SISG 2016

Module 9: Genetic Epidemiology

Instructors: Karen Edwards and Carolyn Hutter

July 18th-20th

Day	Time	Lead	Topics	Details
Monday	8:30-9:00	**Carolyn**	Class Intro	*Intro to class/Review Agenda and topics * Student introductions
	9:00-10:00	**Carolyn**	Epi 101	* Intro to Epidemiology * Measure_of_Association_handout
	10:30-12:00	**Karen**	Overview of Genetic Epi	* Overview of Genetic Epi * Intro to terms, types of variation, genetics 101
	1:30-3:00	**Karen**	Family Studies	* Segregation Analysis * Linkage Analysis
	3:30-5:00	**Carolyn**	Linkage Disequilibrium	* Intro to LD * Haploview and GVS * Imputation
Tuesday	8:30-10:00	**Karen**	Association Studies	* Population Stratification * Odds Ratio Calculations * Power Calculation Resources
	11:00-12:00	**Carolyn**	GWAS	* GWAS overview * "Post-GWAS"
	1:30-3:00	**Carolyn**	GxE	* Concepts and terms * Methods for GxE * In class exercise
	3:30-4:15	**Karen**	Sequencing Studies 1	* Rare variant analysis
	4:15-5:00	**Karen**	Journal Club 1	* Rosenthal et al.
Wednesday	8:30-9:15	**Carolyn**	Journal Club 2	* Nan et al.
	9:15-10:00	**Karen**	Seq. Studies II	* Family studies are new again
	10:30-11:00	**Karen**	Precision Medicine I	* Overview of Precision Medicine * Links to Genetic Epidemiology
	10:30-11:30	**Carolyn**	Precision Medicine II	* Applications in Cancer and Oncology
	11:30-12:00	**Carolyn & Karen**	Wrap-up	* One slide per item

Genetic Epidemiology

SISG Module 9

July 18-20, 2016

Instructors: Carolyn Hutter & Karen Edwards

Big Picture Learning Goals

- Familiarity with major study designs used in genetic epidemiology
- Familiarity with major issues associated with each approach
- Aware of software and web resources used in genetic epidemiology

Course Objectives

- The objective of this course is to provide an introduction to methods and applications of genetic epidemiology.
- Students will be exposed to basic concepts and principles of genetic epidemiology, including:
  - study designs for family based and population based studies
  - analytical methods used in studies of linkage and association
  - modern approaches to gene-environment interactions and rare variant analysis
  - key web resources for analysis and interpretation
  - relevant literature in the field

Class Structure

Day	**Time**	**Lead**	**Topics**	
Monday	8:30-10:00	Carolyn	Class Intro	
		Carolyn	Epi 101	
	10:00-10:30 Break			
	10:30-12:00	Karen	Overview of Genetic Epi	
	12:00-1:30	Lunch		
	1:30-3:00	Karen	Family Studies	
	3:00-3:30 Break			
	3:30-5:00	Carolyn	Linkage Disequilibrium	

Tuesday	**Time**	**Lead**	**Topics**	
AM	8:30-10:00	Karen	Association Studies	
	10:00-10:30 Break			
	10:30-12:00	Carolyn	Genome Wide Association Studies	

Class Structure Continued

Tuesday PM	Time	Lead	Topics	
	12:30-3:00	Carolyn	GxE	
	3:00-3:30 Break			
	3:30-5:00	Karen	Sequencing Studies I /Journal Club I	

Wednesday	Time	Lead	Topics	
	8:30-10:00	Carolyn & Karen	Sequencing Studies II /Journal Club II	
	10:00-10:30 Break			
	10:30-11:30	Carolyn & Karen	Precision Medicine	
	11:30-12:00	Carolyn & Karen	Wrap-up	

- This module will include a combination of lectures, in class tutorials and assignments, small group interactive activities and readings.

Class introductions

- Break into groups of 2-3
- Introduce yourselves to one-another, and you will introduce your group members to the class.
- Items to include:
  - Name
  - “Day-job”
  - Main objective for taking this course
  - Thing most excited to learn in over the next 3 days

Introduction to Epidemiology

Definitions, Objectives and Historic Examples

"And it was so typically brilliant of you to have invited an epidemiologist."

THINK-PAIR-SHARE ACTIVITY

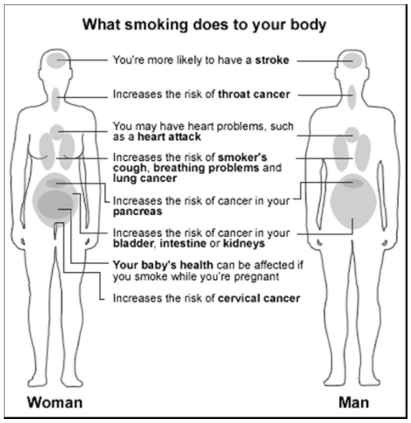
1. Define "Epidemiology"

2. Give an example of what an epidemiologist does

Definitions of Epidemiology

Objectives of Epidemiology

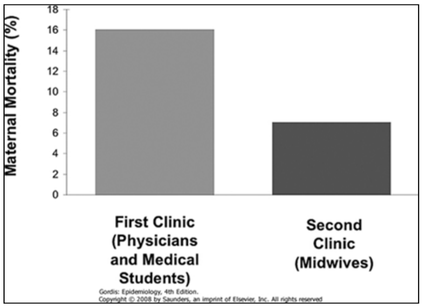
1. Identify disease etiology
2. Determine the burden of disease
3. Study the natural history of disease
4. Evaluate preventive and therapeutic measures
5. Provide foundation for public policy


What smoking does to your body
You're more likely to have a stroke
Increases the risk of throat cancer
You may have heart problems, such as a heart attack
Increases the risk of smoker's cough, breathing problems and lung cancer
Increases the risk of cancer in your pancreas
Increases the risk of cancer in your bladder, intestine or kidneys
Your baby's health can be affected if you smoke while you're pregnant
Increases the risk of cervical cancer
Woman
Man

http://www.webmd.boots.com/a-to-z-guides/tc/smoking-what-will-happen

Ignaz Semmelweis - 1846

- Childbed Fever
  - Major cause of death post childbirth
  - Theories included putrid air, solar influences, etc.
- Obstetrical Clinics of the Allgmeine Krankenhaus
  - 1st Clinic
    - Physicians and Medical Students
    - Performed autopsies at start of the day
    - Mortality: 16%
  - 2nd Clinic
    - Midwives
    - No autopsies
    - Mortality: 7%



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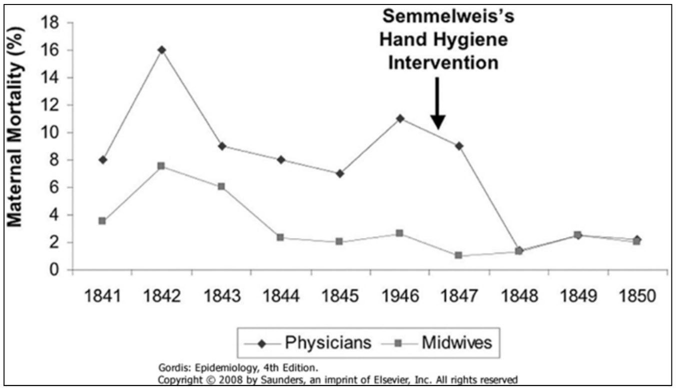
- Suggested transmission of disease from cadavers to women
- Noted a colleague died from similar infection after being punctured during an autopsy
- Implemented policy that physicians and students wash hands and scrub nails after autopsy, before contact with patients:



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Edward Jenner - 1796

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- Smallpox
  - 400,000 people died per year in Europe
  - Devastating when introduced to Americas (biological warfare)
  - Case fatality rate of 40-60%
- Variolation and vaccination
  - Originally infected healthy individuals with material from smallpox patients.
  - Jenner noted that dairy maids, who were exposed to cowpox, did not develop smallpox.

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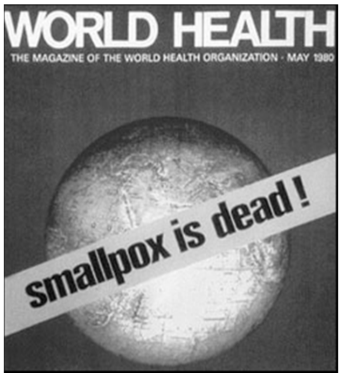
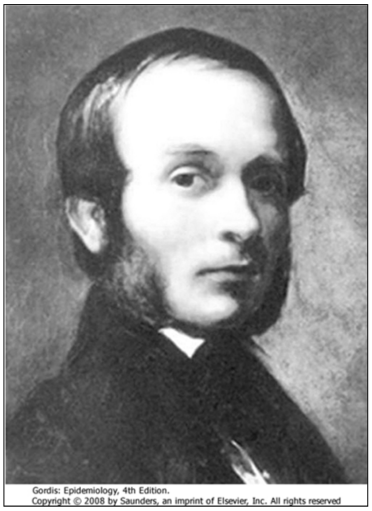
Smallpox Eradication

- 1967
  - ~15 million cases per year
  - ~2 million deaths
  - WHO starts efforts to eradicate smallpox
- 1980
  - WHO certifies that smallpox has been eradicated
  - Last natural case in 1977
  - Last US vaccinations in 1972
- 2001
  - Increased concern about smallpox and bioterrorism
- 2014
  - Small pox found in NIH storage refrigerator



John Snow- 1854

- Cholera
  - Severe bacterial infection
  - Miasmatic theory of disease
- London in the 1800s
  - Multiple cholera pandemics 1831-1854
  - 1949 John Snow published that cholera was caused by water



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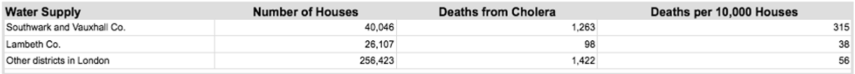
“Father of Modern Epidemiology”

- Combined field work with statistical methods to examine water sources used by people with cholera.

- **Broad Street Pump**
  - Collected records on 83 deaths
  - Noted unexpected non-cases
- **Grand Experiment**
  - Multiple companies supplied same Neighborhoods
  - Noted that the Lambeth Company moved intake to a less polluted source

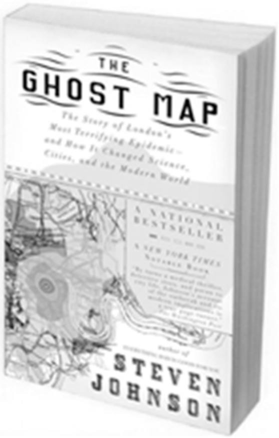
Table 1-5. Deaths from Cholera per 10,000 Houses, by Source of Water Supply, London, 1854

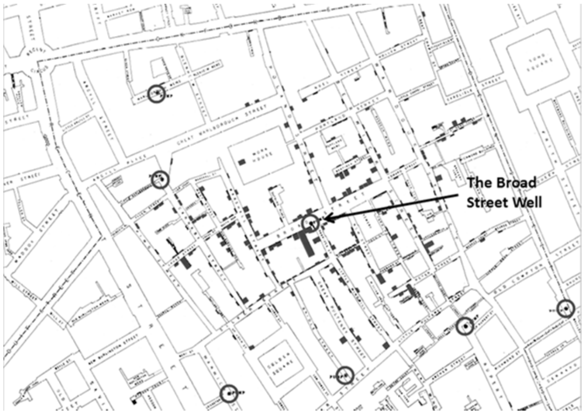


Water Supply	Number of Houses	Deaths from Cholera	Deaths per 10,000 Houses
Southwark and Vauxhall Co.	40,046	1,263	315
Lambeth Co.	26,107	98	38
Other districts in London	256,423	1,422	56

Data adapted from Snow J: On the mode of communication of cholera. In Snow on Cholera: A Reprint of Two Papers by John Snow, M.D. New York, The Commonwealth Fund, 1936.

John Snow’s Map



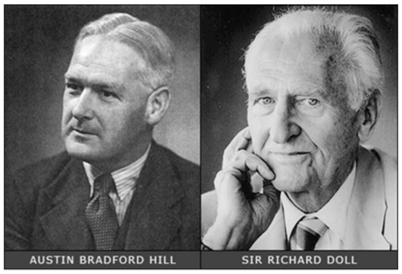


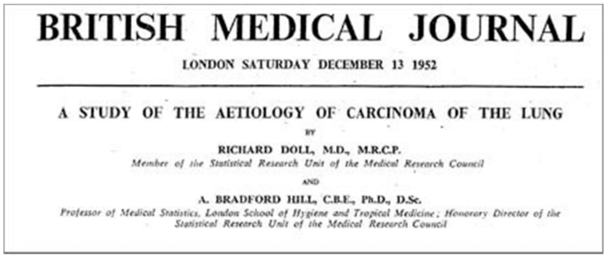
http://scienceblogs.com/significantfigures/index.php/2013/03/11/200-years-of-dr-john-snow-a-significant-figure-in-the-world-of-water/

http://www.youtube.com/watch?v=Pq32LB8j2K8

Richard Doll and Bradford Hill- 1952

- Smoking and lung cancer
  - 1920s health care workers noted that many lung cancer patients also smoked
  - Incidence of lung cancer in men over 45 rose 6 fold from 1930 to 1945
  - Cars or other industrial changes.
- Experimental design
  - Case-control study
    - Looked at hospital patients with and without cancer.
  - Cohort study
    - Prospectively followed >40,000 physicians





BRITISH MEDICAL JOURNAL

LONDON SATURDAY DECEMBER 13 1952

A STUDY OF THE AETIOLOGY OF CARCINOMA OF THE LUNG

BY

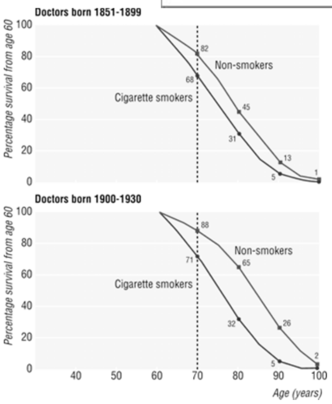
RICHARD DOLL, M.D., M.R.C.P.

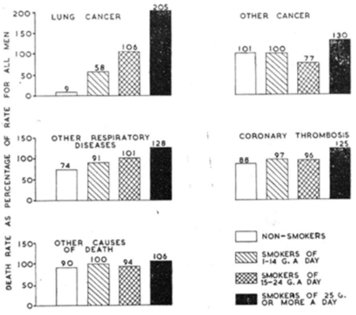
Member of the Statistical Research Unit of the Medical Research Council

AND

A. BRADFORD HILL, C.B.E., Ph.D., D.Sc.

Professor of Medical Statistics, London School of Hygiene and Tropical Medicine; Honorary Director of the Statistical Research Unit of the Medical Research Council



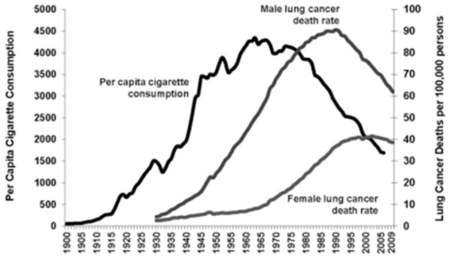


Relationship between death rate, expressed as a percentage of the rate for all men, and the amount smoked, for five disease groups.

BMJ 2004;328:1519

US Lung Cancer Trends

Trends in Tobacco Use and Lung Cancer Death Rates* in the US



*Age-adjusted to 2000 US standard population.

Source: Death rates: US Mortality Data, 1960-2009, US Mortality Volumes, 1930-1959, National Center for Health Statistics, Centers for Disease Control and Prevention. Cigarette consumption: US Department of Agriculture, 1900-2007.

Descriptive and Analytic Epidemiology

Descriptive vs. Analytical Epidemiology

Descriptive Epidemiology

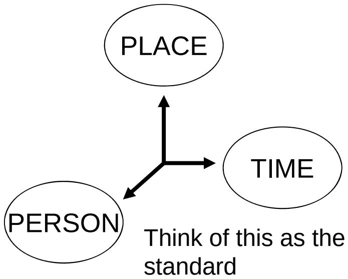
- Includes activities related to characterizing the distribution of diseases within a population

Analytical Epidemiology

- Concerns activities related to identifying possible causes for the occurrence of diseases

Objectives of Descriptive Epidemiology

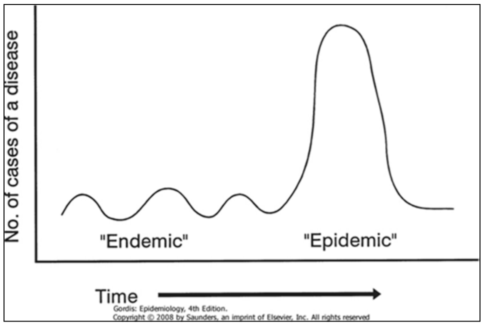
- To evaluate trends in health and disease and allow comparisons among countries and subgroups within countries
- To provide a basis for planning, provision and evaluation of services
- To identify problems to be studied by analytic methods and to test hypotheses related to those problems



Think of this as the standard dimensions used to track the occurrence of a disease.

