FACULTY OF SCIENCES
Master of Statistics: Biostatistics

Masterproef
Longitudinal analysis of differences in white and grey matter brain regions of APP/PS1 and wild type mice, using diffusion kurtosis imaging and diffusion tensor imaging

Promotor :
Prof. dr. Ziv SHKEDY

Promotor :
Dr. LUC BIJNENS
Dr. TOM JACOBS

Leacky Muchene
Master Thesis nominated to obtain the degree of Master of Statistics , specialization Biostatistics

universiteit hasselt
▶▶ **KNOWLEDGE IN ACTION**

Maastricht University

Maastricht University

universiteit hasselt
▶▶ **KNOWLEDGE IN ACTION**

FACULTY OF SCIENCES
Master of Statistics: Biostatistics

Masterproef
Longitudinal analysis of differences in white and grey matter brain regions of APP/PS1 and wild type mice, using diffusion kurtosis imaging and diffusion tensor imaging

Promotor :
Prof. dr. Ziv SHKEDY

Promotor :
Dr. LUC BIJNENS
Dr. TOM JACOBS

Leacky Muchene
Master Thesis nominated to obtain the degree of Master of Statistics , specialization Biostatistics

Maastricht University

universiteit
hasselt
KNOWLEDGE IN ACTION

To my mum Joyce Njeri and dad George Kamau

Acknowledgement

While I have sorely pieced together the information in this report, I cant fail to acknowlegde the collaborative effort of people who worked in the background in making it a success, key among them being;

Throughout the ups and downs that characterized the journey to this ending, the Almighty God guided me, gave me strength to overcome all and gave me a chance to get it right where I erred. Praise be unto Him.

Leacky Muchene

Diepenbeek, September 9, 2012

Table of Contents

List of figures and tables

List of abbreviations

Abstract

The goal of this study was to quantify the longitudinal differences in the brain scans between APP/PS1 and wild type mice strains. Magnetic resonance images (MRI) were obtained using Diffusion Kurtosis Imaging (DKI) and Diffusion Tensor Imaging (DTI) from 34 mice aged between 2 and 8 months at two months intervals, from 15 regions from both white and grey matter in the brain.

Univariate linear mixed-effects models for each DKI or DTI parameter and region as well as joint random effects models for the bivariate DKI and DTI pairs were considered and different covariance structures explored.

Univariate results indicated that while much of the differences are detected as from four months, DKI was more sensitive to differences in grey matter as compared to DTI. Moreover, for the bivariate models, while the correlation between DKI and DTI is region dependent, it is strongest at four months for all regions and lowest at six months.

Keywords: DKI, DTI, Linear Mixed-effects models, longitudinal data, MRI

1   Introduction

Human beings are distinct from most of the other living organisms due to their advanced mental development, which enable them to not only, learn, but also to achieve a lot more brain-controlled functionality (Higbee & Higbee, 2001). Pathophysiological changes in the human brain occur naturally as we age resulting to reduced mental response/performance. There is an increase in the volume of white matter (WM) and a reduction in grey matter (GM) during adolescence and early adulthood, while as we age, both white and grey matter reduce. These structural changes have been associated with the changes in cognitive ability (Falangola, et al., 2008).

Alzheimer's disease (AD) is one common age-related mental disorder. There has been a lot of interest in AD, probably because of the impact it has on the ageing population. The World Alzheimer's report (2011) documents that as of 2009, 36 million people worldwide were living with dementia, with the numbers projected to double every 20 years to 66 million by 2030. AD is therefore one of the most significant social, health and economic crises of the 21st century requiring urgent and sustainable interventions (Prince, Bryce, & Ferri, 2011).

Although not age dependent, AD is a progressive age-related disorder that develops systematically and is characterised by majorly extracellular amyloid proteins deposits as well as intraneuronal neurofibrillary changes. Progression occurs in stages and the pattern is different in both white and grey matter of the brain. The initial onset of dementia is characterized by memory lapses, disorientation, impaired performance on daily tasks, impaired speech and judgement, changes in moods, behaviour and personality amongst others (Agronin, 2007). The difficulty in early diagnosis of AD is partly due to the fact that these symptoms are associated with non-dementia diagnoses as well.

Some of the benefits associated with early diagnosis of AD include; provides a basis for evidence-based treatment, care and support as the disease progresses, enables the patients to

plan ahead when they still have the mental capacity to make important decisions about their future care, early therapeutic intervention can be effective in improving cognitive function, delaying institutionalisation amongst other benefits (Prince, Bryce, & Ferri, 2011).

Due to the predictable nature of spread of amyloid deposits and intraneuronal neurofibrillary changes, the analysis of age related staging of AD is possible as was demonstrated by Braak and Braak (1996). Initially, amyloid protein deposits occur in the less myelinated areas of the basal cortex then spreads to adjoining areas and eventually to the hippocampus. On the other hand, intraneuronal lesions develop initially in the transentorhinal region, spreading in a predictable manner. While either amyloid protein deposits or intraneuronal changes are expected at specific stages of the disease, some individuals exhibit these changes earlier. It could therefore be of interest in making early diagnosis for AD (Braak & Braak, 1997).

Transgenic models of AD are popular in studying the disease since most of the human disease features have been shown to be represented in the mice. Although the mice model falls short of showing the Tau pathology and extensive cell loss, they are excellent for amyloidosis. AD is associated with genetic mutation including the amyloid precursor protein (APP) gene and the presenilins (PS) amongst others (Duff & Suleman, 2004). APP/PS1 mice are double transgenic and have excellent prerequisites for imaging Aβ-amyloid proteins even at a young age (Kießling, 2011).

Magnetic resonance imaging (MRI) is a technique commonly used to study the changes in brain cells structure. In particular magnetic resonance (MR) diffusion tensor imaging (DTI) has been shown to provide unique structural information in characterizing tissue microstructure non-invasively. This has made DTI a popular technique in the diagnosis of brain related complications. DTI relies on measuring the water diffusivity in cells, under the assumption that water diffusivity follows a Gaussian distribution. However, DTI is sensitive to tissue anisotropy and has therefore largely been restricted to use in white matter. Diffusion-

weighted magnetic resonance imaging provides considerably more information unlike the standard DTI metrics: mean diffusivity (MD) and fractional anisotropy (FA) (Falangola, et al., 2008).

Deviations from the Gaussian diffusivity can be attributed to presence of diffusion barriers in cells such as cell membranes and organelles and both intracellular and extracellular water compartments (Falangola, et al., 2008). Diffusion kurtosis imaging (DKI) is a dimensionless measure that has been proposed to capture the deviation of water diffusion profiles from the Gaussian distribution. DKI has been shown to provide better characterisation of changes in the neural tissue structures, with the extra advantage of ease of implementation ((Cheung, et al., 2009); (Falangola, et al., 2008); (Hui, Cheung, Qi, & Wu, 2008)).

DTI is characterised by a matrix of nine values each corresponding to a gradient orientation. In DTI, only six of these tensor values are measured plus the non-diffusion weighted   base value. Diffusivity is then described by three Eigen values describing the axes of an ellipsoid (for anisotropic diffusion). The Eigen values for DTI matrix define the axial diffusivity (AD), radial diffusivity (RD) and MD. The 4th order kurtosis tensor (KT) is fully symmetric with 15 independent components. Orthogonal transformation of KT is used to compute the diffusion kurtosis along the three Eigen vectors directions of the 2nd order DT. The corresponding DKI measures mean kurtosis (MK), axial kurtosis (AK) and radial kurtosis (RK) have been compared with the DTI parameters -for sensitivity in detecting developmental and pathological changes in neural tissues (Hui, Cheung, Qi, & Wu, 2008).

The goal of this study is to quantify the longitudinal differences between two mice strains using DTI and DKI. Focus will be on the mean response as well as the variability of this mean response for eight DKI and six DTI parameters measured at four timepoints in 15 different brain regions of interest (in both white and grey matter). The correlation between DTI and DKI outcomes over time will be explored as well.

2   Data description

The sample comprised of 34 mice of varying ages between two and eight months and of

either the APP/PS1 or wild type (WT) genotype. APP/PS1 mice comprised the experimental

arm, while the control arm comprised of WT mice.

Magnetic resonance imaging of 15 predefined regions in the brain was done for eight DKI

parameters and six DTI parameters. Measurements were scheduled to be done on four

equally spaced time points, corresponding to when the mice were 2, 4, 6 or 8 months old. As

is the case in most longitudinal settings, missingness was reported with only 5 mice having

measurements at all scheduled timepoints. The reason for missingness was due to

unavailability of the mice at the time of measurement. Table 1 provides a summary of the

variables. A comprehensive list of the coding for the parameters and regions of interest is

presented in Appendix 1.

Table 1: Summary of variables in the dataset

variable name	Description
Animalnr_unique	A unique identifier for the mice
Genotype	Mice genotype: APP/PS1 (Experimental arm), WT (Wild type-Control)
Mean_ROI	Mean response for parameter-ROI combination
Parameter	Response indicator for either DKI(8 parameters) or DTI(6 parameters)
ROI	15 regions (on white and grey matter) on which measurements are based
Timepoint	Age at which measurement was taken (2 months,4 months,6 months,8 months)
SD_ROI	Standard deviation of Mean_ROI

2.1   Statistical software

Statistical analysis will be performed using SAS version 9.2 and R version 2.12. All statistical

tests will be performed at 5% significance level, and where applicable, 95% confidence

intervals will be computed.

