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Applied Surrogate Endpoint Evaluation Methods with SAS and R
Evaluation of Magnetic Resonance Imaging as a Biomarker in Alzheimer’s Disease

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17.1 Introduction

17.1.1 Alzheimer’s Disease

Alzheimer’s disease (AD), the most common cause of dementia, is an irreversible age-related condition resulting in an increase in dependency on care providers for basic functioning. Clinical symptoms of sporadic AD manifest mostly in the elderly population (at least 65 years) and include progressive deterioration of specific cognitive functions such as memory, speech, motor skills and perception (McKhann et al., 1984). A proper diagnosis of AD suffers from the lack of diagnostic tools that can accurately distinguish AD from other causes of cognitive impairment especially at an early stage of the disease (Blennow, 2004; Chetelat and Baron, 2003; Galvin and Sadowsky, 2012). Moreover, AD results in multiple pathological changes in the brain, which do not manifest the same way in all patients. The most common AD related pathological changes in the brain include: amyloid-beta protein plaque deposition (Masters et al., 1985; Hardy and Selkoe, 2002), neurofibrillary tangle (hyperphosphorylated tau) formation, and neuro-degeneration (Hol et al., 2003; Serrano-Pozo et al., 2011). How these changes influence the progression of AD is unfortunately not clearly understood since the onset of clinical symptoms of AD occurs much later than the onset of the pathological changes associated with the disease (Agronin, M.E., 2007). Considering the fact that there is no known cure for AD, an early diagnosis of the disease would therefore be preferable in order to allow for the introduction of treatments that may delay the progression of the disease such as a lifestyle intervention or novel therapeutic management of the patients.

From a practical point of view, although the pathological markers of AD are indicative of the disease progression, they can only be measured cross-
Evaluation of MRI as a Biomarker in Alzheimer’s Disease

Sectionally (once per patient). This is due to the fact that pathological histology staining involves post-mortem examination whose acquisition comes too late from a diagnostic point of view (Perl, 2010). Thus, potential biomarkers which can be easily acquired in clinical follow-up of patients would be of interest in early diagnosis of the disease (Hampel et al., 2009). One of the challenges in current AD research is the availability of a suitable animal model, fully representative for the disease pathology. On the other hand, the advantage of using an animal model exhibiting only one pathological indication of AD is that, it enables us to study the influence of one aspect of the disease, without the interaction of other pathological indications. The existing animal models mainly target one or a few aspects of the disease (Duff and Suleman, 2004). An animal model with over-expressed Amyloid Precursor Protein (APP) gene and Presenilin (PS) results in variants of mouse models such as the APP/PS1 mouse model that was used in this experiment (Götz and Götz, 2009).

17.1.2 Magnetic Resonance Imaging and Histology Parameters

Non-invasive neuro-imaging based technologies such as Positron Emission Tomography (PET) scan and Magnetic Resonance Imaging (MRI), if adequately validated, hold the most promise for adoption in both diagnosis and clinical follow-up of disease progression in AD (Dickerson and Sperling, 2005). Using neuro-imaging, differences in brain anatomy, chemistry and physiology can be detected via the measured MRI parameters. Additionally, longitudinal MRI studies enable the assessment of neuro-anatomical changes as the animal ages. MRI technology is highly advanced with different scanning technologies resulting in different measures. Diffusion Tensor Imaging (DTI) has been shown to characterize AD progression in white matter (Alexander et al., 2007; Klohs et al., 2013). While DTI quantifies the diffusivity of water molecules in the brain microstructure, which is hypothesized to follow a Gaussian distribution. Diffusion Kurtosis Imaging (DKI) aims at simultaneously quantifying both the Gaussian (diffusion tensor) and non-Gaussian (diffusion kurtosis) behavior of water. Several studies have reported the superiority of DKI over DTI in detecting AD pathology in both white and grey matter (Hui et al., 2008; Cheung et al., 2009; Veraart et al., 2011).

The degree of neuronal myelination was determined by immunohistochemical visualization of myelin basic protein (MBP), the major protein component of myelin sheaths. In addition, Glial Fibrillary Acidic Protein (GFAP) and Ionizing Calcium-Binding Adaptor molecule 1 (IBA-1) were used as markers for astrocytes and microglia, respectively. Finally, 4G8 labeling was performed to detect amyloid-beta in the brains of APP/PS1 transgenic mice.

In this chapter, we apply the methodology presented in Chapter 4 to evaluate the potential of MRI parameters as biomarkers for histology features in different regions of the brain. Although, the experimental setting we discuss in this chapter is not the same as the clinical trial setting discussed in previous
TABLE 17.1
Summary of the data: Number of animals per age and genotype.

<table>
<thead>
<tr>
<th>Age</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transgenic</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Wildtype</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

chapters, we propose to use the surrogacy framework for the evaluation of MRI parameters as biomarkers for specific histology features, using a similar approach within the normal-normal surrogacy setting. After a description of the case study in Section 17.2, we present an elaborate discussion on the similarity to the surrogacy setting in Section 17.3. A two-stage modeling approach is presented in Section 17.4. Sections 17.5 and 17.6 are devoted to the application of the proposed methodology on the case study, while software issues are discussed in Sections 17.7 and 17.8.

