Special Topics in (Genetic) Epidemiology

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Outline

- Course overview
- Course main topics
- Basics
  - Common epidemiologic study designs
  - Basic genetics

Acknowledgements

- Bhramar Mukherjee (U Michigan)
- Shawn Lee (U Michigan)
- James Dai
- Chongzhi Di
- Charles Kooperberg
- Ross Prentice
- Yingye Zheng

Overview

- This course will review many (but selected) current developments in statistical methods for (genetic) epidemiologic studies
  - Cover some basic models and standard analysis techniques but is not meant to be comprehensive
  - Focus on opportunities and challenges
- Things that are not covered:
  - Data preprocessing and QC steps
  - Software packages

Main topics

- Various topics that are related to observational studies
- Why?
  - A lot of data (genetics, environmental risk factor, biomarkers, various molecular data on the phenotypes)
  - Lots of interesting questions
  - Messy and complex

Topics: Genetic association studies

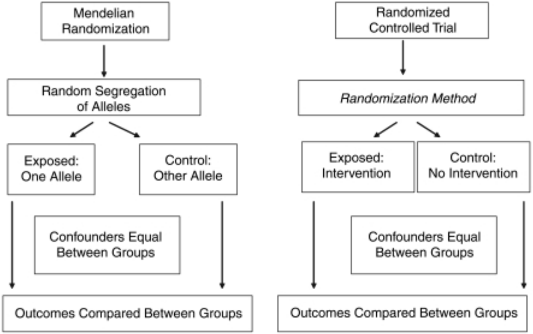
- This course will review many statistical methods in genetic association studies
  - Single variant analysis and confounding; effect size estimation and winner's curse
  - Meta-analysis
  - Set-based (genetic) association analysis
- No biology prerequisites

Topics: Environmental risk factors

- Measurement error (**Ross Prentice**)
- Functional data analysis and applications to high-resolution bio-signal data from wearable devices (**Chongzhi Di**)

Topics: Genetics and Environment

- Gene-environment interaction, genome-wide search strategies and machine learning approaches (**Charles Kooperberg**)
- Mendelian randomization and instrumental variable analysis (**James Dai**)


Mendelian Randomization
Random Segregation of Alleles
Exposed: One Allele
Control: Other Allele
Confounders Equal Between Groups
Outcomes Compared Between Groups
Randomized Controlled Trial
Randomization Method
Exposed: Intervention
Control: No Intervention
Confounders Equal Between Groups
Outcomes Compared Between Groups

Topics: Study design and risk estimation

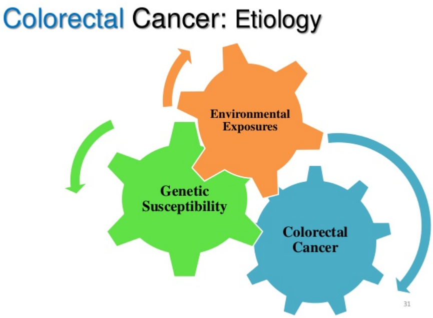
- Current developments of sub-sampling study designs for epidemiologic and biomarker studies (**Yingye Zheng**)
- Absolute risk estimation from case-control and cohort studies

Evaluation

- Credit/No Credit course; audit is welcome
- No homework, but there will be recommended readings
- Final will be a 20-30 minute presentation of a self-chosen topic
  - An in-depth reading of a particular topic covered in this course or related to the research interests of (guest) lecturers

Complex Diseases

- Many complex diseases are contributed by both genes and environment
- Twin studies suggest that about 30% of colorectal cancer risk is due to genetic factors

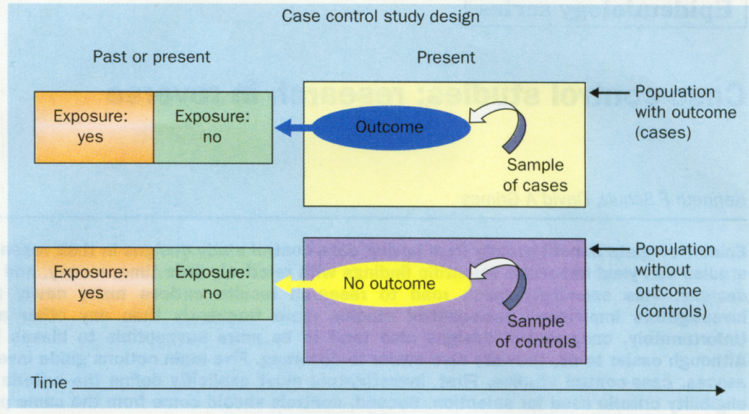


Observational Studies

- Powerful tools for studying disease etiology (risk factors or disease causation)
- Investigator has no control over exposure
- Two common study designs
  - Case-control studies
  - Cohort studies

Case-control studies

- Identify risk factors for a disease/outcome



Schematic diagram of case-control study design

- Schulz KF and Grimes DA 2002. Case-control studies. Lancet 359:431-34.