3 Scope of the study

The main objective of this study will be to quantify the longitudinal differences in the mean response between two mice strains using DTI and DKI. Furthermore, the variability of the mean response will be explored for differences in the two mice strains using DTI and DKI. The correlation between pairs of DTI and DKI variables will be explored through joint modelling of the multivariate longitudinal data.

Specific objectives

In order to meet the overall objective outlined above, the problem will be broken down into specific subsections namely;

i. Univariate analysis of the longitudinal effect of DTI parameters by region for both mean and variance.

ii. Univariate analysis of the longitudinal effect of DKI parameters by region for both mean and variance.

iii. Exploration of the correlation between DTI and DKI pairs of variables through joint modelling of the multivariate longitudinal responses by region.

Chapter 4 will discuss the statistical methodology to be applied, while the results of the analysis will be presented in chapter 5. Chapter 6 is dedicated to discussing some computational challenges while chapter 7 will provide a brief discussion of the foregoing results and a conclusion

4 Statistical methodology

4.1 Exploratory data analysis

As a starting point, exploration of the obtained data will be performed majorly using graphical tools for visualizing the trends, distributional properties, variability amongst others. Summary statistics will be tabulated where appropriate and discussed.

4.2 Univariate longitudinal data analysis

Classical statistical methods such as linear regression analysis and analysis of variance (ANOVA) amongst others presume independence of the responses for a particular subject (Kutner, Nachtsheim, Neter, & Li, 2005). There are instances however; that this assumption is violated particularly due to the way the data is collected. Whenever more than one measurement per subject is collected, the responses from the same subject are no longer independent. There is need for specialized methods for handling such data. Correlated data may either be multivariate data-measurements from different response variables on the same subject, repeated measures- measurements from the left and right ear for each subject, clustered measurements-observations from litters of each mother, longitudinal data-measurements taken on the same subject at different timepoints, or spatial data-measurements taken from different districts in a province (Verbeke & Molenberghs, 2000).

Data for a particular DKI or DTI parameter and region of interest was obtained at four timepoints (equally spaced) hence longitudinal. Like in most longitudinal settings, there was incompleteness in the responses both intermittent and dropout missingness. In their work of 1987, Little and Rubin discussed various missingness mechanisms and ways for handling it. Moreover, Verbeke and Molenberghs (2000) have presented a comprehensive discussion on validity of inferences under ignorability, as well as possible ways of mitigating missingness. For this study, direct likelihood approach will be applied; consequently the missingness mechanism is assumed to be missing at random (MAR) - indicating that the distribution of the missing data is independent of unobserved data (Verbeke & Molenberghs, 2000).

The general linear mixed effects model while assuming the response vector has a Gaussian distribution, allows for the correct handling of the multivariate responses characteristic of longitudinal data. A linear mixed-effects model (LMM) is defined as;

$$\begin{cases} \mathbf{Y}_i = X_i \beta + Z_i b_i + \varepsilon_i \\ \mathbf{b}_i \sim N(\mathbf{0}, D) \\ \varepsilon_i \sim N(0, \Sigma_i) \\ \mathbf{b}_1 ...... \mathbf{b}_N, \varepsilon_1 .... \varepsilon_N \text{ independent} \end{cases} \tag{1}$$

With Y_i the n_i dimensional response vector for subject i, $1 \le i \le N$, N is the number of subjects, X_i and Z_i are the $(n_i xp)$ and $(n_i xq)$ dimensional matrices of known covariates, β is a p-dimensional vector of fixed effects, b_i is a q-dimensional vector of random effects, and ε_i is an n_i-dimensional vector of residual components. D and Σ_i are the covariance matrices for the random and fixed effects respectively.

The univariate models to be considered have an unstructured mean (An estimate at each time point). The resulting general multivariate model fitted in this case can be denoted by

$$\mathbf{Y}_i = X_i \beta + \varepsilon_i \tag{2}$$

$$X_i = \begin{cases} 1 \text{ if APP/PS1} \\ 0 \text{ if WT} \end{cases} \text{ is an } n_i x1 \text{ matrix (Since the model has no intercept)}$$

β is a vector of regression coefficients

Y_i is an n_i dimensional vector of responses for each parameter; i=1,2,..8 for DKI and i=1,2...6 for DTI

The covariance structure for the residuals is modelled flexibly using both unstructured and compound symmetry covariance matrix. The two structures can be denoted as follows;

Unstructured covariance
$$\begin{pmatrix} \sigma^2_1 & \sigma_{12} & \sigma_{13} & \sigma_{14} \\ & \sigma^2_2 & \sigma_{23} & \sigma_{24} \\ & & \sigma^2_3 & \sigma_{34} \\ & & & \sigma^2_4 \end{pmatrix}$$
where σ_{ij} is the covariance between the ith and j^{th} measurement

for a given subject

Compound
symmetry

$$\begin{pmatrix} \sigma^2_i+\sigma^2 & \sigma^2_i & \sigma^2_i & \sigma^2_i \\ & \sigma^2_i+\sigma^2 & \sigma^2_1 & \sigma^2_i \\ & & \sigma^2_i+\sigma^2 & \sigma^2_i \\ & & & \sigma^2_i+\sigma^2 \end{pmatrix}$$ σ^2_i is a subject specific variance component,

while σ^2 is the residual

The model is implemented in *SAS Proc Mixed* with the variance estimation using restricted maximum likelihood (REML). Verbeke and Molenberghs (2000) have extensively discussed the theoretical basis of REML as applied to general linear mixed-effects models. In brief, REML estimation involves breaking down the estimation problem into estimation of fixed effects parameters and variance components parameters simultaneously. This can be achieved by maximizing the REML likelihood function with respect to all parameters simultaneously (Verbeke & Molenberghs, 2000).

Due to the high number of univariate models to be fitted (15*14=210 models), a *SAS* macro titled "*Modelfit*" will be developed and the resulting output saved in a data file for further processing graphically or otherwise. Key findings from this section will be highlighted.

4.2.1   Correction for multiple testing

Estimation of differences between the two genotypes at each time point will be performed, resulting into four comparisons per parameter-region combination. There is need to correct for multiple testing, to guarantee the validity of the resulting inferences (Kutner, Nachtsheim, Neter, & Li, 2005). Correction for multiple comparisons will be achieved using a procedure *False Discovery Rate* (FDR) proposed by Benjamin and Hochberg (1995).

While conventional familywise procedures are considered more conservative that FDR especially for large families of comparisons, FDR is more powerful since it controls the expected proportion of falsely rejected hypothesis under the assumption of the tests being independent. There is a version however, for positively correlated tests (Verhoeven, Simonsen , & McIntyre, 2005).

In correcting for multiple testing, various approaches could be adopted including, correcting for tests within a parameter-region combination (Which was performed in this analysis), correcting for all parameters simultaneously or correcting for all regions simultaneously.

4.3   Joint modelling of multivariate longitudinal data

While classical longitudinal data analysis as described in section 4.2 has been popular, there are instances when joint modelling of multivariate longitudinal data is necessary. Multivariate data poses additional modelling challenges in capturing the correlation between the responses. Methodology for joint modelling of longitudinal data as well as statistical computing capability has expanded tremendously. Possible approaches to simultaneously analyze multivariate outcomes include conditional models, shared-parameter models, methods based on dimension reduction and random effects models amongst others (Rizopoulos, Verbeke, & Molenberghs, 2010). Standard statistical procedures such as SAS Proc Mixed, Proc NLMIXED and Proc GLIMMIX can be used to implement multivariate longitudinal mixed models, with slight modification to the syntax

One popular approach to joint modelling is using random effects. This is a flexible approach that can handle multiple outcomes of varying nature by using Generalized Mixed Models while assuming that conditional on the random effects, the different outcomes are independent. The vector of all random effects is typically given a joint multivariate normal distribution and the correlation between the vectors of random effects, depicts the correlation between the different sets of outcomes (Faes, et al., 2008). Some of the benefits of this approach are its ability to handle unequally spaced (Unbalanced) data and to combine linear, non-linear general and generalized mixed models in joint modelling.

As the number of response vectors increase, so does the complexity in estimating the joint distribution, due to the high number of parameters to estimate. Fieuws and Verbeke (2006), proposed a Pseudo-likelihood based pairwise random effects model for multivariate continuous longitudinal data whose pitfalls they later highlighted (Fieuws & Verbeke, 2004).

Extensions of the pairwise fitting approach to non-Gaussian and the mix between the two were demonstrated by Faes, et al. (2008). For this report, the distribution of the response is Gaussian, which makes it possible to use classical SAS Proc Mixed with only slight modifications on the presentation of the data and syntax. Moreover, for the problem at hand, we shall consider response pairs of DKI and DTI parameters (Bivariate models).

