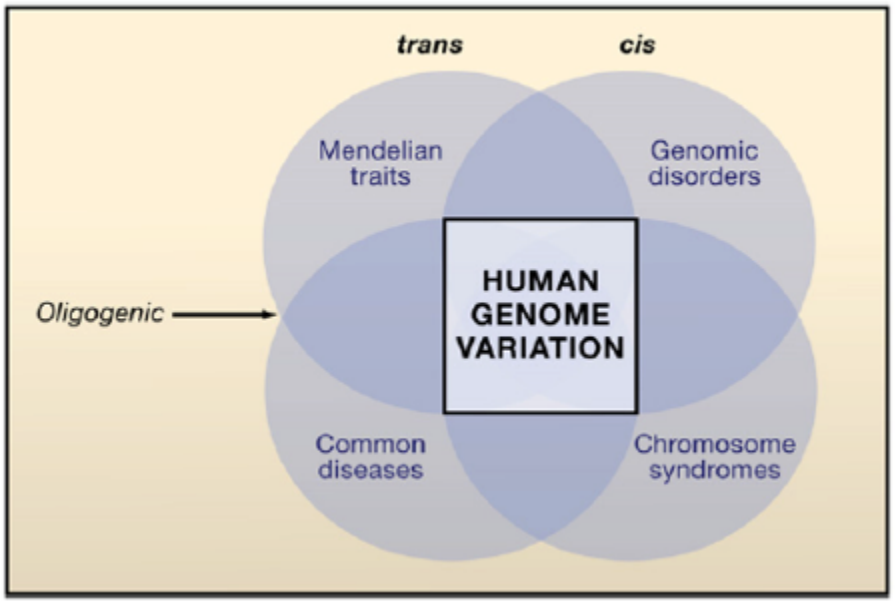
Inheritance Pattern of Copy-Number Variants and Frequency of Second-Site Variants Associated with a Genomic Disorder.

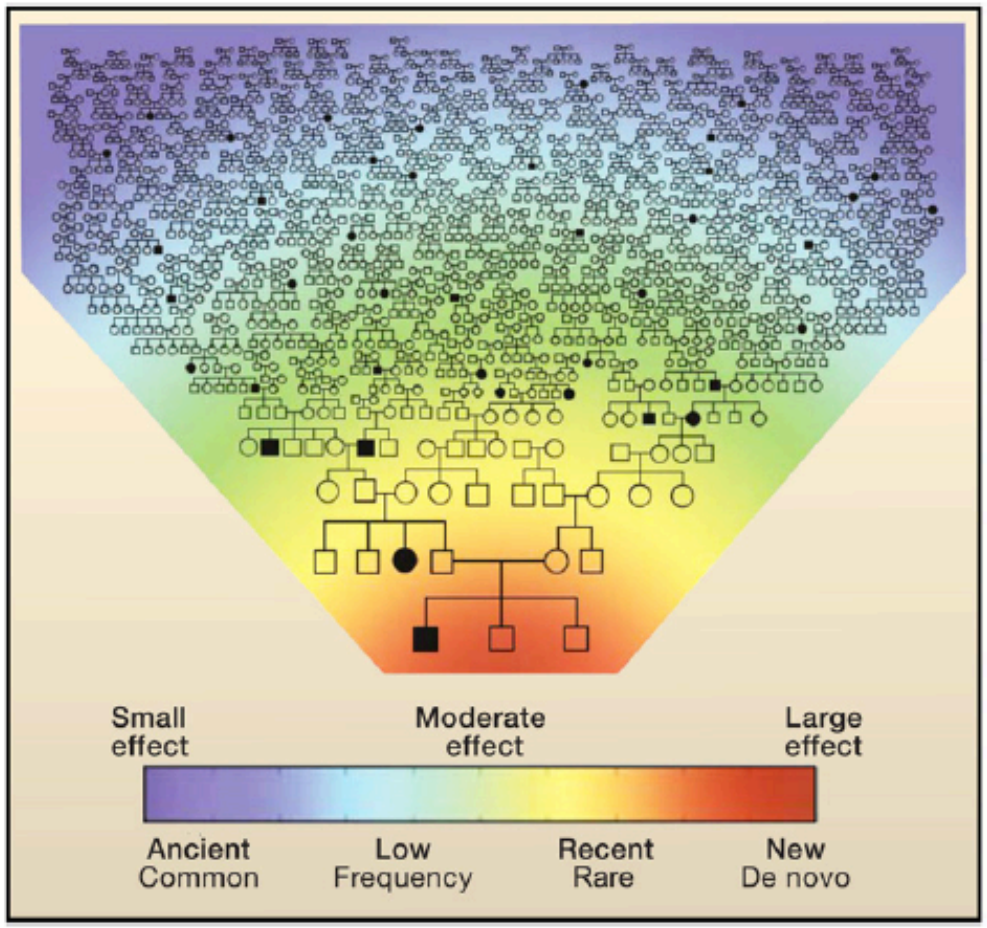


Girirajan S et al. N Engl J Med 2012. DOI: 10.1056/NEJMoa1200395

Clan Genomics and the Complex Architecture of Human Disease

James R. Lupski,[1,2,3,*] John W. Belmont,[1,2] Eric Boerwinkle,[4,5] and Richard A. Gibbs[1,5,*]





Rare Variants – CNVs, SNVs, indels, etc… in Rare AND Common diseases

High Frequencies of De Novo CNVs in Bipolar Disorder and Schizophrenia

Dheeraj Malhotra,[1,2,22] Shane McCarthy,[22] Jacob J. Michaelson,[1,2] Vladimir Vacic,[15,22] Katherine E. Burdick,[23] Seungtai Yoon,[5,22] Sven Cichon,[10,11,12] Aiden Corvin,[17] Sydney Gary,[22] Elliot S. Gershon,[21] Michael Gill,[17] Maria Karayiorgou,[18] John R. Kelsoe,[2,4,20] Olga Krastoshevsky,[19] Verena Krause,[19] Ellen Leibenluft,[7] Deborah L. Levy,[19] Vladimir Makarov,[5,22] Abhishek Bhandari,[1,2,22] Anil K. Malhotra,[6] Francis J. McMahon,[14] Markus M. Nöthen,[10,11,16] James B. Potash,[8] Marcella Rietschel,[13] Thomas G. Schulze,[9] and Jonathan Sebat[1,2,3,4,22,*]