Example: Diet, Activity, and Lifestyle Study (DALS)

- DALS is a population-based case-control study of colon cancer.
- **Case-definition** Participants were recruited from three locations: the Kaiser Permanente Medical Care Program (KPMCP) of California, Utah, and Minnesota. Eligibility criteria included age at diagnosis of primary colorectal cancer between 30 and 79 years in 1991-1994. Individuals with familial adenomatous polyposis, Crohns disease, or ulcerative colitis were excluded.
- **Control-definition** Controls from KPMCP were randomly selected from membership lists. In Utah and Minnesota, controls were randomly selected through random-digit dialing and driver license lists.
- **Matching** Cases and controls were matched by age (5-year) and sex.

Case-Control Data Analysis

- Y: Disease status
- Z: Covariates
- Logistic regression model

$$\Pr(Y = 1|Z) = \frac{\exp(\beta_0 + \beta' Z)}{1 + \exp(\beta_0 + \beta' Z)} \qquad (1)$$

Case-Control Data Analysis

- Bayes' rule and logistic regression model (1) imply that

$$
\begin{aligned}
\frac{\Pr(Z|Y=1)}{\Pr(Z|Y=0)} &= \frac{\Pr(Z,Y=1)/\Pr(Y=1)}{\Pr(Z,Y=0)/\Pr(Y=0)} \\
&= \frac{\Pr(Y=1|Z)\Pr(Y=0)}{\Pr(Y=0|Z)\Pr(Y=1)} \\
&= \frac{\Pr(Y=0)}{\Pr(Y=1)}\exp(\beta_0+\beta' Z) \qquad (2)
\end{aligned}
$$

Case-Control Data Analysis

- Now imagine cases and controls are members of a second, hypothetical population of individuals whose disease probability is π, the proportion of sampled subjects are cases, but the covariate distribution $\Pr(Z|Y=1)$ and $\Pr(Z|Y=0)$ still satisfy (2).
- In this hypothetical population, from Bayes' rule

$$
\begin{aligned}
P_Z \equiv \Pr(Y=1|Z) &= \frac{\pi \Pr(Z|Y=1)}{\pi \Pr(Z|Y=1) + (1-\pi)\Pr(Z|Y=0)} \\
&= \frac{\pi/(1-\pi)\Pr(Z|Y=1)/\Pr(Z|Y=0)}{1+\pi/(1-\pi)\Pr(Z|Y=1)/\Pr(Z|Y=0)} \\
&= \frac{\exp(\beta^* + \beta Z)}{1+\exp(\beta^* + \beta Z)}
\end{aligned}
$$

- $\beta^* = \beta_0 + \log\{\pi/(1-\pi)\} - \log\{\Pr(Y=1)/\Pr(Y=0)\}$
- β_0: Baseline disease probability is not identifiable

Case-Control Data Analysis

- Constraint

$$\int P_Z f(Z) dZ = \pi$$

- It happens that the constraints are satisfied when maximizing the likelihood function based on P_Z. The score equation corresponding to β_0^* solves the same constraint. Suppose the data consist of (Y_i, Z_i), $i = 1, \ldots, n$

$$\sum_{i=1}^{n} Y_i - \sum_{i=1}^{n} P_{Z_i} = 0$$

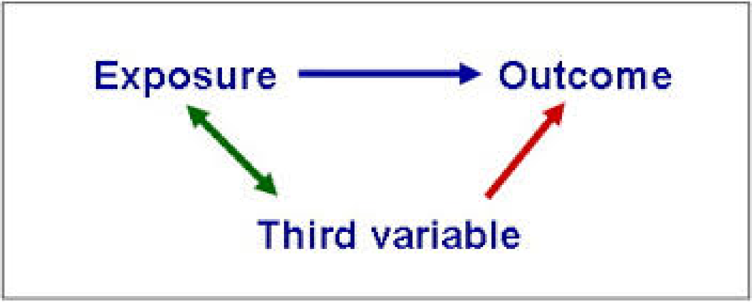
- Despite case-control studies are restrospective in nature, the data can be analyzed as if they were prospectively collected using a logistic model and the odds ratio approximates the relative risk if the disease prevalence is low

Anderson JA(1972). Separate sample logistic discrimination. Biometrika, 59: 19-35.

