BIO 226: APPLIED LONGITUDINAL ANALYSIS

MODERN CASE STUDIES IN MIXED MODELS

Longitudinal Assessement of Air Pollution and Lung Function in Children with Asthma

Mixed Models for Multiple Outcomes

Air Pollution and Lung Function in Children with Asthma

Short-term air pollution exposures can increase airflow obstruction in asthmatic children.

Long-term pollution effects on lung function, and the modifying effects of controller asthma treatment on pollution effects are less understood.

In particular, although ambient air pollution has been linked to reduced lung function in normal children, longitudinal analyses of air pollution effects in asthma are lacking.

In asthmatic children participating in a 4-year clinical trial, Ierodiakonou et al. (2014a, 2014b) examined

- associations of lung function with air pollutants: ozone, carbon monoxide, nitrogen dioxide and sulfur dioxide levels.
- effect modification by budesonide and nedocromil (vs. placebo).
- G $\times$ E interaction: GWAS effect modification of pollution effects.

Ierodiakonou D et al. (2014). Ambient air pollution, lung function and airway responsiveness in children with asthma. Under revision for publication.

Ierodiakonou D et al. (2014). Pathway analysis of a genome-wide gene by air pollution interaction study in asthma. Under revision for publication.

The Childhood Asthma Management Program (CAMP)

CAMP is a randomized clinical trial involving eight cities in North America

- Albuquerque, Baltimore, Boston, Denver, San Diego, Seattle, St. Louis, Toronto.

Children enrolled in CAMP were 5–12 years of age and were hyperresponsive to methacholine at study entry.

1,041 Children entered the randomization phase and 311, 312, 418 children received budesonide, nedocromil, and placebo, respectively.

All subjects were treated and followed for four years with visits at two and four months after randomization and at four-month intervals thereafter (n=13 follow-up visits).

Spirometry, before and after the bronchodilator administration and we considered both pre- and post-BD FEV1 and FVC as outcomes in this current analysis (Final N=1003).

CAMP Analysis as a Case Study

A longitudinal analysis of the association between pulmonary function (e.g. FEV1) and pollution exposure involves several subtle issues:

- 4-month average air pollution varies greatly both cross-sectionally (across people at baseline) and longitudinally (within a subject).
- If we are interested in the longitudinal association between air pollution and FEV1, we have to consider confounders that also vary within a person.
- In the G $\times$ E analysis, it is of interest to have random slopes for something other than time (exposure in this case).
- In estimating the presence of G x E interactions within a genome-wide association study (GWAS) framework, the computing time associated with 474,792 SNP-specific linear mixed models gets prohibitive.
  - The two-stage strategy comes in handy here.

Cross-sectional vs longitudinal associations

For concreteness, consider a random intercept and slopes model. Before Lecture 13, we may have considered the following model

$$Y_{ij} = \beta_1 + \beta AP_{ij} + [\text{confounders}] + b_{1i} + (\beta_2 + b_{2i})\, t_{ij} + \epsilon_{ij}$$

where

- AP_{ij} represents the average of a given air pollutant over the 4-months prior to the spirometry assessment in subject i at time j.
- t_{ij} represents time (in months) since randomization.

We now know it may be important to estimate the cross-sectional and longitudinal associations with time-varying air pollution separately:

$$Y_{ij} = \beta_1 + \beta^{(C)} AP_{i1} + \beta^{(L)} (AP_{ij} - AP_{i1}) + [\text{confounders}] + b_{1i} + (\beta_2 + b_{2i}) t_{ij} + \epsilon_{ij}$$

As discussed in Lecture 13, the estimate of $\beta^{(L)}$ is not subject to confounding by cross-sectional factors that do not vary within a subject (gender, disease status, genotype, etc).

Longitudinal associations and temporal confounding

Our estimate of the longitudinal association, $\beta^{(L)}$, is however subject to confounding by factors that vary across a subject's 13 measurements over time. These could include:

- Time since randomization (by treatment group)
- Seasonality, by city (C_i), as defined by day of the year ($d_{ij} = 1, \ldots, 365$)

We therefore fit the final model

$$\begin{aligned} Y_{ij} \quad = \quad & \beta_1 + \beta^{(C)} AP_{i1} + \beta^{(L)} (AP_{ij} - AP_{i1}) + \omega_1 C_i + \\ & \beta_3 \text{Sine}\,(365.24\pi d_{ij}/365.24) + \beta_4 \text{Cos}\,(2\pi d_{ij}/365.24) + \\ & \omega_2 C_i * \text{Sine}\,(365.24\pi d_{ij}/365.24) + \omega_3 C_i * \text{Cos}\,(365.24\pi d_{ij}/365.24) + \\ & \gamma Trt_i * t_{ij} + b_{i1} + (\beta_2 + b_{2i}) t_{ij} + \epsilon_{ij} \end{aligned}$$

Estimated overall, Cross-sectional, and longitudinal associations between $\mathbf{FEV}_1$ and 4-month long-term averages of two air pollutants (NO_2 and CO).

