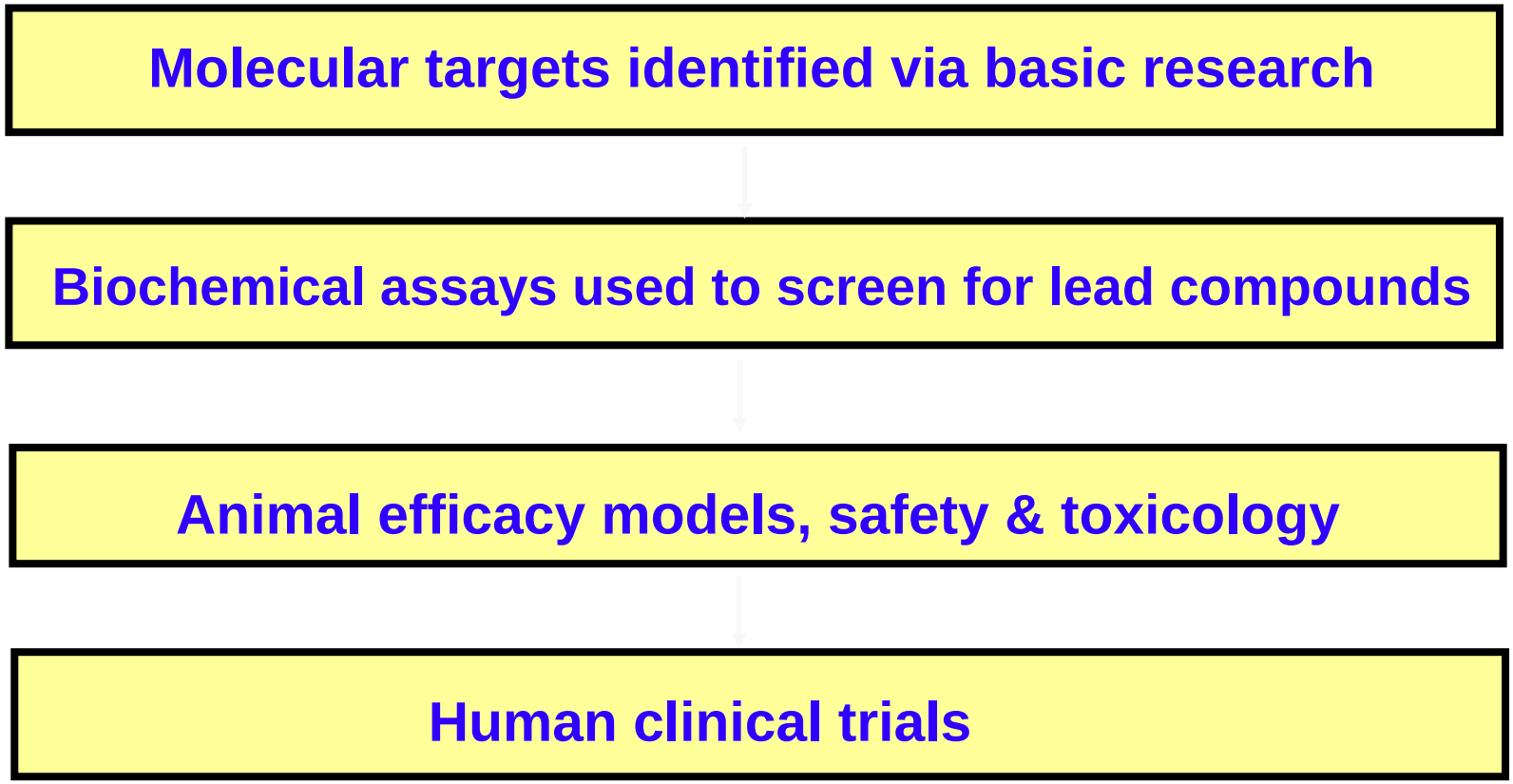
9.914 Special Topics: Genetics, Neurobiology, and Pathophysiology of Psychiatric Disorders
Fall 2008