Prentice RL & Pyke R(1979). Logistic disease incidence models and case-control studies. Biometrika, 66: 403-411.

Confounding

- Exposure of interest may be confounded by a third factor that is associated with exposure and the disease.



Control for confounding

- At the design phase:
  - Sampling cases and controls from the same targeted population
  - Matching controls to cases on factors that are potentially important for disease (e.g., age, sex)
  - If these factors are fixed to be the same in the cases and controls then they can not confound the association
- At the analysis phase:
  - Multivariable adjustment or stratification

Case-Control Designs

- Advantages:
  - Quick and cheap (relatively)
  - Best for rare diseases
  - Evaluation of multiple exposures
- Concerns:
  - Selection bias: Cases and controls selected on criteria related to the exposure
  - Recall bias: Presence of disease may affect ability to recall or report the exposure
  - Beware of reverse causation: the disease results in a change in behavior (exposure)

(Prospective) Cohort Design

- Cohort is a group of people with something in common, usually an expoure or a defined population group, identified before occurrance of disease under investigation
- The study population is followed over a period of time to determine the frequency of disease
- Study the association of exposures with diseases in this group of people

Example: Women's Health Initiative (WHI)

- The Women's Health Initiative (WHI) initiated in 1991 consists of three clinical trials (hormone therapy, dietary modification and calcium/vitamin D) and an observational study
- Investigate major health issues causing morbidity and mortality (e.g., cardiovascular disease, cancer, and osteoporosis) in postmenopausal women
- WHI enrolled more than 160,000 postmenopausal women aged 50 - 79 years (at time of study enrollment) over 15 years

Cohort Data Analysis

- Survival analysis with time to event outcome
- Suppose T is time to the event of interests and Z are covariates
$$\lambda(t|Z) = \lambda_0(t)\exp(\beta' Z)$$
- $\lambda(t|Z) = \lim_{\Delta t \to 0} \Pr(t \leq T < t + \Delta t | T \geq t, Z)$
- Longitudinal analysis with repeated (or longitudinal measurements)
- Suppose Y_{ij} is the measurement (e.g., blood pressure) for the ith subject at t_{ij}th time

$$\mathrm{E}(Y_{ij}) = \beta_0 + \beta_1 t_{ij} + \beta_2' Z_{ij}$$

Subsampling Design

- Case-cohort and nested case-control study designs.
- Commonalities:
  - Sampling from a prospective cohort where disease outcomes and some baseline information are known for all the individuals
  - Include all individuals who develop the disease during follow-up (cases)
- Differences:
  - In case-cohort studies, controls come from a subcohort sampled from the entire cohort at baseline, while in nested case-control designs, controls are sampled from individuals at risk at the times when cases are identified.
- Dr. Yingye Zheng will cover (newest) methods development for subsampling data

Example: WHI

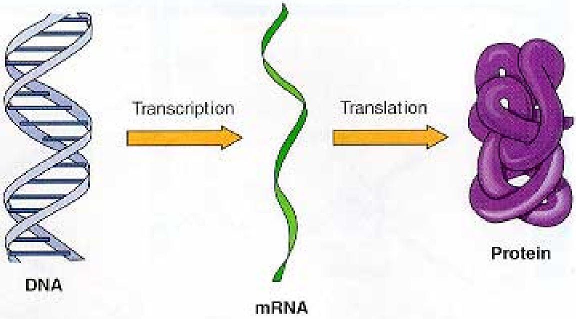
- Cases included colorectal cancer cases from 2009 database
- A control was selected for each case from the risk set at the time of the case's diagnosis
- Additional matching variables: age, race, trial arm/observation, and center
- Exclusion criteria: a prior history of colorectal cancer at baseline, IRB approval not available for data submission into dbGaP, and not sufficient DNA available.