Extending equation 2 for each parameter by adding a random effect parameter, the resulting joint linear mixed models for DTI and DKI pairs can be denoted as follows:

$$
\begin{cases}
\mathbf{Y}_{i1} = X_{i1}\beta + a_i + \varepsilon_{i1} \quad ..... \text{ For DKI parameter} \\
\mathbf{Y}_{i2} = X_{i2}\beta + b_i + \varepsilon_{i2} \quad ..... \text{ For DTI parameter} \\
\mathbf{b}_i = \begin{pmatrix} a_i \\ b_i \end{pmatrix} \sim MVN\left[\begin{pmatrix} \mathbf{0} \\ \mathbf{0} \end{pmatrix}, D = \begin{pmatrix} \sigma^2{}_a & \sigma_{ab} \\ \sigma_{ab} & \sigma^2{}_b \end{pmatrix} \right] \\
\varepsilon_{ij} \sim N(0, \Sigma_{ij}) \\
\varepsilon_{1j}....\varepsilon_{Nj} \text{ independent}
\end{cases}
\tag{3}
$$

All the parameters in equation 3 have the same meaning as before. The matrix of random effects in this case however, is multivariate normal with the correlation between the random effects $\rho = \dfrac{\sigma_{ab}}{\sigma_a \sigma_b}$. If $\rho = 0$, the two response vectors are independent and $\rho = 1$ simplifies the bivariate model to a shared random effect model. One of the advantages of using the random effects model formulation is that a wide array of covariance structures can be implemented, over and above the fact that different inter-marker and intra-marker correlations can be specified, unlike while using the Kroneker Product covariance structure formulation (Gao , Thompson, Xiong, & Miller, 2006).

The correlation between the random effects will be of interest since its effect on the computation of the variance-covariance matrix of a subject determines the nature and strength of correlation between the two biomarkers. For instance, if the correlation between

the random effects is zero, then the responses in the joint model are independent and the problem reduces to univariate analysis per response.

Two covariance structures- unstructured and compound symmetry were applied and the results compared. An unstructured covariance structure for the bivariate model implies an unstructured covariance within a response and between the two responses as shown below.

	Y1	Y2
Y1	$\begin{array}{ccccc} & 1 & 2 & 3 & 4 \\ 1 & \sigma^2_1 & \sigma_{12} & \sigma_{13} & \sigma_{14} \\ 2 & & \sigma^2_2 & \sigma_{23} & \sigma_{24} \\ 3 & & & \sigma^2_3 & \sigma_{34} \\ 4 & & & & \sigma^2_4 \end{array}$	$\begin{array}{cccc} 1 & 2 & 3 & 4 \\ \sigma^2_1 & \sigma_{12} & \sigma_{13} & \sigma_{14} \\ & \sigma^2_2 & \sigma_{23} & \sigma_{24} \\ & & \sigma^2_3 & \sigma_{34} \\ & & & \sigma^2_4 \end{array}$
Y2		$\begin{array}{cccc} \sigma^2_1 & \sigma_{12} & \sigma_{13} & \sigma_{14} \\ & \sigma^2_2 & \sigma_{23} & \sigma_{24} \\ & & \sigma^2_3 & \sigma_{34} \\ & & & \sigma^2_4 \end{array}$

Note: $\sigma_{ij} \neq \sigma_{ij}$ for $Y_k \neq Y_{k'}$

While unstructured covariance allows for estimation of different values for correlation between the two responses, compound symmetry assumes a constant correlation between the two responses. Under compound symmetry therefore, the resulting variance matrix would

have blocks of the form $\begin{pmatrix} \sigma^2_i+\sigma^2 & \sigma^2_i & \sigma^2_i & \sigma^2_i \\ & \sigma^2_i+\sigma^2 & \sigma^2_1 & \sigma^2_i \\ & & \sigma^2_i+\sigma^2 & \sigma^2_i \\ & & & \sigma^2_i+\sigma^2 \end{pmatrix}$ in the diagonal blocks (Possibly

unique matrices for each response) and a constant correlation matrix $\begin{pmatrix} \sigma^2_k & \sigma^2_k & \sigma^2_k & \sigma^2_k \\ & \sigma^2_k & \sigma^2_k & \sigma^2_k \\ & & \sigma^2_k & \sigma^2_k \\ & & & \sigma^2_k \end{pmatrix}$

between the two responses.

5 Results

5.1 Exploratory data analysis

There was a maximum of 12 mice of each genotype available at each timepoint with the distribution of mice being lowest at 8 months (Table 2). At all timepoints, the sample sizes in the two mice genotypes were relatively well balanced albeit with low sample sizes for 6 and 8 months.

Table 2: Sample size in each genotype per timepoint

Genotype	Time point			
	2 months	4 months	6 months	8 months
WT	12	12	6	5
APP/PS1	10	12	5	7
Total	22	24	11	12

5.1.1 Exploratory results for Mean_ROI

In this section, the Mean_ROI for a particular parameter – ROI will be referred to as the response. Boxplots of the response for each parameter and region combination indicated no significant differences between the genotypes at most timepoints. There was however more variability from the average response value in both genotypes for regions 1-5(majorly white matter) as compared to regions 11-15 (grey matter). Moreover, for most parameters, there was more variability in the WT mice as compared to APP/PS1 mice in each region. Results are presented here for DKI_MD and DTI_MD for the four timepoints (Figure 1). For some regions, there were a few outlying observations.

Figure 1: Boxplots for DKI_MD and DTI_MD at each timepoint
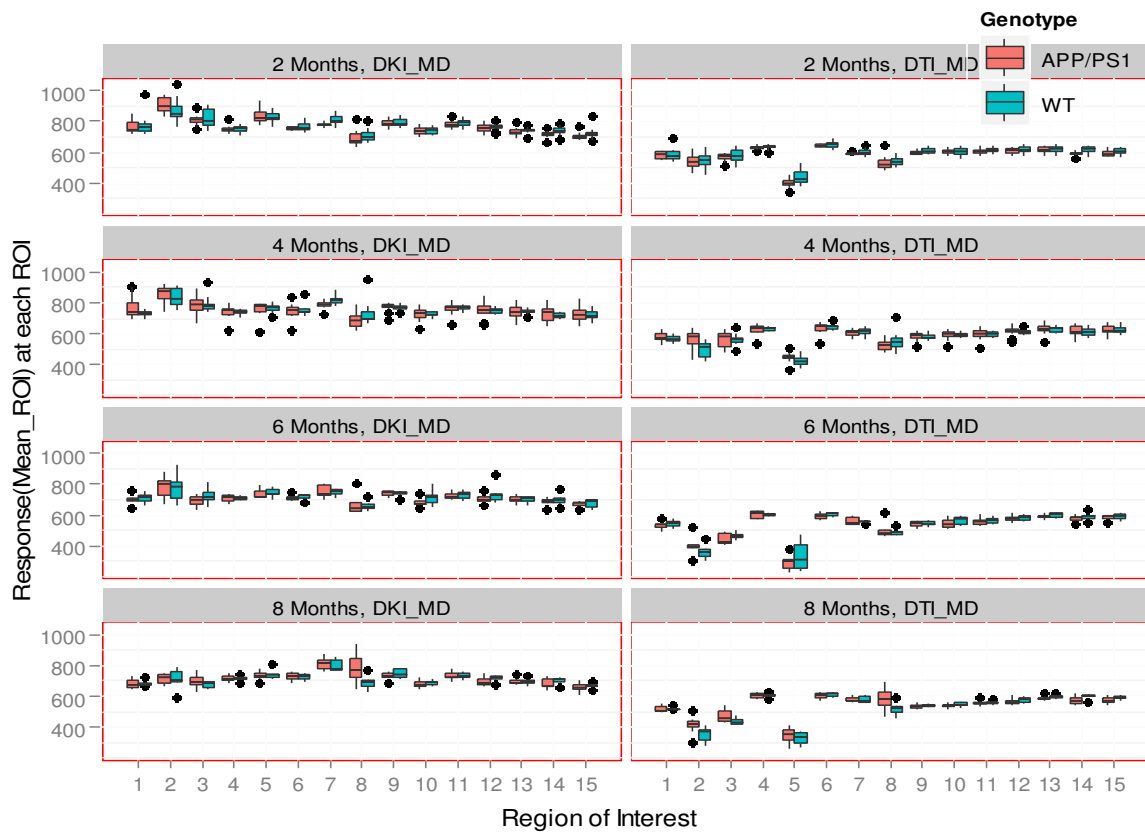
Subject specific as well as average evolution profiles were obtained from which it was

observed that the evolution of the response varied with parameter as well as region of

interest. From the subject specific profiles for DKI_MD and DTI_MD in Figure 2, within a

region, there was no much difference in the evolution profiles between the two genotypes.

Moreover, the evolution profiles for a particular parameter were dependent on the region of

interest with regions 1-5 having a higher decline in response over time.

For most parameter-region of interest combinations, the population averaged evolution

profiles indicated lack of a significant difference in the profiles for APP/PS1 and WT mice. For

the few that exhibited a difference in the profiles, the general trend was that there was clearly

no difference in response for the two genotypes at 2 months and at 8 months, while the

profiles were well separated and changed direction at 4 and 6 months - hence crossing. In

particular, while at 4 months APP/PS1 mice recorded a higher value than WT mice, at 6

months, WT mice reported a higher mean value, resulting in the characteristic crossing of profiles. The population averaged profiles for DKI_MD and DTI_MD are presented in (Figure 3).