	Model (1)		Model (2)			
AP_{ij}	$\widehat{\beta}$ (SE)	p	$\widehat{\beta}^{(C)}$ (SE)	p	$\widehat{\beta}^{(L)}$ (SE)	p
NO_2	-38.5 (26.2)	0.142	83.6 (80.7)	0.301	-52.7 (27.7)	0.057
CO	-1.21 (0.43)	0.002	0.73 (1.62)	0.654	-1.36 (0.44)	0.002

G × E Model

Another aim of the study was to assess whether there exists genetic susceptibility to air pollution in children with asthmatics.

Genome-wide single nucleotide polymorphisms (SNP) genotyping for CAMP subjects was performed on Illumina's HumanHap550 Genotyping BeadChip ⇒ 474,792 analyzed SNP values.

For each SNP, we are interested in the model:

$$\begin{aligned} Y_{ij} &= \beta_1 + \beta^{(C)} AP_{i1} + \cdots + b_{i1} + (\beta_2 + b_{2i})\, t_{ij} + \\ &\quad \left(\beta_1^{(L)} + \beta_2^{(L)} SNP_i + b_{3i}\right)(AP_{ij} - AP_{i1}) + \epsilon_{ij} \end{aligned}$$

We approximated that running this model separately for 474,792 SNPs would take 22 days!

Alternative two-stage G × E Analysis Approach

We therefore take the two-stage approach:

$$\begin{aligned} Y_{ij} &= \beta_1 + \beta^{(C)} AP_{i1} + \cdots + b_{i1} + (\beta_2 + b_{2i})\, t_{ij} + \\ &\quad \left(\beta^{(L)} + b_{3i}\right)(AP_{ij} - AP_{i1}) + \epsilon_{ij} \end{aligned}$$

Repeatedly run linear regressions on the BLUPs $\widehat{b}^{*}_{3i}$:

$$\widehat{b}_{3i} = \gamma_0 + \gamma_1 SNP_i + e_{ij},$$

for each SNP location separately.
Running 472,792 linear regressions took just a couple of days.

* Sikorska et al. (2013). Fast linear mixed model computations for genome-wide association studies with longitudinal data. *Statistics in Medicine*, 32: 165–180.

Replicated NO_2 × SNP interactions in two ethnicities

SNP	Race	Minor allele	CHR	$\widehat{\gamma}_1 * IQR_{NO2}$	p
rs4672884	AA	A	2	1.12	0.007
	Cauc.			0.68	1.98E-05
	Combined				3.3E-06
rs3769767	AA	A	2	1.19	0.036
	Cauc.			0.91	5.38E-06
	Combined				1.4E-06
rs4378142	AA	A	23	1.24	0.016
	Cauc.			0.70	2.82E-07
	Combined				4.8E-08

Can subsequently run pathway-based gene enrichment analyses on the resulting 470,000+ $\widehat{\gamma}_1$ and associated p-values.

Mixed Effects Multiple Outcomes Model

In many studies, multiple interrelated outcomes that are thought to collectively represent some underlying biologic construct are recorded.

Examples include:

- Markers of systemic inflammation: CRP, ICAM, VCAM, IL-6
- Multiple interrelated birth defects: growth, microcephaly, etc.
- Neurobehavioral development as measured by assessment subscales
- Cognitive Domains

Consider a study design in which multiple outcomes are measured at a single time point.

The overwhelmingly most popular approach to analyzing such data is the run an ordinary regression model for each outcome separately.

One typically assesses by eye how the associations between an exposure/treatment and each outcome varies by outcome.

One disadvantage of this approach is that one should really account for multiple testing using some form of multiple comparisons adjustment, which can results in a loss of power.

More importantly, scientific interest often focuses on the association between the exposure/treatment and the underlying biologic processes (neurodevelopment) instead of any single endpoint.

To address these issues, one could consider mixed models to pool evidence of an exposure/treatment effect across multiple outcomes.

This borrowing of information across outcomes has the potential to yield more power tests of an effect.

Specifically, we can use mixed models to formally

- specify and estimate a global, or average, effect based on the information in all the outcomes.
- assess the amount of heterogeneity in the associations between outcome and exposure

This pooling of information can be highly beneficial if there small to moderate effects of exposure across a range of outcomes.

Mixed Effects Multiple Outcomes Model

Let Y_{ij} denote endpoint j on subject i.

If the outcomes are on different scales, it is better to use scaled version $Y^*_{ij} = Y_{ij}/SD(Y_{ij})$.