Modern Paradigm of Drug Discovery

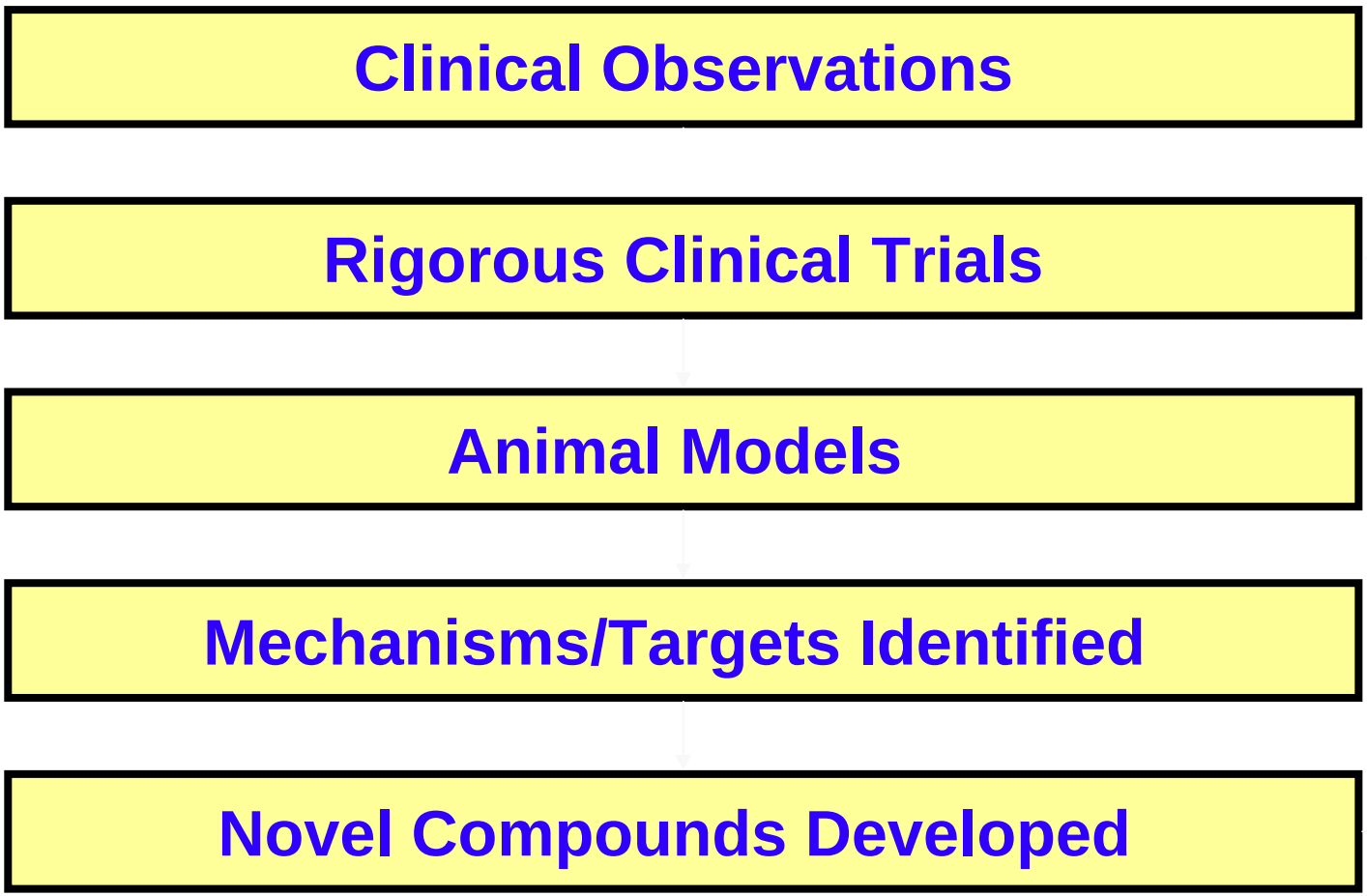


Example of Paradigm for Atherosclerosis

The Development of Statins

- ***1948*** – In Framingham, MA, a large study shows an association between cholesterol and coronary risk
- ***1958*** – HMG-CoA reductase shown to be the rate-limiting enzyme in cholesterol biosynthesis
- ***1973 – 1974*** – Discovery of role of LDL receptors in regulatory HMG CoA reductase activity and LDL levels in blood
- ***1978*** – An inhibitor of HMG-CoA reductase discovered
- ***1979 – 1980*** – Clinical trials begun
- ***1987*** – Cholesterol lowering demonstrated, 1st medicine approved
- ***1994*** – Long-term effectiveness studies show decreases in heart attacks and mortality

History of the Discovery of Medicines for Serious Mental Illness



Summary: Historical Discovery Paradigms in Mental Health

- Serendipity
- Compounds in search of clinical use
- Refinements based on clinical experience
- Mechanisms identified after clinical efficacy

Mechanistically Distinct Drugs

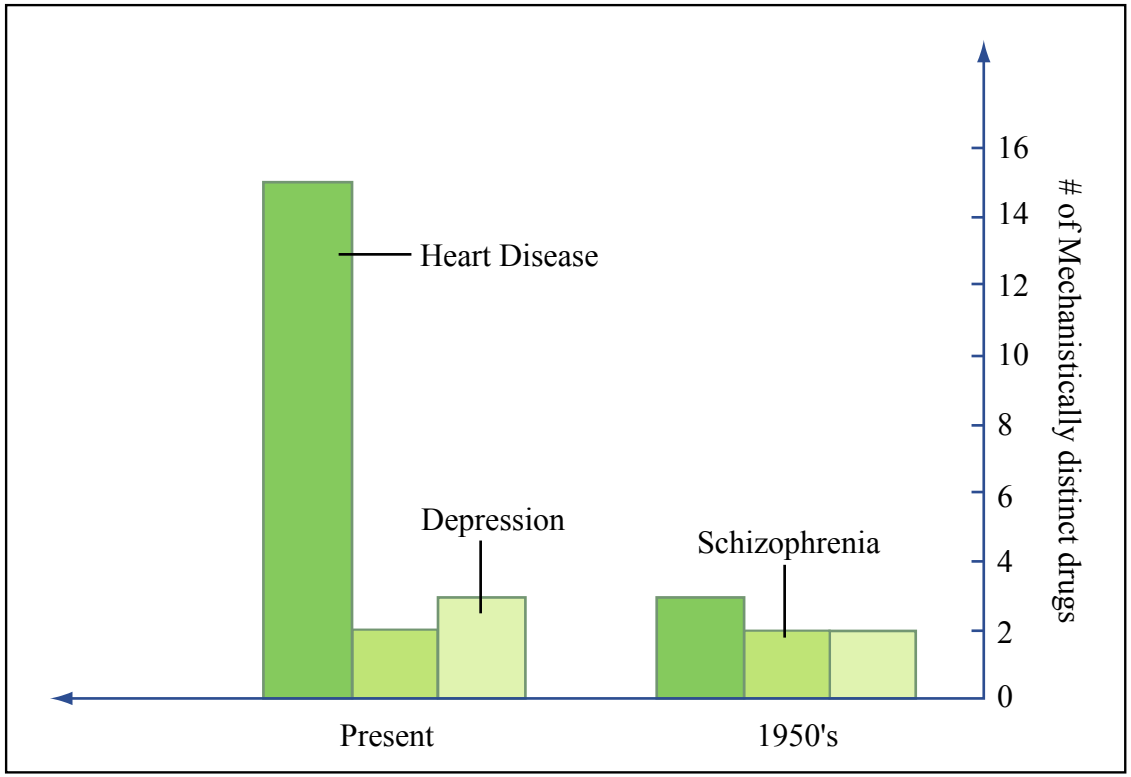


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Table 1

Paradigms for Treating Complex Medical Illnesses Drugs Have a Single Target

To achieve increased efficacy, defined ratios of single mechanism drugs are used together