17.2 The AD Mouse Model for MRI and Histology Data

Data from an APP/PS1 transgenic mouse model experiment is used to evaluate if MRI parameters can be used as biomarkers for histology. Up to five cohorts of mice aged 2, 4, 6, 8 and 10 months were scanned (sample sizes are given in Table 17.1). The experiment comprised two mice genotypes: (1) transgenic APP/PS1 mice which over-expressed the KM670/671NL APP mutation and the L166P PS1 mutation and (2) wildtype mice which represents a healthy control group.

Diffusion weighted data were acquired on a 7T Bruker Pharmascan system: 28 slices with resolution (0.2136 × 0.214 × 0.2)mm³; 3 x (20 diffusion gradient directions), δ = 5ms, Δ = 12ms, 7 b-values (400 − 800 − 1200 − 1600 − 2000 − 2400 − 2800)s/mm². Seven diffusion parameter maps were estimated (Veraart et al., 2013), including Axial Kurtosis (MRI-AK), Radial Kurtosis (MRI-RK), Mean Kurtosis (MRI-MK), Axial Diffusivity (MRI-AD), Radial Diffusivity (MRI-RD), Mean Diffusivity (MRI-MD) and Fractional Anisotropy (MRI-F A). In addition, four pathological histology stains were applied: GFAP and IBA-1 staining for neuroinflammation, MBP staining for myelination and 4G8 staining for amyloid-beta. For all four histology stains, the histology feature used in the analyses presented in this chapter was percentage of area stained.

In total, data was available from 23 brain Regions of Interest (ROI) covering both the white and grey matter. For each region, the averages of each of the seven MRI parameters and four histology stains (each quantified by the
percentage of area stained) are available for each animal. Figures 17.1 and 17.2 show an example of an MRI parameter (MRI-AK) versus GFAP percentage of area stained measured in the motor cortex region.
Panel a and b in Figure 17.3 show an illustrative example in which a histology feature is plotted against a specific MRI parameter at 2 and 8 months, respectively. The effect of the disease progression is translated to a shift in

FIGURE 17.2
The motor cortex region. Panels a and b: the residuals after the group means for each age group were subtracted. Blue symbols: wildtype mice. Red symbols: transgenic mice.

17.3 Two Levels of Surrogacy
Panel a and b in Figure 17.3 show an illustrative example in which a histology feature is plotted against a specific MRI parameter at 2 and 8 months, respectively. The effect of the disease progression is translated to a shift in
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FIGURE 17.3
Illustrative example: The effect of AD progression on an MRI parameter and a specific histology feature at two time points for simulated data. The solid line connects means of the two genotypes. Blue symbols: wildtype mice. Red symbols: transgenic mice.

both the MRI parameter and histology feature in the transgenic group. Panel a and b of Figure 17.4 show the data at 2 and 8 months respectively, which will be analyzed in this chapter. Note that the slope of the lines connecting the means of the two clouds in panels b of Figures 17.3 and 17.4 corresponds to the relative effect (RE), discussed in Chapter 3.

Figures 17.5 and 17.6 illustrate two aspects of the association between an
FIGURE 17.4
Illustrative example: The effect of AD progression on an MRI parameter and a specific histology feature at two time points for real data. The solid line connects means of the two genotypes. Blue symbols: wildtype mice. Red symbols: transgenic mice.

MRI parameter and a given histology feature: the effect of AD progression (characterized by continual amyloid-beta deposition) and the correlation between the two variables. Panels a and b show the data and the residuals after subtracting the means, respectively. From Figure 17.5, the AD progression
effect can be seen clearly in panel a, while panel b indicates that, conditional on AD progression effect, the two variable are not correlated. Using surrogacy terminology, Figure 17.5b indicates that, on an individual level, MRI is a poor biomarker for histology. A second illustrative example is shown in Figure 17.6a and b. For this example, the AD progression effect (shown in Figure 17.6a) has the same magnitude of the effects as in the first example. Note that the relative effects in the two examples are similar. Figure 17.6b reveals a substantial difference between the two examples on an individual level. For the second example, after adjusting for the AD progression effects, MRI and histology are correlated (Figure 17.6b). Hence, at an individual level, MRI is a good biomarker for histology. Note that we can use the adjusted association, discussed in Chapter 3, to evaluate MRI as a biomarker for histology at an individual level.

In the next example, presented in Figure 17.7 and 17.8, we “translate” the two aspects of the association between MRI and histology into two surrogacy measures: individual-level surrogacy and disease-level surrogacy. The latter corresponds to the trial-level surrogacy, discussed in Chapter 4. The examples presented in Figures 17.5 and 17.6 correspond to a single trial setting and allows us to evaluate the quality of MRI as a biomarker for histology only at individual level. On the other hand, the animal model for AD allows us to estimate surrogacy measures in both levels since MRI parameters and histology features are measured at 5 times points. Figure 17.7 shows a scenario in which an MRI parameter and a histology feature are not correlated, given the progression effect of AD but the disease effects (on both MRI and histology), shown in panel (c), are correlated. This suggests a scenario for which the disease-level surrogacy is high while individual-level surrogacy is low. In other words, the effect of AD progression on histology features can be predicted using the AD progression effects observed on MRI parameters while at individual level, MRI values are not predictive for histology features. A scenario in which MRI parameters and histology features are associated at both disease and individual levels is shown in Figure 17.8.
FIGURE 17.5
Illustrative examples demonstrating the effect of AD progression on an MRI parameter and a histology feature at two time points. Without correlation between MRI and histology parameters. Larger symbols denote the group means. Blue symbols: wildtype mice. Red symbols: transgenic mice.
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**FIGURE 17.6**