Cohort Studies

- Advantages:
  - Temporality can be established
  - Several outcomes related to exposure can be studied simultaneously
  - Best for relatively common diseases or rare exposures
- Concerns:
  - Large population is needed; time consuming and expensive
  - Study itself may alter people's behavior
  - Cohort subjects may differ from the general population because of eligibility criteria and characteristics related to their self-selection

Genetics-Central Dogma

- The central dogma describes the flow of genetic information in cells from DNA to messenger RNA (mRNA) to protein.



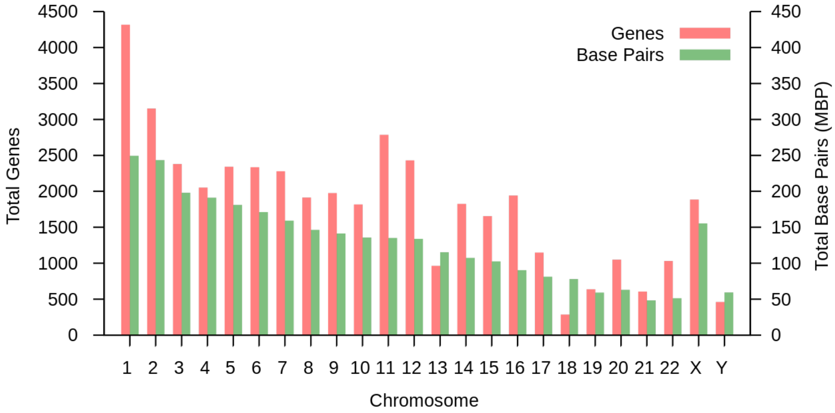
Central Dogma of Gene Expression.

Through the production of mRNA (transcription) and the synthesis of proteins (translation), the information contained in DNA is expressed.

Human Genome

- 3 billion base pairs
- 22 autosomes and 2 sex chromosomes
- Approximately 20,000 protein-coding genes
- Protein-coding sequences account for only a very small fraction of the genome ($\sim$ 1.5%)
- Other types of genes
  - Non-coding RNA : $\sim$20, 000
  - Pseudogenes : $\sim$14, 000

Human Chromosomes



DNA Mutations

- DNA mutations can occur because
    - DNA damage from environmental agents
    - Mistakes occur when a cell copies its DNA in preparation for cell devision
- Mutations are essential to evoluation; they are the raw material of genetic variation

DNA Mutations

- Substitution

  $CTGGAG$

  $CTGGTG$

- Insertion

  $CTGGG$

  $CTGGCTGG$

- Deletion

  $CTGGCTGG$

  $CTGGG$

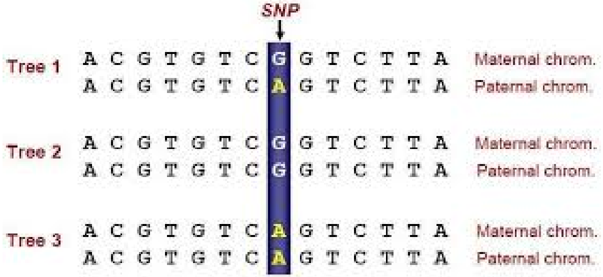
- Frameshift

  $ATG \quad TCG \quad AAT$

  $TGT \quad CGA \quad AT$

Single Nucleotide Polymorphism (SNP)

- SNP is the most common form of polymorphisms

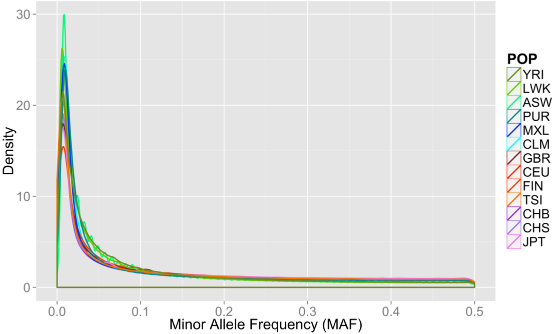

SNP
Tree 1
A C G T G T C G G T C T T A Maternal chrom.
A C G T G T C A G T C T T A Paternal chrom.
Tree 2
A C G T G T C G G T C T T A Maternal chrom.
A C G T G T C G G T C T T A Paternal chrom.
Tree 3
A C G T G T C A G T C T T A Maternal chrom.
A C G T G T C A G T C T T A Paternal chrom.