Therapeutic Area	Mechanism of Action	Named Drugs
Hypertension	ACE inhibition; All inhibition	Captopril, Vasotec; Cozaar
	Diuretics	Diuril
	Calcium channel blockers	Nifedipine Diltiazem Verapamil
Atherosclerosis	HMG CoA reductase inhibition (statins)	Mevacor Zocor
	Inhibition of cholesterol absorption	Zetia

Table 2

Antipsychotics

Therapeutic Area	Mechanism of Action	Named Drugs
Atypical Antipsychotics	Very mixed pharmacology D_2 /5HT2A blockade + others	Clozaril Risperidol
Typical Antipsychotics	D_2 receptor Blockade	Haloperidol

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Table 3

Current Pharmacological Treatments of Schizophrenia

	D1	D2	D3	D4	5-HT1A	5-HT2A	5-HT2C	α1	α2	M1	H1
Clozaril	141	83	200	20	640	2.5	9	4	12	2	23
Risperadol	75	0.3	14	7	488	0.2	26	2	3	>1000	155
Olanzapine	31	11	--	27	>1000	5	11	19	228	2	7
Quetiapine	455	160	--	--	>1000	295	>1000	7	87	120	11
Zotepine	29	8	6	39	260	3	--	6	540	250	21
Ziprazadone	9	3	7	32	37	0.3	0.5	2	400	>1000	510
Aripiprazole		0.4			4.4	3.4	15	47		>1000	67

Table 4

Treatments for Bipolar Illness

Therapeutic Area	Mechanism of Action	Named Drugs
Bipolar Illness	? GSK 3 Beta, ? IMPase	***Lithium***
	? Histone deacetylase(s)	***Valproate***
	? Sodium channel blockade	***Lamictal***
	D_2 and 5-HT2A receptor	***Atypical antipsychotics***

Limitations to Improving on Treatments for Psychotic Illness

- No molecular cause or molecular understanding of either schizophrenia or bipolar illness
  - No chemical, physical, or biological measurement in a patient used to make diagnosis
- No animal models based upon known human cause
- No cell culture assays
- Several theories: are they sufficiently supported by unambiguous data so that companies will invest in new approaches to treatment?

Mental Retardation: a Complicated Biological Brain Disease

- ~ 3% of World's population is mentally retarded (~180,000,000)
- Comparable to total affected by bipolar illness and schizophrenia
- 30 - 40% of mental retardation can be attributed to defined chromosomal and genetic abnormalities

  (~ 50 - 70 million patients)

Fragile X Mental Retardation – 1 Gene (FMR1)

Figures removed due to copyright restrictions.

Fragile X Syndrome is a Null Mutation Lacking FMR1, mRNA, and FMRP

Molecular Basis of Fragile X Syndrome

Expansion to Full Mutation Alleles

↓

Transcriptional Silencing of FMR1

↓

Absence of the RNA-Binding Protein FMRP

↓

Translational over-expression of select mRNAs

↓

Fragile X Syndrome Phenotype

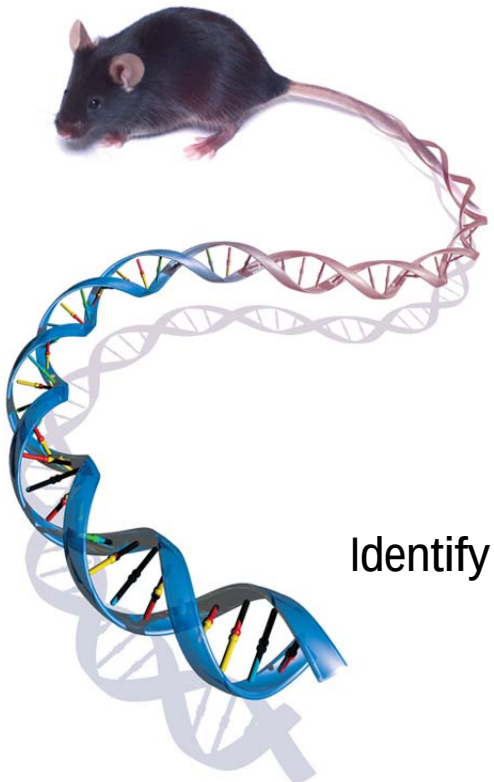
mGluR 1/5 is a Druggable Target

Pharmacological Data with mGluR 5 Antagonist in Models of Fragile X Syndrome

- Prevention of audiogenic induced seizures in mouse model of Fragile X null
- Prevention of lethality in Drosophilia model of Fragile X null