Illustrative examples demonstrating the effect of AD progression on an MRI parameter and a histology feature at two time points. With correlation between MRI and histology parameters. Larger symbols denote the group means. Blue symbols: wildtype mice. Red symbols: transgenic mice.
17.4 Evaluation of MRI Parameters as a Biomarker for Histology Features

17.4.1 A Joint Model for MRI and Histology

The analysis presented in this section consists of a region/MRI/histology-specific model. Hence, for each region, 4 × 7 models are fitted. Each model is used to evaluate one MRI parameter as a biomarker for one histology feature. The observation unit for the analysis is \((X_{ij}, Y_{ij}, Z_j)\) with \(X_{ij}\) being the histology feature for the \(j^{th}\) animal, \(j = 1, \ldots, N_i\) at age \(i, i = 1, \ldots, I\), \(Y_{ij}\) is the MRI parameter of the \(j^{th}\) animal at age \(i\) and \(Z_j\) is an indicator variable for the genotype the animal belongs to given by

\[
Z_j = \begin{cases} 
1, & \text{APP/PS1 Transgenic,} \\
0, & \text{Wildtype.} 
\end{cases}
\]

We assume that the mean structure for MRI and histology parameters respectively, is given by

\[
E (X_{ij} | Z_j) = \mu_{Xi} + \alpha_i Z_j, \\
E (Y_{ij} | Z_j) = \mu_{Yi} + \beta_i Z_j.
\]  

(17.1)

Here, \(\mu_{Xi}\) and \(\mu_{Yi}\) are the age-specific means for the MRI feature and histology parameter, respectively, in the wildtype mice group. Note that for the wildtype mice group, we assume that the histology feature is constant over time since the disease pathology does not vary a lot for these young ages (2-8 months) in the wildtype mice. Thus, the mean structure in (17.1) can be simplified by having only one parameter for histology staining in wildtype mice, i.e, \(\mu_{Yi} = \mu_Y\). The age-specific parameters \(\alpha_i\) and \(\beta_i\) correspond to the disease effect on MRI and histology at a given age, respectively. Further, we assume that the two endpoints (histology and MRI) follow a bivariate normal distribution with genotype-specific covariance matrices, that is,

\[
\begin{pmatrix} 
X_{ij} \\
Y_{ij} 
\end{pmatrix} \sim N \left( \begin{pmatrix} \mu_{Xi} + \alpha_i Z_j \\
\mu_{Yi} + \beta_i Z_j \end{pmatrix}, \Sigma \right).
\]  

(17.2)

Here, \(\Sigma\) is a 2 × 2 genotype-specific covariance matrix given for transgenic and wildtype mice, respectively, by

\[
\Sigma_T = \begin{pmatrix} 
\sigma^2_A & \sigma_{Ah} \\
\sigma_{Ah} & \sigma^2_h 
\end{pmatrix} \quad \text{and} \quad \Sigma_W = \begin{pmatrix} 
\sigma^2_W & \sigma_{Wh} \\
\sigma_{Wh} & \sigma^2_h 
\end{pmatrix}.
\]  

(17.3)

The joint model specified in (17.1) allows us to model two sources (or aspects) of the association between a specific histology feature and an MRI parameter: (1) the association between the disease evolution effects (with respect to
FIGURE 17.7
Illustration of simulated individual- and disease-level surrogacy using the AD animal model for a scenario with low individual-level surrogacy. The solid lines in panel a connect the means of the transgenic and wildtype groups at each age. The slope of these lines is equal to the RE (at each age). Panel b shows the association between a histology feature and the MRI parameter. Panel c presents the disease effects $\beta$ on a histology feature versus the disease effects $\alpha$ on an MRI parameter. Blue symbols: wildtype mice. Red symbols: transgenic mice.
FIGURE 17.8
Illustration of simulated individual and disease-level surrogacy using the AD animal model for a scenario with high individual-level surrogacy. The solid lines in panel a connect the means of the transgenic and wildtype groups at each age. The slope of these lines is equal to the RE (at each age). Panel c presents the disease effects $\beta$ on a histology feature versus the disease effects $\alpha$ on an MRI parameter. Blue symbols: wildtype. Red symbols: transgenic.
age) of the two endpoints and (2) the association between the two endpoints adjusted for the time evolution of the disease. In what follows, we show that the two sources of association can be interpreted as individual and disease-level surrogacy. The latter is similar to the trial-level surrogacy discussed in Chapter 4.

### 17.4.2 Genotype-specific Individual-level Surrogacy

Based on the covariance matrices specified in (17.3), we can derive the adjusted correlation between an MRI parameter and a specific histology feature for each genotype given by

\[
\rho_T = \frac{\sigma_{Ahm}}{\sqrt{\sigma_{Ah}^2 \times \sigma_{Am}^2}}, \quad \text{and} \quad \rho_W = \frac{\sigma_{Whm}}{\sqrt{\sigma_{Wh}^2 \times \sigma_{Wm}^2}}. \quad (17.4)
\]

The genotype-specific adjusted correlations \( \rho_W \) and \( \rho_T \) measure the association between the two endpoints adjusted for the time evolution of the disease and can be interpreted in the same way as the adjusted association in the surrogacy model presented in Chapter 3. A large absolute values of the adjusted correlation implies a better surrogacy at an individual level. Note that in contrast with the models discussed in Chapter 4, we do not assume that the association between MRI and histology is equal in the two groups.

