Outline

1. Introduction: longitudinal studies, advantage and challenge.
2. Exploratory data analysis (EDA) and summary statistics.
3. Methods based on (general) linear models, for continuous outcomes.
   - Weighted least-square (WLS).
   - Maximum likelihood (ML) and restricted maximum likelihood (REML).
   - Linear mixed models (LMM).
4. Methods based on generalized linear models, for binary, count and categorical outcomes.
   - Generalized linear model (GLM), quasi-likelihood (QL).
   - Generalized estimating equations (GEE).
   - Generalized linear mixed models (GLMM).
   - Transition models.
5. Special topics:
   - Time-dependent covariates and causal inference.
   - Missing data.
   - Sample size considerations.

Introduction

Longitudinal study

In a longitudinal study, each subject is measured multiple times, often over a considerable time interval, as opposed to *cross-sectional* data, where a single outcome is measured for each individual.

Examples

1. Orthodontic measurements
2. Multicenter AIDS Cohort Study (MACS).
3. Indonesian Children's Health Study (ICHS).
4. Analgesic crossover trial.
5. Epileptic seizures.

Data example 1: Orthodontic Measurements

- Orthodontic measurements were taken from 27 children (16 boys and 11 girls) every two years from age 8 to 14.
- Note that here the data are *balanced*, that is, the subjects were measured at the same times with no missing data. Unbalanced data (due to design or missing data) is more common in biomedical studies and introduces extra technical difficulties.
- The following table presents the data for 11 girls in the "wide" form, i.e., one subject per row, with multiple columns representing multiple measurements of the same variable. Alternatively the data can also be presented in the "long" form, that is one row for each time point at which one measurement is taken.

Table 1: Orthodontic measurements over time for 11 girls.

Subject \ Age	8	10	12	14
01	21.0	20.0	21.5	23.0
02	21.0	21.5	24.0	25.5
03	20.5	24.0	24.5	26.0
04	23.5	24.5	25.0	26.5
05	21.5	23.0	22.5	23.5
06	20.0	21.0	21.0	22.5
07	21.5	22.5	23.0	25.0
08	23.0	23.0	23.5	24.0
09	20.0	21.0	22.0	21.5
10	16.5	19.0	19.0	19.5
11	24.5	25.0	28.0	28.0

Data example 2: MACS: CD4+ Cell Number

- HIV attacks CD4+ cell which regulates the body's immuno-response to infectious agents; An uninfected individual has around 1100 cells per milliliter of blood.