Some Definitions

- **Endemic**
  - Habitual presence of a disease in a given area
  - Usual prevalence of disease within such an area
- **Epidemic**
  - Occurrence in a community or region of a group of illnesses of similar nature in excess of normal expectancy.
  - Derived from common or propagated source.
  - Often called "outbreak"
- **Pandemic**
  - Epidemic over a wide geographic area.



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Key Measures in Epidemiology

Morbidity Measures

- Incidence (new cases)
  - Cumulative incidence (proportion)
  - Incidence rate (new cases per unit time)
- Prevalence (Existing cases)
  - Existing cases
  - Point prevalence (proportion)
  - Period prevalence

Mortality Measures

- Mortality rate (number of deaths per unit time)
- Cumulative mortality (proportion)
- Case-fatality rate (proportion of subjects with disease that die from that disease)
- Proportionate mortality (proportion of people who die, who died from a specific disease- be careful when using this measure)

The denominator is a key part of measures in epidemiology.

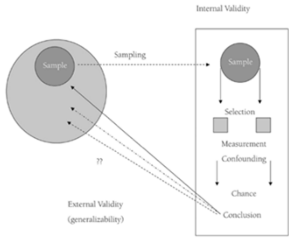
Goal in Analytical Epidemiology

- Test a hypothesis about relationship between exposure(s) and disease(s)

- Consider Internal Validity
  - Ideal: Free from bias in design, implement, analyze and interpretation
  - Reality: We need to address biases

- Consider External Validity
  - Ideal: Generalizable
  - Reality: Applies to study population, infer more broadly

Figure 1. The architecture of clinical research

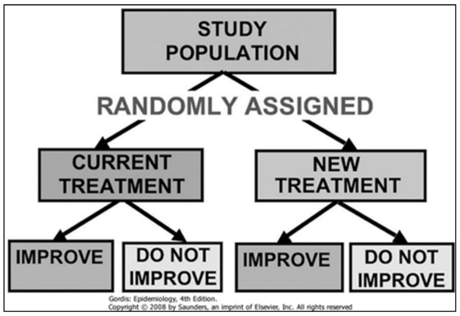


Source: Reproduced with permission from Fletcher et al. (1996).

Study Designs for Analytical Epidemiology

Randomized Trials

- RCTs
  - Randomized Clinical Trials
  - Randomized Control Trials
- Often used for studies of treatment/drugs in relation to prognosis
- Can also be used in other settings, including studies of risk and prevention
- Key elements:
  - Study population is defined
  - Study population is randomly assigned to two (or more) study "arms"
  - Outcomes are compared for the different arms



STUDY POPULATION

RANDOMLY ASSIGNED

CURRENT TREATMENT

NEW TREATMENT

IMPROVE

DO NOT IMPROVE

IMPROVE

DO NOT IMPROVE

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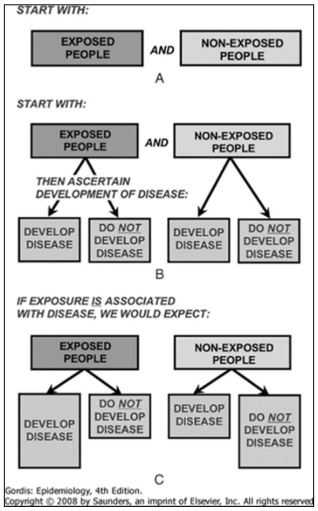
Cohort and Case-Control Studies

- Observational study designs used in epidemiology
- Consider strengths, limitations and sources of bias
- Cohort Study
  - Steps: Define cohort, take baseline measurements and exposure status, ascertain outcome information, compare incidence in exposed and unexposed
  - Can be prospective or retrospective
- Case-Control Study
  - Steps: Identify cases, identify/select controls, collect exposure history prior to disase onset, compare odds of exposure in cases to odds in controls
  - Key step is identifying an appropriate control group

Cohort



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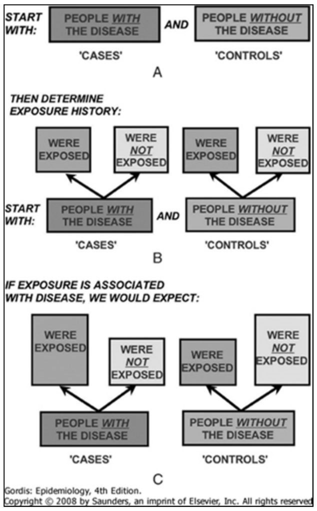
Case-Control



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Cohort Study

Strengths

- Temporal sequence
- Allows measurement of incidence rate and risk
- Can examine multiple outcomes
- Allows examination of rare exposures
- Minimizes information bias for exposure
- Minimizes survivor bias

Limitations

- Ineffective for rare diseases
- Often requires large sample
- Often requires long time to complete (lag-time)
- Expensive
- Attrition/sensitive to loss to follow-up
- May have differential ascertainment of outcome
- Confounding can occur

Case-Control Study

Strengths

- Can examine multiple exposures
- Allows examination of rare diseases
- Minimizes information bias for exposure
- Applicable when long lag time
- Compared to cohort studies, they are often smaller and require less time and money

Limitations

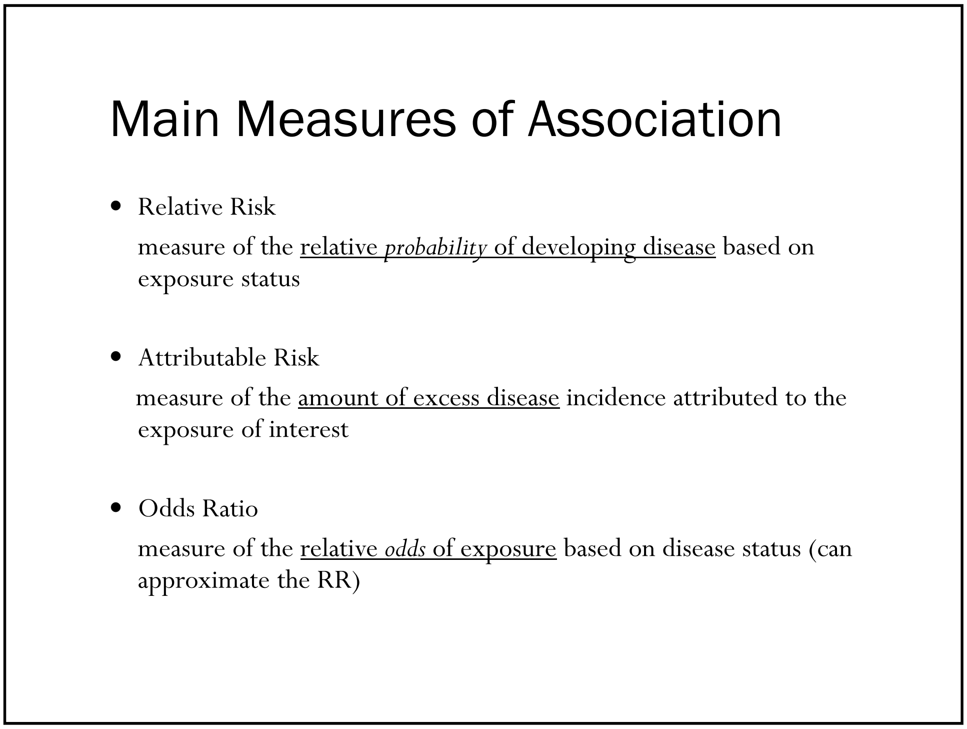
- Control selection can be difficult
- Recall limitations and recall bias
- Sample size issues for rare exposures
- Cannot directly estimate incidence rates, relative risks or attributable risk.

Measures of Association

Note: Some slides in this lecture come from:

http://www.teachepi.org/documents/courses/fundamentals/Pai_Lecture4_Measures%20of%20Effect%20and%20Impact.pdf

Others from University of Washington EPI 420 materials



Main Measures of Association

- Relative Risk

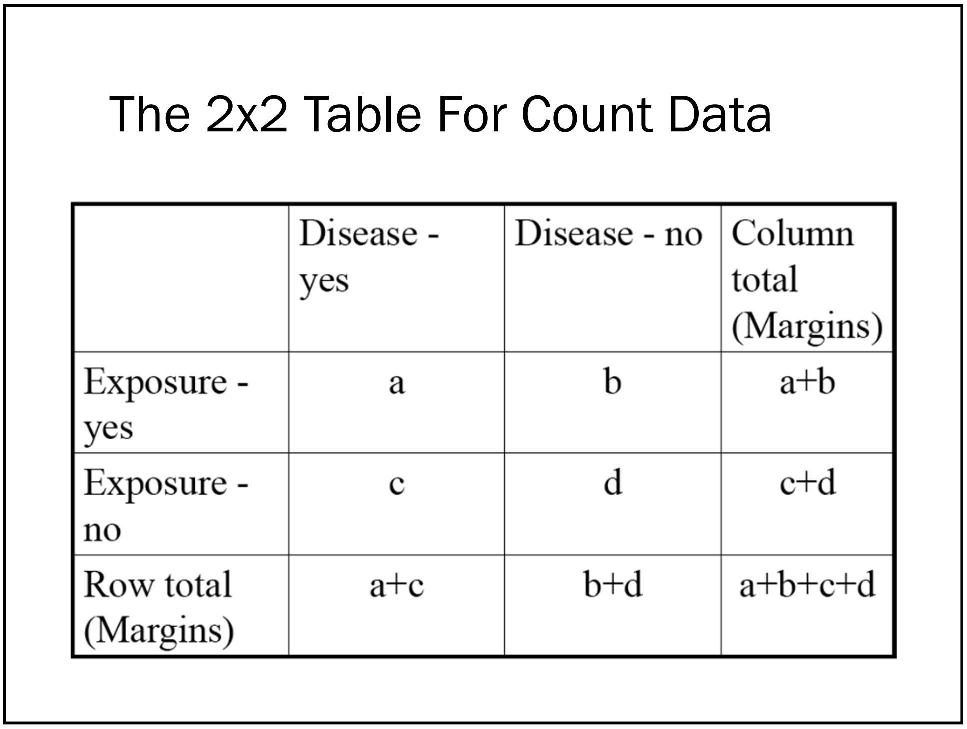
  measure of the relative *probability* of developing disease based on exposure status

- Attributable Risk

  measure of the amount of excess disease incidence attributed to the exposure of interest

- Odds Ratio

  measure of the relative *odds* of exposure based on disease status (can approximate the RR)



The 2x2 Table For Count Data

	Disease - yes	Disease - no	Column total (Margins)
Exposure - yes	a	b	a+b
Exposure - no	c	d	c+d
Row total (Margins)	a+c	b+d	a+b+c+d

Relative Risk (RR) For Count Data

- Used in Randomized trials Cohort studies
- Based on cumulative incidence measure
- AKA: Risk Ratio
- If no association RR=1

	Disease		
Exposure	+	-	Total
+	a	b	a+b
-	c	d	c+d

$$\textbf{RR =} \quad \frac{a/(a+b)}{c/(c+d)} = \frac{\text{(Incidence of Disease in Exposed)}}{\text{(Incidence of Disease in Unexposed)}}$$

Attributable Risk (AR) for Count Data

- Used in Randomized trials and Cohort studies
- AKA: Risk Difference*
- Difference in risk between exposed and unexposed
- If no association AR=0

	Disease		
Exposure	+	-	Total
+	a	b	a+b
-	c	d	c+d

$$\textbf{AR =} \quad a/(a+b) - c/(c+d) = \text{Incidence}_{\text{(exposed)}} - \text{Incidence}_{\text{(unexposed)}}$$

* Note: Some argue that you should use the term risk difference when testing for association, and only use “Attributable Risk” for when you have established causality.

Odds Ratio (OR) for Count Data

- Used primarily in Case Control Studies (also in Cohort)
- AKA: Relative Odds
- Good *estimate* of RR
- If no association OR=1

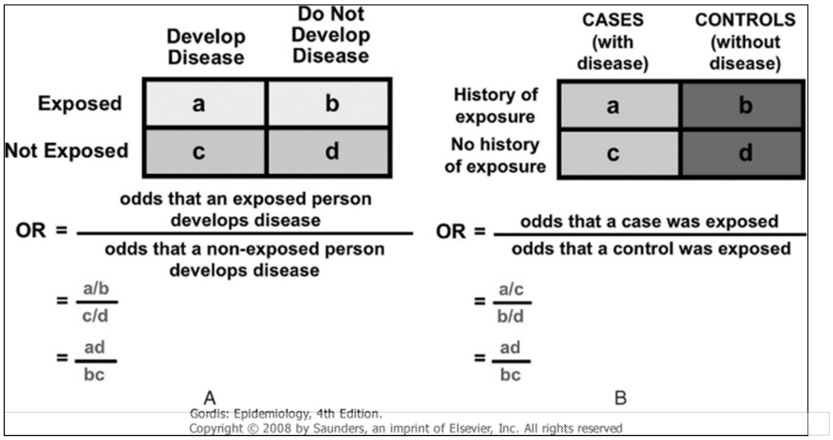
	Disease		
Exposure	+	-	Total
+	a	b	a+b
-	c	d	c+d

$$\textbf{OR} = \frac{a/c}{b/d} = \frac{a*d}{b*c} = \frac{\text{(Odds of Exposure among Cases)}}{\text{(Odds of Exposure among Controls)}}$$

What are Odds?

- Odds: Ratio of ways an event can occur to ways the event can not occur.
  - Odds of 1:1 indicate both options are easily likely.
  - When rolling dice, probability of getting a 2 is 1/6
  - Odds of getting a 2 is 1:5.
- Used in epidemiology, because of situations where we calculate odds ratios
  - Case-control studies
  - Logistic regression
- If P=probability of event, then odds= $\dfrac{P}{1-P}$
- If P is very small, 1-P≈1 and odds= $\dfrac{P}{1-P} \approx \dfrac{P}{1} = P$

Odds Ratio in Cohort Study and Case-Control Study Reduce to Same Calculation



	Develop Disease	Do Not Develop Disease
Exposed	a	b
Not Exposed	c	d

$$OR = \frac{\text{odds that an exposed person develops disease}}{\text{odds that a non-exposed person develops disease}}$$

$$= \frac{a/b}{c/d}$$

$$= \frac{ad}{bc}$$

A

	CASES (with disease)	CONTROLS (without disease)
History of exposure	a	b
No history of exposure	c	d

$$OR = \frac{\text{odds that a case was exposed}}{\text{odds that a control was exposed}}$$

$$= \frac{a/c}{b/d}$$

$$= \frac{ad}{bc}$$

B

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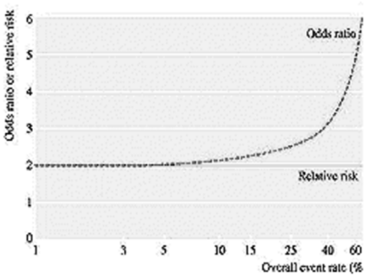
Odds Ratio Estimates Relative Risk When Disease is Rare

- The OR will be a good estimate of the RR if the outcome is rare.
- If the outcome is common, and association is positive, then the OR will overestimate the RR
- This overestimation can be quite large for common outcomes.



BMJ 1997;No 7121 Volume 315

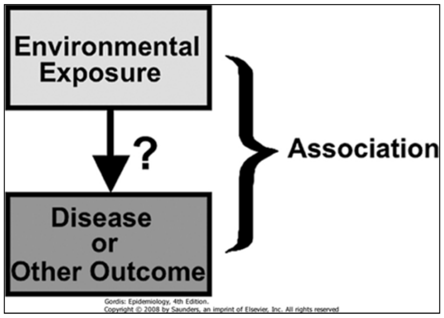
Confidence Intervals and p-values

- Presentation so far has focused on point estimates
- Gives information on magnitude of association
- Statistical software will also provide estimate of confidence intervals and p-values
- Important to consider precision and statistical significance, along with estimate of magnitude of association.

Bias, Confounding, and Causal Inference

Association and Causality

- An exposure and outcome are associated if there is a differential distribution:
  - Incidence of outcome differs for exposed and unexposed group; or
  - Prevalence of exposure differs between cases and controls
- An exposure is causal for the outcome if the presence (or absence) of the exposure directly or indirectly influences whether the outcome occurs.