Figure 2: Subject Specific Profiles by Region and Genotype for DKI_MD and DTI_MD
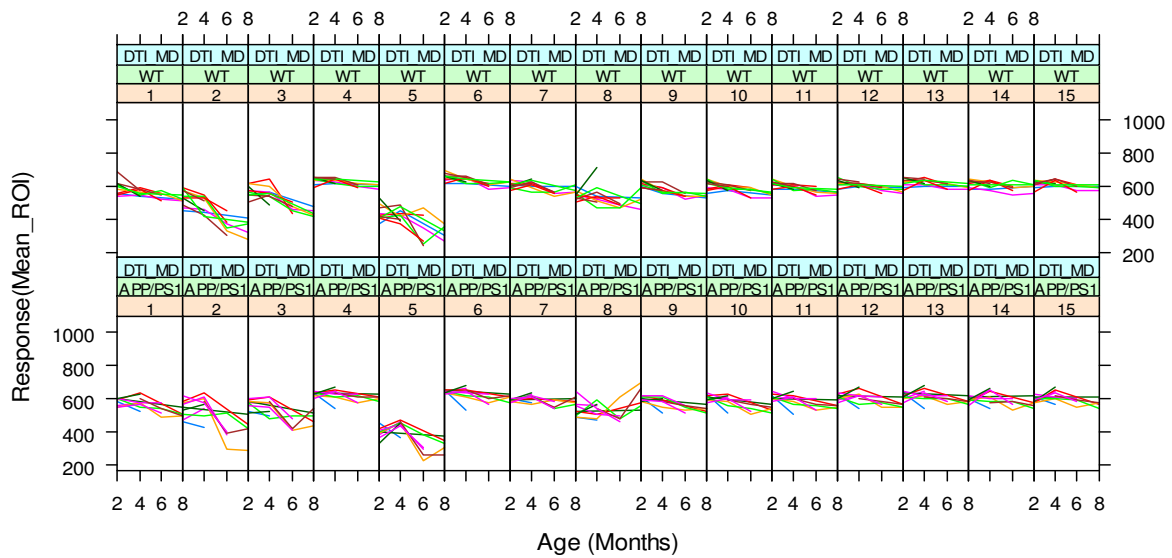
Figure 3: Profiles by Region for DKI_MD and DTI_MD
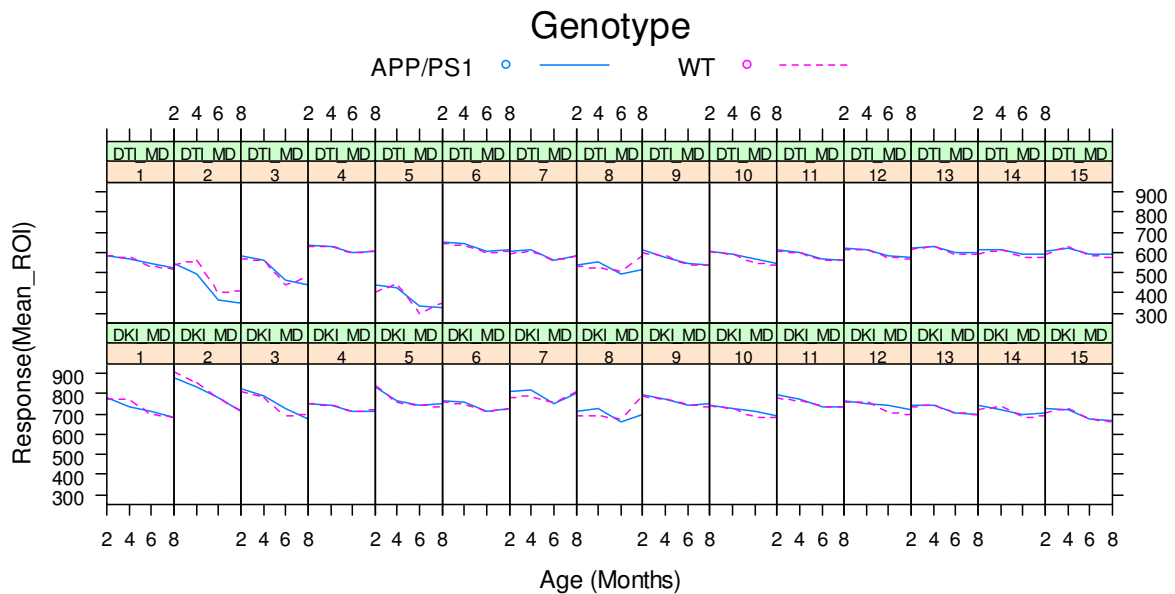
From the results of section 5.1.1, it was clear that while there seems not to be significant differences in the population averaged evolution profiles in most regions of interest, DKI parameters were more sensitive to differences within regions 1-5 as compared to DTI parameters. In particular, DKI_RK, DKI_MK, DKI_AD and DKI_K23 profiles suggested differences in regions 1-5, while only DT_RD and DTI_L3 had signs of differences in the profiles of the two genotypes.

5.1.2   Exploratory results for SD_ROI
Histograms of the standard deviation indicated that the distribution of the standard deviation for a given parameter and region at any time point was right skewed. Logarithmic transformation of the standard deviation was performed, making it more symmetric in line with the Gaussian data assumption for linear mixed-effects modelling performed in this report. The Gaussian assumption on the log transformed standard deviation (Henceforth in this section referred to as the response) was further confirmed with a Kolmogorov-Smirnov test for each parameter at each timepoint.

Boxplots for the response (Log transformed SD) indicated that while the variability of the response was region dependent, there was more variability in the response in regions 1-5, with minimal differences between the genotypes per region (Figure 4). Outliers were also present in some regions, which on further analysis were identified to be mainly from subjects 6, 16 and 27 at 4 months and 6, 20, 25 and 34 at 6 months.

Figure 4: Boxplots at each timepoint for Parameters DKI_MD and DTI_MD
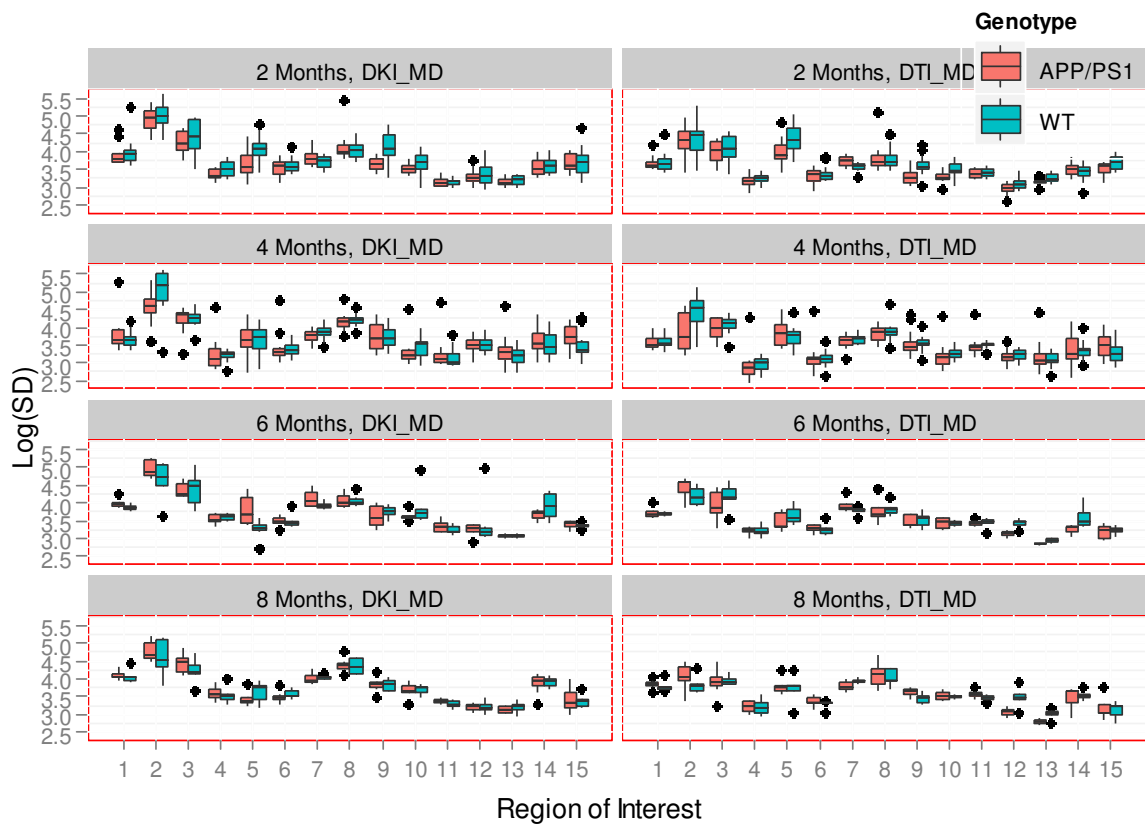
The population averaged profiles for log (SD) indicated that there were minimal differences in evolution between the two genotypes. The trend however varied with parameter and region of interest; with regions 1-5 a lot more variability within the genotypes. From Figure 5, it was evident that although there was minimal variability in APP/PS1 profiles, WT mice exhibited much variability in the profiles over time.