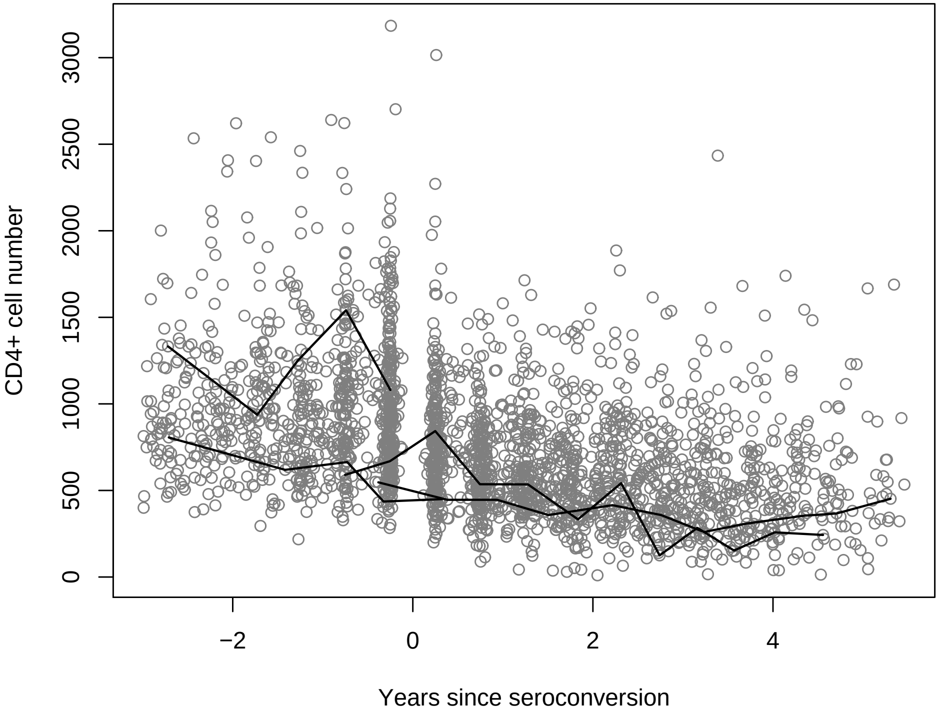


Figure 1: CD4+ cell numbers and time since seroconversion.

- In Figure 1, 2376 values of CD4+ cell counts are plotted against time since seroconversion (detectable HIV antibodies) for 369 infected men enrolled in the Multicenter AIDS Cohort Study (MACS).
- Highly unbalanced with irregular observation times and numbers.
- Goals:
  - Characterize the time course of CD4+ cell depletion.
  - Identify factors which predict CD4+ cell changes.
  - Characterize the degree of heterogeneity across men in the rate of progression.

Data example 3: Indonesian Children's Health Study (ICHS)

- Study the causes and effects of Vitamin A deficiency (as indicated by respiratory or diarrhoeal infection and xerophthalmia, "dry eyes") in pre-school children in Indonesia.
- Over 3000 children examined for up to six visits to assess whether they suffer from respiratory infection (RI). Weight and height are also measured.
- Binary responses (infection yes/no).
- Goals:
  - Whether vitamin A deficient children are at increased risk of respiratory infection.
  - Estimate the degree of heterogeneity in the risk of disease among children

Table 2: Summary of 1200 observations of respiratory infection (RI), xerophthalmia and age on 275 children from the ICHS

		Age						
Xerophthalmia	RI	1	2	3	4	5	6	7
No	No	90	236	330	176	143	65	5
	Yes	8	36	39	9	7	1	0
Yes	No	0	2	18	15	8	4	1
	Yes	0	0	7	0	0	0	0

Data example 4: Analgesic Crossover Trial

- Three-period crossover trial of an analgesic drug for pain relieving.
- Three levels of analgesic (placebo, low, and high) were given to each of the 86 women.
- Women were randomized to one of the six possible orders for administering the three treatment levels.
- Ignoring the order of treatment, pain was relieved for 26% with placebo, 71% with low dose, and 80% with high dose
- A cross-over study, the treatment changes each time.
- Binary outcome.
- Need assessment of carry-over effects.

Table 3: Number of patients for each treatment and response sequence in three-period cross-over trial of analgesic treatment for pain from primary dysmenorrhoea.

	Response sequence in periods 1, 2, 3 (0= no relief; 1= relief)								
	000	100	010	001	110	101	011	111	Total
PLH	0	0	2	2	1	0	9	1	15
PHL	2	1	0	0	0	0	9	4	16
LPH	0	1	1	1	0	8	3	1	15
LHP	0	1	1	1	8	0	0	1	12
HPL	3	0	0	0	1	7	2	1	14
HLP	1	5	0	0	4	3	1	0	14
Total	6	8	4	4	14	18	24	8	86

Data example 5: Epileptic Seizures

- Clinical trial of 59 epileptics.
- For each patient, the number of epileptic seizures was recorded during a baseline period of eight weeks.
- Patients were randomized to be treated with the anti-epileptic drug progabide or placebo.
- Number of seizures was then recorded in four consecutive two-week intervals.
- Does progabide treatment reduce the rate of epileptic seizures?
- Count data.