Start with the simple random intercepts model that accommodates correlation among individuals:

$$Y_{ij} = \beta_0 + \beta_1 E_i + b_i + [confounders] + \epsilon_{ij}$$

This model treats the multiple outcomes as repeated measures that are correlated within an individual.

Assumes that the effect of exposure is the same across all outcomes.

We can expand this model to allow the effects of exposure to potentially vary across outcomes using additional *outcome-specific random effects*:

$$Y_{ij} = (\beta_1 + a_{1j}) + (\beta_2 + a_{2j})\, E_i + [confounders] + b_i + \epsilon_{ij}$$

- $b_i \sim N\left(0, \sigma_b^2\right)$, $\epsilon_i \sim N\left(0, \sigma^2\, I\right)$
- $a_j = \begin{pmatrix} a_{1j} \\ a_{2j} \end{pmatrix} \sim N\left(\mathbf{0}, G\right), \begin{bmatrix} g_{11} & g_{12} \\ g_{12} & g_{22} \end{bmatrix}$

- b_i, a_j, ϵ_{ij} are all independent of one another.

This model yields parameters that are easy to interpret:

$$Y_{ij} = (\beta_1 + a_{1j}) + (\beta_2 + a_{2j})\, E_i + [confounders] + b_i + \epsilon ij$$

Interpretation:

β_1: the average intercept across all outcomes.

β_2: the overall (global) effect of exposure averaged across outcomes.

$(\beta_1 + a_{1j})$: the intercept for outcome j.

$(\beta_2 + a_{2j})$: the exposure effect for outcome j.

g_{11} in G is the variation in the intercepts across the outcomes.

g_{22} in G is the variation in the intercepts across the slopes.

Like the subject-specific random effects we used in standard linear mixed effects models, the **BLUPs**,

$$\left(\widehat{\beta}_1 + \widehat{a}_{1j}\right) \quad \text{and} \left(\widehat{\beta}_2 + \widehat{a}_{2j}\right)$$

use

$$\widehat{a}_j = E(a_j | Y; \widehat{\beta}, \widehat{G}, \widehat{\sigma}_b^2; \widehat{\sigma}^2)$$

and are therefore a weighted average of the outcome specific effects and the average effect across all outcomes.

This averaging produces outcome-specific effect estimates that are shrunk back towards the overall average effect (shrinkage).

$g_{22} = 0$ means all $a_{2j} = 0$ and the exposure effects are constant across all outcomes.

A large g_{22} means the exposure effects are heterogeneous and therefore vary across outcomes.

Example: Effects of environmental manganese (Mn) exposure on adolescent neurodevelopment

The Public Health Impact of Manganese Exposure (PHIME) study was designed to investigate long-term Mn exposure timing and neurodevelopment in chilldren.

Cross-sectional study of 11-14 year olds residing near ferroalloy industry in Brescia, Italy.

Subjects recruited from three well-characterized communities in Brescia that differ in the timing and intensity of environmental Mn exposure from current or historic ferromanganese alloy plant operations.

Neurobehavioral test battery: Conners Behavior Rating Scale:

- Family Problems, Emotional Problems, Conduct Problems, Cognitive Problems/Inattention, Anger Control Problems, Hyperactivity, ADHD Index, and DSM-IV Symptom Subscales (2).
- Higher scores indicate more neurobehavioral difficulities.

In our analysis we'll investigate the association between neurobehavioral development, as characterized by these 9 sub scales collectively, and Mn exposure as measured in saliva.

We will control for children's age, sex, SES, and Pb exposure (blood).

Long Form of PHIME Data

OBS	ID	Y	T	E
1	1	-0.33	1	11.1
2	1	-0.21	2	11.1
3	1	-0.35	3	11.1
4	1	0.24	4	11.1
5	1	0.30	5	11.1
6	1	-0.52	6	11.1
7	1	-0.54	7	11.1
8	1	-0.28	8	11.1
9	1	-0.33	9	11.1
10	2	0.004	1	25.1
11	2	0.70	2	25.1
12	2	1.35	3	25.1
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SAS Code

```
proc mixed data=longcass covtest;
class id t;
model cass = saliva_Mn_ugl blood_Pb_ugl
                        child_age_yr child_sex ses_3cat /s chisq;
random intercept / subject=id;
random intercept saliva_Mn_ugl / subject=t type=un s;
title 'CASS multiple outcomes model';
run;
```

* This model is much more computational intensive to run than the previous mixed models we have considered. In some situations involving large datasets it may be necessary to use `type=vc` for the outcome-specific random effects, which fits:

$$g_j = \begin{pmatrix} g_{1j} \\ g_{2j} \end{pmatrix} \sim N\left(\mathbf{0}, \begin{bmatrix} g_{11} & 0 \\ 0 & g_{22} \end{bmatrix}\right)$$