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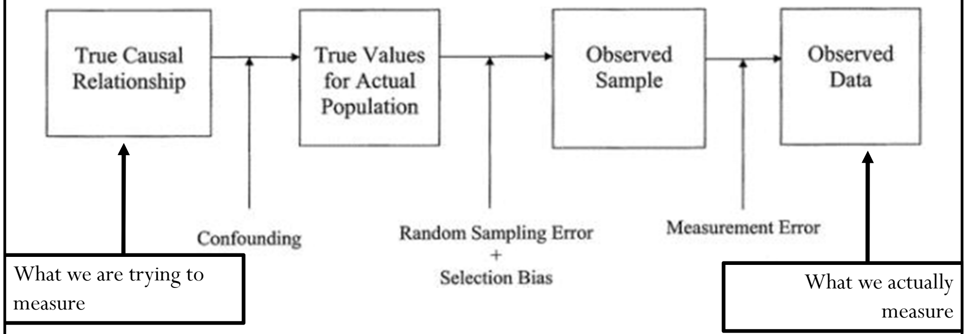
Sources of Bias in Epidemiology



What we are trying to measure

What we actually measure

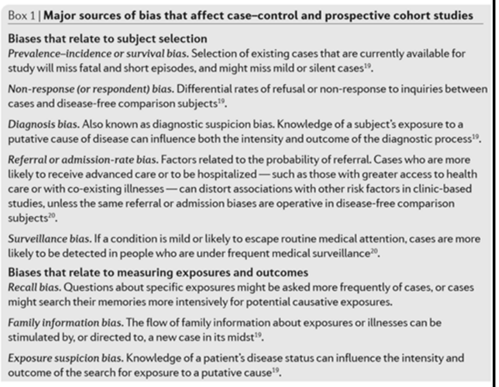
Bias = Systematic error in the design, conduct or analysis of a study that results in a <u>mistaken estimate of an exposure's effect on the risk of disease</u>

Phillips, C.V. 2003. Epidemiology. 14(4):459-466.

Sources of Bias in Epidemiology

- **Selection Bias**
  - Arises from issues in case/control ascertainment
- **Information Bias**
  - Arises from measurement error or misclassification in assessing factors of interest.
- **Confounding***
  - Arises when there is an extraneous disease risk factor that is also associated with exposure and not in the causal pathway.

*Some argue confounding is not technically a bias



Box 1 | **Major sources of bias that affect case–control and prospective cohort studies**

Biases that relate to subject selection

Prevalence–incidence or survival bias. Selection of existing cases that are currently available for study will miss fatal and short episodes, and might miss mild or silent cases[19].

Non-response (or respondent) bias. Differential rates of refusal or non-response to inquiries between cases and disease-free comparison subjects[19].

Diagnosis bias. Also known as diagnostic suspicion bias. Knowledge of a subject's exposure to a putative cause of disease can influence both the intensity and outcome of the diagnostic process[19].

Referral or admission-rate bias. Factors related to the probability of referral. Cases who are more likely to receive advanced care or to be hospitalized — such as those with greater access to health care or with co-existing illnesses — can distort associations with other risk factors in clinic-based studies, unless the same referral or admission biases are operative in disease-free comparison subjects[20].

Surveillance bias. If a condition is mild or likely to escape routine medical attention, cases are more likely to be detected in people who are under frequent medical surveillance[20].

Biases that relate to measuring exposures and outcomes

Recall bias. Questions about specific exposures might be asked more frequently of cases, or cases might search their memories more intensively for potential causative exposures.

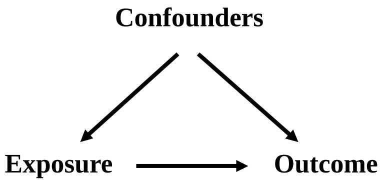
Family information bias. The flow of family information about exposures or illnesses can be stimulated by, or directed to, a new case in its midst[19].

Exposure suspicion bias. Knowledge of a patient's disease status can influence the intensity and outcome of the search for exposure to a putative cause[19].

Manolio et al. Nat Rev Genet. 2006. 7: 812-820.

Confounding

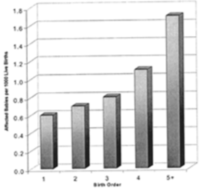
- Confounding is a key topic in epidemiology
- A confounder is often defined as a factor that is:
  1. A risk factor for disease
  2. Associated with exposure
  3. Not a direct result of exposure
- Confounding can lead to "spurious" associations



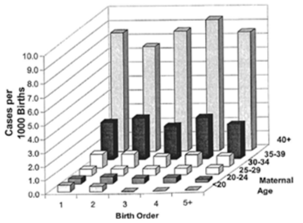
Example of Confounding

- Birth order and Down syndrome
- Birth order is associated with Down syndrome, later order children with higher risk
  - Maternal age is associated with birth order
  - Maternal age is associated with Down Syndrome
- Stratifying on maternal age, there is no longer evidence of an association between birth order and Down syndrome

Association between birth order and Down syndrome



Data from Stark and Mantel (1966) Source: Rothman 2002



Data from Stark and Mantel (1966) Source: Rothman 2002

Approaches to Handling Confounding

In Design of Study

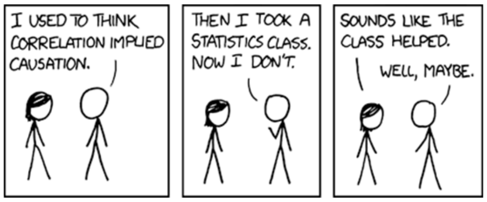
- Randomization
- Restriction
- Matching
  - Group Matching
  - Individual Matching

In Analysis of Data

- Standardization
- Adjustment
- Stratification

Guidelines for Judging Whether an Association is Causal

- Temporal relationship (exposure should proceed outcome)
- Strength of association (size of odds ratio or relative risk)
- Dose-response relationship
- Cessation of exposure leads to reduction in outcome
- Replication of finding (multiple independent studies)
- Biological plausibility
- Consistency with other knowledge
- Consideration of alternative explanations (ability to rule them out)
- Specificity of the association


I USED TO THINK CORRELATION IMPLIED CAUSATION.
THEN I TOOK A STATISTICS CLASS. NOW I DON'T.
SOUNDS LIKE THE CLASS HELPED.
WELL, MAYBE.

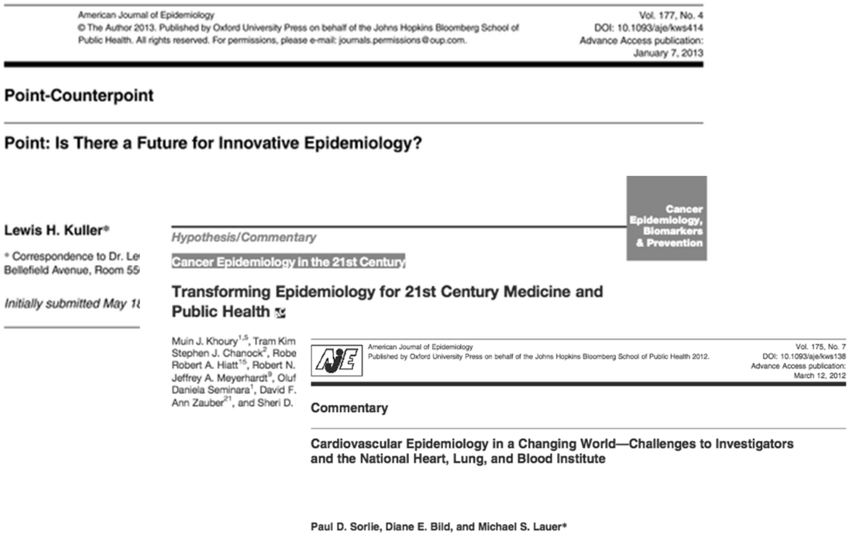
What is Meant by Interaction?

- **Biological Interaction**
  - The interdependent operation of two or more biological causes to produce, prevent or control an effect
  - Two causes interact on a biological level to cause a disease or outcome

- **Statistical Interaction**
  - The observed joint effects of two factors differs from that expected on the basis of their independent effects
  - Deviation from additive or multiplicative joint effects

- **Effect Modification** (or Effect Measure Modification)
  - Differences in the effect measure for one factor at different levels of another factor
  - Example: OR differs for males vs. females; AR differs for pre-menopausal and post-menopausal women, etc.

Future Directions in Epidemiology



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Advance Access publication:
January 7, 2013

Point-Counterpoint

Point: Is There a Future for Innovative Epidemiology?

Lewis H. Kuller*

* Correspondence to Dr. Le
Bellefield Avenue, Room 55

Initially submitted May 1

Cancer
Epidemiology,
Biomarkers
& Prevention

Hypothesis/Commentary

Cancer Epidemiology in the 21st Century

Transforming Epidemiology for 21st Century Medicine and Public Health

Muin J. Khoury[1,5], Tram Kim
Stephen J. Chanock[2], Robe
Robert A. Hiatt[15], Robert N.
Jeffrey A. Meyerhardt[9], Oluf
Daniela Seminara[1], David F.
Ann Zauber[21], and Sheri D.

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Advance Access publication:
March 12, 2012

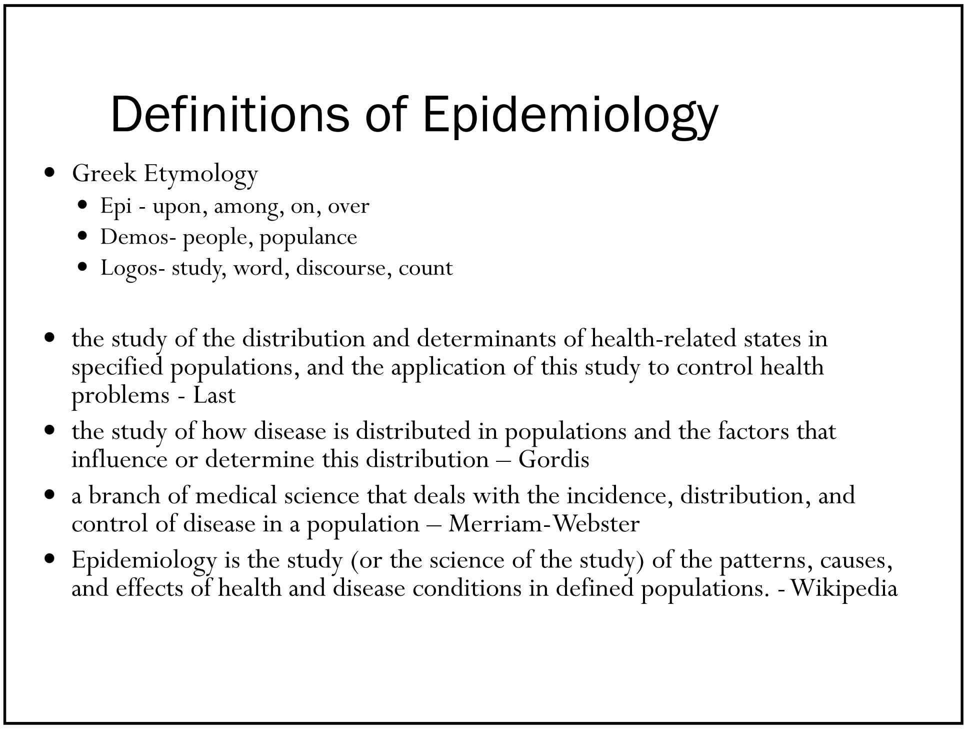
Commentary

Cardiovascular Epidemiology in a Changing World—Challenges to Investigators and the National Heart, Lung, and Blood Institute

Paul D. Sorlie, Diane E. Bild, and Michael S. Lauer*

Summary

- Epidemiology is the study of the distribution and determinants of health-related states in populations
- Historic examples demonstrate objectives of epidemiology
- Study design is a key component of epidemiology
- Relative risks, risk differences and odds ratios are used to measure association
- It is important to consider and address bias in epi studies
- Selection bias and information bias are two main classes of bias
- Understanding confounding and effect modification are important in studies of association
- Future directions are transforming the field of epidemiology



Definitions of Epidemiology

- Greek Etymology
  - Epi - upon, among, on, over
  - Demos- people, populance
  - Logos- study, word, discourse, count

- the study of the distribution and determinants of health-related states in specified populations, and the application of this study to control health problems - Last
- the study of how disease is distributed in populations and the factors that influence or determine this distribution – Gordis
- a branch of medical science that deals with the incidence, distribution, and control of disease in a population – Merriam-Webster
- Epidemiology is the study (or the science of the study) of the patterns, causes, and effects of health and disease conditions in defined populations. - Wikipedia

Genetic Epidemiology

Introduction

Karen L. Edwards, Ph.D.

Professor

Department of Epidemiology and

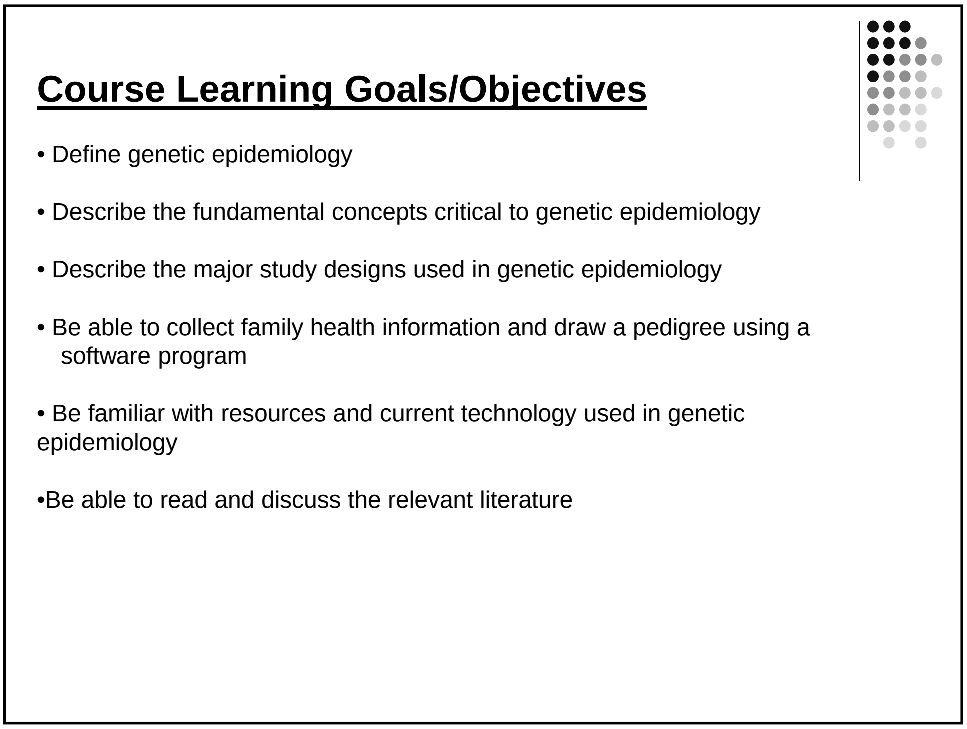
Genetic Epidemiology Research Institute

School of Medicine

University of California, Irvine
Irvine, CA

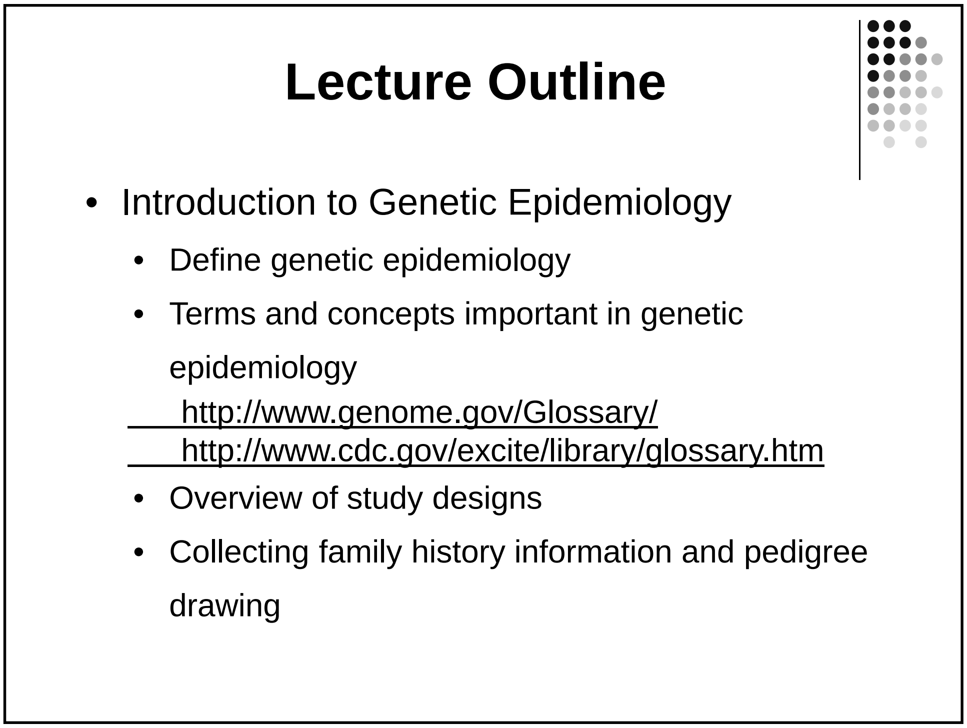
Big Picture Learning Objectives

- Familiarity with major study designs used in genetic epidemiology
- Familiarity with major issues associated with each approach
- Aware of software and web resources used in genetic epidemiology