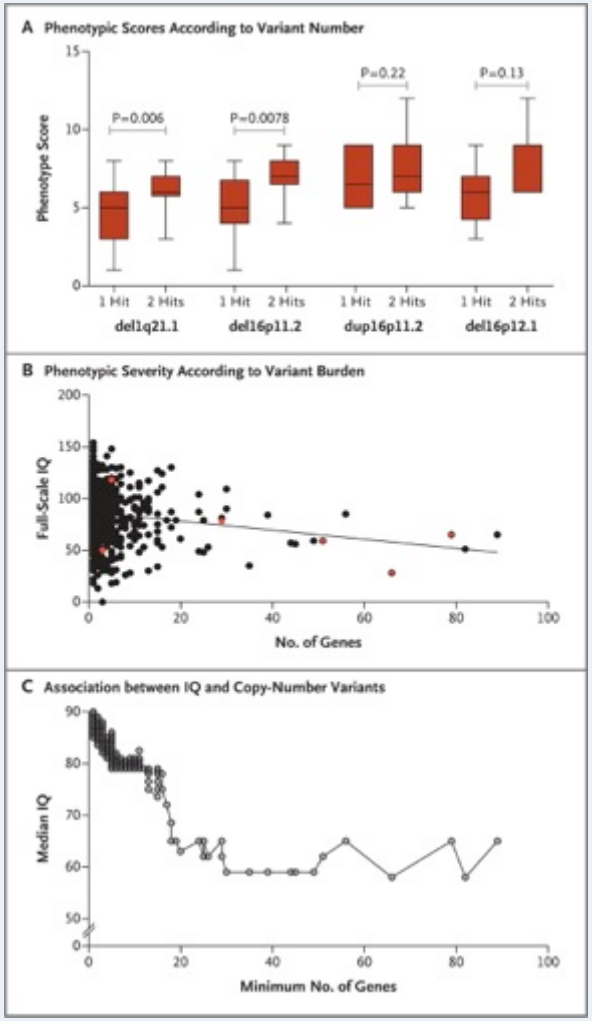
Deep resequencing of GWAS loci identifies independent rare variants associated with inflammatory bowel disease

Manuel A Rivas[1–3], Mélissa Beaudoin[4,23], Agnes Gardet[5,23], Christine Stevens[2,23], Yashoda Sharma[6], Clarence K Zhang[6], Gabrielle Boucher[4], Stephan Ripke[1,2], David Ellinghaus[7], Noel Burtt[2], Tim Fennell[2], Andrew Kirby[1,2], Anna Latiano[8], Philippe Goyette[4], Todd Green[2], Jonas Halfvarson[9], Talin Haritunians[10], Joshua M Korn[2], Finny Kuruvilla[2,11], Caroline Lagacé[4], Benjamin Neale[1,2], Ken Sin Lo[4], Phil Schumm[12], Leif Törkvist[13], National Institute of Diabetes and Digestive Kidney Diseases Inflammatory Bowel Disease Genetics Consortium (NIDDK IBDGC)[14], United Kingdom Inflammatory Bowel Disease Genetics Consortium[14], International Inflammatory Bowel Disease Genetics Consortium[14], Marla C Dubinsky[15], Steven R Brant[16,17], Mark S Silverberg[18], Richard H Duerr[19,20], David Altshuler[1,2], Stacey Gabriel[2], Guillaume Lettre[4], Andre Franke[7], Mauro D'Amato[21], Dermot P B McGovern[10,22], Judy H Cho[6], John D Rioux[4], Ramnik J Xavier[1,2,5] & Mark J Daly[1,2]

Evolution and Functional Impact of Rare Coding Variation from Deep Sequencing of Human Exomes

Jacob A. Tennessen,[1*] Abigail W. Bigham,[2*†] Timothy D. O'Connor,[1*] Wenqing Fu,[1] Eimear E. Kenny,[3] Simon Gravel,[3] Sean McGee,[1] Ron Do,[4,5] Xiaoming Liu,[6] Goo Jun,[7] Hyun Min Kang,[7] Daniel Jordan,[8] Suzanne M. Leal,[9] Stacey Gabriel,[4] Mark J. Rieder,[1] Goncalo Abecasis,[7] David Altshuler,[4] Deborah A. Nickerson,[1] Eric Boerwinkle,[6,10] Shamil Sunyaev,[4,8] Carlos D. Bustamante,[3] Michael J. Bamshad,[1,2‡] Joshua M. Akey,[1‡] Broad GO, Seattle GO, on behalf of the NHLBI Exome Sequencing Project

Phenotypic Variation Associated with Additional Large Copy-Number Variants.