Paradigm for new treatments for autism, bipolar disorder, and schizophrenia

http://www.genome.gov/pressDisplay.cfm?photoID=40



Identify risk genes

Understand functional consequences of variant gene

Develop cell-based assays

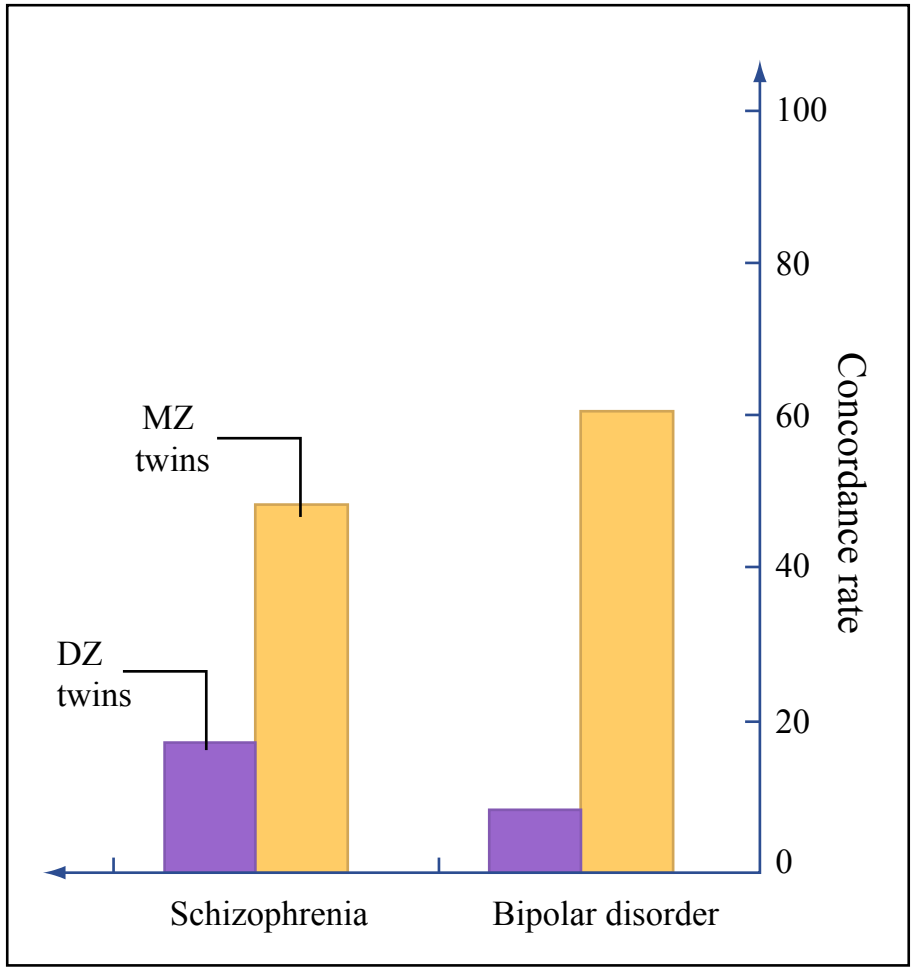
Develop mouse model with a quantifiable phenotype; a consequence of variant gene

Identify chemicals that reverse cell-based and animal-based phenotype

Human trials in genetically defined variant

Test in broader group of patients with the illness

Schizophrenia and bipolar disorder are the most heritable adult psychiatric diseases and are biological brain diseases.



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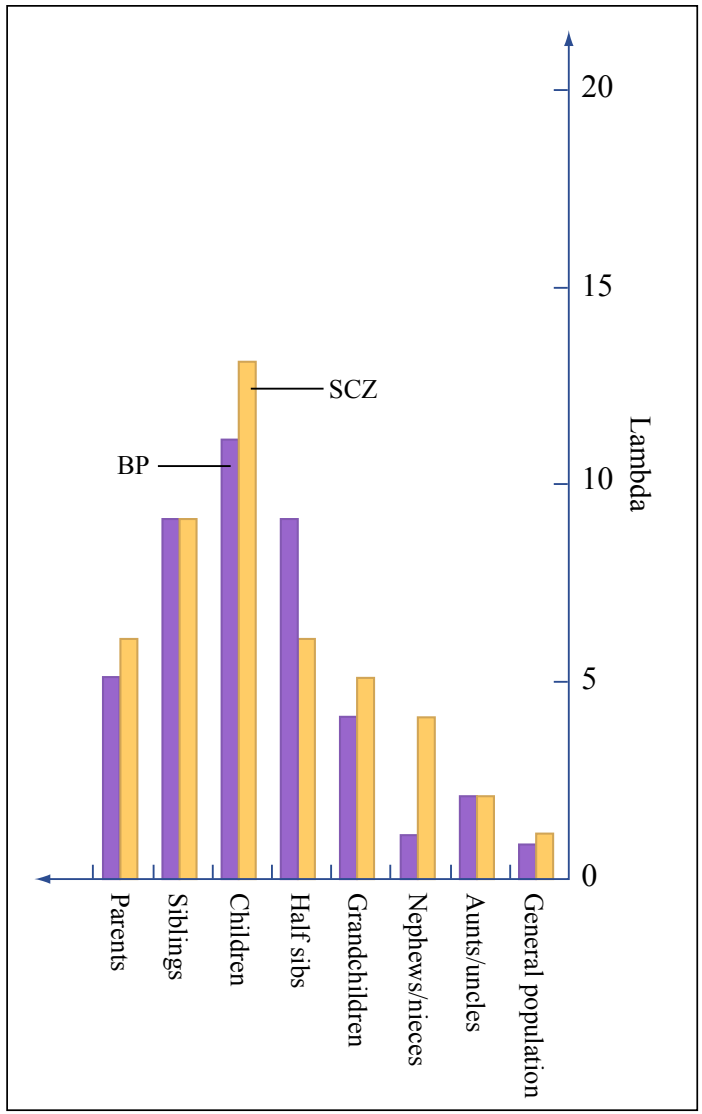
From Gottesman 1991