Figure 5: Population averaged Profiles for Log (SD) by Region for DKI_MD and DTI_MD
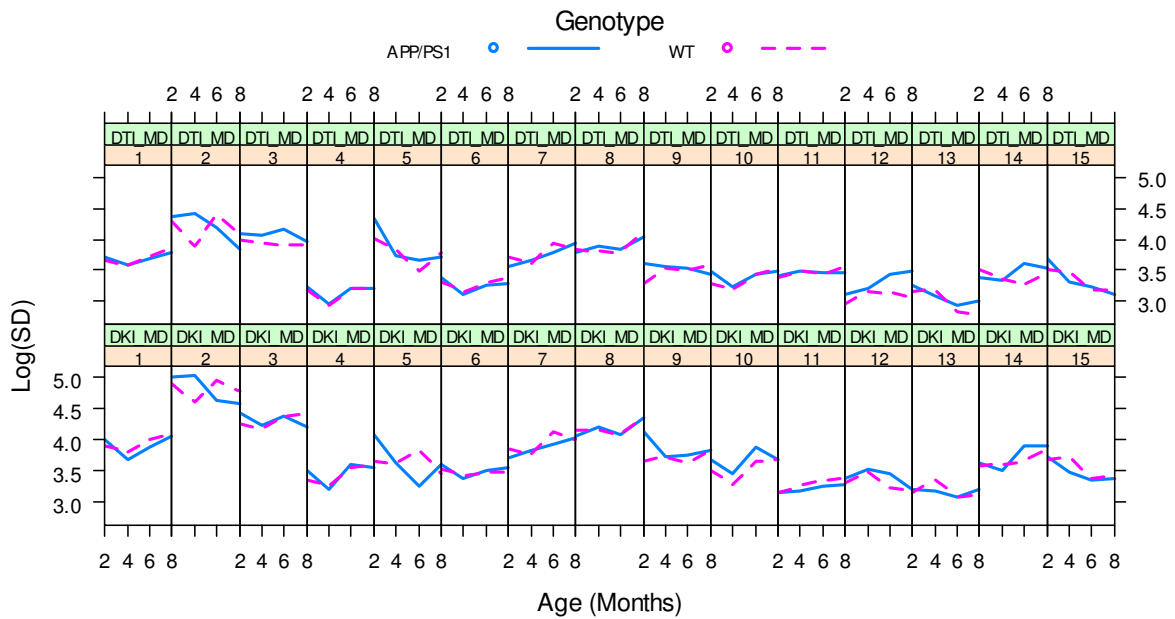
5.2 Univariate longitudinal analysis by parameter and region

Classical models for longitudinal data were fitted for each parameter and region combination.

Since interest was in quantifying the differences in responses across various timepoints, a

model with unstructured mean was fitted with both unstructured and compound symmetry

covariance structures. While unstructured covariance makes no assumptions on the association

between responses of the same subject, compound symmetry imposes a particular structure

on correlated responses, by assuming homogeneity of variance and constant correlations

within a subject. Models were fit for both the mean and the log transformed standard

deviation.

5.2.1 Univariate models for Mean_ROI

5.2.1.1 Unstructured covariance
This model provides an estimate of the mean response at each time point as well as estimates of the difference in mean response between the two genotypes at each time point (By using ESTIMATE statement in SAS Proc Mixed). Confidence intervals for the estimated differences were obtained and the results summarized graphically. Convergence of the models was however a challenge, with only models with parameters DKI_MK, DKI_AD and DTI_FA attaining convergence in all the regions.

Figure 6 presents the estimated difference at each time point with the corresponding confidence intervals for parameters DKI_MK, DKI_AD and DTI_FA in 9 selected regions. For different parameter –region combinations, at each time point, most of the confidence intervals included zero-an indication of no significant difference between the genotypes images. Confidence intervals for grey matter regions such as the cortex were however, narrower as compared to those for white mater regions for all DKI parameters, while for DTI, there was no much difference in the width of the confidence intervals between WM and GM regions.

Figure 6: Estimated differences (With 95% Confidence intervals) per timepoint
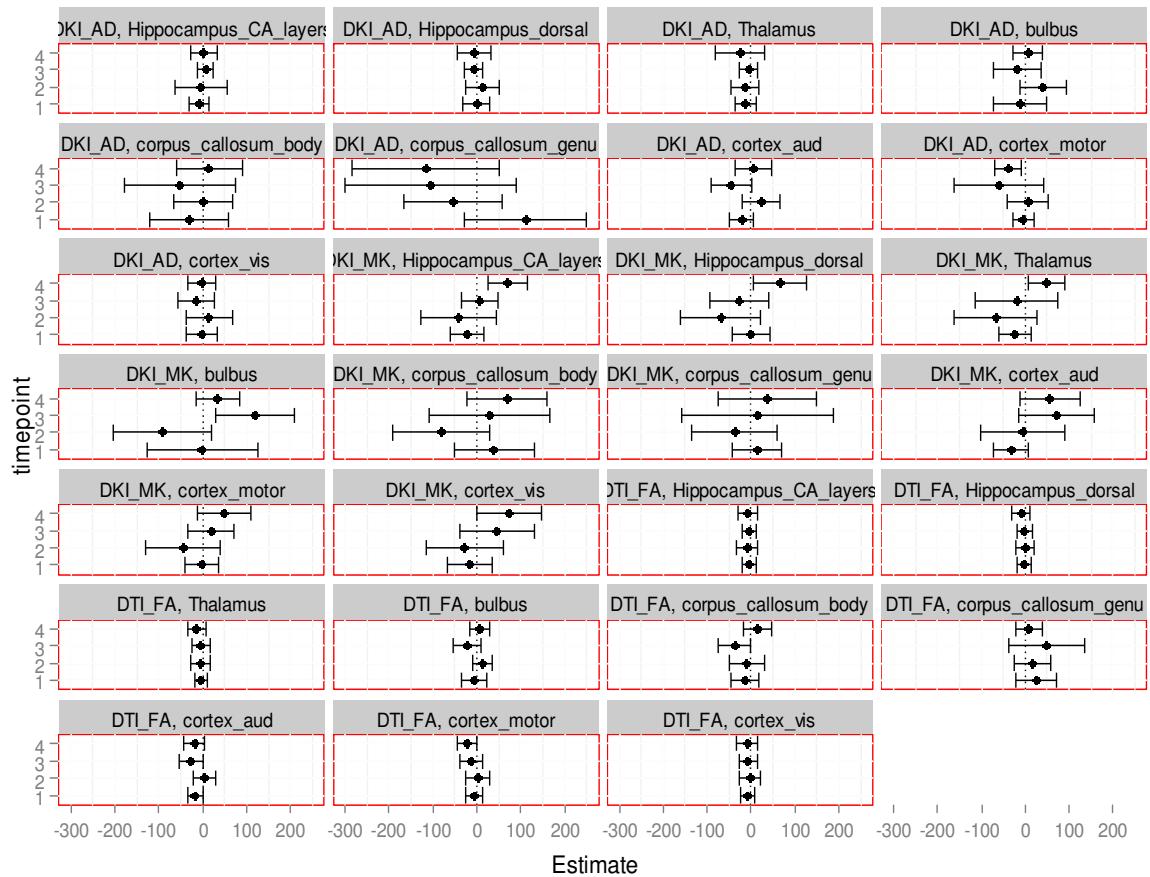
These confidence intervals are however based on tests not adjusted for multiplicity and may therefore be misleading. On adjusting for multiplicity using FDR, significant differences were detected in some regions for different parameters as summarized in Figure 7. While much of the difference is detected at six and eight months, most of the DKI parameters detected differences in most of the GM regions as compared to DTI parameters.

Figure 7: Significant (P-value <0.05) FDR Adjusted P-values
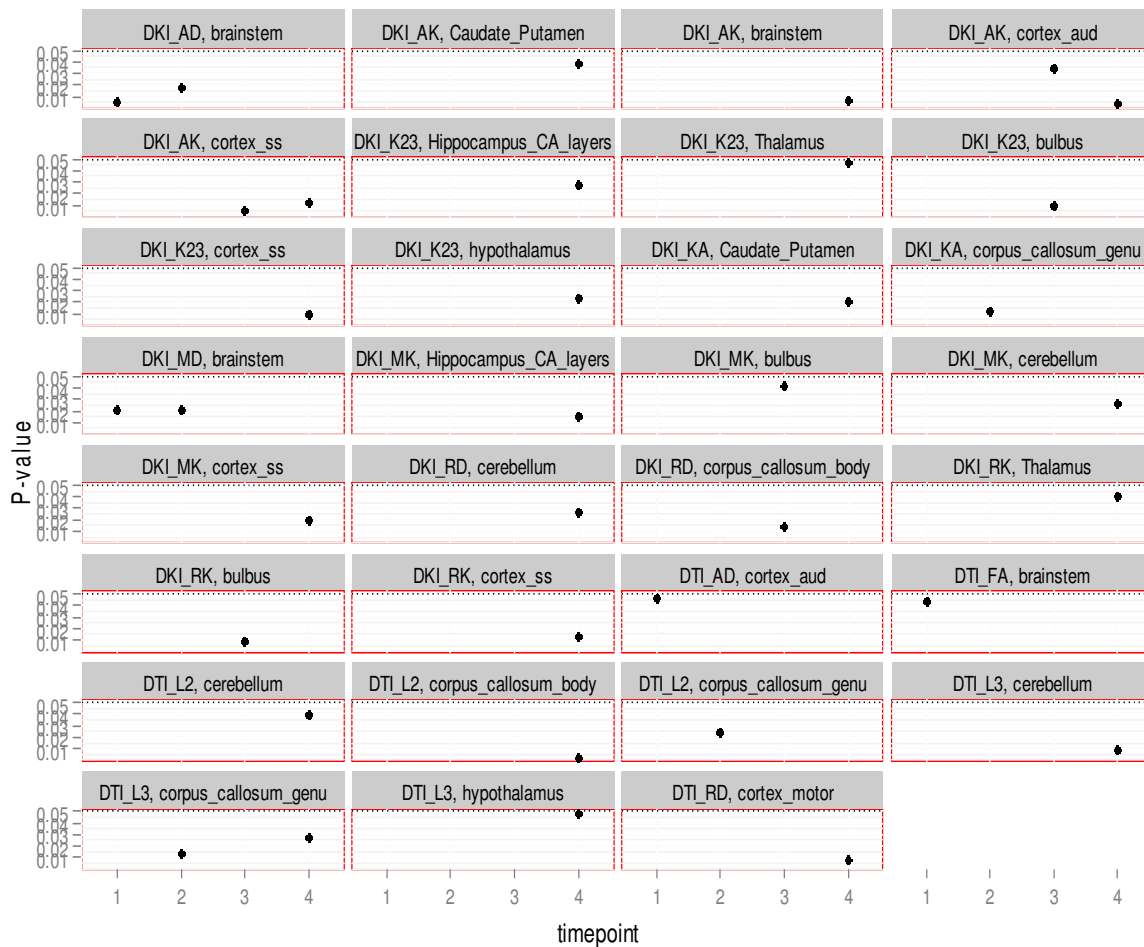
5.2.1.2   Compound symmetry