### 17.4.3 Disease-level Surrogacy

The joint model specified in (17.1) allows us to estimate the age and genotype specific parameters \((\alpha_1, \alpha_2, \alpha_3, \alpha_4, \alpha_5)\) and \((\beta_1, \beta_2, \beta_3, \beta_4, \beta_5)\). Our aim is to establish a relationship between \(\alpha_i\) and \(\beta_i\) and in particular, to assess whether AD evolution observed for the MRI parameter is predictive for the AD evolution observed for a particular histology feature. In other words, we wish to evaluate whether an MRI parameter can be used as a biomarker for a given histology feature in an AD mouse model at a disease level. Disease-level surrogacy can be measured using \(R^2\) obtained from the regression model in a similar way as done in Chapter 4 for trial-level surrogacy. From (17.5), \(\eta\) and \(\gamma\) are regression coefficients, while \(\varepsilon_i\) denotes the measurement error for the regression model:

\[
\hat{\beta}_i = \eta + \gamma \hat{\alpha}_i + \varepsilon_i, \quad i = 1, \ldots, I. \quad (17.5)
\]
TABLE 17.2
The motor cortex region: Parameter estimate (standard error) of AD progression effects. $\beta$: the disease effect on GFAP percentage of area stained. $\alpha$: the disease effects on MRI-AK.

<table>
<thead>
<tr>
<th>Age</th>
<th>$\beta$ (s.e.)</th>
<th>$\alpha$ (s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>-0.35 (0.51)</td>
<td>-0.01 (0.02)</td>
</tr>
<tr>
<td>4</td>
<td>1.58 (0.51)</td>
<td>0.03 (0.02)</td>
</tr>
<tr>
<td>6</td>
<td>5.43 (0.53)</td>
<td>0.03 (0.02)</td>
</tr>
<tr>
<td>8</td>
<td>11.83 (0.53)</td>
<td>0.05 (0.02)</td>
</tr>
<tr>
<td>10</td>
<td>15.43 (0.92)</td>
<td>0.08 (0.03)</td>
</tr>
</tbody>
</table>

17.5 Application to the MRI Project Data: Examples of Region-specific Models

The joint model specified in (17.1) was applied for each combination of the seven MRI parameters and the four histology stains in each of the 23 ROI (shown in Figure 17.15). In this section, we discuss the results in the motor cortex (Section 17.5.1) and the caudate-putamen (Section 17.5.2) regions. Note that, as explained in Section 17.4, the joint model (17.1) is formulated with a constant effect of histology in the wildtype mice ($\mu_{Y_i} = \mu_Y$).

17.5.1 The Motor Cortex: GFAP Staining and MRI-AK

The observed data for GFAP percentage of area stained and MRI-AK are shown in Figure 17.9(a) where an age-dependent shift of MRI and histology measurements for older transgenic mice is observed. Parameter estimates obtained for the joint model are presented in Table 17.2. The estimated regression model, shown in Figure 17.9(b) is given by $\beta_i = 0.064 + 192.6756\alpha_i$. The surrogacy measure at disease level $R^2_D = 0.91$ indicates that MRI-AK is a good predictive biomarker for GFAP staining. At an individual level, after adjusting for the disease effect, there is low correlation between the residuals ($\rho_A = \rho_W = 0.13$) indicating a low individual-level surrogacy (Figure 17.10a and b).

Figure 17.11(a) presents the disease-level surrogacy measures for all seven MRI parameters and four histology stains in the motor cortex region. Note that the MRI-AK parameter is found to be predictive for 4G8 staining ($R^2_D = 0.94$) and GFAP staining ($R^2_D = 0.91$), while it has relatively low predictive value for the IBA-1 ($R^2_D = 0.60$) and MBP ($R^2_D = 0.47$) staining. MRI-MD was found to be predictive at disease-level for 4G8 ($R^2_D = 0.87$), GFAP ($R^2_D = 0.90$), and IBA-1 ($R^2_D = 0.76$) stainings, respectively. In addition,
FIGURE 17.9

MRI-RD was predictive for MBP staining with $R_D^2 = 0.83$. The results for individual-level surrogacy are shown in Figure 17.11 which reveals that MRI parameters were not predictive for histology at an individual-level in both transgenic (Figure 17.11(b)) and wildtype mice (Figure 17.11(c)).
17.5.2 The Caudate-putamen: GFAP Staining and MRI-AK

A similar analysis was conducted for the caudate-putamen region. The observed data for GFAP percentage of area stained and MRI-AK shown in Figure 17.12a indicates an age-dependent shift in histology values for transgenic mice and a relatively small shift in MRI-AK values. The estimated disease-level surrogacy is relatively low at $R_D^2 = 0.596$ (See Figure 17.12b). Moreover,
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FIGURE 17.11
The motor cortex region: Individual and disease-level surrogacy for all MRI parameters and histology stains (percentage of area stained).
TABLE 17.3
The caudate-putamen region: Parameter estimate of AD progression effects for GFAP percentage of area stained with MRI-AK. $\beta$: disease effect on GFAP percentage of area stained. $\alpha$: disease effect on MRI-AK.