Families are at Increased Risk



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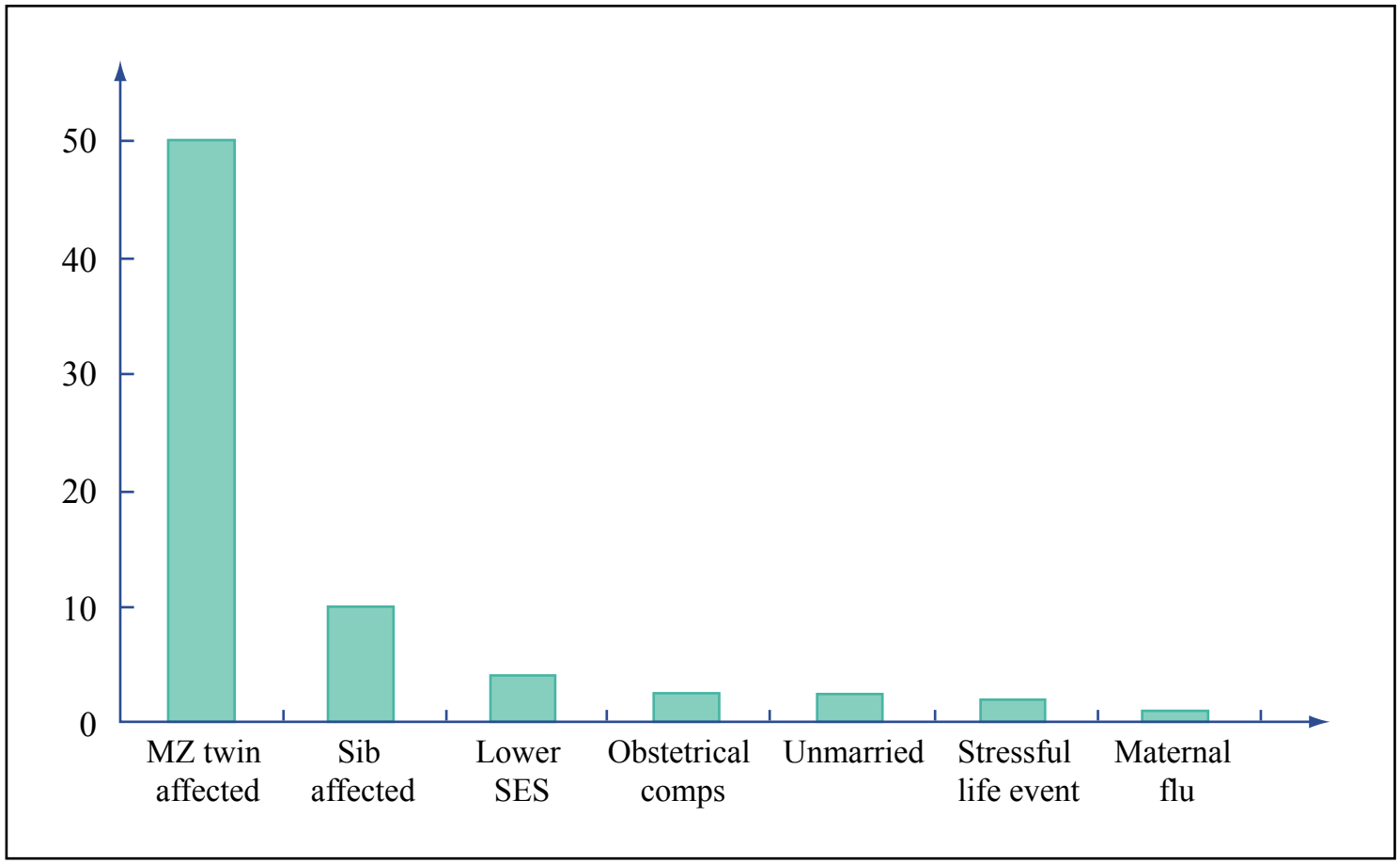
From: Tsuang and Faraone 1990

Relative Risk of Schizophrenia



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From McGuffin, Owen and Gottesman 2004

Categories of Genetic Illnesses

- Single genes determine
  - Mendelian
- Multiple genes determine
  - Complex

Types of Genetic Alterations

- Single Base Changes
  - Letters of the DNA Alphabet
- Deletions of parts of genes
  - Varying sizes
- Rearrangements of genes
- Copy number variations
  - Increase or decrease
  - Large or small

Two Kinds of Inheritance

- Altered gene present in a parent
- Gene alteration during development from sperm and egg-new, de novo