Convergence was attained for all models under this structure. As in the previous section, without adjusting for multiple tests, most of the confidence intervals contained zero hence not significant. The estimates with their confidence interval for parameters DKI_MD, DKI_RD, DTI_MD and DTI_RD in nine of the 15 WM and GM regions of interest are presented in Figure 8, from which it is evident that WM regions have wider confidence intervals as compared to GM regions in both DKI and DTI. For the parameters presented here however, within the same region, DKI provides wider confidence intervals as compared to DTI.

Figure 8: Estimated differences (With 95% Confidence intervals) per timepoint
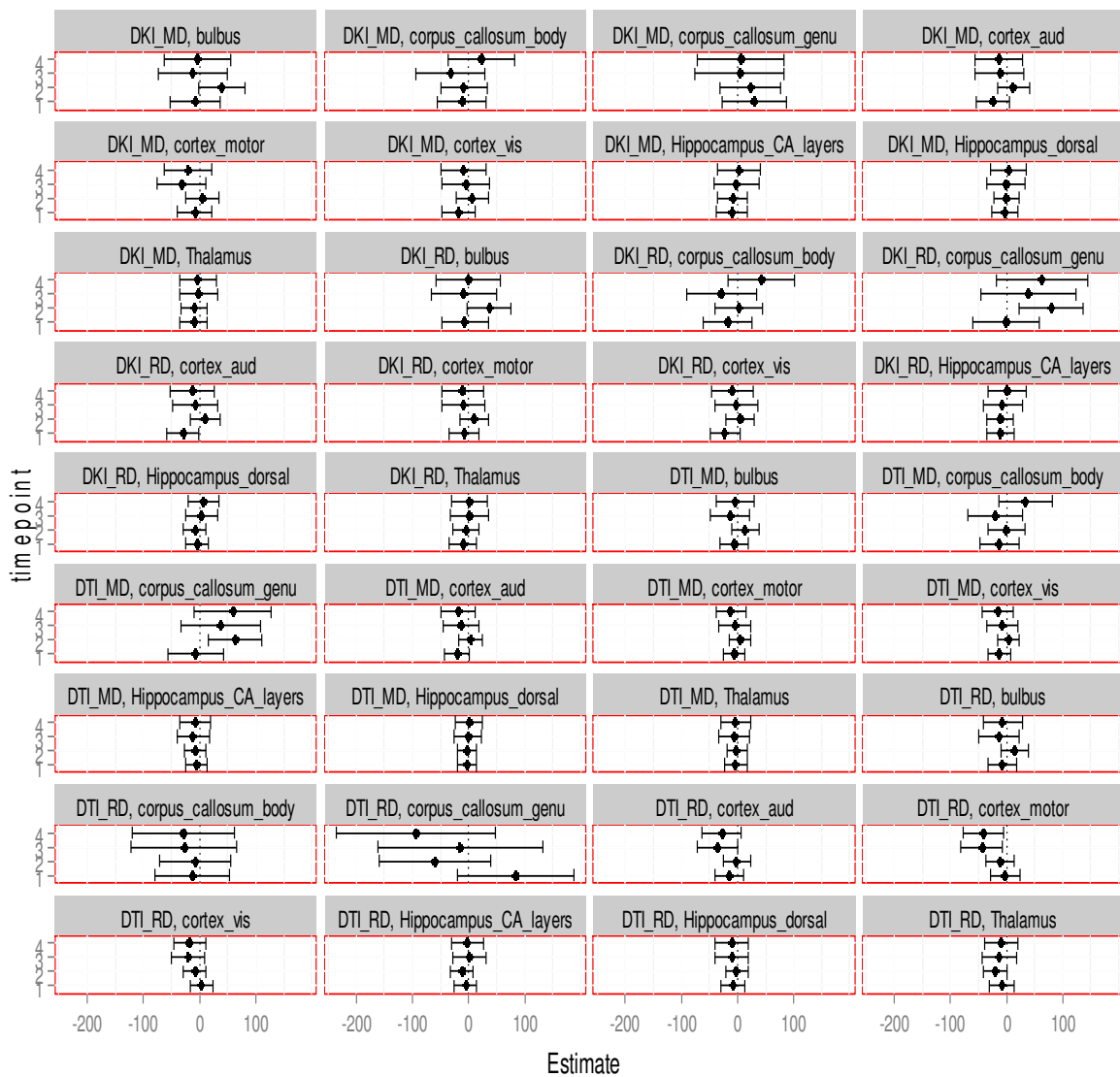
Upon adjusting for multiplicity using FDR, some significant differences were detected

especially at six and eight months for four DKI and four DTI parameters in different regions as

indicated in Figure 9. The differences were mainly detected in the grey matter sub-regions.

Figure 9: Significant (P-value <0.05) FDR Adjusted P-values
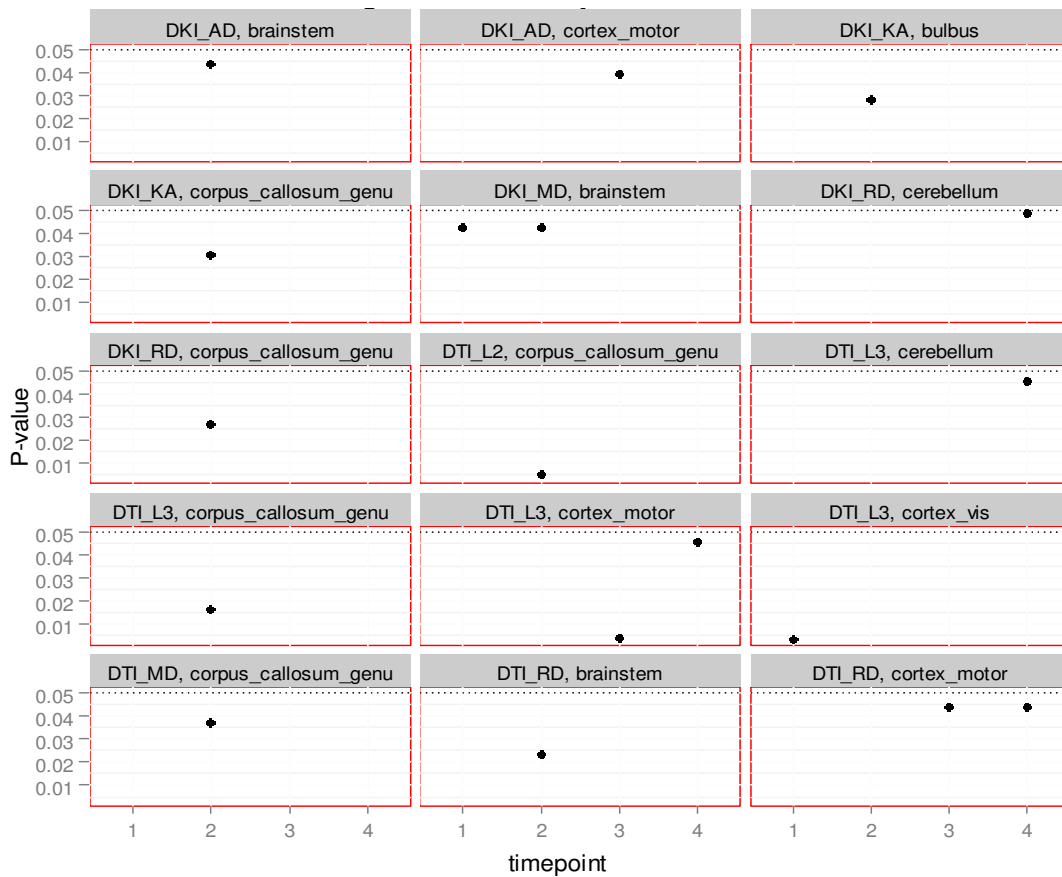
5.2.2 Univariate models for the variability

In modelling the variability of the mean response, a logarithmic transformation of the standard deviation was used since it was more symmetric. Like in the previous section, unstructured and compound symmetry covariance structures were considered.