<table>
<thead>
<tr>
<th>Age</th>
<th>$\beta$ (s.e.)</th>
<th>$\hat{\alpha}$ (s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.35 (0.43)</td>
<td>-0.0001 (0.02)</td>
</tr>
<tr>
<td>4</td>
<td>1.05 (0.43)</td>
<td>0.02 (0.02)</td>
</tr>
<tr>
<td>6</td>
<td>3.99 (0.46)</td>
<td>-0.013 (0.024)</td>
</tr>
<tr>
<td>8</td>
<td>8.75 (0.46)</td>
<td>0.0001 (0.024)</td>
</tr>
<tr>
<td>10</td>
<td>16.37 (0.80)</td>
<td>0.09 (0.03)</td>
</tr>
</tbody>
</table>

although a large disease effect on histology is observed over time as shown in Table 17.3, the disease effect on MRI is relatively small, hence the low association between MRI and histology disease effects. Similar patterns can be observed for individual-level surrogacy of MRI-AK as a biomarker for GFAP percentage of area stained as shown in Figure 17.13a and b.

Figure 17.14a shows the surrogacy measures for all MRI parameters and histology stains (percentage of area stained) in the caudate-putamen region. For the 4G8 staining, the highest surrogacy measures at a disease level was found for MRI-AD and MRI-MD ($R^2_D = 0.62$ and $R^2_D = 0.61$, respectively). The MRI parameters with the highest disease-level surrogacy measures in the caudate-putamen region were MRI-MD, MRI-MK and MRI-RD for the GFAP staining with $R^2_D = 0.83$, $R^2_D = 0.87$, and $R^2_D = 0.86$, respectively. Note that for the MBP staining, all MRI parameters except for MRI-AD ($R^2_D = 0.35$), are good biomarkers at disease-level with $R^2_D \geq 0.72$. For transgenic mice, individual-level surrogacy was very low for all MRI parameters and histology stains as shown in Figure 17.14b. On the other hand, individual-level surrogacy was found to be relatively high in the wildtype mice for MBP staining using MRI-AK ($\hat{\rho}_w = 0.95$), MRI-MK ($\hat{\rho}_w = 0.94$), MRI-RD ($\hat{\rho}_w = 0.94$) and MRI-RK ($\hat{\rho}_w = 0.71$).

17.6 The Surrogacy Map of the Brain

The joint model allows us to evaluate the surrogacy pattern in the brain for all combinations of MRI parameters and histology stains. Figure 17.15 shows a heatmap of the disease-level surrogacy in the 23 ROI. Clearly, surrogacy is highly dependent on the region, MRI parameter and histology stain. For regions such as amygdala and olfactory bulb, none of the MRI parameters is useful as a biomarker for any of the histology stains. GFAP percentage
FIGURE 17.12

of area stained can be predicted by several MRI parameters in the caudate-putamen, cerebellum, and several cortex regions. For IBA-1 staining, good disease-level surrogacy was observed in the septal nucleus region using all MRI parameters (apart from MRI-AD and MRI-AK). Relatively high level of surrogacy of MRI parameters with 4G8 staining was observed in the cortex regions. Thus, since these four histology stains evaluate different aspects of the disease morphology, there is need to evaluate surrogacy at the different brain
regions using MRI parameters with high surrogacy level for the particular histology stains. A surrogacy map for individual-level surrogacy in transgenic mice is presented in Figure 17.16a. Overall, a low individual-level surrogacy is observed, an indication that prediction of histology at an individual level is not practical using MRI parameters. For the wildtype mice, however, individual-level surrogacy was relatively high in some brain regions, MRI parameters and histology stains (see Figure 17.16b).
FIGURE 17.14
The caudate-putamen region: Individual and disease-level surrogacy map for all MRI parameters and histology stains (percentage of area stained).
FIGURE 17.15
Disease level surrogacy in 23 regions of the brain ($R^2_D$ for each MRI parameter and histology percentage of area stained). White fill: surrogacy measure not computed.

17.7 Implementation in SAS

In this section, we discuss the implementation of the joint model discussed in Section 17.4 in SAS using data from the motor cortex with a pair of MRI-AK (biomarker) and GFAP percentage of area stained (true endpoint).
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Individual level surrogacy in 23 regions of the brain. ($\hat{\rho}_r$ and $\hat{\rho}_w$, respectively, for each MRI parameter and histology percentage of area stained). White fill: surrogacy measure not computed.
17.7.1 Data Structure

The joint model, discussed in Section 17.4, was fitted using procedure MIXED in SAS 9.4. For each subject, measurements for both MRI and histology were available. Hence, data for MRI and histology parameters for a single subject appeared in subsequent rows. A partial print of the data is given below:

```
proc print data=MriHistData;
run
```

```
animalid  age  genotype  response   endpoint
1         2     TRANSGENIC 0.001128658 MRI
1         2     TRANSGENIC 0.126652588 Histology
2         2     WILDTYPE  0.189814745 Histology
2         2     WILDTYPE  0.001124777 MRI
```

17.7.2 Common Parameter for Histology in the Wildtype Group

As mentioned in Section 17.4, from a biological point of view, it is assumed that histology values of wildtype mice should remain constant between the age of 2-10 months, since there is no significant disease pathology (due to ageing) progression. Hence, in the model for histology, wildtype mice have a single parameter which does not change with age. In SAS, this can be achieved by defining a common parameter `CommonInt` for histology in wildtype using the following code:

```
data MriHistData;
set MriHistData;
commonInt=age;
if treatment='WILDTYPE' and endpoint='Histology'
   then commonInt=0;
run;
```