DNA-based frequency variants and disease susceptibility

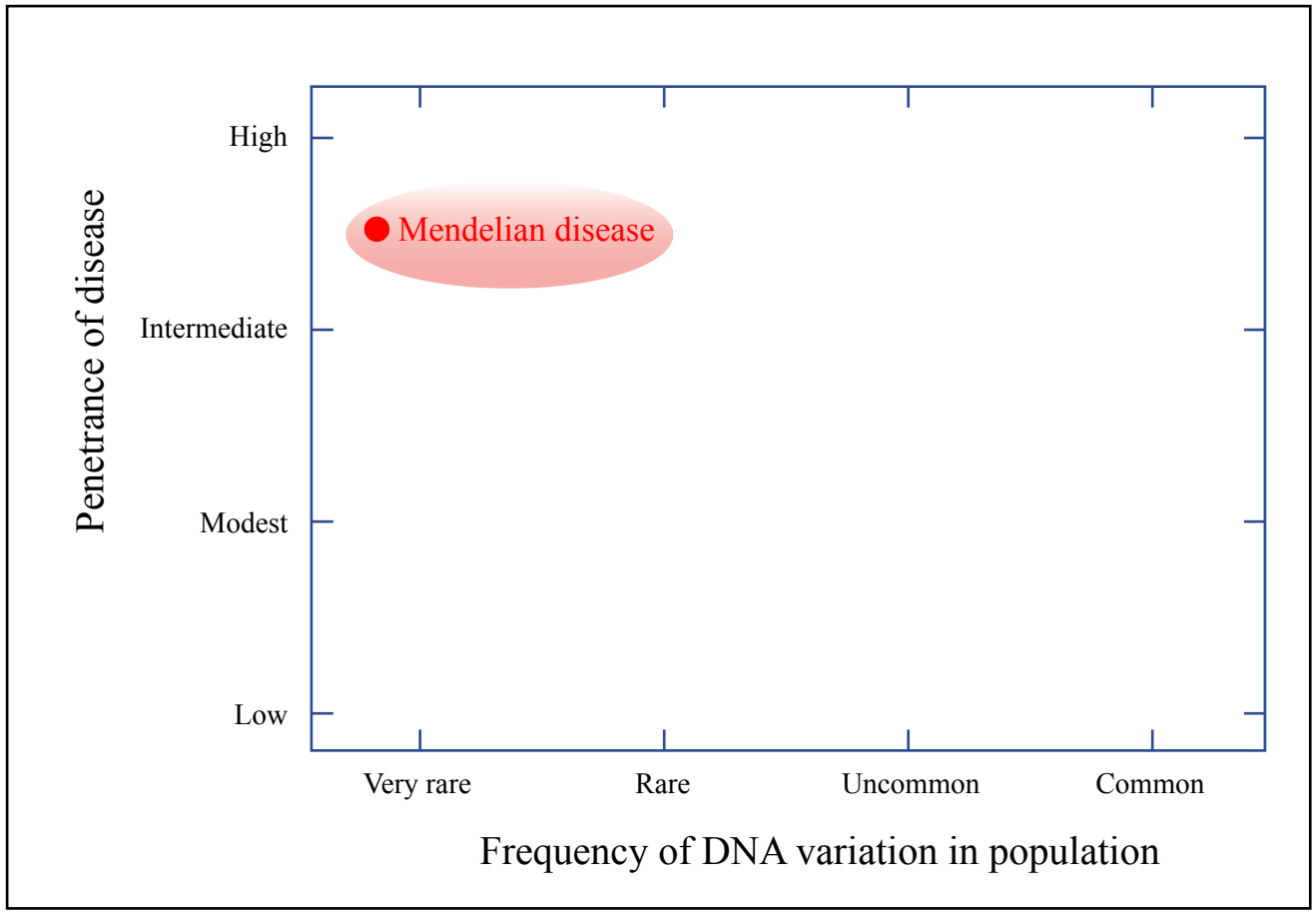

Figure by MIT OpenCourseWare.

McCarthy ☐et al., Nat Genet Rev 2008

An Ancestor in a Family

- Trace the presence of disease in extended family
- Look for genetic markers segregating or tracking with the disease
- Strong effect genes: when a single gene defect can cause a disease

Limited Numbers of Ancestors in a Population

- Search for genes in populations
  - Cases vs. controls
- Genetic markers that associate with the illness based upon inheritance from small number of ancestors in a population
  - Single base changes
  - Copy number variations