SNP

- Locus : Specific location of variant on a chromosome.
- Allele : One of a number of alternative forms of the same gene (variant) in a specific locus.
- Previous example: A vs G
- Suppose G is a major allele and A is a minor allele
- Homozygote : individuals with identical pairs of alleles
  - GG : major allele homozygote
  - AA : minor allele homozygote
- Heterozygote: individuals with two different alleles
  - AG

SNP

- Number of dbSNP $>$ 60 millions
- The density plot of the minor allele frequencies



Inheritance

- Inheritance mechanism makes the following characteristics
  - One locus: Hardy-Weinberg Equilibrium
  - Multiple loci: Linkage Disequilibrium

Hardy-Weinberg Law

- Hardy-Weinberg equilibrium (HWE) means the frequency of a diploid genotype is the product of the frequencies of its consitituent alleles
- Suppose a locus A has two alleles, A_1 and A_2
- p is the frequency of allele A_1 and $1 - p$ is the frequency of allele A_2
- Genotype frequency?

$$\Pr(A_1 A_1), \quad \Pr(A_1 A_2), \quad \Pr(A_2, A_2)$$

- At HWE
  - p^2 is the frequency of $A_1 A_1$ homozygotes
  - $2p(1 - p)$ is the frequency of $A_1 A_2$ heterozygotes
  - $(1 - p)^2$ is the frequency of $A_2 A_2$ homozygotes

Properties of HWE

- HWE occurs when the two alleles of an individual are random draws from the population. One generation of random mating produces HWE.
- Allele frequency of the next generation

$$\Pr(A_1) = \Pr(A_1A_1) + \Pr(A_1A_2)/2 = p^2 + p(1-p) = p$$

- Allele frequency doesn't change
- A variety of factors can disturb the equilibrium
  - Inbreeding and other forms of non-random mating
  - Subdivision of the population
  - Natural selection and genetic drifts

HWE

- HWE had a profound effect in early genetics
- In genetic association studies, HWE is primarily used to check genotyping quality
  - Association studies assume that samples are unrelated individuals in HWE.
  - Genotype calls are very precise with error rate $\sim 10^{-3}$.
  - Genotyping technology may be affected by sample preparation, DNA quality, lab conditions, and SNP conditions. Badly called SNPs may be out of HWE.

Genotype QC

- Example: Suppose 10% of A_1A_2 was mis-called to A_1A_1.
- Probabilities of observed genotypes are

$$\overset{o}{\Pr}(A_1A_1) = p^2 + 2p(1-p)*0.1, \quad \overset{o}{\Pr}(A_1A_2) = 2p(1-p)*0.9$$

- Based on the observed genotypes we can calculate the allele frequency

$$p' = (p^2 + 2p(1-p)*0.1) + p(1-p)*0.9 = p + 0.1p(1-p)$$

- By HWE, the probability of A_1A_1 should be $p^2 + 2p(1-p)*0.1 + 0.1^2p^2(1-p)^2$.

Inferrinng population substructure

- HWE has also been used for deriving population substructure
- Suppose in the sample there are two sub-populations Pop1: Pop2 = 1:1
- The allele frequency is: 0.7(Pop1) and 0.3(Pop2)

$$
\begin{aligned}
\Pr(A_1 A_1) &= (0.7 * 0.7 + 0.3 * 0.3)/2 = 0.29 \\
\Pr(A_1 A_2) &= (2 * 0.3 * 0.7 + 2 * 0.3 * 0.7)/2 = 0.42
\end{aligned}
$$

- The allele frequency is 0.29+0.42/2 = 0.50
- Under HWE, $\Pr(A_1 A_1) = 0.25$ and $\Pr(A_1 A_2) = 0.50$