5.2.2.1 Unstructured covariance

For all DKI and DTI parameters, convergence was not attained in at least one region of interest. However, where the models converged successfully, there was no significant difference in the genotypes with regards to the results not adjusted for multiplicity. Figure 10 presents the estimated differences with the corresponding confidence intervals in nine selected regions for parameters DKI_MD, DKI_RD, DTI_MD and DTI_RD.

Figure 10: Estimated differences (With 95% Confidence intervals) per timepoint
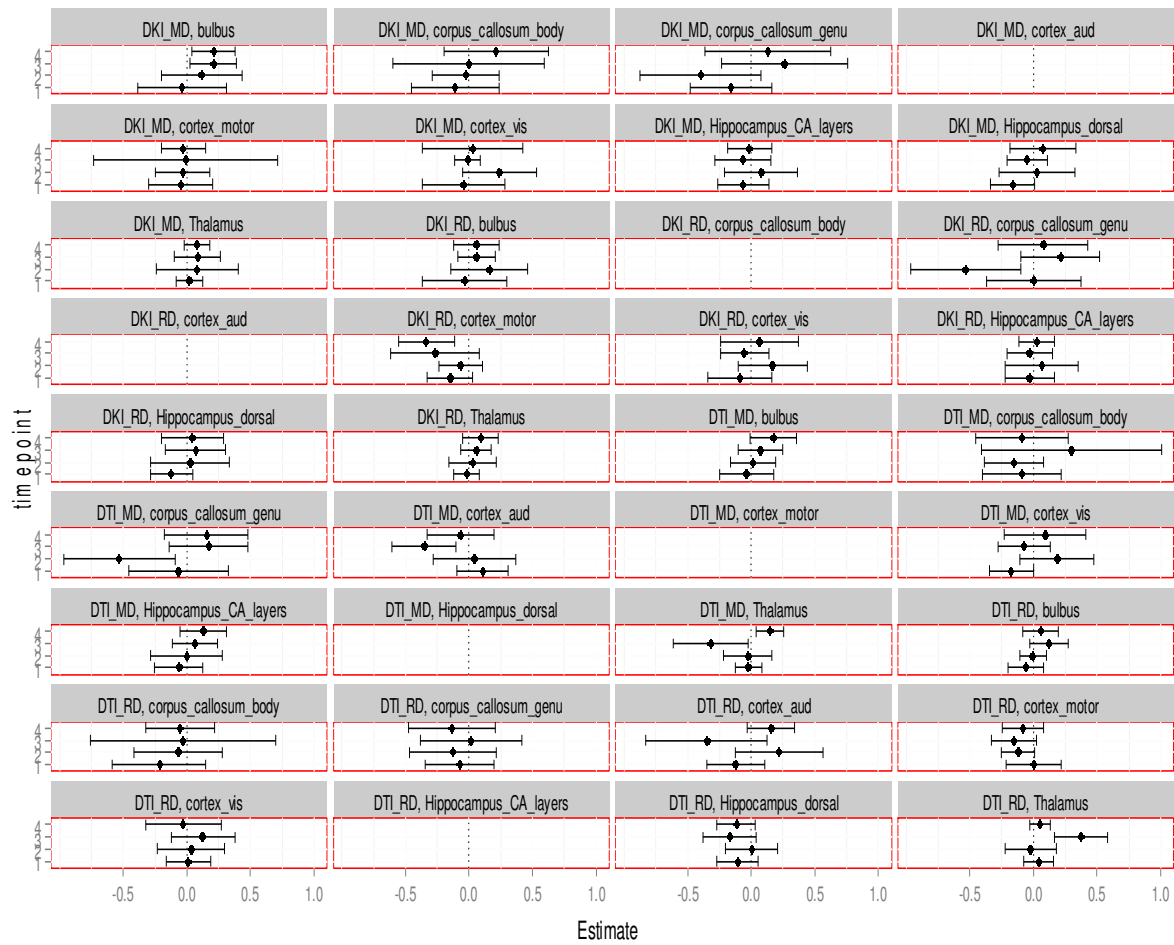
The confidence intervals included zero, with those of white matter regions being wider than the ones from grey matter regions.

Upon adjusting for multiplicity using FDR as before, for all parameters, significant differences were detected in at least one region and timepoint, as summarized in Figure 11.

Figure 11: Significant (P-value <0.05) FDR Adjusted P-values
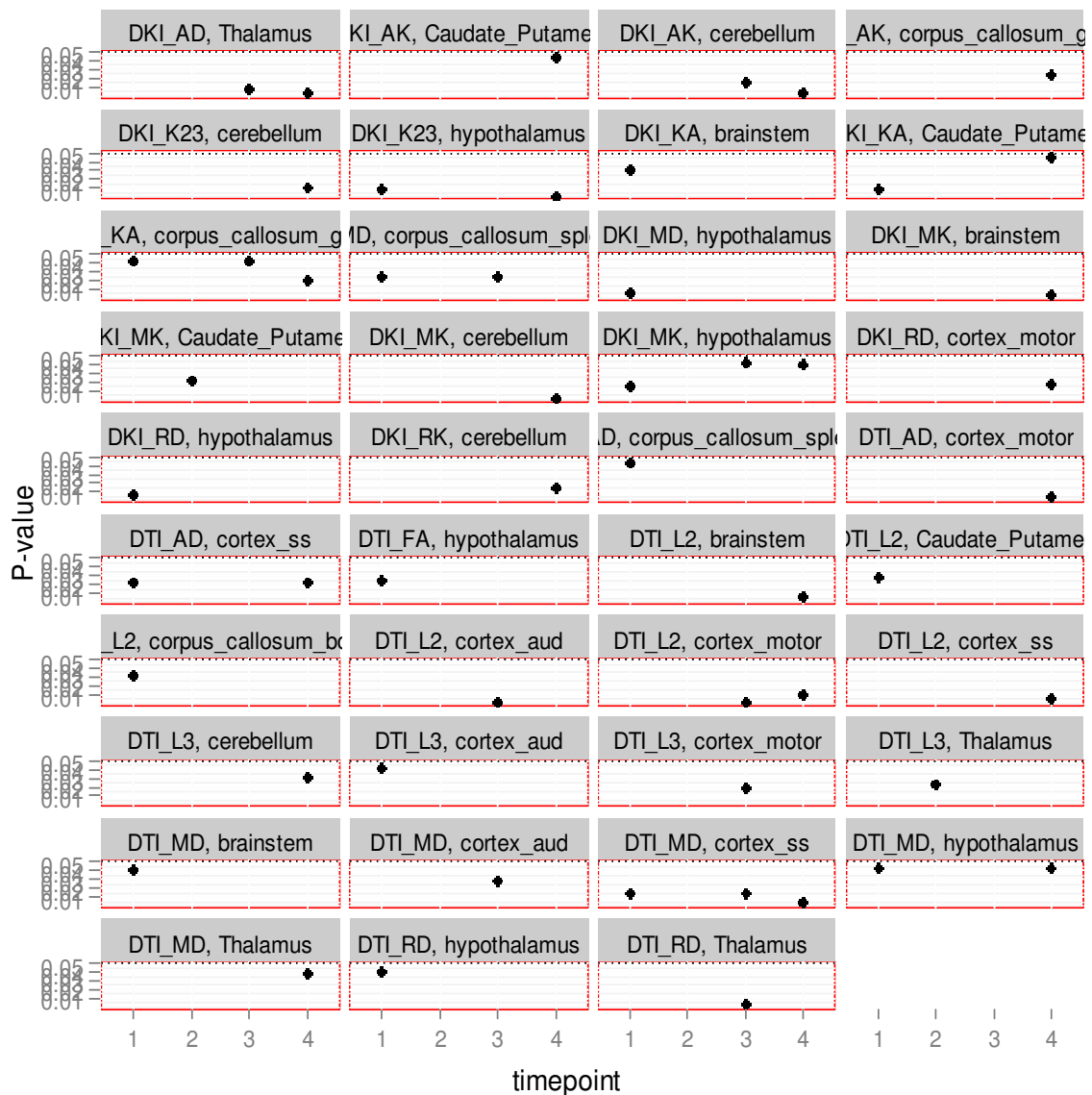
5.2.2.2 Compound symmetry

Upon simplifying the covariance structure, convergence was attained in all regions unlike in section 5.2.2.1. The unadjusted results however, didn't detect any significant differences between the genotypes as can be seen in Figure 12 for some selected parameters.