Course Learning Goals/Objectives

- Define genetic epidemiology
- Describe the fundamental concepts critical to genetic epidemiology
- Describe the major study designs used in genetic epidemiology
- Be able to collect family health information and draw a pedigree using a software program
- Be familiar with resources and current technology used in genetic epidemiology
- Be able to read and discuss the relevant literature



Lecture Outline

- Introduction to Genetic Epidemiology
  - Define genetic epidemiology
  - Terms and concepts important in genetic epidemiology

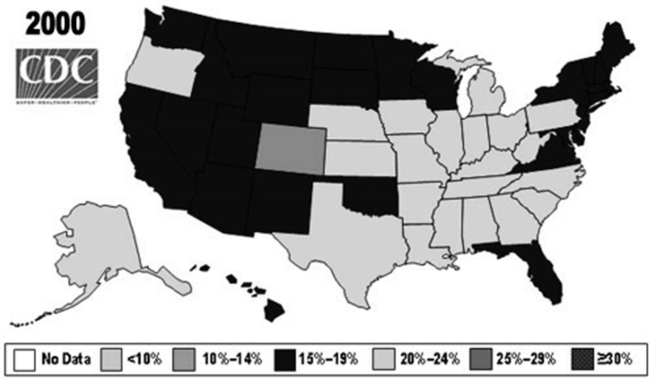
    http://www.genome.gov/Glossary/

    http://www.cdc.gov/excite/library/glossary.htm
  - Overview of study designs
  - Collecting family history information and pedigree drawing

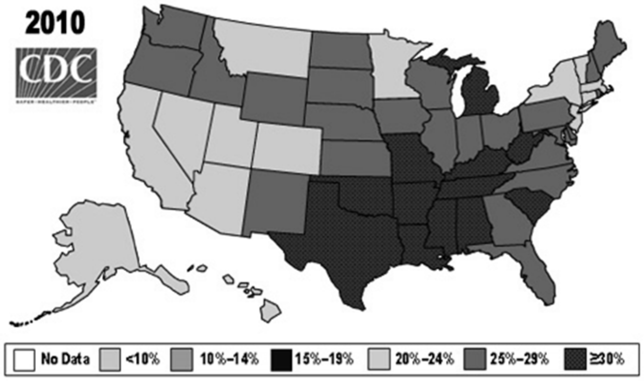
Genetic Epidemiology

- Goals
  - To discover and characterize genetic susceptibility to health and disease in human populations
  - To identify interactions between genetic and environmental factors
- Use family based studies and studies of unrelated individuals
- Apply principals of epidemiology, biostatistics and genetics/genome science
- Rapidly evolving field

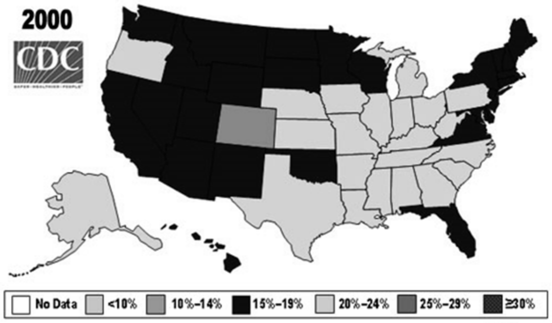
Percent of Obese (BMI ≥ 30) in U.S. Adults

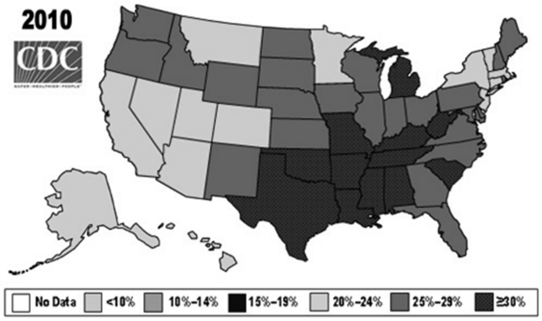


Percent of Obese (BMI ≥ 30) in U.S. Adults

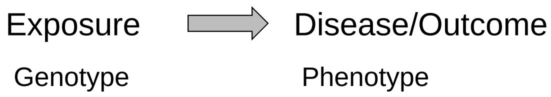


Percent of Obese (BMI ≥ 30) in U.S. Adults





Genetic Epidemiology



Exposure → Disease/Outcome

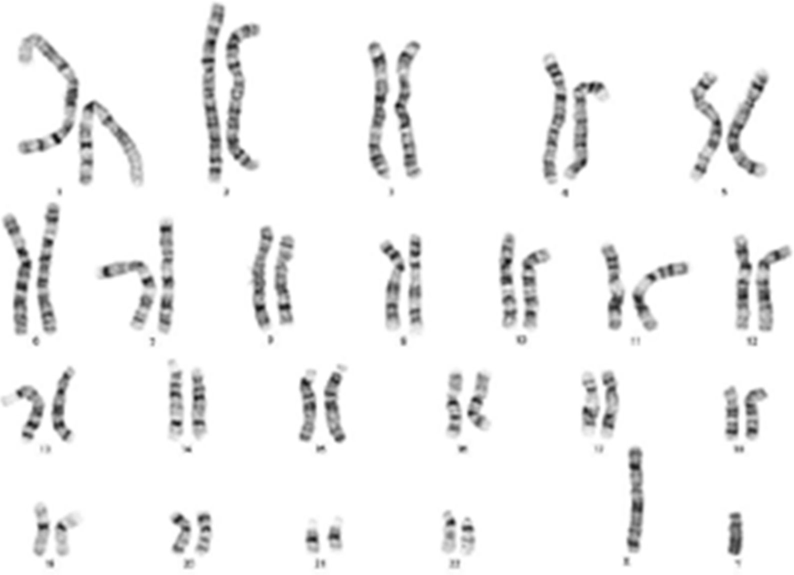
Genotype

Phenotype

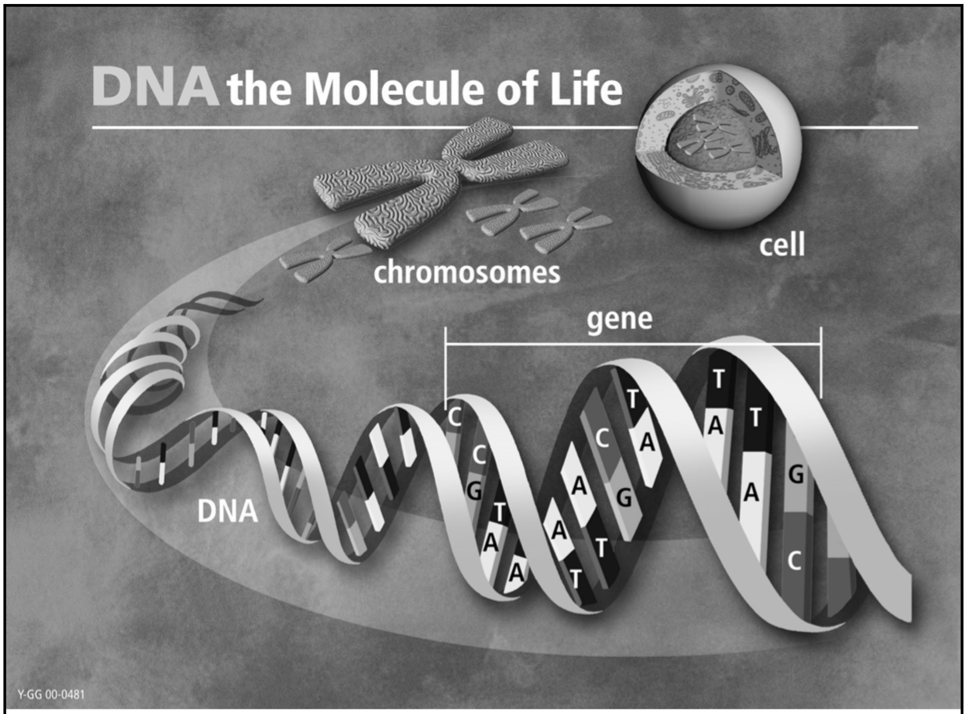
Phenotype and Genotype

- Phenotype and Genotype are the key components in genetic epidemiologic studies

- Phenotype (trait) – observed characteristics that are usually the focus of a genetic epidemiologic study
  - Not always a direct reflection of genotypes
  - Examples: Blood pressure, body weight, cholesterol level, eye color, heart disease, diabetes, Parkinson's disease, cancer, longevity
  - Quantitative vs. qualitative (discrete) trait



DNA the Molecule of Life



Y-GG 00-0481

Chromosomes, DNA, Genes

- Chromosomes are made up of DNA and are long strands of “genes”
  - Humans have about 20,000 genes in their genome
  - Genes have both coding (exon), noncoding (intron) and regions upstream that affect expression (promoter region)
    - Promoter region – a sequence of DNA found near beginning of a gene and needed to turn a gene on or off
    - Exons – contain stretches of DNA that code for proteins
    - Introns come in between the exons – intervening sequences, do not code for proteins
  - Genes control growth, development, health and disease
  - Genes are turned on and off in different patterns and at different stages of development = gene regulation

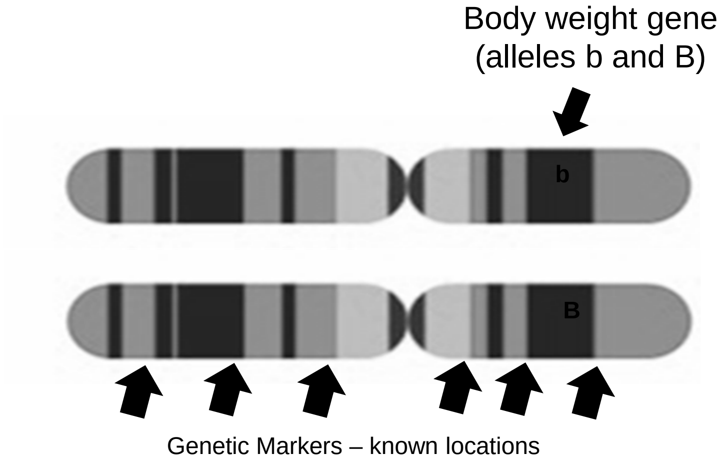
Genotype

- The unique genetic information of an individual
- Each gene has its own specific location on the chromosome
- Genes come in pairs, one version of each gene is inherited from your mother and one from your father (allele)
- Variations in the underlying DNA can result in differences between individuals, and may underlie a specific phenotype
  - Different ways of measuring the genotype and alleles
  - Single nucleotide polymorphism – most SNPs are not themselves functional, but mark the functional variations that affect disease risk
  - Sequencing is now common
- Factors that affect the expression of the gene (such as environment) are also important to consider (Gene x environment (GxE))

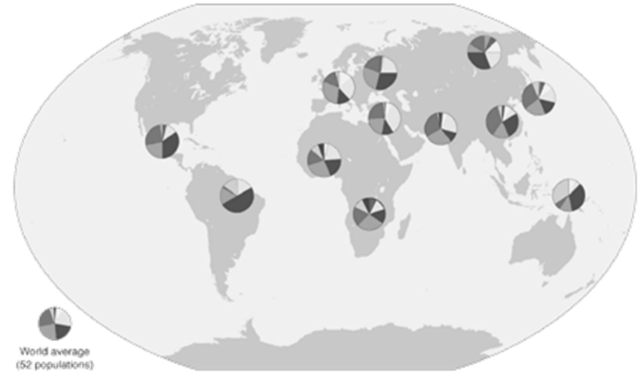
Genotype

- Each person inherits 1 chromosome from their biologic father and one from their biologic mother
- 23 pairs of chromosomes (a total of 46 chromosomes)
  - 22 of the pairs look the same in males and females
  - The sex chromosomes differ in males (X, Y) and females (X, X)
- Genotype – the unique genetic information from an individual
  - The genetic contribution to the phenotype
  - Genotype can refer to a collection of genes or the two alleles of a particular gene
  - Humans have about 20,000 genes

A pair of chromosomes



Allele frequencies vary across populations



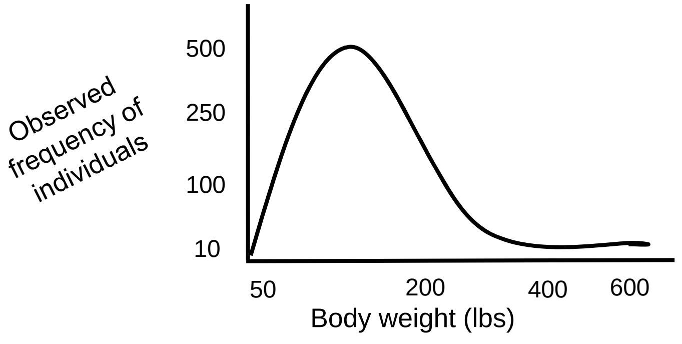
Humans on the move. Worldwide genetic variation at a neutral marker. Allele frequencies of one randomly chosen microsatellite marker reveal common alleles shared in all populations and the gradual and arbitrary differences in allele frequencies across geographic regions. Populations shown in this example are Yoruba and Bantu (Africa); French, Russians, Palestinians, and Pakistani Brahui (Eurasia); Han Chinese, Japanese, and Yakut (East Asia); New Guineans (Oceania); and Maya and Karitianans (America). From King and Motulsky (2002), Science, 298: 2342-2344.

Identifying genetic effects: Overview

Approaches to understanding genetic influences: Overview of Genetic Epidemiologic Studies

Question	Approach
Is there evidence for genetic influences on a quantitative trait?	Commingling
Is there familial aggregation?	Family Study
Is the familial aggregation caused by genetic factors?	Twin Study
Is there a major gene? Is it dominant or recessive ?	Segregation Study
Where is this major gene in the human genome?	Linkage Analysis
Is there linkage with DNA markers under a specific genetic model?	A. Parametric Approach
Is there an increased allele sharing for affected relatives (sib pairs) or for relatives with similar phenotype	B. Allele Sharing Approach (sib-pair analyses)
Where is the exact location of this gene and which polymorphism is associated with disease?	Association Study (population and family)

- Most human traits have a skewed distribution – which could be consistent with a genetic effect
- Body weight as our example

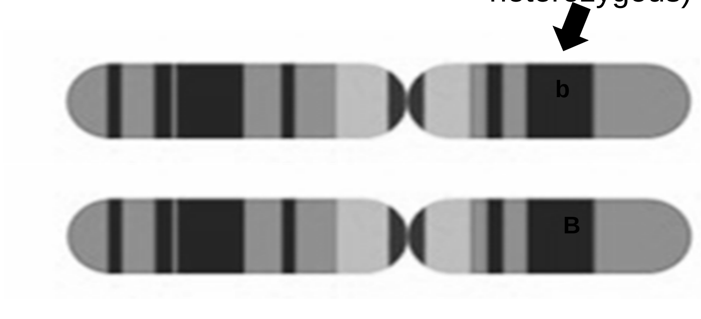


Basic idea behind Commingling Analysis:

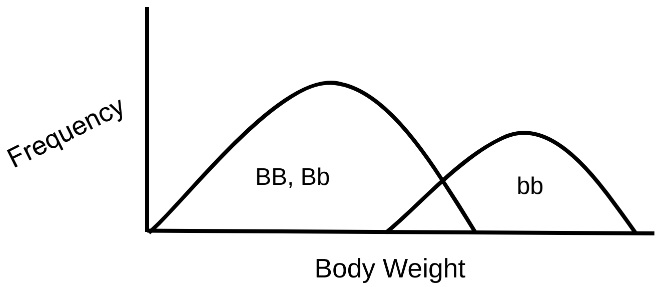
- If a single gene has an effect on the variation in a quantitative trait (body weight), each genotype has a particular distribution associated with it
- The overall population distribution results from the commingling of these genotype-specific sub-distributions
- Assume a gene with alleles B and b: have 3 possible genotypes in the population:
  - BB - homozygous
  - Bb - heterozygous
  - bb - homozygous

A pair of chromosomes from an individual person

Body weight gene (alleles b and B - heterozygous)



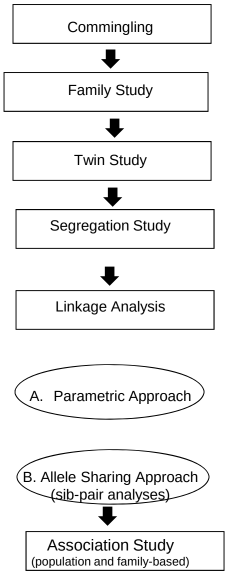
- Is the frequency distribution of body weight consistent with the influence of a gene with either dominant or recessive effects?