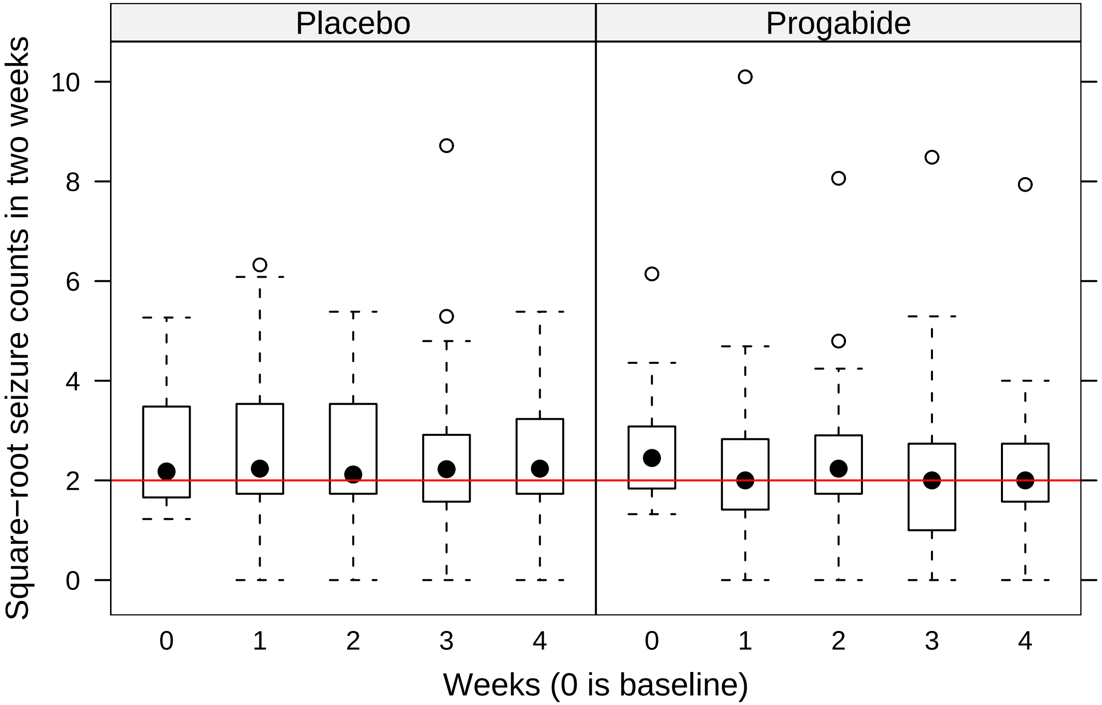
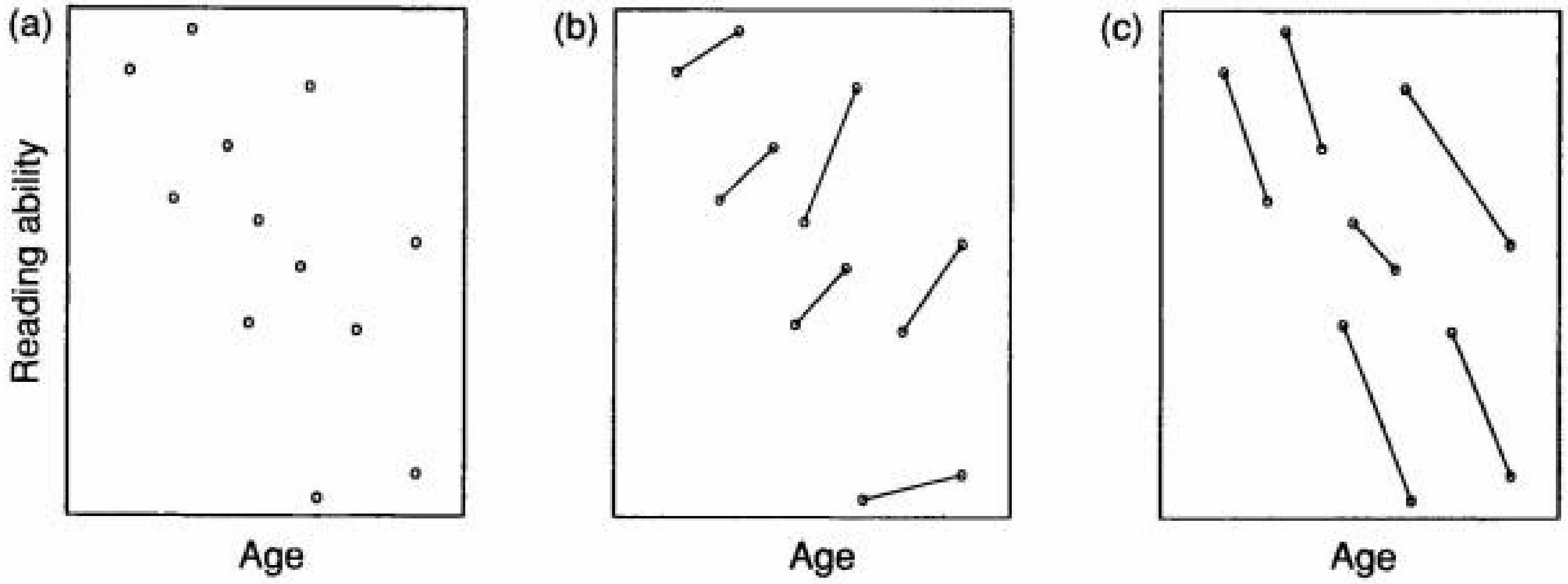