Girirajan S et al. N Engl J Med 2012. DOI: 10.1056/ NEJMoa1200395

The NEW ENGLAND JOURNAL of MEDICINE

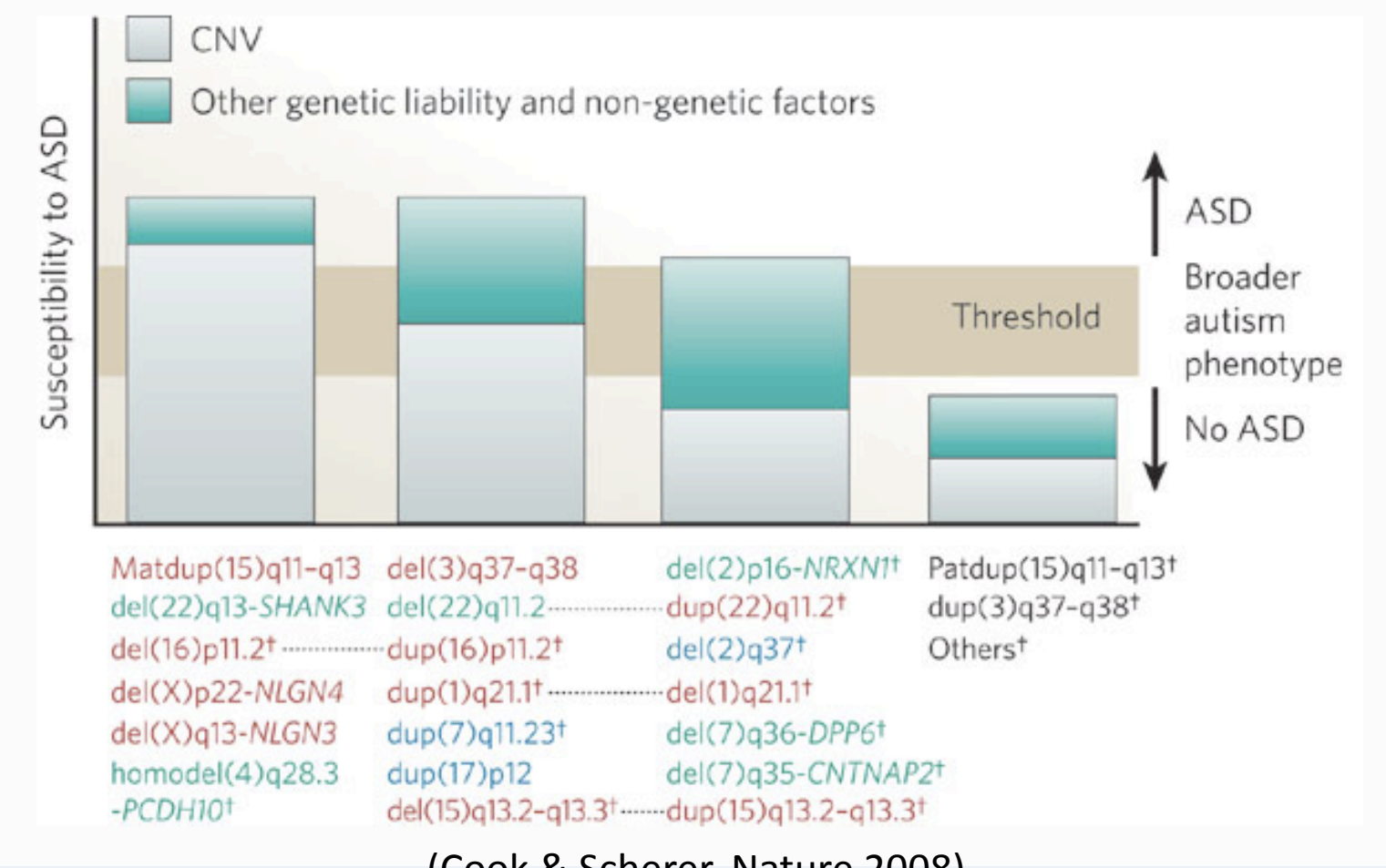
CNVs in Schizophrenia

- del 22q11 & schizophrenia (Murphy 1999)
- GLUR7 (gain), CACNG2 (loss) and AKAP5 (gain) in brain tissue (n=35) (Wilson 2006)
- del 15q13.3 and 1q21.1 (n=3391) (Stone 2008)
  - Also found 1.15x more CNVs<100kb
- Novel CNVs found (n=150) (Walsh 2008)
  - 5% of controls vs 15% of cases vs 20% of early-onset scz
- CNVs >1 Mb were 2.26x more common in cases (Kirov 2009)
- In the last couple of years:
  - 1q21, 15q11.2, 15q13.3, 16p11.2, 22q12, Neurexin 1, etc….

CNVs in Bipolar

- dup GSK3beta (Wnt signaling, Li target) (Lachman 2007)
- CNVs>100kb in 16.2% of cases vs 12.3% of controls (Zhang 2009)
- More pending….

CNVs & Autism Susceptibility



(Cook & Scherer, Nature 2008)

& Autism

- Deletion or duplication at 16p11.2 in ~1% of ASD (Weiss 2008)
  - Del in 5/512 (Boston), 3/299 (Iceland) vs. 2/18,834 controls (Iceland)
  - Dup in 7/751 (AGRE), 4/512 (Boston)
- Neurexin 1 (Kim 2008)
- Contactin 4 (Fernandez 2008)
- Ubiquitin pathway genes (UBE3A, PARK2, RFWD2, FBXO40) (Glessner 2009)
  - Also found neuronal cell adhesion genes (NLGN1 & ASTN2)
- SHANK3 deletions in up to 1% of ASD
  - Associated with lower functioning autism? (Sykes 2009)
  - Located at 22q13.3 & encodes a scaffold protein
- Others: DOCK4, AUTS1, AUTS5
- 15q13.3 microdeletion
- Maternally inherited 15q11-13 in 3-5%
  - Paternal inheritance of dup15q11-13 less severe

Psychological Medicine (2011), **41**, 19–32.
doi:10.1017/S003329171000070X

REVIEW ARTICLE

Rethinking the genetic architecture of schizophrenia

K. J. Mitchell[1]* and D. J. Porteous[2]

[1] *Smurfit Institute of Genetics, Trinity College Dublin, Ireland*
[2] *Medical Genetics Section, University of Edinburgh Molecular Medicine Centre, Institute of Genetics and Molecular Medicine, Edinburgh, UK*

BRITISH JOURNAL OF PSYCHIATRY **(2007), 190, 194–199. doi: 10.1192/bjp.bp.106.025585**

Schizophrenia: a common disease caused by multiple rare alleles†

JON M. McCLELLAN, EZRA SUSSER and MARY-CLAIRE KING

Molecular Psychiatry (2009) 14, 740–745

www.nature.com/mp

LETTERS TO THE EDITOR

GWAS for psychiatric disease: is the framework built on a solid foundation?

Mitchell and Porteous

"A primary justification for any genetic study of a condition of uncertain etiology must surely be to shed light on the biological causes…. Focused studies on individual cases, single families or genetically homogeneous populations do not currently attract the same cash or cachet as consortium-based GWAS studies, but promise greater returns in terms of biological insight and etiological understanding."