PROC MIXED output

Cov Parm	Subject	Estimate	Standard Error	Z Value	Pr > Z
Intercept	ID	0.4623	0.03860	11.98	<.0001
Intercept	t	0.000084	0.000755	0.11	0.4558
saliva_Mn_ugl	t	3.779E-7	0	.	.
Residual		0.4580	0.01212	37.79	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	0.8115	0.5459	8	1.49	0.1755
saliva_Mn_ugl	0.004145	0.001224	8	3.39	0.0096
blood_Pb_ugl	0.006649	0.002523	2863	2.64	0.0085
child_age_yr	-0.07272	0.04279	2863	-1.70	0.0893
child_sex	-0.09001	0.07702	2863	-1.17	0.2427
ses_3cat	-0.1316	0.05560	2863	-2.37	0.0180

Solution for Random Effects

Effect	ID	t	Estimate	Std Err Pred	DF	t Value	Pr > |t|
Intercept	1001		-0.6005	0.2337	2863	-2.57	0.0102
Intercept	1002		0.1339	0.2241	2863	0.60	0.5503
Intercept	1003		0.04543	0.2237	2863	0.20	0.8391
Intercept	1004		-0.2835	0.2298	2863	-1.23	0.2174
Intercept	1005		1.0618	0.2208	2863	4.81	<.0001
Intercept	1006		-0.4116	0.2246	2863	-1.83	0.0669
Intercept	1007		0.3315	0.2231	2863	1.49	0.1375
Intercept	1008		-0.2563	0.2277	2863	-1.13	0.2604
Intercept	1009		-0.8956	0.2330	2863	-3.84	0.0001
Intercept	1011		-0.3261	0.2318	2863	-1.41	0.1595
Intercept	1012		2.7721	0.2200	2863	12.60	<.0001
Intercept	1013		-0.2055	0.2372	2863	-0.87	0.3865
Intercept	1014		-0.1533	0.2249	2863	-0.68	0.4956
Intercept	1015		0.4954	0.2260	2863	2.19	0.0285
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Solution for Random Effects

Effect	ID	t	Estimate	Std Err Pred	DF	t Value	Pr > |t|
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Intercept		1	0.000596	0.008913	2863	0.07	0.9467
Intercept		2	-0.00159	0.008913	2863	-0.18	0.8582
Intercept		3	0.000525	0.008913	2863	0.06	0.9530
Intercept		4	-0.00042	0.008913	2863	-0.05	0.9628
Intercept		5	-0.00278	0.008913	2863	-0.31	0.7550
Intercept		6	0.001157	0.008913	2863	0.13	0.8967
Intercept		7	-0.00303	0.008913	2863	-0.34	0.7343
Intercept		8	0.001611	0.008913	2863	0.18	0.8566
Intercept		9	0.003927	0.008913	2863	0.44	0.6595
saliva_Mn_ugl		1	0.000176	0.000534	2863	0.33	0.7420
saliva_Mn_ugl		2	0.000264	0.000534	2863	0.49	0.6216
saliva_Mn_ugl		3	0.000442	0.000534	2863	0.83	0.4080
saliva_Mn_ugl		4	-0.00030	0.000534	2863	-0.57	0.5716
saliva_Mn_ugl		5	0.000017	0.000534	2863	0.03	0.9753
saliva_Mn_ugl		6	-0.00059	0.000534	2863	-1.11	0.2680
saliva_Mn_ugl		7	0.000218	0.000534	2863	0.41	0.6826
saliva_Mn_ugl		8	-0.00023	0.000534	2863	-0.43	0.6705
saliva_Mn_ugl		9	4.673E-6	0.000534	2863	0.01	0.9930

We compare to what we would have gotten if we had analyzed each outcome separately using an ordinary regression model for each outcome.

SUBSCALE	Effect	SE	P-val*
Family	0.0041	0.0016	0.0105
Emotional	0.0057	0.0016	**0.0003**
Conduct	0.0055	0.0016	**0.0004**
Inatt.	0.0037	0.0016	0.0242
Anger	0.0042	0.0016	0.0108
Hyper	0.0019	0.0016	0.2251
ADHD	0.0057	0.0015	**0.0003**
DSM-1	0.0031	0.0017	0.0639
DSM-2	0.0035	0.0016	0.0332
Pooled ($\widehat{\beta_2}$)	0.0041	0.0012	0.0096

* Bonferroni correction for multiple testing compare outcome-specific effects to $\alpha = 0.05/9 = .0056$.

PHIME Analysis: Summary

In this analysis we addressed multiple testing issues associated with estimating exposure effects on a set of multiple, interrelated outcomes.

Our multiple outcome model indicated strong evidence of a global (across outcomes) association between neurobehavioral development and salivary Mn concentrations in adolescents.

Because we estimated zero variation in the outcome-specific effects, our analysis suggests that this effect is common across the different Connor's sub scales, as opposed to domain specific.