DNA-based frequency variants and disease susceptibility

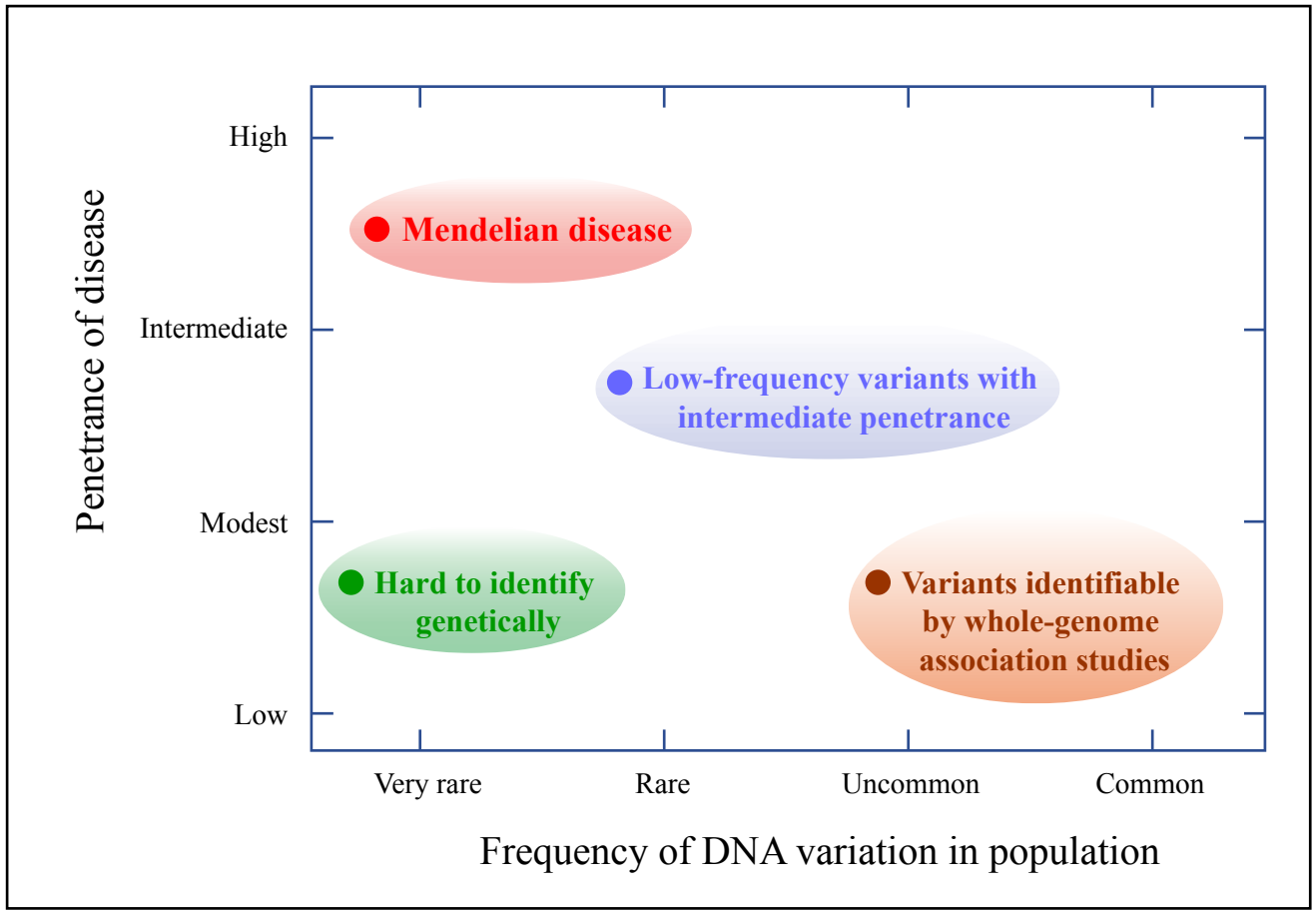

Figure by MIT OpenCourseWare.

McCarthy □et al., Nat Genet Rev 2008

Summary

Current and evolving methods in human genetics allow sound technical approaches to any of these categories of genetic changes conferring risk for, or causing a genetically based illness.

Based on neuroscience, pharmacology, biochemistry, animal models, and human genetics, what can we conceive as new approaches to therapy of psychotic illness?

Schizophrenia

Hypotheses of pathogenesis based upon data in humans without human genetics

- Efficacy of dopamine 2 receptor antagonists and sophisticated receptor occupancy studies
- NMDA glutamate receptor “hypofunction”
  - Ketamine studies in humans and animals
  - NR1 receptor knockdown mice
- Biochemistry of DARPP-32 in relationship to pharmacology of psychomimetic drugs
  - M. Flagolet, et al. PNAS 2003; 100: 16006-11.
  - D. Gerber, et al. PNAS 2003; 100: 8993-8.
  - P. Svenningsson, et al. Science 2003; 302: 1412-15.
  - D. Gerber, et al. New Eng. J of Medicine 2004; 350: 1047-8.

Biochemistry and Pharmacology of Treatments of Schizophrenia

- “Deficiency of GABA production”
  - In certain interneurons that regulate firing of pyramidal neurons
  - Histology of postmortem brain
- Clues from Clozaril
  - Allosteric muscarinic 1 receptor agonist

Possible Implications for New Treatments of Schizophrenia Based on Pathogenesis Hypotheses, Biochemistry, or Pharmacology

- Improve functioning of NMDA receptor
  - Agonists at glycine, serine allosteric site of receptor
    - Glyt1 transport inhibitors
    - ? DAAO inhibitors: issue of anatomical distribution
    - Serine transporter inhibitors
- Enhance function selectively at GABA $\alpha_{2,3}$ receptors
- Allosteric agonist at muscarinic 1 receptor
  - Based upon pharmacology of Clozaril and its primary metabolite desmethylclozaril
    - “Allosteric Site Agonists Allow Unprecedented Selectivity for M1 Receptors” (Lazareno, S. et al. (1998) *Mol. Pharmacol*. **53**, 573-589)
    - Work by Acadia Pharmaceuticals and Herb Meltzer, and Sur *et al*.

Genetics of Schizophrenia in Humans

- Reported Risk Genes
  - G72
  - DISC1
  - Capon
  - Trace amine receptor
  - GRM3 glutamate transporter
  - Dysbindin
  - Proline dehydrogenase
  - Catechol o methyl transferase
  - Neuregulin 1
  - RGS4
  - Calcineurin
  - Palmitoylation enzyme

Genetics of Schizophrenia in Humans

- No clear sequence defined risk variant in common forms of schizophrenia comparable in certainty to
  - Current:
    - APOe4 or presenilin in Alzheimer's disease
    - complement factor H in age-related macular degeneration
    - serine protease in regulating LDL receptor levels
    - Fragile X polymorphism and role of FMR gene product
  - Historical
    - 5 alpha reductase deficiency in male pseudohermaphrodites with small prostate gland
    - Gleevec for CML

Hypotheses for Treatment Based on Genetics of Humans

- Finding: VAL MET polymorphism of catechol o methyl transferase
- More active enzyme →
  - ↓ dopamine in prefrontal cortex →
  - ↓ "cognitive functioning" and possible abnormality in cortical thalamic pathways
- ? Specific inhibitor of COMT
  - Issue: estrogen metabolism pathway
- ? How important is COMT in pathogenesis of schizophrenia
- ? How important is COMT polymorphism in response to atypical neuroleptic treatment

Genetics of Schizophrenia in Humans

- DISC1 Gene truncation
  - Can this be used to devise new treatment?
  - Issue: generality in schizophrenia unclear

- ? PAF receptor antagonist that was brain penetrant

Genetics of Schizophrenia in Humans

- No practical utility in diagnosis or treatment at this time

Meta-Analyses Results:
Bipolar disorder 2500+ Patients; 600+ Families
(Badner & Gershon, Molecular Psychiatry, 2002; Segurado et al., AJHG, 2003)

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State of Genetics Research in Bipolar Illness