The association between MRI and histology is modeled using the REPEATED statement. The option GROUP=genotype allows for genotype-specific covariance matrices (17.3). The estimated disease effects on both MRI and histology are output by passing the solution option and stored in a dataset named fixedeffects using the SOLUTIONF option in the ODS OUTPUT command. The complete SAS code used to fit the joint model is:

```
proc mixed data=surrogate;
```
FIGURE 17.17
SAS fixed effects parameter estimates.

class genotype animalid endpoint commonint(ref='0');
model response=commonint*endpoint commonint*endpoint*genotype / solution noint;
repeated endpoint / subject=animalid*commonint
type=un group=genotype R=1,4 rcorr=1,4;
ods output solutionf=fixedeffects;
run;

Parameter estimates for the disease progression effects (in both endpoints) are shown in Figure 17.17. The individual-level surrogacy for wildtype and transgenic mice is computed using the estimated covariance matrices (Fig-
To compute disease-level surrogacy, a linear regression model is fitted using PROC GLM in SAS.

```sas
proc glm data=fixedeffectsProcessed;
model effectstrue=effectssurrogate;
run;
```

In the above code, `effectstrue` corresponds to the estimated disease effects on the true endpoint $\hat{\beta}$, while `effectssurrogate` corresponds to the estimated disease effects on the surrogate endpoint $\hat{\alpha}$. The reported measure for disease-level surrogacy corresponds to $R^2$ obtained from the linear regression model (Figure 17.19).

**FIGURE 17.18**
SAS covariance matrix estimates for individual-level surrogacy.
17.7.3 Age-specific Parameters for Histology in the Wild-type Model

Rather than assume a common histology parameter in wildtype mice, an age-specific parameter can be specified by substituting `commonint` with `age`. In order to obtain age and genotype-specific estimates for the disease-effect on both MRI and histology, we include in the model an interaction term `age*endpoint*genotype`.

```
proc mixed data=MriHistData;
  class genotype animalid endpoint age;
  model response=age*endpoint age*endpoint*genotype
    / solution noint;
  repeated endpoint / subject=animalid*age type=un
    group=genotype R=1,4 rcorr=1,4;
  ods output solutionf=fixedeffects;
run;
```

17.8 Implementation in R

The proposed model can easily be implemented in R using the `gls` function from the `nlme` package.
17.8.1 Common Parameter for Histology in the Wildtype Group

We adopt a dummy coding for the variables of interest as shown in the partial print of the `MriHistData.dummy` data object (Figure 17.20).
Evaluation of MRI as a Biomarker in Alzheimer’s Disease

> head(MriHistData.dummy, 10)

# Partial print of the MriHistData.dummy data object.

FIGURE 17.20
Partial print of the MriHistData.dummy data object.
The model can be fitted using the following R code:

```r
library(nlme)
fit <- gls(response~-1+endpoint+mu_SURRO_wt4+mu_SURRO_wt6+
mu_SURRO_wt8+mu_SURRO_wt10 +beta_TRANS_TRUE_2
+alpha_TRANS_TRUE_4+alpha_TRANS_TRUE_6+alpha_TRANS_TRUE_8
+alpha_TRANS_TRUE_10+beta_TRANS_SURRO_2
+beta_TRANS_SURRO_4 +beta_TRANS_SURRO_6
+beta_TRANS_SURRO_8 +beta_TRANS_SURRO_10,
data=MriHistData.dummy,
correlation=corSymm(form = ~ 1| animalid ),
weight=varIdent(form=~1|endpoint*genotype))
```

By specifying `endpoint` in the right hand side of the `formula`, we allow for a common parameter estimate for histology (true endpoint) in wildtype as well as a parameter for MRI (surrogate endpoint) at 2 months for wildtype mice. The variables `alpha_TRANS_TRUE_2` - `alpha_TRANS_TRUE_10` correspond to $\beta_1$ - $\beta_5$ while `beta_TRANS_SURRO_2` - `beta_TRANS_SURRO_10` correspond to $\alpha_1$ - $\alpha_5$ in (17.1).

The argument `correlation=`... allows for the specification of correlated outcomes within a subject. Further, we specify an unstructured correlation using the `corSym` construct. In order to define heterogeneous variances, that is, endpoint and genotype-specific variance covariance matrices as defined in (17.3), the argument `weight=varIdent(...)` is used. The output for the disease progression parameters is shown in Figure 17.21.