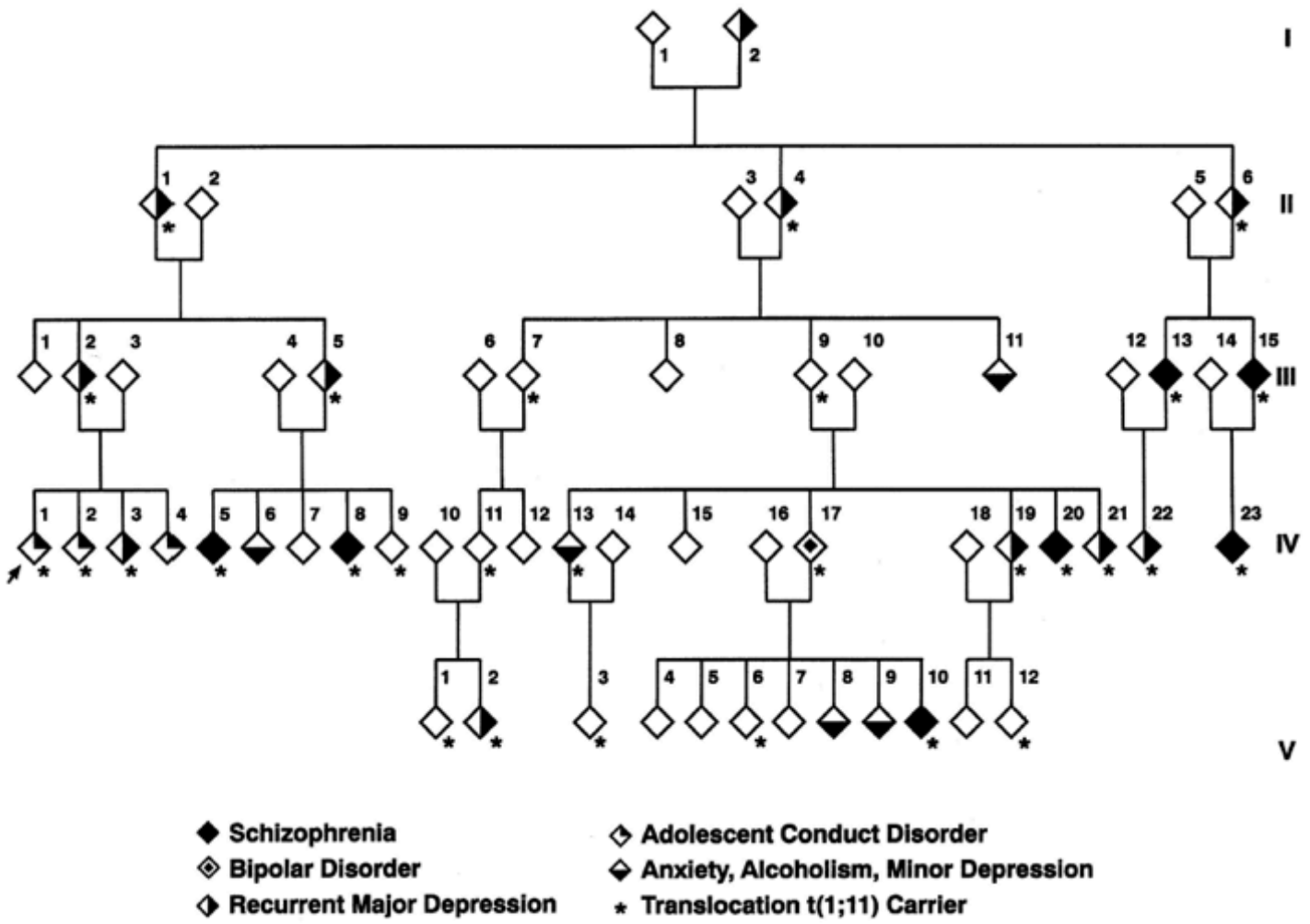
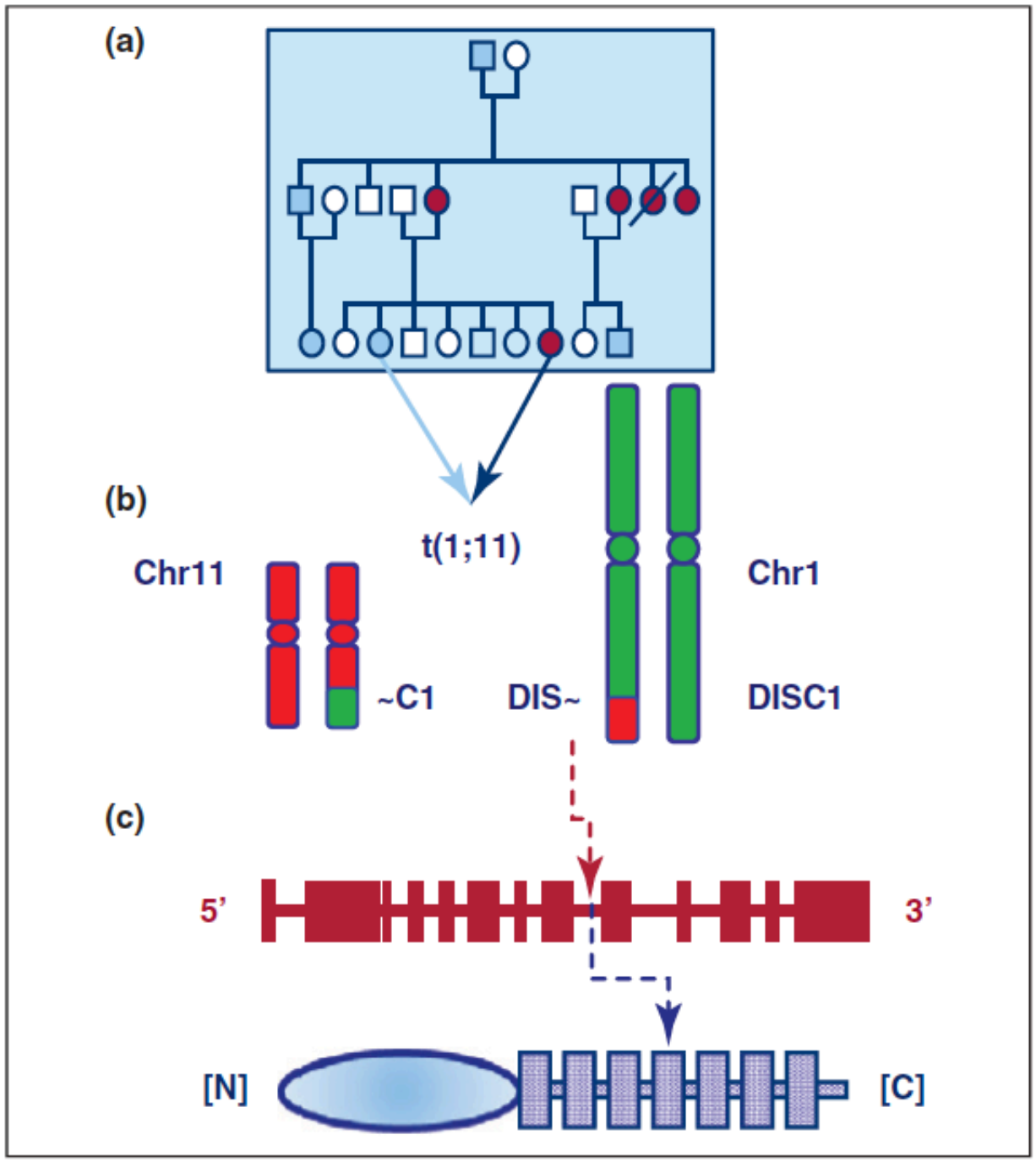