- **?** Possible BDNF haplotype
- **?** Possible disc gene mutation
- **?** Polymorphism in G72 gene
- **?** GRK polymorphism
- **?** Polymorphism in a stress response gene
- **?** Region on chromosome 6
- **?** NPAS3

Pathogenesis in Animal Models of Bipolar Disorder

Amphetamine / chlordiazepoxide treatment of rats and mice

- Lithium amelioration in rodents and humans

Biochemistry of Drugs to Treat Bipolar Disorder

- **Lithium:**
  - Inhibition of glycogen synthase kinase beta
    - Genetic GSK Beta +/- heterozygotes (Klein, et al)
  - Inhibition of Inositol monophosphatase
    - ? Role of pituitary adenlycyclase activating peptide (PACAP hormone)
- **Valproate:**
  - Inhibition of histone deacetylases
  - Large gene family
- **Lamotrigine:**
  - ? Na channel inhibitor
  - Rash not mechanism based

Issues Up to Now with Genetics Studies

?

Issues Up to Now with Genetics Studies

- Markers used in linkage studies and genome scans
  - 400-800 microsatellites, small numbers of SNPs compared to ~$10x10^6$ common variants and >$100x10^6$ rare variants
- Size of studies: Few hundred patients in diseases widely acknowledged to be heterogeneous categories
- Has endophenotype not been precise enough?
  - In medical genetics, common patient clinical findings provide impetus for delineation of a syndrome and subsequent discovery of molecular cause. Then redefine syndrome based upon shared molecular structure among patients (Lupski)
  - Mitochondrial metabolic syndrome with tRNA anitcodon mutation
    - Stochastic distribution of symptoms
  - Hutchinson Gilford Progenia: 9 different syndromes from mutations in a single gene

Clustering of Hypertension, Hypercholesterolemia, & Hypomagnesemia

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What can be done to improve chances for defining with certainty risk genes for bipolar disorder and schizophrenia?

Is it worth investing?

- Newer intellectual and technical approaches
  - Various Technologies to search comprehensively for common variants
  - Various genome array technologies to search for structural genomic changes
  - Emerging technologies to look for rare variants of genes

Common Variants that Influence Human Diseases

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HLA: other autoimmune dz.

ApoE4: Alzheimers

Factor V^{leiden}: DVT

HFE: Hemachromatosis

CCR5: HIV infection

CTLA4: Graves disease

NOD2, 5q31: IBD

PTPN22: RA, T1DM, etc

ApoA5: triglycerides

LPL: lipid levels

G6PD: malaria

Ret: Hirschprungs

Complement Factor H: AMD

PPARG: Type 2 Diabetes

Examples of Concrete Contributions of Genetics to Medical Treatment and Diagnosis

Discovery	Practical Importance
LDL receptor mutations	• Development of statins
5 alpha reductase deficiency	• Treatment of benign prostate hypertrophy
Deficiencies of drug metabolizing enzymes	• Use of certain antidepressants • Chemotherapy of glioblastoma • Variation in response to warfarin therapy
Genetic alteration in CML	• Gleevec • Chemotherapy of childhood leukemia

Examples of Concrete Contributions of Genetics to Medical Treatment and Diagnosis (continued)

Discovery	Practical Importance
Her2 amplification in breast cancer	Antibody to Her2
Amyloid precursor mutations in Alzheimer's	Numerous ongoing approaches to therapy to alter natural history
Fragile X Syndrome	New approach binding antagonism of metabotropic Glu5 receptor
pcskg gene in regulating LDL receptor levels	An inhibitor would synergize with statins

The Value of Genetic Information: Understanding Why Disease Happens

Age-related macular degeneration

- Little previously known about molecular causes of disease

- Using LD-based approaches, Complement Factor H found as major cause of the disease (35% frequency, 2.5- fold risk per allele)

Common Variant Drug Targets

Locus Definite Targets	Trait	Drug	rs number	Allele	Risk allele frequency	Statistical eridence	Effect size	Reference
HMGCR	LDL cholesterol	Statins	rs 12654264	A/T	39%	$1x10^{-20}$	4 mg/dL difference in LDL cholesterol between homozygote classes	Kathiresan, nature geneties, in press
PPARG	Type 2 diabetes	Thiazoli	rs 1801282	P12A	87%	$2x10^{-6}$	Odds ratio 1.14 per allele 95% Cl (1.08-1.20)	Diabetes genatics initiative, Science 2007
KCNJ11	Type 2 diabetes	Sulfony lureas	rs 215	E23K	35%	$5x10^{-11}$	Odds ratio 1.14 per allele 95% Cl (1.10-1.19)	Frayling, Nature reviews genetics, 2007

Questions for Psychiatric Geneticists

- Should there be large (>10,000) samples for new searches for risk genes for bipolar illness and schizophrenia?

- Can the field risk not using new technologies on such large sample numbers to try to achieve discoveries with concrete practical application to diagnosis and therapy and refine syndromes based upon shared genetic and molecular structures?

- If this is not done, what are the chances of quantal improvement in our understanding of bipolar illness and schizophrenia?

Drug Discovery and Mental Illness

- Partially effective well-documented treatments for mental illnesses have been available for many decades
- Limited understanding of the disease mechanisms and how existing treatments work has impeded the discovery of more effective medicines
- Drug discovery must move toward a new paradigm based on understanding disease mechanisms, defining biochemical targets, and a sufficiently large and rigorous clinical trial infrastructure to allow iterative clinical trials to determine effective new mechanism of action drugs
- Once new mechanisms are found, long term outcome trials to document benefits and risks of long term treatment

Risk Factors Discovered in Diabetes Mellitus

Gene	Variant	Frequency	Odds Ratio
PPARg	Pro12Ala	85%	1.2
Kir6.2	Glu23Lys	25%	1.25