The estimated correlation estimates for disease-level surrogacy (17.4) are shown in the panel below.

```r
Transgenic.covmat <- getVarCov(fit, individual=1)
cov2cor(Transgenic.covmat )
Marginal variance covariance matrix
[,1] [,2]
[1,] 1.00000 0.13295
[2,] 0.13295 1.00000

Wildtype.covmat <- getVarCov(fit, individual=4)
cov2cor(Wildtype.covmat)
Marginal variance covariance matrix
[,1] [,2]
[1,] 1.00000 0.13355
[2,] 0.13355 1.00000
```

To obtain the disease-level surrogacy, a linear regression is fitted to the the disease progression effects and the model $R^2$ obtained.
**Evaluation of MRI as a Biomarker in Alzheimer’s Disease**

> `round(summary(fit)$tTable,3)`

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>Std.Error</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>endpointtrue</td>
<td>0.730</td>
<td>0.380</td>
<td>1.923</td>
<td>0.058</td>
</tr>
<tr>
<td>endpointsurrogate</td>
<td>0.695</td>
<td>0.022</td>
<td>31.281</td>
<td>0.000</td>
</tr>
<tr>
<td>mu_SURRO_wt4</td>
<td>-0.001</td>
<td>0.031</td>
<td>-0.036</td>
<td>0.971</td>
</tr>
<tr>
<td>mu_SURRO_wt6</td>
<td>0.002</td>
<td>0.031</td>
<td>0.076</td>
<td>0.939</td>
</tr>
<tr>
<td>mu_SURRO_wt8</td>
<td>0.006</td>
<td>0.031</td>
<td>0.202</td>
<td>0.840</td>
</tr>
<tr>
<td>mu_SURRO_wt10</td>
<td>0.009</td>
<td>0.031</td>
<td>0.286</td>
<td>0.775</td>
</tr>
<tr>
<td>alpha_TRANS_TRUE_2</td>
<td>-0.381</td>
<td>0.632</td>
<td>-0.604</td>
<td>0.548</td>
</tr>
<tr>
<td>alpha_TRANS_TRUE_4</td>
<td>1.545</td>
<td>0.632</td>
<td>2.444</td>
<td>0.017</td>
</tr>
<tr>
<td>alpha_TRANS_TRUE_6</td>
<td>5.390</td>
<td>0.654</td>
<td>8.237</td>
<td>0.000</td>
</tr>
<tr>
<td>alpha_TRANS_TRUE_8</td>
<td>11.793</td>
<td>0.654</td>
<td>18.022</td>
<td>0.000</td>
</tr>
<tr>
<td>alpha_TRANS_TRUE_10</td>
<td>15.390</td>
<td>0.997</td>
<td>15.436</td>
<td>0.000</td>
</tr>
<tr>
<td>beta_TRANS_SURRO_2</td>
<td>-0.010</td>
<td>0.024</td>
<td>-0.400</td>
<td>0.690</td>
</tr>
<tr>
<td>beta_TRANS_SURRO_4</td>
<td>0.024</td>
<td>0.024</td>
<td>0.982</td>
<td>0.329</td>
</tr>
<tr>
<td>beta_TRANS_SURRO_6</td>
<td>0.030</td>
<td>0.024</td>
<td>1.250</td>
<td>0.215</td>
</tr>
<tr>
<td>beta_TRANS_SURRO_8</td>
<td>0.050</td>
<td>0.024</td>
<td>2.057</td>
<td>0.043</td>
</tr>
<tr>
<td>beta_TRANS_SURRO_10</td>
<td>0.080</td>
<td>0.028</td>
<td>2.877</td>
<td>0.005</td>
</tr>
</tbody>
</table>

**FIGURE 17.21**

*R gls output for the surrogacy model.*

```r
summary(lm(alpha~beta, data=fixedEffectsProcessed))$r.squared

[1] 0.9101327
```

**17.8.2 Age-specific Parameters for Histology in the Wildtype Group**

A partial print of the dataset for the model with age-specific parameters for histology in the wildtype group is shown below.

> `head(MriHistData)`

<table>
<thead>
<tr>
<th>animalid</th>
<th>age</th>
<th>genotype</th>
<th>endpoint</th>
<th>response</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>1</td>
<td>10 TRANSGENIC</td>
<td>true</td>
<td>17.4715576</td>
</tr>
<tr>
<td>81</td>
<td>1</td>
<td>10 TRANSGENIC</td>
<td>surrogate</td>
<td>0.8114934</td>
</tr>
<tr>
<td>31</td>
<td>2</td>
<td>10 TRANSGENIC</td>
<td>true</td>
<td>16.5277908</td>
</tr>
<tr>
<td>82</td>
<td>2</td>
<td>10 TRANSGENIC</td>
<td>surrogate</td>
<td>0.7101217</td>
</tr>
<tr>
<td>32</td>
<td>3</td>
<td>10 TRANSGENIC</td>
<td>true</td>
<td>14.3902328</td>
</tr>
<tr>
<td>83</td>
<td>3</td>
<td>10 TRANSGENIC</td>
<td>surrogate</td>
<td>0.8296881</td>
</tr>
</tbody>
</table>

The model is fitted using `age` and `endpoint` as factor variables. The interaction term `endpoint:age` denotes the age-specific average readout for
> round(summary(fit)$tTable,3)