Commingling Analysis Summary:

- Why is it used: To provide preliminary evidence for a single gene that influences a quantitative trait (e.g. body weight, blood pressure, cholesterol level, blood glucose level).
- A statistical modeling approach that does not measure the genotype, but assumes genetic principals in the model
- Unrelated individuals – faster and easier

Overview of Genetic Epidemiologic Study Design



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Where is the disease causing gene and which polymorphism is associated with disease?	Association Study (population and family-based)

General comments about twin studies

- One of the first approaches used to evaluate evidence for genetic influences on traits
- Evaluate both genetic and environmental influences on traits
- Measure of interest is the heritability of the trait
  - Proportion of total variance in the quantitative trait due to additive genetic effects
  - Population specific
- Evaluate evidence for genetic influences on different types of traits
  - qualitative traits – diabetes
  - quantitative traits – blood glucose

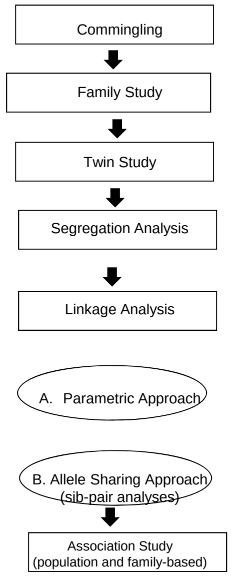
A twin approach to unraveling epigenetics

Jordana T. Bell and Tim D. Spector
Trends Genet. 2011 March ; 27(3): 116–125.

Abstract

The regulation of gene expression plays a pivotal role in complex phenotypes, and epigenetic mechanisms such as DNA methylation are essential to this process. The availability of next generation sequencing technologies allows us to study epigenetic variation at an unprecedented level of resolution. Even so, our understanding of the underlying sources of epigenetic variability remains limited. Twin studies have played an essential role in estimating phenotypic heritability, and these now offer an opportunity to study epigenetic variation as a dynamic quantitative trait. High monozygotic twin discordance rates for common diseases suggest that unexplained environmental or epigenetic factors could be involved. Recent genome-wide epigenetic studies in disease-discordant monozygotic twins emphasize the power of this design to successfully identify epigenetic changes associated with complex traits. We describe how large-scale epigenetic studies of twins can improve our understanding of how genetic, environmental and stochastic factors impact upon epigenetics, and how such studies can provide a comprehensive understanding of how epigenetic variation affects complex traits.

Overview of Genetic Epidemiologic Study Design



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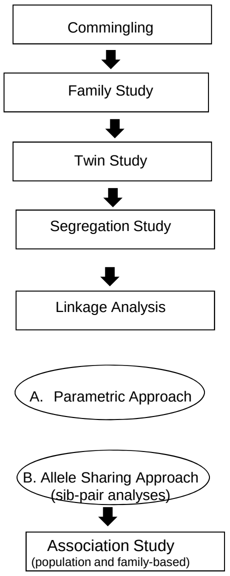

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Association Studies

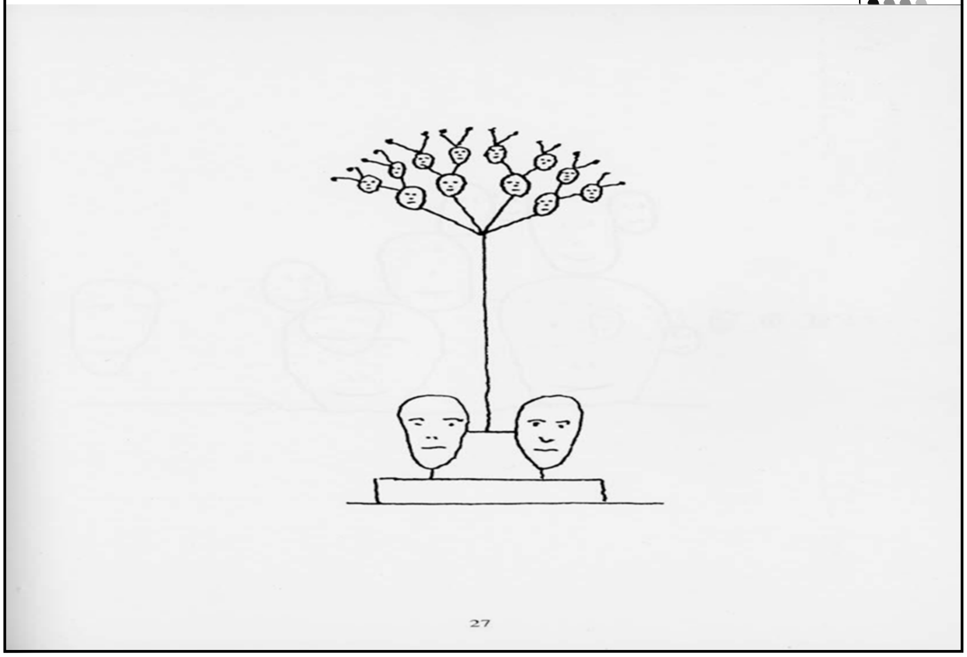
- Evaluate the association between a particular genetic variant and the trait (disease) in a population
- Focuses on unrelated individuals – usually case-control study
- Risk is typically estimated by the odds ratio (OR)
  - Compares the frequency of the genetic variant in those with disease to those without the disease
  - Measure of the strength of an association
    - OR=1 is no effect, OR>1 is increased risk, OR< is decreased risk
- A follow-up or replication study is an important, but challenging aspect of association studies

Summary

- Genetic Epidemiology - the genetic and environmental aspects of disease in human populations
- Use a variety of study designs to identify and evaluate evidence of genetic effects and impact on disease risk in populations
- Integrates epidemiology, genetics, genomics and biostatistics

Genetic Epidemiology

Collecting Family Data



Collecting Family Data

- Collecting family data is time consuming and expensive
- Need for complete and extended pedigrees
  - Local relatives vs. all relatives
  - Collection of phenotype data
- Need for accurate description of biologic relationships
- Confidentiality and IRB issues in collecting family data

Collecting family data

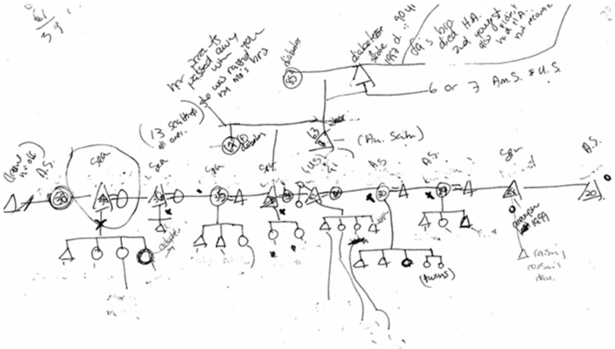
- IRB Issues
  - Confidentiality of information
    - Publication of pedigree information, genetic status
  - Sensitive information
    - Non-paternity, adoptions, abortions, medical conditions
- General approaches to data collection
  - Proband contact
  - Individual family members as contacts

Collecting family data

- Phenotype Information
  - Survey
    - Proband only

      Pro: quick and inexpensive

      Con: lack of knowledge about some relatives
    - Relatives
    - Critical information
      - ▪ Mother and Father ID for ALL related individuals
  - Measurements
    - Collecting blood / tissue samples
    - Physical measurements (height, weight, etc)
    - Tools for standardized measures (www.phenxtoolkit.org)
      - ▪ PhenX Tool Kit – a catalog of high-priority measures for consideration and inclusion in genetic epi studies


her parents passed away when you[ng]
so was raised by mo's bro
(13 siblings all over.)
diabetes
diabetes
diabetes stroke 1997 d.
fa's bro died H.A.
2nd youngest also died H.A.
6 or 7 Am.S. & U.S.
(Am. Sch.)
(drew in old) A.S.
Sea
Sea
Sea
Sea
(U.S.)
A.S.
A.S.
Sea
A.S.
diabetes
(twins)
raising cousin's dau.

Family Studies: Family Health History, Segregation and Linkage Analysis

Karen L. Edwards, Ph.D.

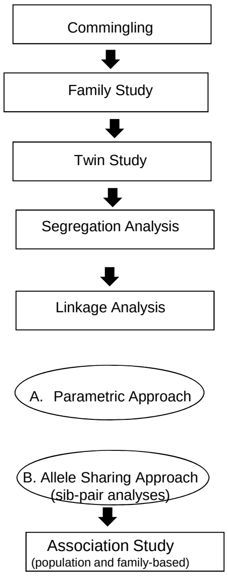
Professor

Dept of Epidemiology and

Genetic Epidemiology Research Institute

School of Medicine
University of California Irvine
Seattle, WA

Overview of Genetic Epidemiologic Study Design



Question	Approach
Is there evidence for genetic influences on a quantitative trait?	Commingling
Is there familial aggregation? higher risk in relatives or higher correlation in relatives	Family Study
Is the familial aggregation caused by genetic factors? MZ twins concordance rate or correlation higher than DZ twins	Twin Study
Is there a major gene? Is it dominant or recessive ? (likelihood of Mendelian models higher than environmental or polygenic model)	Segregation Analysis
Where is this major gene in the human genome?	Linkage Analysis
Is there a linkage with DNA markers under a specific genetic model?	A. Parametric Approach
Is there an increased allele sharing for affected relatives (sib pairs) or for relatives with similar phenotype	B. Allele Sharing Approach (sib-pair analyses)
Where is the disease causing gene and which polymorphism is associated with disease?	Association Study (population and family-based)

Family Health History: Application to public health

Advantages:

- Reflects multiple genetic, environmental, behavioral factors and interactions
  - No genetic test can do this
- Family history is a predictor of most diseases (diabetes, cancers, CVD)
- Effective (public health) interventions exist for many of these diseases
  - Quitting smoking, maintaining ideal body weight, diet, exercise
- Overcomes one of the most important barriers - getting people interested in learning and talking about their health

Goal: Use family history information to motivate behavior change and promote a healthy lifestyle for primary prevention of disease

- More personalized health messages that " fit within pre-existing beliefs about current health status, possible causes and risk factors, course of the disease, magnitude of and potential consequences of the risk, and ways to reduce the risk" See Claassen *et al. BMC Public Health 2010,* ***10:248***

Genetic Epidemiology

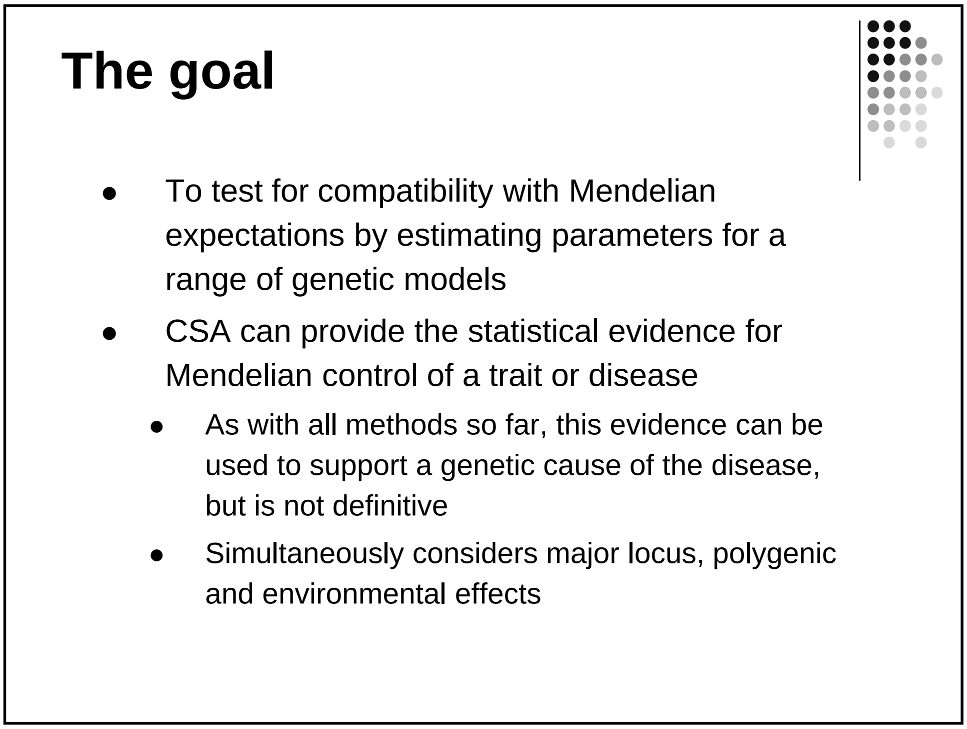
Segregation Analysis

Complex Segregation Analysis (CSA)

- A modeling approach used to determine whether there is evidence for a single gene that underlies a trait or disease
  - Also provides information on mode of inheritance
    - Dominant, Recessive or Codominant
- General method for evaluating the transmission of a trait within pedigrees
  - Mendelian transmission

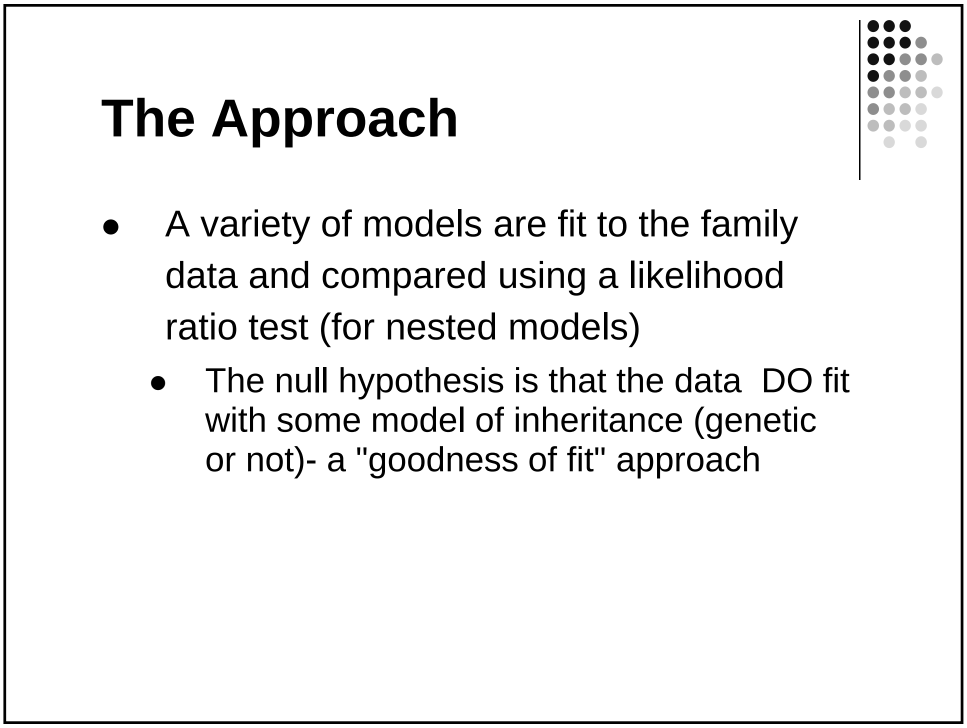
CSA, cont

- Information from CSA is useful in model based (parametric) linkage methods
  - LOD method linkage analysis depends on the specification of a reasonable model, including an approximation of the mode of inheritance
    - Assumes the existence of a Mendelian trait



The goal

- To test for compatibility with Mendelian expectations by estimating parameters for a range of genetic models
- CSA can provide the statistical evidence for Mendelian control of a trait or disease
  - As with all methods so far, this evidence can be used to support a genetic cause of the disease, but is not definitive
  - Simultaneously considers major locus, polygenic and environmental effects



The Approach

- A variety of models are fit to the family data and compared using a likelihood ratio test (for nested models)
  - The null hypothesis is that the data DO fit with some model of inheritance (genetic or not)- a "goodness of fit" approach

The Models

- The models are formed by estimating and restricting a specified set of parameters
  - The most general model, where all parameters are estimated
  - Single locus models with no polygenic inheritance and differing modes of inheritance
  - Polygenic model, with no single locus effect
  - Mixed model, both single gene and polygenic components
  - Nongenetic model or "environmental model"

Parameters: single locus component

- Means (*u*) for each subdistribution
- Variance of each subdistribution
- Allele frequencies
- Transmission probabilities - should conform to Mendelian expectations
  - t_1 **=** P(AA parents transmits A allele to offspring) = 1.0
  - t_2= P(Aa parents transmits A allele to offspring) = 0.5
  - t_3 = P(aa parents transmits A allele to offspring) = 0.0

Parameters : Polygenic component

- Heritability (h^2)
  - proportion of variance due to additive genetic effects
  - Not a single major gene
  - Can reflect “residual genetic effects” not accounted for by a single major locus
  - Sometimes referred to as multifactorial component