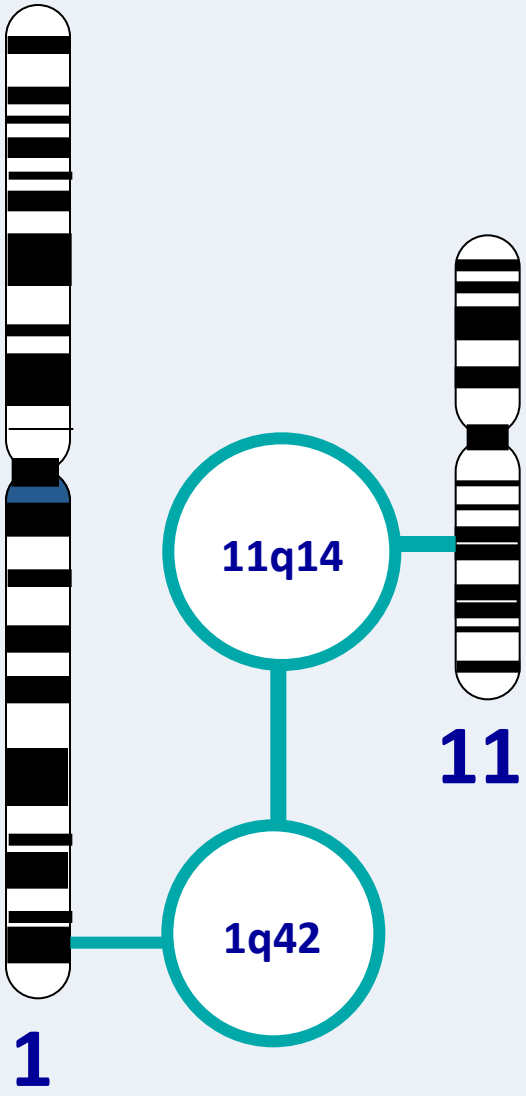