<table>
<thead>
<tr>
<th>Term</th>
<th>Value</th>
<th>Std.Error</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>endpointtrue:month2</td>
<td>0.143</td>
<td>0.882</td>
<td>0.162</td>
<td>0.872</td>
</tr>
<tr>
<td>endpointsurrogate:month2</td>
<td>0.693</td>
<td>0.022</td>
<td>30.955</td>
<td>0.000</td>
</tr>
<tr>
<td>endpointtrue:month4</td>
<td>0.111</td>
<td>0.882</td>
<td>0.126</td>
<td>0.900</td>
</tr>
<tr>
<td>endpointsurrogate:month4</td>
<td>0.691</td>
<td>0.022</td>
<td>30.900</td>
<td>0.000</td>
</tr>
<tr>
<td>endpointtrue:month6</td>
<td>1.776</td>
<td>0.882</td>
<td>2.015</td>
<td>0.047</td>
</tr>
<tr>
<td>endpointsurrogate:month6</td>
<td>0.701</td>
<td>0.022</td>
<td>31.318</td>
<td>0.000</td>
</tr>
<tr>
<td>endpointtrue:month8</td>
<td>0.190</td>
<td>0.882</td>
<td>0.216</td>
<td>0.829</td>
</tr>
<tr>
<td>endpointsurrogate:month8</td>
<td>0.699</td>
<td>0.022</td>
<td>31.266</td>
<td>0.000</td>
</tr>
<tr>
<td>endpointtrue:month10</td>
<td>1.432</td>
<td>0.882</td>
<td>1.624</td>
<td>0.108</td>
</tr>
<tr>
<td>endpointsurrogate:month10</td>
<td>0.706</td>
<td>0.022</td>
<td>31.558</td>
<td>0.000</td>
</tr>
<tr>
<td>endpointtrue:month2:genotypeTRANSGENIC</td>
<td>0.206</td>
<td>1.016</td>
<td>0.203</td>
<td>0.840</td>
</tr>
<tr>
<td>endpointsurrogate:month2:genotypeTRANSGENIC</td>
<td>-0.008</td>
<td>0.024</td>
<td>-0.313</td>
<td>0.755</td>
</tr>
<tr>
<td>endpointtrue:month4:genotypeTRANSGENIC</td>
<td>2.164</td>
<td>1.016</td>
<td>2.130</td>
<td>0.036</td>
</tr>
<tr>
<td>endpointsurrogate:month4:genotypeTRANSGENIC</td>
<td>0.026</td>
<td>0.024</td>
<td>1.065</td>
<td>0.290</td>
</tr>
<tr>
<td>endpointtrue:month6:genotypeTRANSGENIC</td>
<td>4.347</td>
<td>1.030</td>
<td>4.222</td>
<td>0.000</td>
</tr>
<tr>
<td>endpointsurrogate:month6:genotypeTRANSGENIC</td>
<td>0.027</td>
<td>0.024</td>
<td>1.092</td>
<td>0.278</td>
</tr>
<tr>
<td>endpointtrue:month8:genotypeTRANSGENIC</td>
<td>12.340</td>
<td>1.030</td>
<td>11.984</td>
<td>0.000</td>
</tr>
<tr>
<td>endpointsurrogate:month8:genotypeTRANSGENIC</td>
<td>0.052</td>
<td>0.024</td>
<td>1.222</td>
<td>0.037</td>
</tr>
<tr>
<td>endpointtrue:month10:genotypeTRANSGENIC</td>
<td>14.698</td>
<td>1.275</td>
<td>11.527</td>
<td>0.000</td>
</tr>
<tr>
<td>endpointsurrogate:month10:genotypeTRANSGENIC</td>
<td>0.078</td>
<td>0.028</td>
<td>2.776</td>
<td>0.007</td>
</tr>
</tbody>
</table>

FIGURE 17.22
R gls output for the age-specific surrogacy model.

MRI and histology in wildtype mice, while the three-way interaction term endpoint:genotype:age denotes the age-specific disease effect (transgenic compared to wildtype mice).

fit2 <- gls(response~endpoint:age+endpoint:genotype:age -1,
             data=MriHistData,
correlation=corSymm(form = ~ 1|animalid),
weight=varIdent(form=~1|endpoint*genotype))

Parameter estimates are shown in Figure 17.22. Note that in this case, the coefficients for histology in wildtype are more imprecise (since at each age, only two observations are available). The estimation of disease-level surrogacy follows as in the previous case of the model with a common histology parameter in wildtype mice.
17.9 Concluding Remarks

The joint model specified in Section 17.4 was developed in order to model the association between MRI and histology, taking into account the disease progression effects on both endpoints. The observation unit that we have used in this chapter is the triplet \((\text{Genotype}_j, \text{MRI}_{ij}, \text{Histology}_{ij})\). Figure 17.23 illustrates the two sources of association presented in this chapter. For a given age, the effect of the disease on MRI \(\alpha_i\) and the effect of the disease on histology \(\beta_i\) is represented by the shift in the distribution of both MRI and histology parameters as illustrated in panel b. Panel a illustrates the genotype-specific association in the residuals after adjusting for the disease effects \(\alpha_i\) and \(\beta_i\).

We have shown that, using a two-stage approach, we can estimate a
genotype-specific adjusted association $\hat{\rho}_W$ and $\hat{\rho}_A$ using the joint model (17.1) in the first stage, while the prediction of the disease progression effects on histology can be done in the second stage using linear regression model for $\hat{\beta}_i$ and $\hat{\alpha}_i$. Although, the experimental setting discussed in this chapter is completely different from the one discussed in Chapter 4, the same association structure (as illustrate in Figure 17.23(a)) implies that the same modelling approach can be used in order to evaluate the quality of MRI as a biomarker for histology. We have shown that the use of MRI as a biomarker for histology depends on the brain region, MRI parameters and histology staining.

The case studies presented in this chapter posed two challenges with regards to sample size: (1) there were only five age groups, which implies that, estimation of the linear regression line in the second stage is based on only five observations and (2) there were only two control mice at each age group. Therefore, the genotype-specific coefficients $\mu_{Y_i}$ in (17.2) are based on two observations, hence they may have higher variability.