Model Testing

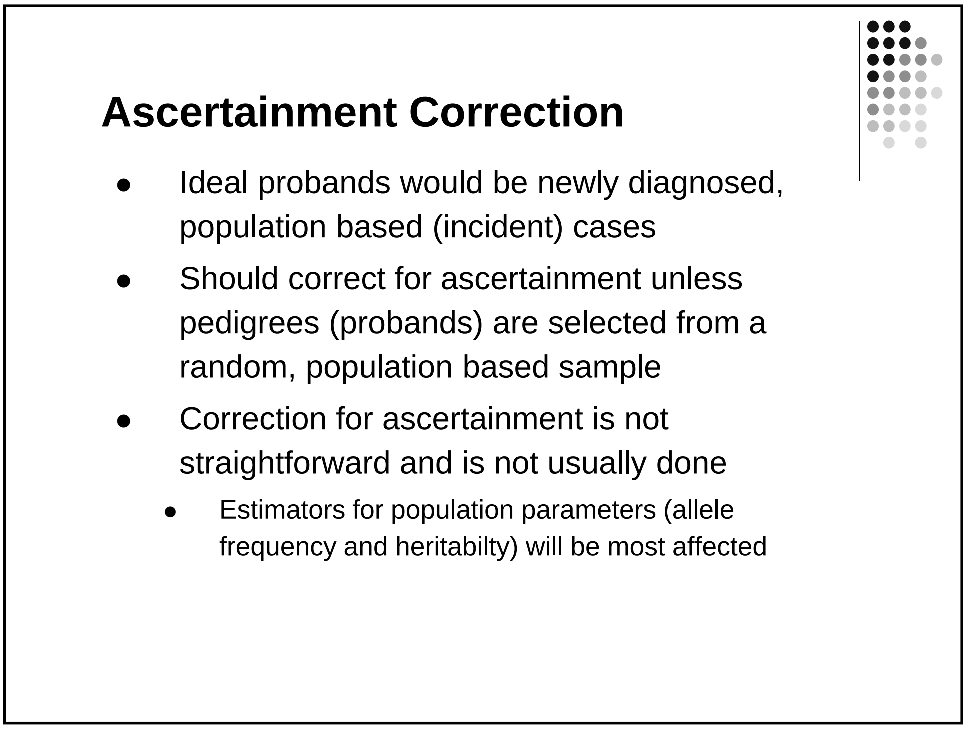
- Hypothesis testing for nested models using the LRT (likelihood ratio test)
- LRT = -2 [In L(reduced model) - In L(full model)]
  - LRT is distributed as a chi square with the degrees of freedom (df) equal to the difference in the number of estimated parameters
  - The likelihood of each model is proportional to the probability of the data, given the model and family structure

Model testing, cont

- To compare non-nested models
  - use the AIC to compare (not test) models to support a particular model over another
    - AIC= -2(ln likelihood) + 2(number of estimated parameters)
    - Calculate the AIC for each competing model and select the one with the smallest AIC as being the most parsimonious

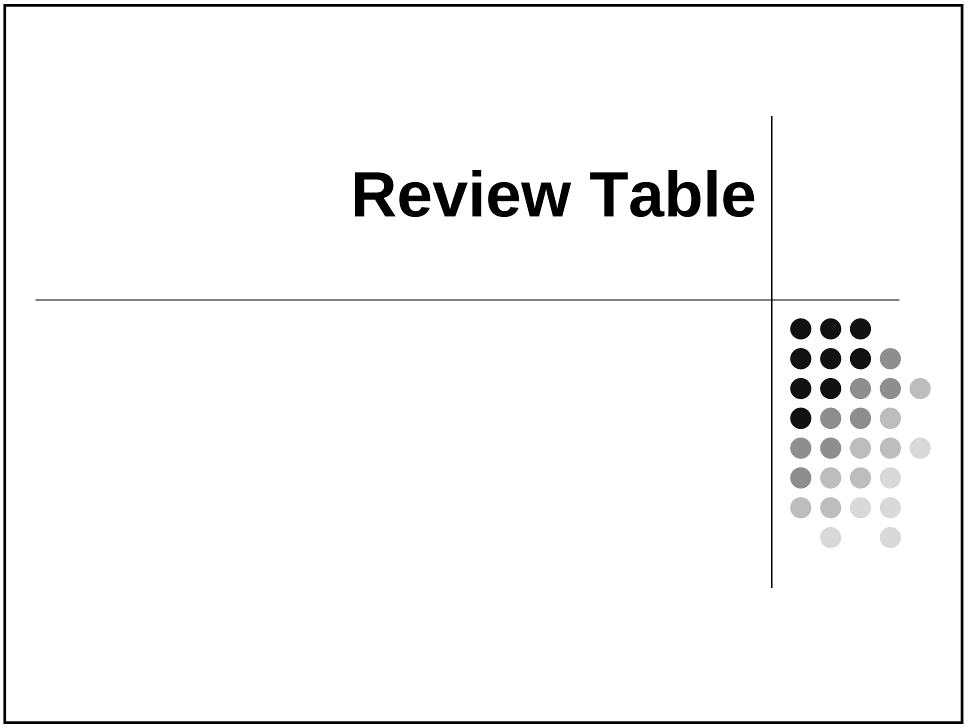
Interpretation: Inferring A Major Gene

- To infer a major gene
  - reject nongenetic models
  - accept a major gene model (single or mixed model)
  - should always test transmission probabilities in CSA of quantitative traits to safeguard against false inference of a major gene

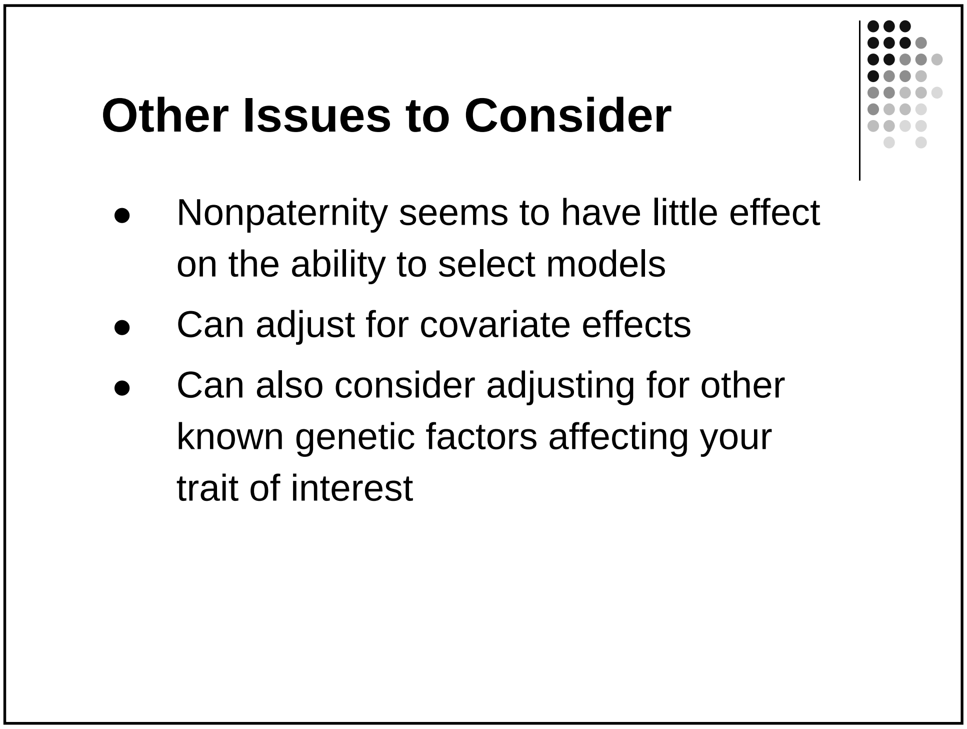


Ascertainment Correction

- Ideal probands would be newly diagnosed, population based (incident) cases
- Should correct for ascertainment unless pedigrees (probands) are selected from a random, population based sample
- Correction for ascertainment is not straightforward and is not usually done
  - Estimators for population parameters (allele frequency and heritabilty) will be most affected

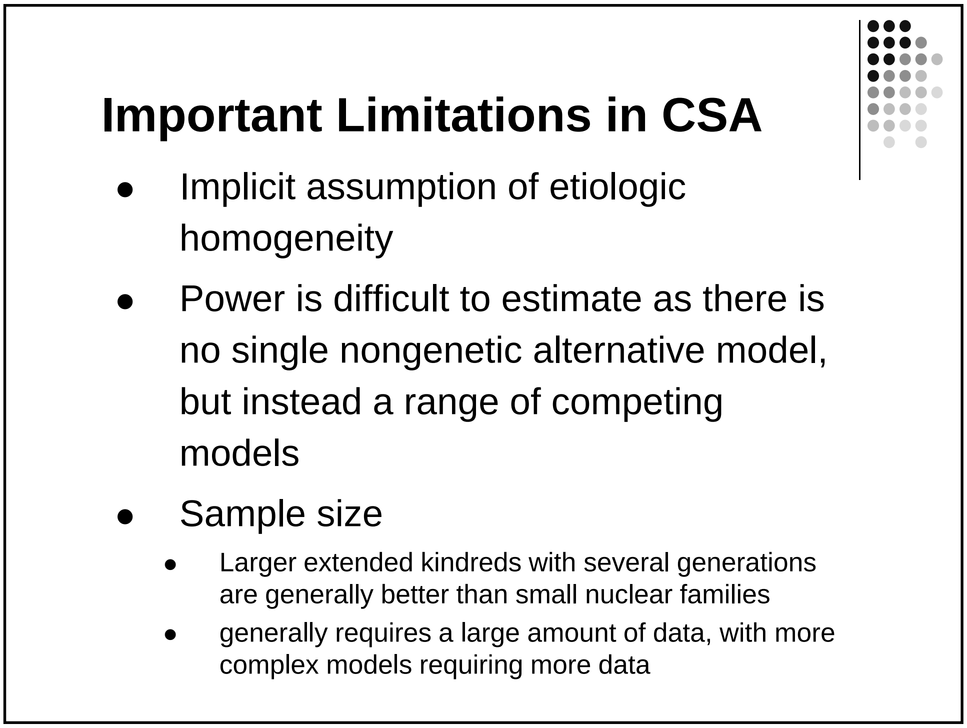


Review Table



Other Issues to Consider

- Nonpaternity seems to have little effect on the ability to select models
- Can adjust for covariate effects
- Can also consider adjusting for other known genetic factors affecting your trait of interest

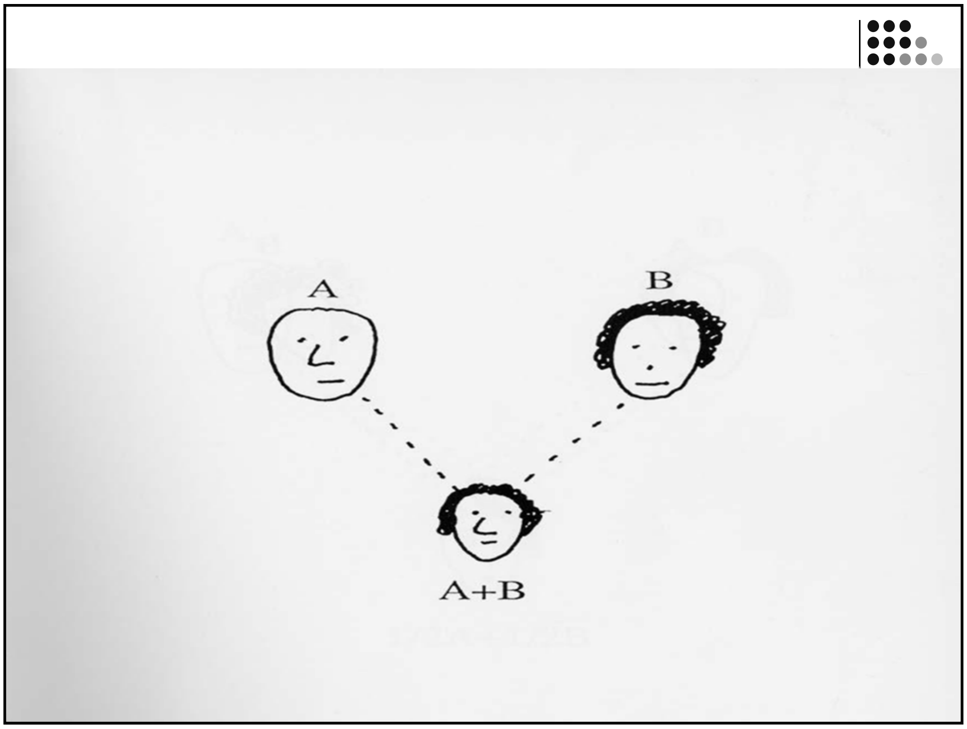


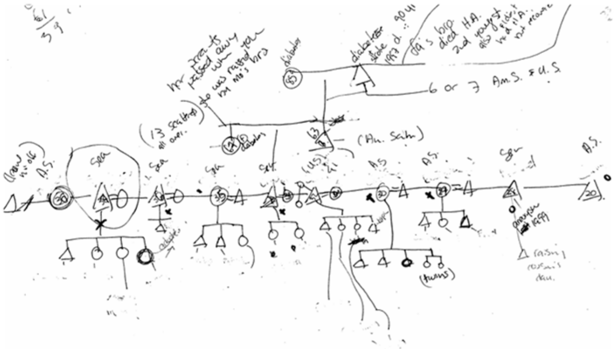
Important Limitations in CSA

- Implicit assumption of etiologic homogeneity
- Power is difficult to estimate as there is no single nongenetic alternative model, but instead a range of competing models
- Sample size
  - Larger extended kindreds with several generations are generally better than small nuclear families
  - generally requires a large amount of data, with more complex models requiring more data

Summary of CSA

- Does not require genotype data
- Can be time consuming to complete analyses
- Information from CSA is useful for a variety of reasons
  - Preliminary data, estimates for linkage analyses, choice of phenotype
- Assumes the existence of a Mendelian trait





Standardized Human Pedigree Nomenclature: Update and Assessment of the Recommendations of the National Society of Genetic Counselors.
Authors: Bennett, French, Resta, Lochner Doyle

- Standard format and nomenclature for drawing pedigrees
- Pedigrees convey lots of information
  - Picture is worth a 1000 words
  - Sensitive information and how to display?

J Genet Counsel (2008) 17:424–433

Bennett article - some key points

- A medical pedigree is a graphic presentation of a family's health history and genetic relationships

- A pivotal tool in the practice of medical genetics / genetic epi research

- Interpreting a pedigree should be a standard competency of all health professionals

- Pedigrees should not contain information about which a subject had no prior knowledge.
  - a person who had presymptomatic or susceptibility genetic testing through research should not find out about increased or decreased disease risk status from a publication

In Class Exercise: Pedigree Drawing

Let me start with my great-great grandparents: Jim and Ann Flight.

They had two children: Kathy, and Gerry.

Kathy died in a car accident along with her father Jim.

Gerry married Kate Doe.

Kate and Gerry had one child, Kathy

Kathy Flight married David Dewey and they had my dad, Bob. My dad took his mother's maiden name because David had an affair with someone named Maggie Braun.

After Jim's death, Ann married Paul Wright. Ann and Paul had one child: Tom Wright.

Tom Wright married Kaisa Stone.

Tom and Kaisa had one daughter: Heather. Heather Wright was wed to Peter Meter and had one child, Jean. Jean married Bob Flight and they had me Jane Flight.

In Class Exercise: Collecting Family History Information

Think about your own family history

- Do you know the vital status of your immediate family members, what about more distant relatives?
- Do you know the DOB and DOD for your immediate family members, what about more distant relatives?
- What health conditions run in your family?
  - Do you know age or date of onset?
  - How confident are you in this information?