Figure 1 Part of the family with a (1;11)(q42;q14.3) translocation. Karyotype analysis has been performed on 87 members of this family, and clinical psychiatric data were obtained from 69 of those family members. Shown are 58 of the family members for whom carrier status is known and whose psychiatric phenotype has been defined through follow-up by direct interview, general-practice contact, or hospital case-note review.



Trends Mol Med. 2011 Oct 18.
DISC1 at 10: connecting psychiatric genetics and neuroscience.
Porteous DJ, Millar JK, Brandon NJ, Sawa A.

The t(1;11) breakpoint & linkage to psychosis

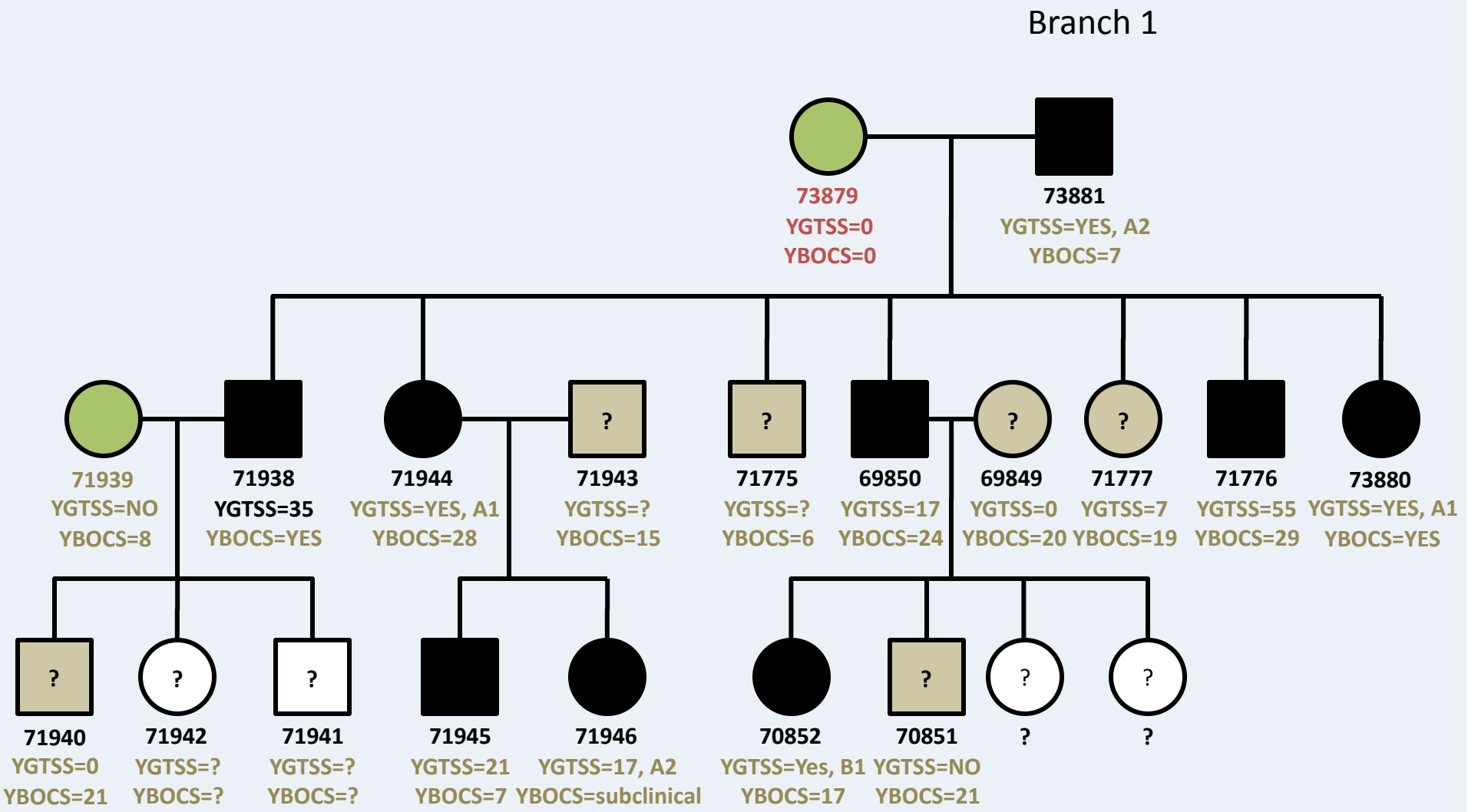


	t(1;11) YES N=36	t(1;11) NO N=35
LOD = 3.4		
Schizophrenia	7	0
Bipolar affective disorder	1	0
Recurrent major depression	10	0
MLOD = 7.1		
Conduct disorder with anxiety in adolescence	2	1
Minor depression	1	3
Alcoholism	0	1

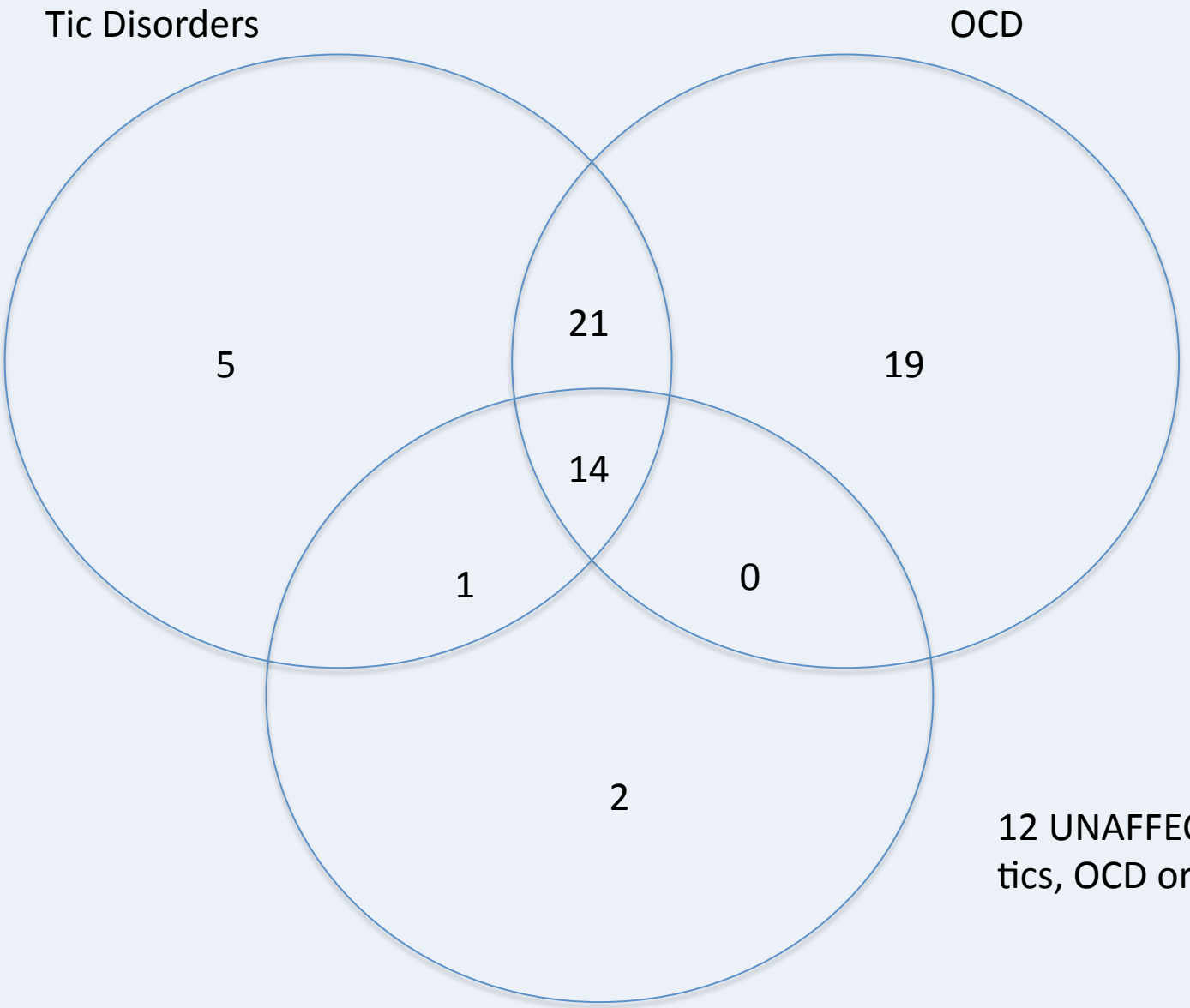
Dominant, variable penetrance, broad diagnosis

