Measuring periciliary liquid depth in newborn pigs with cystic fibrosis disease and in control pigs

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Research Team

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• Michael V. Rector, Research Assistant, Carver College of Medicine, University of Iowa
• Dr. Michael J. Welsh, Professor, Howard Hughes Medical Institute, Carver College of Medicine, University of Iowa
Introduction

• What is cystic fibrosis (CF)
• How is CF caused
  – CFTR protein
• Primary problem: lung disease and mucus in the airways
The Lung

Trachea → Segment → Slide → Airway Epithelia

Picture by Dr. David Stoltz
Mucociliary Clearance

• Mucociliary clearance is by airway surface liquid and cilia.

• When CFTR is absent, water is transported out of the epithelium

Picture by Dr. David Stoltz
Hypothesis

• In CF pigs, PCL depth is reduced through the sodium ion transport channel in the epithelial cells at the airway surface.
Objectives

- To model the data from the experiment measuring PCL in newborn pigs
- To estimate of the variance components
- To examine if there is a difference in the Periciliary Liquid (PCL) depth between CF and Non-CF newborn pigs
Justification

- PCL depth is hard to measure in humans
- PCL depth was measured in pigs
- The pig model was generated by researchers at the University of Iowa (mice with CF genotype do not get sick)
Pigs litter  (Pictures taken from Dr. Welsh laboratory)
PCL depth

(Pictures taken from Dr. Welsh laboratory)
Experimental Methods

• Pigs with mutated CFTR gene
• Airway surface was analyzed in 16 pigs; 5 of them newborns with CF and 8 newborn controls, as well as 3 additional pigs.
• Each trachea was divided into 4 segments, and 5 slides were made from each segment.
• 20 measurement of PCL depth (μm) were made by each of 3 independent observers on each slide.
• Over 18,000 measurements to analyze.
Hierarchical Model: Fixed Effect with Nested Random Effects
How the data looks

![Excel spreadsheet showing data](image)
Statistical Methods

• No cf effect: \( y_{ijklm} = \mu + \delta_l + e_i + e_{i(j)} + e_{ij(k)} + e_{ijklm} \)
  \( l=1,...,3; \ i=1,...,16; \ j=1,...,4; \ k=1,...,5; \ m=1,...,20 \)

• cf effect: \( y_{ijklm} = \mu + \theta \, cf_i + \delta_l + e_i + e_{i(j)} + e_{ij(k)} + e_{ijklm} \)
  where cf\(_i\) is 1 for pigs with CF and 0 for non-CF

• Plot all the data (19,199 measurements)

• Plot means and medians of \( y_{ijklm} \) (the 20 measurements by each observer on each slide)
Analysis

• Analyze mean of $y_{ijklm}$ over 20 measurements
• Analyze medians, similarly
• Alternative analysis uses all 19,199 measurements
• Some measurements missing, the data is unbalanced.
• We used lmer method of R, with REML
• We import the data into R, from the Excel files
library(lme4)

means <- read.table("H:\\Observers_means_Long_pigs.txt",header=T)

attach(means)

Seg <- factor(means$segment)
Slide <- factor(means$slide)
Pig <- factor(means$pig)
Obs <- factor(means$v)

cf <- rep(1:length(measurement))

if (Pig==1) { cf[1] <- 0 }
if (Pig==4) { cf[4] <- 0 }
if (Pig==5) { cf[5] <- 0 }
if (Pig==6) { cf[6] <- 0 }
if (Pig==11) { cf[11] <- 0 }
if (Pig==12) { cf[12] <- 0 }
if (Pig==14) { cf[14] <- 0 }
if (Pig==16) { cf[16] <- 0 }

meanspigs <- lmer(measurement ~ Obs + (1|Pig/Seg/Slide))

summary(meanspigs)
Results
Example of data on one CF pig

Pig = M13
Example of data for Non-CF pig

Pig = D4
CF pig: means (left) and medians (right)
Non-CF pig: means (left) and medians (right)
## Analysis results using library lme4

<table>
<thead>
<tr>
<th>Source of Random variability</th>
<th>$\sigma$ estimate with means</th>
<th>$\sigma$ estimate with medians</th>
<th>$\sigma$ estimate with all the data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between pigs ($\sigma_A^2$)</td>
<td>0.5383</td>
<td>0.5523</td>
<td>0.7138</td>
</tr>
<tr>
<td>Between Segments ($\sigma_B^2$)</td>
<td>0.4181</td>
<td>0.4152</td>
<td>0.4554</td>
</tr>
<tr>
<td>Between Slides ($\sigma_C^2$)</td>
<td>0.5852</td>
<td>0.6229</td>
<td>0.5940</td>
</tr>
<tr>
<td>Residual between Observers on the same slide ($\sigma^2$)</td>
<td><strong>0.3843</strong></td>
<td><strong>0.4209</strong></td>
<td><strong>1.2503</strong></td>
</tr>
<tr>
<td>Residual between and within observers on the same slide</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Analysis results using library lme4 for CF and Non-CF comparison with means

<table>
<thead>
<tr>
<th>Source of Random variability</th>
<th>$\sigma$ estimate with means</th>
<th>$\sigma$ estimate with medians</th>
<th>$\sigma$ estimate with all the data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between pigs $(\sigma_A)^2$</td>
<td>0.5673</td>
<td>0.5820</td>
<td>0.5673</td>
</tr>
<tr>
<td>Between Segments $(\sigma_B)^2$</td>
<td>0.4182</td>
<td>0.4153</td>
<td>0.4182</td>
</tr>
<tr>
<td>Between Slides $(\sigma_C)^2$</td>
<td>0.5851</td>
<td>0.6229</td>
<td>0.6033</td>
</tr>
<tr>
<td>Residual between Observers on the same slide $(\sigma^2)$</td>
<td>0.3843</td>
<td>0.4209</td>
<td>1.2891</td>
</tr>
</tbody>
</table>
Discussion of random effects confidence intervals

Means

Medians

All the data
### Discussion

<table>
<thead>
<tr>
<th>Confidence Interval</th>
<th>All data</th>
<th>Data medians</th>
<th>Data means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-0.6909, 0.7240)</td>
<td>(-0.7282, 0.7222)</td>
<td>(-0.6912, 0.7244)</td>
</tr>
</tbody>
</table>

Same answer!
Conclusion

- There is not enough evidence to reject the null hypothesis of no difference p-value is 0.96.
- There is no statistically significant difference in the reduction of the PCL depth through the sodium ion transport channel in the epithelial cells at the airway surface.
- If there is a difference it is less than 0.7 μm.
What the study suggests

This study suggests that the PCL depth is no different in pigs with CF than non-CF newborn pigs, or, if there is a difference it is less than 0.7 mm with 95% confidence.
Future work

• To measure sodium concentrations of the epithelial cells at the airway surface to determine if there is significant difference between the CF and Non-CF pigs.
• To find a better way, to measure PCL
• Possibly image processing
Acknowledgements

• Dr. David Stoltz, Assistant Professor, Carver College of Medicine, University of Iowa
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• Ming Yang, Graduate Student, Department of Biostatistics, University of Iowa
Reference

More references

THANKS!
Questions...?
Pigs litter (Pictures taken from Dr. Welsh laboratory)
Lung disease
(Pictures taken from Dr. Welsh laboratory)
Airway
(Pictures taken from Dr. Welsh laboratory)
Airway obstruction
(Pictures taken from Dr. Welsh laboratory)