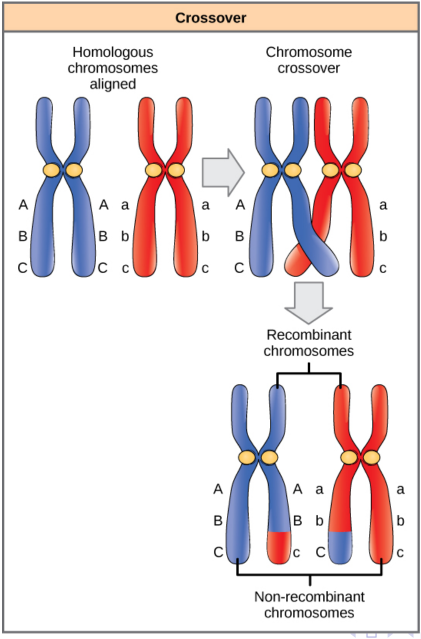
Test for HWE

- Compare observed genotypes vs expected genotypes from HWE
- Pearson χ^2 test:

$$T = \sum_{j}^{3} \frac{(O_j - E_j)^2}{E_j}$$

- Test statistic follows χ_1^2 distribution
- Fisher exact test can be used

Recombination



Recombination

- Introduce genetic diversity.
- Crossovers more likely to occur between genes that are further away; likelihood of a recombination event is proportional to the distance
- Allows for mapping genes

Linkage disequilibrium (LD)

- Two loci A (two alleles A_1 and A_2), B (two alleles B_1 and B_2)
- Haplotype (alleles that are located closely together and that tend to be inherited together)
- Frequency of haplotype and allele

Haplotype	Frequency
A_1B_1	x_{11}
A_1B_2	x_{12}
A_2B_1	x_{21}
A_2B_2	x_{22}

Allele	Frequency
A_1	$p_1 = x_{11} + x_{12}$
A_2	$p_2 = x_{21} + x_{22}$
B_1	$q_1 = x_{11} + x_{21}$
B_2	$q_2 = x_{12} + x_{22}$

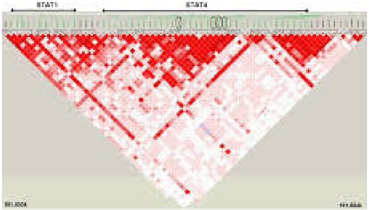
- Under no LD

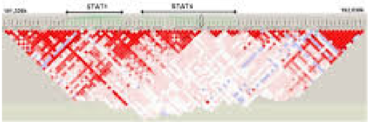
$$D = x_{11} - p_1 q_1 = 0$$

- When only genotypes are available, EM algorithm can be used to estimate haplotype frequencies

Linkage Disequilibrium (LD)

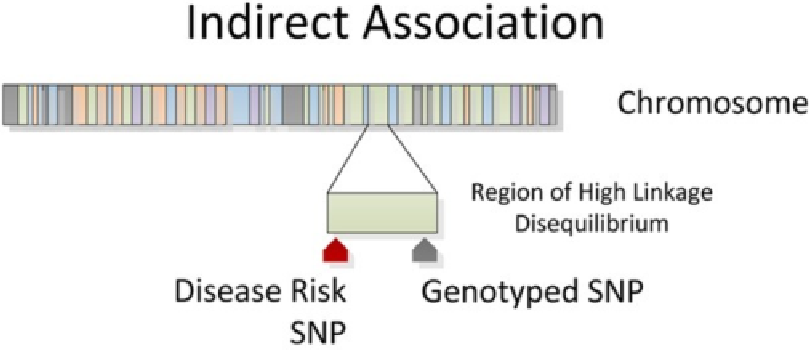
- LD block structure for a particular region.





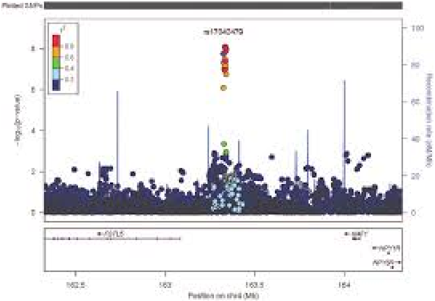
LD

- LD is an extremely important feature in genetic data
- It allows to investigate diseases with fewer markers



LD

- LD makes it hard to adjust for the multiple tests and to find causal variants.



Summary

Today we cover

- Course overview
- Epidemiologic study design
- Genome, DNA-mutation
- Hardy-Weinberg equilibrium
- Linkage disequilibrium

Recommended to read

- Thomas, D. C. (2004). Statistical methods in genetic epidemiology. Oxford University Press.
- Weiss, N. S., & Koepsell, T. D. (2014). Epidemiologic methods: studying the occurrence of illness. Oxford University Press.
- Breslow, N. E. & Day, N. E. (1980). Statistical Methods in Cancer Research. International Agency for Research on Cancer