Slide courtesy of David Porteous

Phenotyping

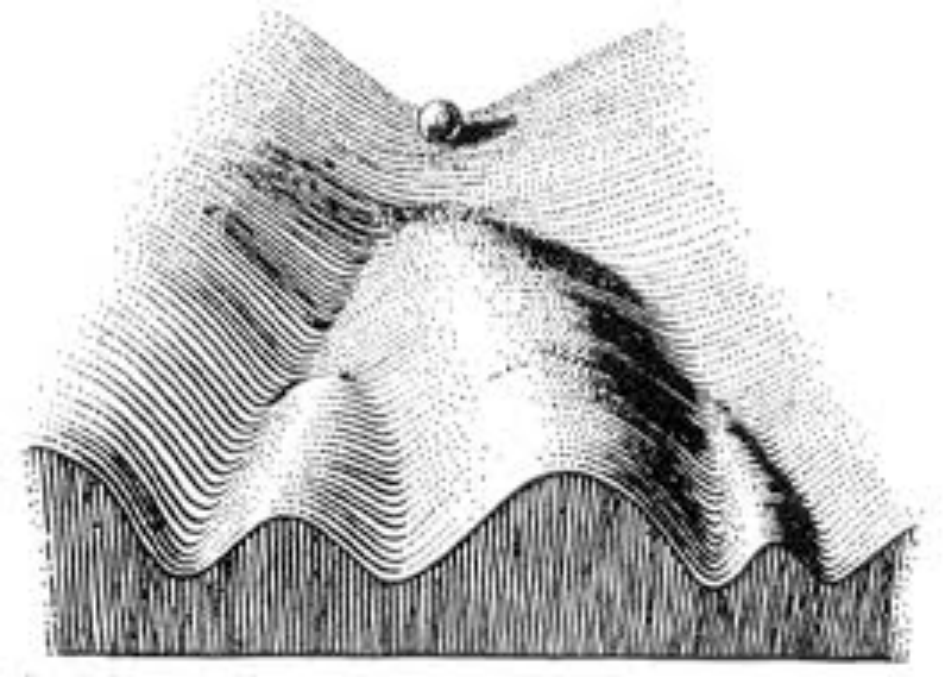


Large Pedigree with 74 collected and phenotyped members



12 UNAFFECTED with tics, OCD or ADHD

Waddington claimed that canals form in the epigenetic landscape during evolution, and that this heuristic is useful for understanding the unique qualities of biological robustness.



The canalisation metaphor suggests that phenotypes are very robust to small perturbations, for which development does not exit the canal, and rapidly returns back down, with little effect on the final outcome of development. But perturbations whose magnitude exceeds a certain threshold will break out of the canal, moving the developmental process into uncharted territory. Strong robustness up to a limit, with little robustness beyond, is a pattern that could increase evolvability in a fluctuating environment.

“Biological Indeterminacy”

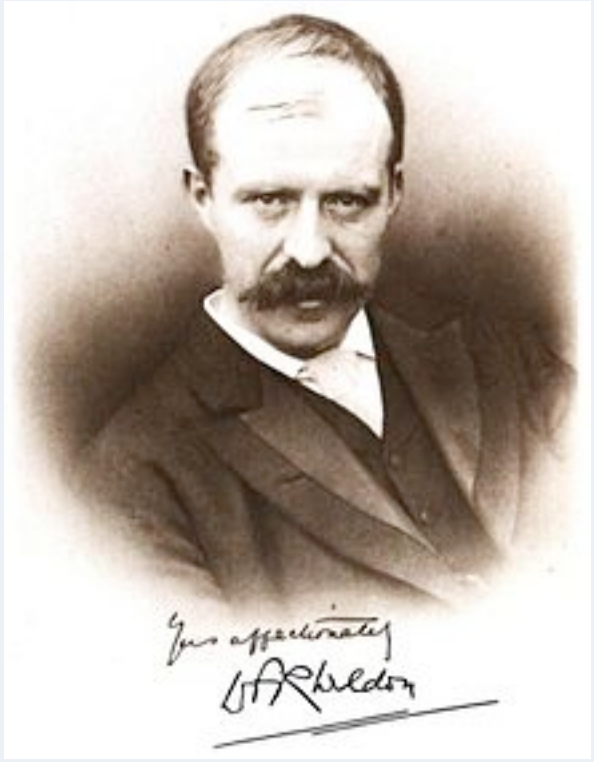
- Bateson became famous as the outspoken Mendelian antagonist of Walter Raphael Weldon, his former teacher, and Karl Pearson who led the biometric school of thinking. This concerned the debate over saltationism versus gradualism (Darwin had been a gradualist, but Bateson was a saltationist). Later, Ronald Fisher and J.B.S. Haldane showed that discrete mutations were compatible with gradual evolution: see the modern evolutionary synthesis.

Biological Indeterminacy. Greenspan RJ. Sci Eng Ethics. 2012 Jul 3

Beyond our Kuhnian inheritance

A recent lecture by Prof Greg Radick questions our scientific inheritance, through textbook histories of genetics and Thomas Kuhn's legacy