Figure 2: Boxplots of square-root transformed seizure counts per two weeks for epileptics at baseline and for four subsequent two-week periods after the patients were randomized to either placebo or progabide treatment.

Longitudinal vs. Cross-sectional Studies

- Example: A cross-sectional study found that older people smoke more. Possible explanations:
  - People tend to smoke more when they get older.
  - Older people grew up in an environment where the harm of smoking was less widely accepted.
- Longitudinal studies can distinguish the effect due to aging (*i.e.*, changes over time within subject) from cohort effects (*i.e.*, difference between subjects at baseline).


(a)
(b)
(c)
Reading ability
Age
Age
Age

Longitudinal vs. Cross-sectional Studies (cont.)

- Investigation of individual-level changes.
- Each subject can serve as his/her own control. Influence of genetic make-up, environmental exposures, and maybe unmeasured characteristics tend to persist over time.
- Distinguish the degree of variation in Y across time within a subject from the variation in Y between subjects. With repeated values, one can borrow strength across time for the person of interest as well as across people.
- Increased power, by repeated measurements. The repeated measurements from the same subject are rarely perfectly correlated. Hence, longitudinal studies are more powerful than cross-sectional studies.

Correlated Data

In a *regression analysis*, we model the **mean** of a response $(Y_1, \cdots, Y_n)$ as a function of covariates $(x_1, \cdots, x_n)$, where the subscripts $1, \cdots, n$ denote *study units*. We typically assume that:

$$Pr(Y_1, \cdots, Y_n | x_1, \cdots, x_n, \boldsymbol{\beta}) = Pr(Y_1 | x_1, \boldsymbol{\beta}) \cdots Pr(Y_n | x_n, \boldsymbol{\beta}).$$

That is, the Y's are *conditionally independent* given the covariates $\boldsymbol{x}$ (and the parameters $\boldsymbol{\beta}$). However, in general, the Y's are *not* independent marginally, i.e., $\text{Cor}(Y_1, Y_2) \neq 0$ or $Pr(Y_2|Y_1) \neq \text{Pr}(Y_2)$.

Longitudinal data is a special case of *correlated data* where $\boldsymbol{Y} | \boldsymbol{X}, \boldsymbol{\beta}$ are *not* independently distributed.