Draw your pedigree, indicating as much of the following as possible

- vital status, health conditions, age at onset or death

Genetic Epidemiology

Linkage Analysis

Linkage Analysis, overview

- Linkage
  - Location of genetic loci sufficiently close together on a chromosome that they do not segregate independently
  - linkage is a property of loci (not alleles), and evaluation involves all alleles at the marker locus
  - the specific alleles segregating in one family may differ from alleles at the same locus segregating in a <u>different</u> family

Linkage vs. Association

- Linkage
  - Cosegregation of a disease or trait with a specific chromosomal region in multiple families
  - Genetic linkage is the tendency of two loci to be inherited together (e.g. loci are on the same chromosome)
  - Property of two loci (genes or locations)
- Association
  - Presence of a disease or trait with a specific allele in a gene or marker (in unrelated subjects) – probably due to linkage disequilibrium

Linkage Analysis –background

- The aim of linkage analysis is to infer the relative position of two or more loci
  - Examining patterns of allele sharing or cosegregation of marker and disease in relatives
  - The location of one locus is known (the marker), the other is unknown (the disease causing gene)
  - Alleles of loci on the same chromosome can violate Mendels’s law of independent assortment (linkage)

- Evidence of linkage between a known marker and a putative gene for a disorder is the ultimate statistical evidence for a genetic component in disease etiology

General Approaches to Linkage Analysis

- Genome Wide Scan
  - Isolate a gene solely on the basis of it's chromosomal location, without regard to it's biochemical function.
  - This is often referred to as the "positional genetic" approach (i.e. genome screens are often referred to positional cloning)

- Candidate gene approach
  - Select candidate genes based on their function or other known properties

Required data for family studies

- At least pairs of related individuals
- Accurate pedigree structure / biological relationships
  - Nuclear family vs. extended kindred

- Phenotype data – quantitative or categorical
- Genotype data
    - Location of markers (marker map)

Genetic Markers

- A genotype (measurable "trait") that is genetically determined, can be accurately classified, has a simple, unequivocal pattern of inheritance (*and polymorphic).*

- Types of genetic markers
  - Polymorphic markers – lots of alleles / variation
    - Variable number of tandem repeats (VNTR)
    - Microsatellites, (e.g. CA repeats), very polymorphic
  - Single nucleotide polymorphisms (SNP's) - 2 allele markers, very common
  - Sequence data – exome or whole genome

Statistical Analysis: LOD based Linkage Analysis

- Involves comparison of likelihoods of observing the segregation pattern of 2 loci under specific models, including
  - Under the null hypothesis of no linkage
    - Independent assortment – loci recombine as if on different chromosomes
  - Alternative hypotheses of linkage
    - differ in the extent of crossing over (i.e. different values of recombination events)

LOD Score

- LOD score = log (base 10) of the odds of linkage vs. no linkage (not an odds ratio!)
  - LOD score ≥ 3, supports linkage, corresponds to a genome-wide type 1 error rate of 0.05 (depends on number of markers tested)
  - LOD score ≤ -2, used to exclude a chromosomal region
    - Exclusion mapping
- add LOD scores from all families to obtain LOD score for your sample
  - Assumes families are independent

Linkage Mapping of CVD Risk Traits in the Isolated Norfolk Island Population

Abstract: To understand the underlying genetic architecture of cardiovascular disease (CVD) risk traits, we undertook a genome-wide linkage scan to identify CVD quantitative trait loci (QTLs) in 377 individuals from the Norfolk Island population. The central aim of this research focused on the utilization of a genetically and geographically isolated population of individuals from Norfolk Island for the purposes of variance component linkage analysis to identify QTLs involved in CVD risk traits.

-The ancestral origins of the Norfolk Island are well documented and originated from divergent founding paternal and maternal lineages, European and Tahitian, respectively.

-1,574 residents

-Exhaustive genealogical documents indicate that the population grew from a limited number of initial founders (nine males, twelve females) and in relative isolation in the early generations of population expansion

- Evidence of the Island's strict immigration laws are obvious by the limited numbers of surnames, resulting in the worlds only telephone directory which includes nicknames to differentiate between individuals with the same name

Hum Genet. 2008 December ; 124(5): 543–552. doi:10.1007/s00439-008-0580-y.

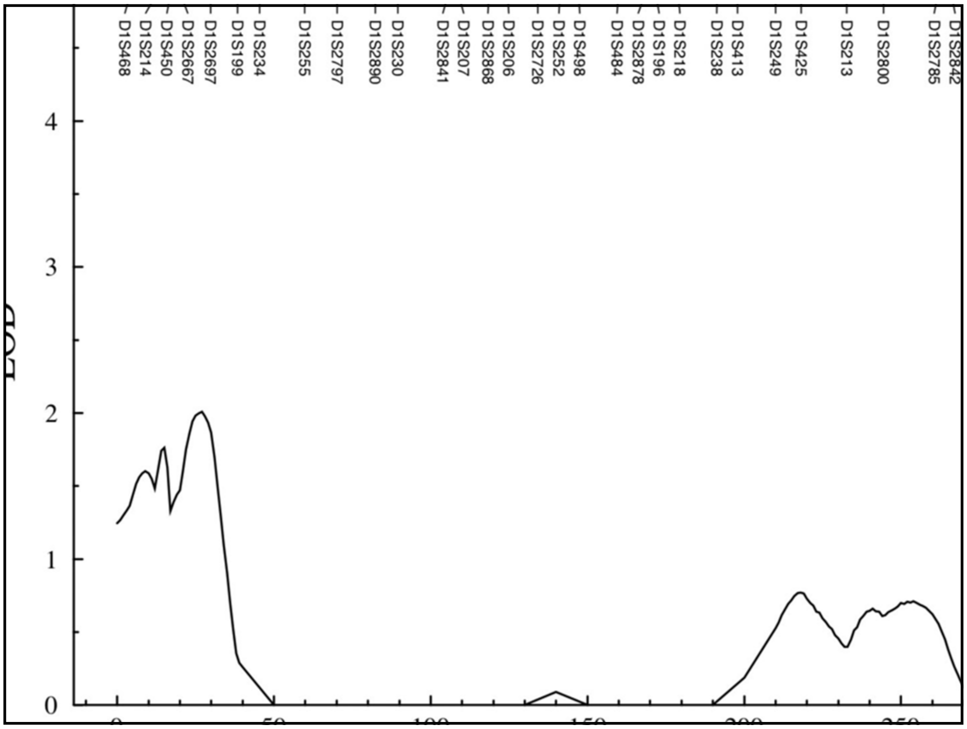
Linkage Mapping of CVD Risk Traits in the Isolated Norfolk Island Population

The Norfolk Island genealogy dates back approximately ten generations to the initial founders and contains 6379 individual entries linked together within 2185 nuclear families. The complexity of the island's heritage is evident considering 5750 individuals reside within a single multifamily pedigree exhibiting 1661 marriages and 1233 founders.

Methods: Substantial evidence supports the involvement of traits such as systolic and diastolic blood pressures (SBP and DBP), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), body mass index (BMI) and triglycerides (TG) as important risk factors for CVD pathogenesis. In addition to the environmental influences of poor diet, reduced physical activity, increasing age, cigarette smoking and alcohol consumption, many studies have illustrated a strong involvement of genetic components in the CVD phenotype through family and twin studies. We undertook a genome scan using 400 markers spaced approximately 10cM in 600 individuals from Norfolk Island. Genotype data was analyzed using the variance components methods of SOLAR.

Results: Our results gave a peak LOD score of 2.01 localizing to chromosome 1p36 for systolic blood pressure and replicated previously implicated loci for other CVD relevant QTLs.

Hum Genet. 2008 December ; 124(5): 543–552. doi:10.1007/s00439-008-0580-y.



Sib-Pair Linkage Analysis

- Sib pairs are generally easier to collect, tend to be more closely matched for age and environment than other relative pairs
- Qualitative trait: under linkage, Affected relative pairs should share alleles IBD (inherited from a common ancestor within the pedigree), more often than expected under Mendelian expectations
- Quantitative trait: relative pairs should show a correlation between the magnitude of their phenotypic difference and the number of alleles shared IBD

Quantitative sib-pair linkage

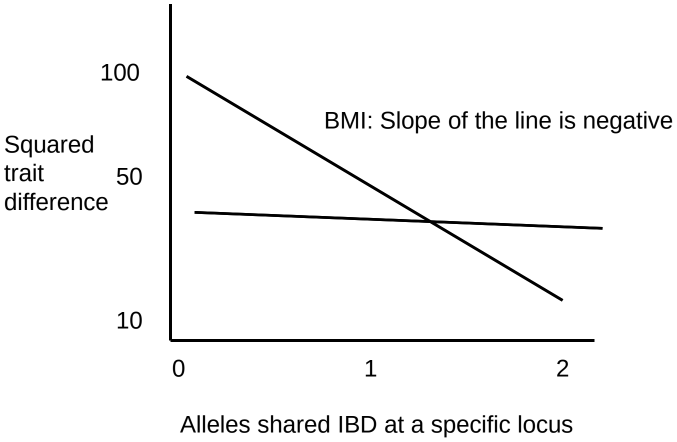
- A regression approach
  - Regress the squared within-pair difference of a quantitative trait on the number of marker alleles shared IBD
- Null hypothesis - the slope of the squared within pair difference is zero
- The alternative hypothesis is that under linkage, the slope is negative.

Identity by descent vs. Identity by state

- IBS- two alleles at a given locus are identical in state if they represent the same allelic variant at that locus

- IBD- two alleles at a given locus are IBD if they were transmitted from a common ancestor –ie they represent copies of the same ancestral DNA

Quantitative Sib-pair linkage results



Linkage Disequilibrium

Outline

- Linkage disequilibrium (LD)
  - Definition of linkage disequilibrium
  - Importance of disequilibrium
  - Measures of disequilibrium
- SNP selection
  - Public resources
  - Tag SNP selection programs
- Imputation

Definitions

SNP1: rs3822050 and SNP2: rs10517002

- **Allele**
  - Different versions of DNA sequence at a given location

SNP1: **C** and **T**

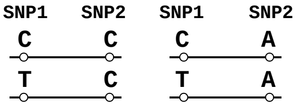
SNP2: **C** and **A**

- **Genotype**
  - The two alleles in an individual at a given locus

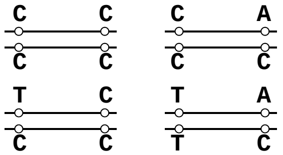
SNP1: **C/C, C/T** or **T/T**

SNP2: **C/C, C/A** or **A/A**

- **Haplotype**
  - A series of alleles along a single chromosome



- **Diplotype**
  - a set of haplotype pairs in an individual



What is Linkage Disequilibrium?

Linkage Disequilibrium: Two loci that are in linkage disequilibrium are inherited together more often than would be expected by chance.

Zondervan & Cardon, 2004

Systematic studies of common genetic variants are facilitated by the fact that individuals who carry a particular SNP allele at one site often predictably carry specific alleles at other nearby variant sites. This correlation is known as **linkage disequilibrium**

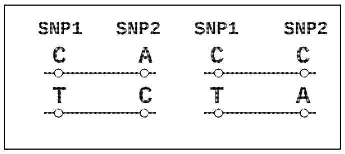
The international HapMap consortium, 2005

Linkage Disequilibrium refers to the nonindependence of alleles at different sites.

Pritchard and Przeworski 2001

Linkage Equilibrium

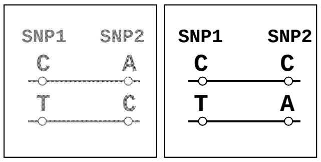
SNP1: **C/T**
SNP2: **C/A**


SNP1 SNP2 SNP1 SNP2
C A C C
T C T A

haplotype frequencies in population match what is expected based on allele frequencies
Example: frequency of C-A haplotype equals frequency of C allele at SNP 1 * frequency of A allele at SNP 2

Linkage Disequilibrium

SNP1: **C/T**
SNP2: **C/A**

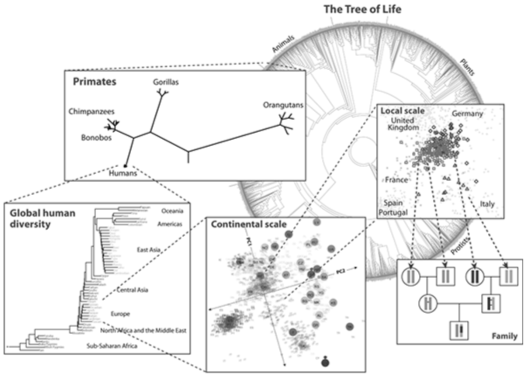

SNP1 SNP2 SNP1 SNP2
C A C C
T C T A

haplotype frequencies in population differ from what is expected based on allele frequencies

It is a Matter of Scale

"*Nothing in biology makes sense except in the light of evolution*"

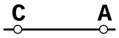
-Theodosius Dobzhansky, 1973



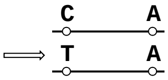
Novembre J, Ramachandran S. 2011.
Annu. Rev. Genomics Hum. Genet. 12:245–74

Current Haplotypes Arose from Ancient Mutation Events

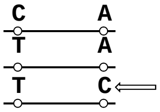
1. Ancestral state has no variation at either SNP position.



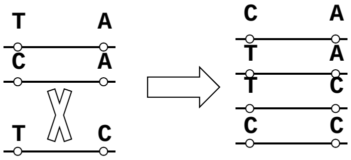
2. Mutation leads to first SNP



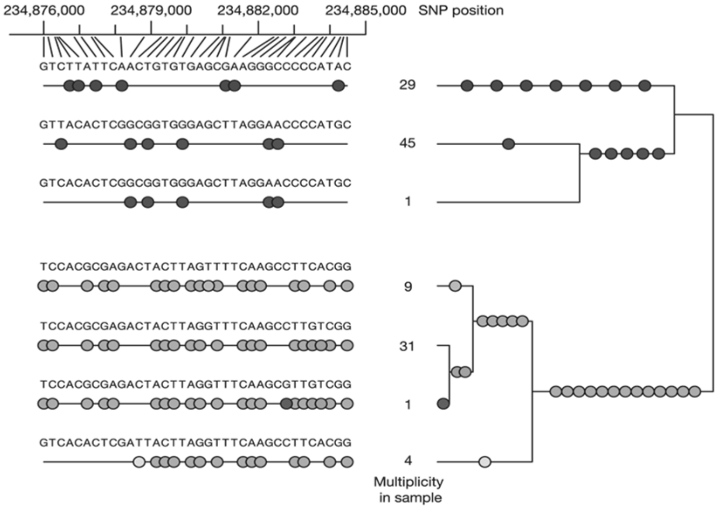
3. Asecond mutation leads to second SNP



4. Recombination or recurrent mutation needed for all four haplotypes

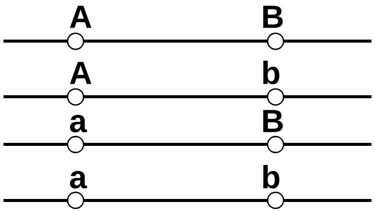


Haplotypes


234,876,000
234,879,000
234,882,000
234,885,000
SNP position
GTCTTATTCAACTGTGTGAGCGAAGGGCCCCCATAC
29
GTTACACTCGGCGGTGGGAGCTTAGGAACCCCATGC
45
GTCACACTCGGCGGTGGGAGCTTAGGAACCCCATGC
1
TCCACGCGAGACTACTTAGTTTTCAAGCCTTCACGG
9
TCCACGCGAGACTACTTAGGTTTCAAGCCTTGTCGG
31
TCCACGCGAGACTACTTAGGTTTCAAGCGTTGTCGG
1
GTCACACTCGATTACTTAGGTTTCAAGCCTTCACGG
4
Multiplicity
in sample

The International HapMap Consortium. Nature | Vol 437 | 27Octob

Focus on Pairwise LD



	A	a	
B	p_{AB}	p_{aB}	$\mathbf{p_B}$
b	p_{Ab}	p_{ab}	$\mathbf{p_b}$
	$\mathbf{p_A}$	$\mathbf{p_a}$	

If loci are independent, then we expect

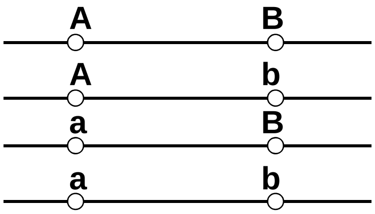
$$p_{AB}= p_A * p_B$$

$$p_{Ab}= p_A * p_b$$

$$p_{AB}= p_A * p_B$$

$$p_{AB}= p_A * p_B$$

Measuring LD for pairs of sites- D



	A	a	
B	p_{AB}	p_{aB}	$\mathbf{p_B}$
b	p_{Ab}	p_{ab}	$\mathbf{p_b}$
	$\mathbf{p_A}$	$\mathbf{p_a}$	

One important measure of LD is

$$D_{AB} = p_{AB} - p_A p_B$$

Notice that D=0 if and only the two sites are independent

A disadvantage of D is that the range of possible values depends greatly on the marginal allele frequencies.

Measuring LD for pairs of sites- D’

Lewontin (1964) proposed an adjusted statistic that has range [-1, 1]:

	A	a	
B	p_{AB}	p_{aB}	p_B
b	p_{Ab}	p_{ab}	p_b
	p_A	p_a	

D’ = D/max(D), where max(D) is dependent on the marginal allele frequencies

If $D_{AB}>0$: $D'_{AB} = D_{AB}/(\min(P_aP_B, P_AP_b))$
If $D_{AB}<0$: $D'_{AB} = D_{AB}/(\min(P_AP_B, P_aP_b))$

Properties of D’

- D’ favored in medical genetics
  - D’=0 implies independence
  - |D’|<1 implies that there has been recombination between the two sites in the history of the sample (or recurrent mutation)
  - |D’=1| implies “complete LD”
    - No historic recombination
    - Neither site has experienced recurrent mutation or gene conversion
    - Genotypes not perfectly correlated (unequal allele frequency)
    - D’ inflated in smaller samples

Measuring LD for pairs of sites- r^2

Along with D', the other most widely used statistic is r^2:

$\mathbf{r^2 = D_{AB}^2 / (p_A * p_B * p_a * p_b)}$

r^2 has range [0,1]. Its value is 1 if just 2 of the 4 haplotypes are present.