http://www.guardian.co.uk/science/the-h-word/2012/aug/28/thomas-kuhn

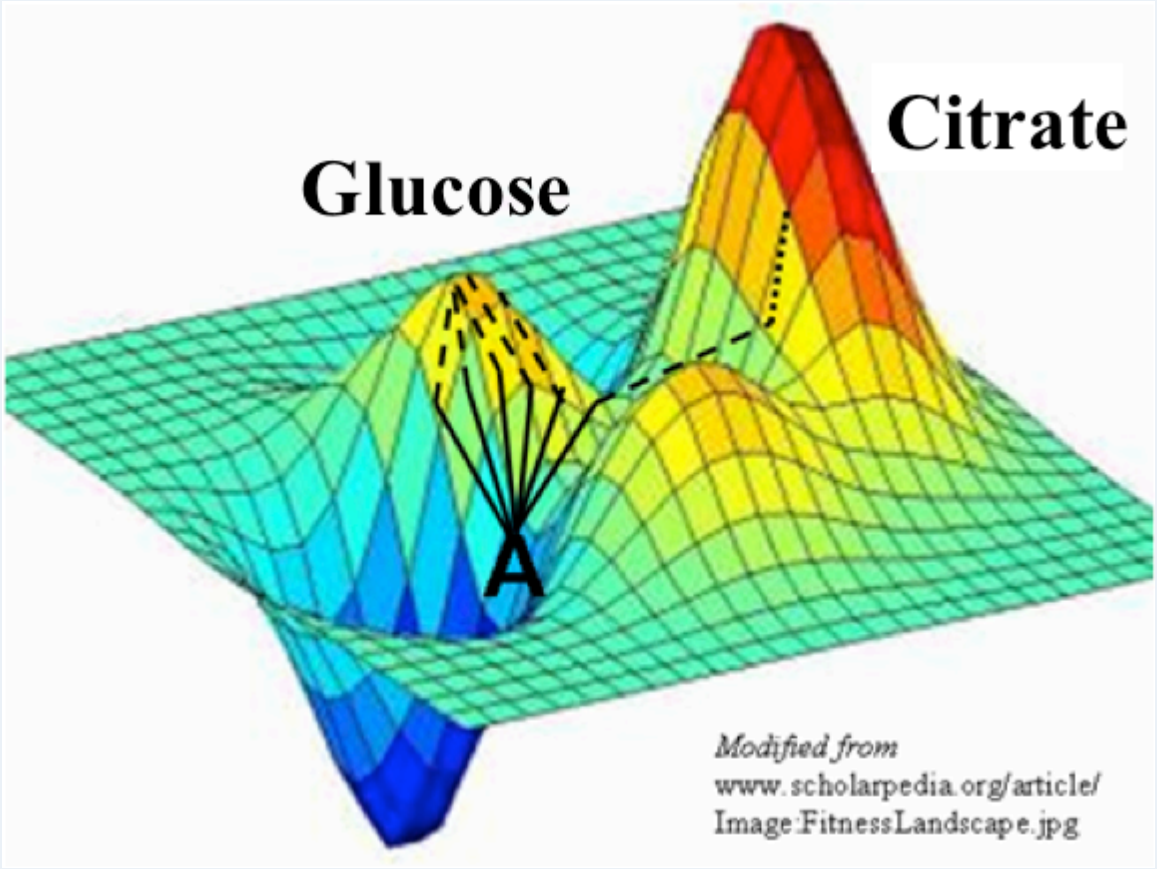


Walter Frank Raphael Weldon

Vs.

William Bateson

Forthcoming by Greg Radick. Scholarly edition of W. F. R. Weldon's Theory of Inheritance (1904-1905), coedited with Annie Jamieson.



E. coli adapting to low glucose conditions, in the context of media containing citrate. "Finally, novel functions often emerge in rudimentary forms that must be refined to exploit the ecological opportunities. This three-step process — in which potentiation makes a trait possible, actualization makes the trait manifest, and refinement makes it effective — is probably typical of many new functions." - Lemski

Genomic analysis of a key innovation in an experimental Escherichia coli population.
Blount ZD, Barrick JE, Davidson CJ, Lenski RE.
Nature. 2012 Sep 19. doi: 10.1038/nature11514

Genotype First, Phenotype Second AND Longitudinally

Human Molecular Genetics, 2010, Vol. 19, Review Issue 2 ***R176–R187***
doi:10.1093/hmg/ddq366
Advance Access published on August 31, 2010

Phenotypic variability and genetic susceptibility to genomic disorders

Santhosh Girirajan and Evan E. Eichler*

Department of Genome Sciences, Howard Hughes Medical Institute, University of Washington School of Medicine, PO Box 355065, Foege S413C, 3720 15th Avenue NE, Seattle, WA 98195, USA

Genome-Wide Association Study of Multiplex Schizophrenia Pedigrees

Am J Psychiatry Levinson et al.; AiA:1–11

"Rare CNVs were observed in regions with strong previously documented association with schizophrenia, but with variable patterns of segregation. This should serve as a reminder that we still know relatively little about the distribution of these CNVs in the entire population (e.g., in individuals with no or only mild cognitive problems) or about the reasons for the emergence of schizophrenia in only a minority of carriers, so great caution is required in genetic counseling and prediagnosis."

Penetrance Issues

- We do not really know the penetrance of pretty much ALL mutations in **humans**, as we have not systematically sequenced or karyotyped any genetic alteration in **Thousands to Millions** of **randomly** selected people, nor categorized into ethnic classes, i.e. clans.

- There is a **MAJOR** clash of world-views, i.e. do single mutations drive outcome predominately, or are the results modified substantially by genetic background and/or environment? i.e. is there really such a thing as genetic determinism for **MANY** mutations?

Clinical Validity?

This is SO complex that the only solid way forward is with a “networking of science” model, i.e. online database with genotype and phenotype longitudinally tracked for thousands of volunteer families.

PatientsLikeMe

Industrialization of Genome Sequencing – Just like what happened in development of MRI

- 09/11/12
  Illumina Announces Expedited Individual Genome Sequencing Service (IGS)

- 09/11/12
  Illumina and Partners HealthCare Announce Alliance to Introduce Next-Generation Sequencing Clinical Interpretation and Reporting Tools

- 09/11/12
  Illumina Launches TruSightTM Targeted Sequencing Content Sets

Lyon and Wang *Genome Medicine* 2012, **4**:58
http://genomemedicine.com/content/4/7/58

Genome Medicine

REVIEW

Identifying disease mutations in genomic medicine settings: current challenges and how to accelerate progress

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For now, more effort should be placed on the following:

- Implementing Standards for a “clinical-grade” exome, and promoting the “networking of science” model.
- Focusing on rare, highly penetrant mutations running in families, with cascade carrier testing of even more relatives as needed.
- The genomic background is much more constant in families.
- The environmental background is sometimes more constant in families.
- This allows one to figure out penetrance of rare variants in these families, along with other issues, such as somatic mosaicism.

Conclusions from Learning Objectives

1. Participants will learn about the current state of genetics, including genome wide association studies (GWAS), Copy Number Variants (CNVs) and next generation sequencing.
2. Some Case Illustrations will be presented.
3. A review of current progress in psychiatric genetics will be presented.

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