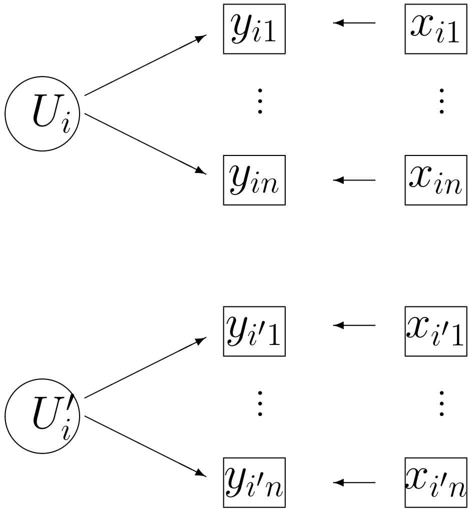
Examples of other correlated data

- Clustered data: multi-center studies, kids in the same classroom. Subjects in the same cluster are correlated.
- Familial data and social networks: complex correlation patterns.
- Multiple outcomes data: exchangeable or complex corrleations
- Time-series data: typically a few subjects with many observations over a long period of time. The emphasis is typically on *prediction*, i.e., using past time-course pattern (cyclic?) to predict the future.
- Spatial data: There is in essence only one subject: the earth. Similar to time-series, only with a higher dimension (2D or 3D) and without the directionality.
- Recurrent event data: the observation times are random and are the outcome variables. For univariate survival data, the event can only occur once.

Characteristic of Longitudinal Data

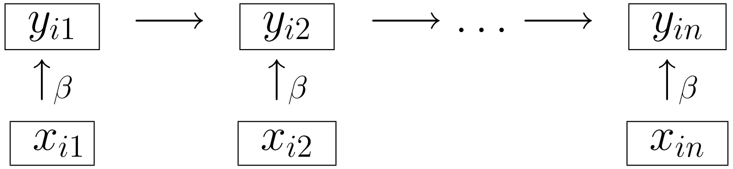
- Individuals are measured repeatedly over time
- When the measurements are taken is not of primary interest and is considered fixed by design. The fact that people have different number of observations and at different times is often ignored under certain assumptions.
- Small number of observations per subject but relatively large number of subjects.
- The variability can be divided into three components:
  1. Heterogeneity between individuals.
  2. Serial correlation, measurements closely spaced are more similar.
  3. Measurement error.

Random effects/latent variable



$$Y_{ij} = x_{ij}\beta + U_i + \epsilon_{ij}; \quad U_i : \text{ unobserved}$$

Serial correlation



By virtual of replication, in subjects and in time, it is possible to distinguish between them.

Notations

We will mostly follow the notation in our textbook (DHLZ).

- Vectors: $\boldsymbol{x}, \boldsymbol{Y}, \boldsymbol{\beta}$. When we say a n-vector, it is an array with n rows and 1 column (i.e., a column vector):

$$\boldsymbol{x} = (x_1, \cdots, x_n)^T = \begin{pmatrix} x_1 \\ x_2 \\ \vdots \\ x_n \end{pmatrix}.$$

- Matrices (upper-case): $\boldsymbol{\mathsf{X}}, \boldsymbol{\mathsf{\Sigma}}$.
- *Parameters* are represented by Greek letters.
- Random variables are upper-cased: $Y_i, \boldsymbol{Y}$, for scalar and vectors, respectively.
- Observed (non-random) variables are lower-cased: $x_i, \boldsymbol{x}$, for scalar and vectors, respectively.
- Let $i = 1, \cdots, m$ index *subjects*.
- For each subject i, there are n_i observations at times t_{ij}, $j = 1, \cdots, n_i$.
- $\boldsymbol{x}_{ij}$ is a p-vector that are the covariates for observation j of subject i. $\boldsymbol{\mathsf{X}}_i$ is a $n_j \times p$ matrix of all the covariates for i and $\boldsymbol{\mathsf{X}}$ is all the covariates.
- The outcome for subject i is denoted by the n_i-vector $\boldsymbol{Y}_i = (Y_{i1}, \ldots, Y_{in_i})^T$ with mean $\boldsymbol{\mu}_i$ and $n_i \times n_i$ covariance matrix $\boldsymbol{\mathsf{V}}_i$ where $v_{ijk} = \mathrm{Cov}(Y_{ij}, Y_{ik})$. The correlation matrix is $\boldsymbol{\mathsf{R}}_i$.
- $\boldsymbol{Y} = (\boldsymbol{Y}_1, \ldots, \boldsymbol{Y}_m)^T$ is an N-vector with $N = \sum_{i=1}^m n_i$.