	A	a	
B	p_{AB}	p_{aB}	p_B
b	p_{Ab}	p_{ab}	p_b
	p_A	p_a	

r^2 is intimately connected to the power of association mapping [Pritchard & Przeworski 2001]

Properties of r^2

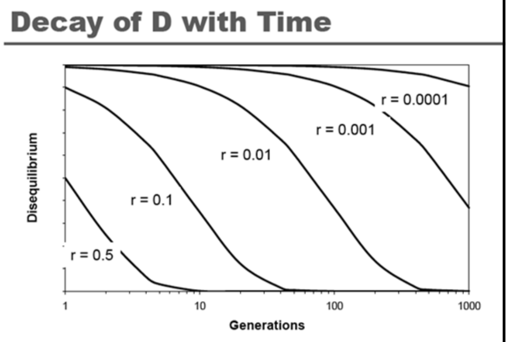
- r^2 favored in population genetics
  - r^2 =0 implies independence
  - r^2 =1 implies "perfect LD"
    - Marker loci have identical allele frequencies
    - Genotype is perfectly correlated
  - Related to power if (N_2=N_1/r^2)
    - where N_1 is sample size needed for directly genotyped SNP, N_2 is sample size needed to test tagged SNP and r2 is the LD between the directly genotyped SNP and the tagged SNP).
    - Assume need 1,000 for directly genotyped SNP, examples of sample size needed for tagged SNPs, depending on r^2
      - r^2=1.0, N_1= N_2=1,000
      - r^2=0.2, N_2 = 1,000/0.2 = 5,000

What factors affect LD?

- Mutation
- Historical recombination
- Natural selection
- Founder effects
- Migration
- Random drift
- Population admixture

LD over time

- Recombination assorts SNPs on haplotypes.
- Under assumption of random mating and a large population, LD will break down over time.



Decay of D with Time

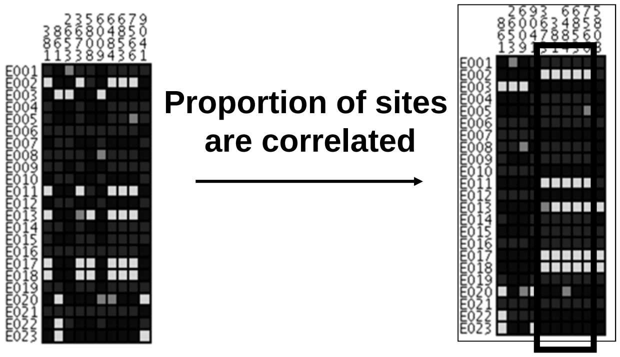
Applications of LD

- LD is the *sine qua non* of genetic association studies:
  - We are interested in testing for an association between disease status and causal mutations
  - If all polymorphisms were independent at the population level, association studies would have to examine every one of them.
  - Instead we can test a subset and get information on all of them.

- LD is also used in studies of human history, natural selection and the biology of recombination

LD Across a Gene

Genotype at one site can predict genotype at another site



Proportion of sites are correlated

SNP Selection

- We use information about allele frequencies and LD across the genome to make informed choices as to which variants to genotype
  - Identify SNPs in region of interest
  - Interested in minimal set of SNPs needed to capture variation in region.

Identify variation for your region

- Option 1: sequence individuals in your sample for the entire gene/region of interest
- Option 2: sequence a subset of individuals to identify variation in your region
- Option 3: Use public databases to identify known variation in your region

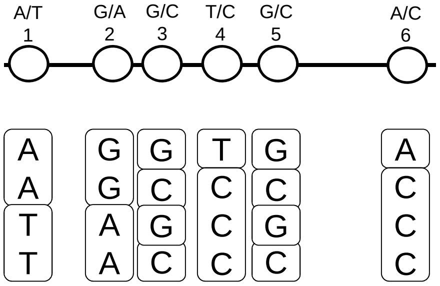
SNP Database Resources

- NCBI SNP Database, dbSNP
  - http://www.ncbi.nlm.nih.gov/SNP/
- International HapMap Project
  - http://www.hapmap.org/
- NHLBI Program for Genomic Applications (http://www.nhlbi.nih.gov/resources/pga/)
  - SeattleSNPs (http://pga.mbt.washington.edu/)
  - InnateImmunity (http://innateimmunity.net/)
- 1,000 genomes project
  - http://www.1000genomes.org
- Exome variant server (EVS)
  - http://evs.gs.washington.edu/EVS/

Tag SNPs

- tagSNPs
  - SNPs are selected based on their pair wise ability to predict genotype of untyped SNPs
  - Based on an r2 concept of LD structure
  - Example program: LDSelect
- haplotype-tagging SNPs (htSNPs)
  - SNPs are selected to optimize resolution of existing haplotypes
  - Based on a D' concept of LD structure
  - Example program: Haploview, HaploBlockfinder
- Multi-marker tagSNPs
  - Use tagSNP concept, but extend past pair wise LD
  - Example program: tagger

Tag SNPs – using r^2 information

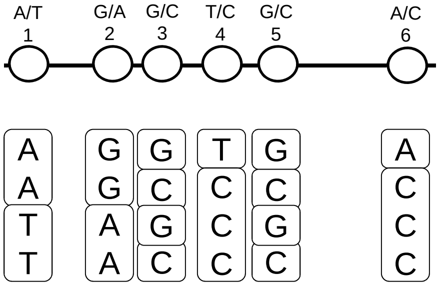


A/T 1	G/A 2	G/C 3	T/C 4	G/C 5	A/C 6
A	G	G	T	G	A
A	G	C	C	C	C
T	A	G	C	G	C
T	A	C	C	C	C

Think-Pair-Share Exercise:
Which SNPs are in high LD?
How many SNPs would you need to genotype to effectively capture the variation across the region?

After Carlson *et al.* (2004) *AJHG* **74**:106

Tag SNPs – using r^2 information



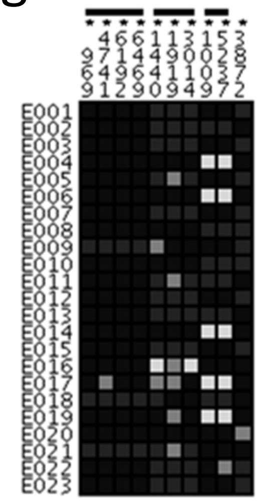
A/T 1	G/A 2	G/C 3	T/C 4	G/C 5	A/C 6
A	G	G	T	G	A
A	G	C	C	C	C
T	A	G	C	G	C
T	A	C	C	C	C

Tags:

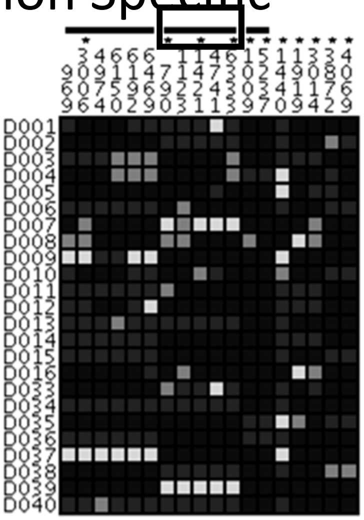
Test for association:

After Carlson *et al.* (2004) *AJHG* **74**:106

Tag SNPs are Population Specific



European-Americans
CRP



African-Americans
CRP

Thousand Genomes and GVS Tutorial

Limitations of tag SNPs

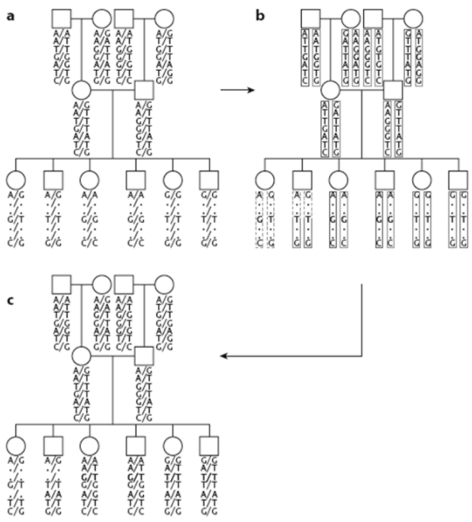
- Ultimately, we are interested in identifying common polymorphisms that are causally associated with disease risk, we cannot determine if signal is from the tagSNP or from a correlated SNP.

- What happens if your tagSNP fails in the genotyping/QC stage?

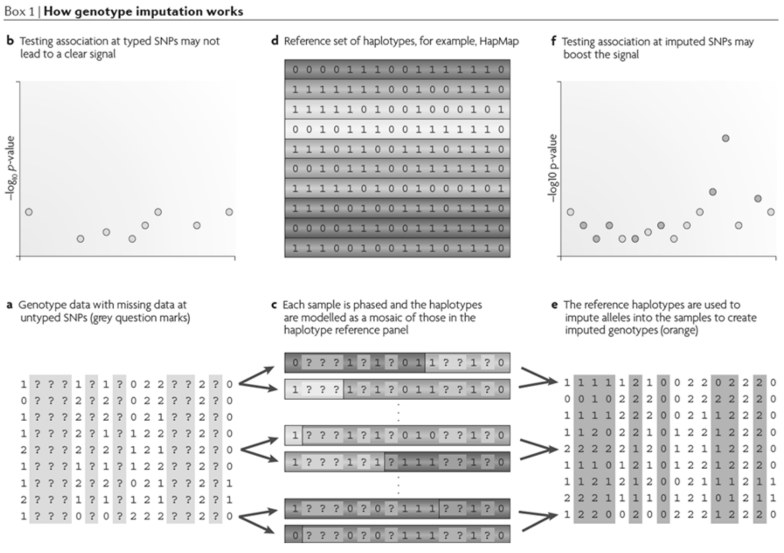
Imputation

- We also use LD information to impute genotype information.
- Common example is in genome-wide association studies.
  - Example: SNPs on a GWAS chip can be used to infer information on all variants in HapMap and 1000 genomes data
- Recent literature focuses on appropriate reference populations (see for example Eur J Hum Genet. 2015 Jul;23(7):975-83.)

Imputation with family data



Imputation with Population Data


Box 1 | **How genotype imputation works**

b Testing association at typed SNPs may not lead to a clear signal

d Reference set of haplotypes, for example, HapMap

```
0 0 0 0 1 1 1 0 0 1 1 1 1 1 1 0
1 1 1 1 1 1 1 0 0 1 0 0 1 1 1 0
1 1 1 1 1 0 1 0 0 1 0 0 0 1 0 1
0 0 1 0 1 1 1 0 0 1 1 1 1 1 1 0
1 1 1 0 1 1 0 0 1 1 1 0 1 1 1 0
0 0 1 0 1 1 1 0 0 1 1 1 1 1 1 0
1 1 1 1 1 0 1 0 0 1 0 0 0 1 0 1
1 1 1 0 0 1 0 0 1 1 1 0 1 1 1 0
0 0 0 0 1 1 1 0 0 1 1 1 1 1 1 0
1 1 1 0 0 1 0 0 1 1 1 0 1 1 1 0
```

f Testing association at imputed SNPs may boost the signal

a Genotype data with missing data at untyped SNPs (grey question marks)

```
1 ? ? ? 1 ? 1 ? 0 2 2 ? ? 2 ? 0
0 ? ? ? 2 ? 2 ? 0 2 2 ? ? 2 ? 0
1 ? ? ? 2 ? 2 ? 0 2 1 ? ? 2 ? 0
1 ? ? ? 2 ? 1 ? 1 2 2 ? ? 2 ? 0
2 ? ? ? 2 ? 2 ? 1 2 1 ? ? 2 ? 0
1 ? ? ? 1 ? 1 ? 1 2 2 ? ? 2 ? 0
1 ? ? ? 2 ? 2 ? 0 2 1 ? ? 2 ? 1
2 ? ? ? 1 ? 1 ? 1 2 1 ? ? 2 ? 1
1 ? ? ? 0 ? 0 ? 2 2 2 ? ? 2 ? 0
```

c Each sample is phased and the haplotypes are modelled as a mosaic of those in the haplotype reference panel

```
0 ? ? ? 1 ? 1 ? 0 1 1 ? ? 1 ? 0
1 ? ? ? 1 ? 1 ? 0 1 1 ? ? 1 ? 0
               .
               .
               .
1 ? ? ? 1 ? 1 ? 0 1 0 ? ? 1 ? 0
1 ? ? ? 1 ? 1 ? 1 1 1 ? ? 1 ? 0
               .
               .
               .
1 ? ? ? 0 ? 0 ? 1 1 1 ? ? 1 ? 0
0 ? ? ? 0 ? 0 ? 1 1 1 ? ? 1 ? 0
```

e The reference haplotypes are used to impute alleles into the samples to create imputed genotypes (orange)

```
1 1 1 1 1 2 1 0 0 2 2 0 2 2 2 0
0 0 1 0 2 2 2 0 0 2 2 2 2 2 2 0
1 1 1 1 2 2 2 0 0 2 1 1 2 2 2 0
1 1 2 0 2 2 1 0 1 2 2 1 2 2 2 0
2 2 2 2 2 1 2 0 1 2 1 1 2 2 2 0
1 1 1 0 1 2 1 0 1 2 2 1 2 2 2 0
1 1 2 1 2 1 2 0 0 2 1 1 1 2 1 1
2 2 2 1 1 1 1 0 1 2 1 0 1 2 1 1
1 2 2 0 0 2 0 0 2 2 2 1 2 2 2 0
```

Nature Reviews Genetics 11, 499-511 (July 2010)

Imputation Programs

- IMPUTE2
  - http://mathgen.stats.ox.ac.uk/impute/impute_v2.html
- Beagle
  - http://faculty.washington.edu/browning/beagle/beagle.html
- MaCH/minimac
  - http://genome.sph.umich.edu/wiki/MaCH:_1000_Genomes_Imputation_Cookbook
  - http://genome.sph.umich.edu/wiki/Minimac

Example MaCH

- Uses a hidden Marcov-model
  - Iteratively update the phase of each individuals genotype data conditional on haplotype estimates of other samples.

$$P(G_i|D_{-i}, \theta, \eta) = \sum P(G_i|Z, \eta)P(Z|D_{-i}, \theta)$$

    - G_i is the observed genotype of individual *i*,
    - D_{-i} is estimated haplotypes of all other individuals
    - Z are the hidden states
    - θ is the crossover parameter between hidden states
    - η is the error parameter

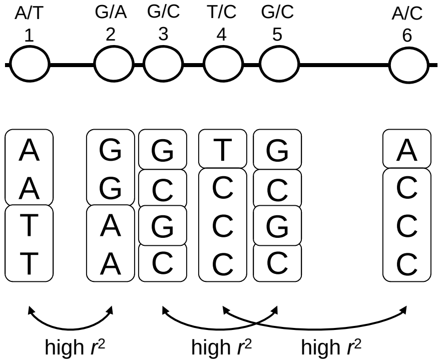
Imputation Output

- A “best guess” genotype (i.e. TT)
- Probability of each genotype (i.e. pr(TT), pr(TA), pr(AA))
- A “dosage”. If T is 0 and A is 1, then people are on a scale from 0 to 2 (where 0=TT, 1=TA and 2=AA).
    - dosage=pr(TA)+2*pr(TT)
- A quality score (typically an “information” or r2 measure) that captures the uncertainty in the imputation.

Summary

- Linkage disequilibrium (LD) refers to the nonindependence of alleles at different sites in the genome
- LD is shaped by population genetic forces
- We exploit LD information in genetic epidemiology
    - – Selecting tagSNPs for association studies
    - – Imputation in GWAS studies
- LD complicates interpretation of association studies

Tag SNPs – using r^2 information



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

Tags:

SNP 1
SNP 3
SNP 6

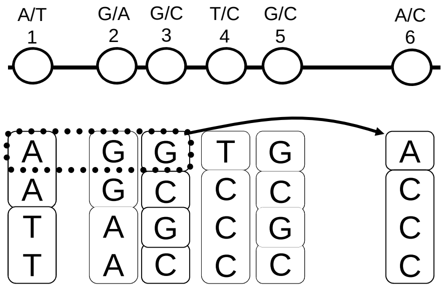
3 in total

Test for association:

SNP 1 captures 1 & 2
SNP 3 captures 3 & 5
SNP 6 captures 4 & 6

After Carlson *et al.* (2004) *AJHG* **74**:106

Picking tag SNPs using multimarker r2



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

Tags:

SNP 1
SNP 3

2 in total

Test for association:

SNP 1 captures 1+2
SNP 3 captures 3+5
SNP 1 and 3 in combo also captures 4 and 6

http://www.broad.mit.edu/mpg/tagger