Review of Linear Model Theory

- A classic linear model can be written as:

$$\mathrm{E}(\boldsymbol{Y} \mid X) = \boldsymbol{\mu} = X\boldsymbol{\beta} \tag{1}$$

$$\mathrm{Var}(\boldsymbol{Y} \mid X) = \boldsymbol{\Sigma} = \sigma^2 \boldsymbol{I}, \tag{2}$$

where $\boldsymbol{Y}$ is a m-vector, $\boldsymbol{\beta}$ a p-vector (p is the number of regression parameters) and X is a $m \times p$ *design matrix*. $\boldsymbol{I}$ is the identity matrix.

- The *method of least squares* aims to minimize the quadratic loss function (sum of squared errors):

$$(\boldsymbol{Y} - X\boldsymbol{\beta})^T(\boldsymbol{Y} - X\boldsymbol{\beta}).$$

- The OLS (ordinary least squares) solution is

$$\hat{\boldsymbol{\beta}} = \left(X^T X\right)^{-1} X^T \boldsymbol{Y}.$$

- It is also the MLE of $\boldsymbol{\beta}$ if we assume $\boldsymbol{Y}$ has a multivariate normal distribution

$$\boldsymbol{Y} \mid X \sim \mathcal{N}(X\boldsymbol{\beta}, \sigma^2 \boldsymbol{I}).$$

- It follows then that $\hat{\boldsymbol{\beta}}$ is also mutlivariate normal with mean $\boldsymbol{\beta}$ and variance $\sigma^2(X^T X)^{-1}$.
- An unbiased estimator of σ^2 is:

$$\hat{\sigma}^2 = \frac{\mathrm{RSS}}{m-p} = \frac{1}{m-p}(\boldsymbol{Y} - X\hat{\boldsymbol{\beta}})^T(\boldsymbol{Y} - X\hat{\boldsymbol{\beta}}).$$

Note that it is *not* the MLE. (More on this later.)

Analysis of Longitudinal Data

The main challenge involves how to take into account the correlation structure.

- Summary statistics based approach: calculate a univariate summary statistic for the multiple measurements and then it can be analyzed as a function of covariates.
  - Simple and especially useful for exploratory analysis.
  - Lost of information, underestimation of uncertainly.
  - Cannot deal with time-dependent covariates.
- Marginal approach: models the marginal mean responses:

$$\mathrm{E}(\boldsymbol{Y}_i) = X_i\boldsymbol{\beta} \tag{3}$$
$$\mathrm{Var}(\boldsymbol{Y}_i) = V_i(\boldsymbol{\alpha}), \tag{4}$$

  where both $\boldsymbol{\beta}$ and $\boldsymbol{\alpha}$ must be estimated, and V_i may also depend on some covariates.
- Conditional approach (random effects model, hierarchical model, multi-level model): assumes correlation arises because of heterogeneity in subjects, that is,

$$\boldsymbol{Y}_i \,|\, \boldsymbol{b}_i \sim \mathcal{N}\big(X_i\boldsymbol{\beta} + Z_i\boldsymbol{b}_i, \sigma^2 I\big) \tag{5}$$
$$\boldsymbol{b}_i \sim \mathcal{N}\big(\mathbf{0}, \tau^2 I\big) \tag{6}$$

- Transition model:

$$\mathrm{E}(Y_{ij} \,|\, Y_{ij-1}, \cdots, Y_{i1}, \boldsymbol{x}_{ij}) = \boldsymbol{x}_{ij}^T\boldsymbol{\beta} + \alpha Y_{ij-1}. \tag{7}$$

Further Reading

- Chapter 1 of Diggle et al (2002).
- Optional: Chapter 1 of Hedeker and Gibbons (2006) and Chapter 5 of Diggle et al (2002).