Figure 12: Estimated differences (With 95% Confidence intervals) per timepoint
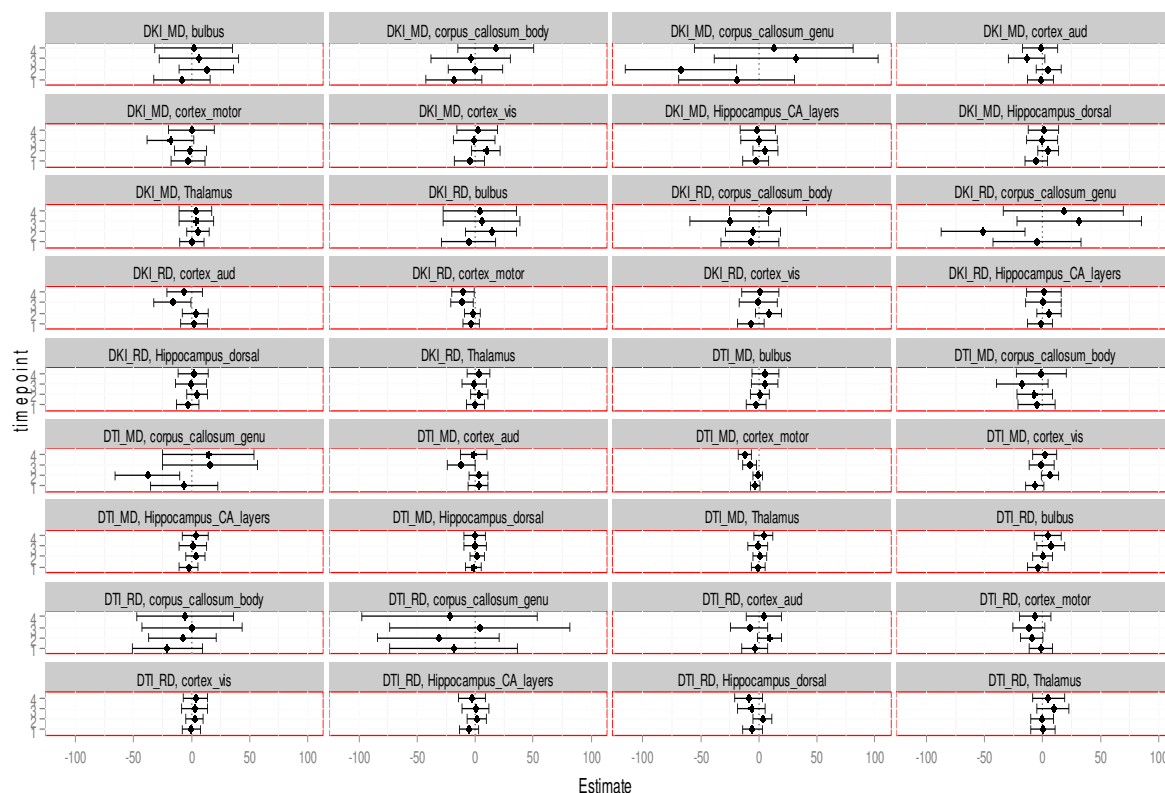
P-values adjusted for multiplicity detected significant differences at different timepoints as summarized in Figure 13.

Figure 13: Significant (P-value <0.05) FDR Adjusted P-values
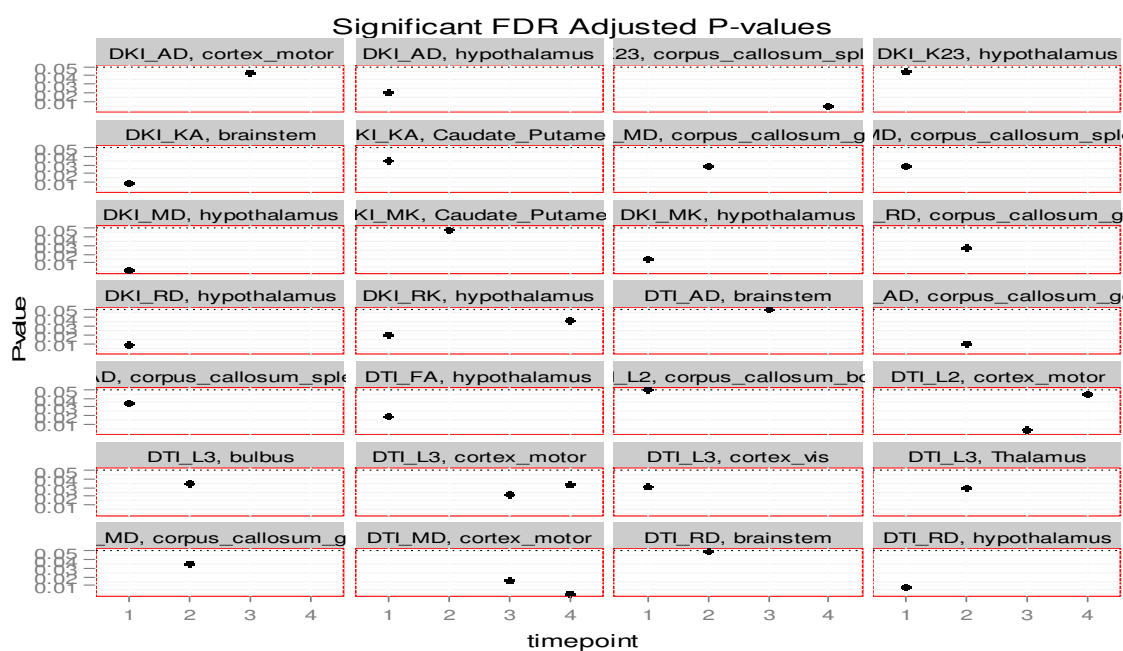
5.3 Joint modelling of bivariate longitudinal DKI and DTI pairs by region

This step was performed only on the corresponding parameter pairs in both DTI and DKI in order to assess the suitability of DKI as a biomarker for the corresponding DTI parameter. Out of the eight DKI and six DTI parameters, only MD, RD and AD parameter pairs from both DKI and DTI were considered, resulting into three bivariate models of the form provided in model 3.

The correlation within a biomarker for a particular subject can be modelled through either (or both) of the variance components of the variance covariance matrix $V_i = Z_i D Z'_i + \sum_i$. In this case, the residuals were assumed to be uncorrelated conditional on the random effects. This assumption can later be relaxed by allowing the residuals to be correlated, through one of the many correlation structures available. Serial correlation can also be accounted for if need be. Additionally, heterogeneous residuals were specified for the response vectors pair, thus assuming $\sum_{i,DKI} \neq \sum_{i,DTI}$.

Various between and within biomarker correlation structures were explored starting with unstructured and simplifying further by imposing additional assumptions on the correlation structure. In particular a compound symmetry structure allowing for the estimation of a constant correlation parameter within each biomarker was fit. As will be discussed in section 5.4, two options for unstructured in PROC Mixed were implemented; UN (Variances bounded) and UNR (Correlations bounded). Models (a-c) were implemented for each region separately, and a summary of key results per model follow.

5.3.1 DKI_MD vs DTI_MD

An unstructured variance-covariance matrix not only allowed for the estimation of unique variance components at each timepoint within a biomarker, but also provided timepoint specific estimates for the correlation between the biomarkers, thus enabling us to study the evolution of the association between the biomarkers. Models for all regions converged under CS, while for UNR the joint model for the brainstem (Region seven) did not attain

convergence. UN however had a high convergence failure rate with convergence being only attained in regions 1, 2, 3, 5, 7 and 9. Comparison of the Akaike Information Criterion (AIC) values for models with these three covariance structures (UN, UNR and CS) revealed very little gain/loss in AIC values across the models. For demonstration purposes, results for the parameter estimates for fixed effects as well as the covariance estimates for the random effects of the second subject (ROI=bulbus) under UNR are first presented in Table 3 and Table 4.

Table 3: Estimated V Correlation Matrix for Subject 2 (UNR)

		DKI				DTI			
	Timepoint	2	4	6	8	2	4	6	8
DKI	2	1	0.3736	0.2758	0.351	0.3876	0.391	0.5558	0.5596
	4		1	0.329	0.4187		0.4664	0.663	0.6676
	6			1	0.309			0.4894	0.4928
	8				1				0.6272
DTI	2					1	0.4839	0.6878	0.6926
	4						1	0.6938	0.6986
	6							1	0.9931
	8								1

Table 4: Parameter estimates for DKI_MD and DTI_MD from the Joint Model

Response	Genotype	Timepoint	Estimate	Standard Error	Lower CL	Upper CL
DKI_MD	APP/PS1	2 months	768.5	16.669	735.22	801.79
	APP/PS1	4 months	780.06	17.6372	744.84	815.27
	APP/PS1	6 months	708.18	16.9991	674.24	742.12
	APP/PS1	8 months	672.46	9.4622	653.57	691.35
	WT	2 months	778.9	15.7609	747.43	810.37
	WT	4 months	730.04	17.9229	694.26	765.83
	WT	6 months	722.2	16.1752	689.9	754.49
	WT	8 months	692.85	10.2579	672.37	713.33
DTI_MD	APP/PS1	2 months	579.92	9.8884	560.17	599.66
	APP/PS1	4 months	584.77	9.8491	565.1	604.43
	APP/PS1	6 months	538.21	12.1362	513.98	562.44
	APP/PS1	8 months	510.62	6.7648	497.11	524.13
	WT	2 months	587.77	9.502	568.8	606.74
	WT	4 months	563.19	10.1149	543	583.39
	WT	6 months	552.26	11.5593	529.18	575.33
	WT	8 months	527.29	7.3185	512.68	541.9

From the block correlation matrix in Table 3, the diagonal blocks comprise of the intra-marker correlation matrices while the off diagonal block is the inter-marker correlation matrix. The diagonal elements of the inter-marker correlation matrix provides the correlation between DKI and DTI parameters at corresponding timepoints, thus enabling us to study the evolution of the association between the two markers. Evolution profiles for the association between the DKI_MD and DTI _MD biomarker pair in the 15 regions of interest are presented in Figure 14 for the first two subjects, from which it was observed that correlation for a particular region of interest is highest at four months and lowest at six months in most regions. Save for a few regions whose correlation increases linearly over time, the correlation at eight months is slightly lower than that at 2 months. A similar trend is observed for the correlations with the markers.

Figure 14: Correlation Profiles for the bivariate model for MD
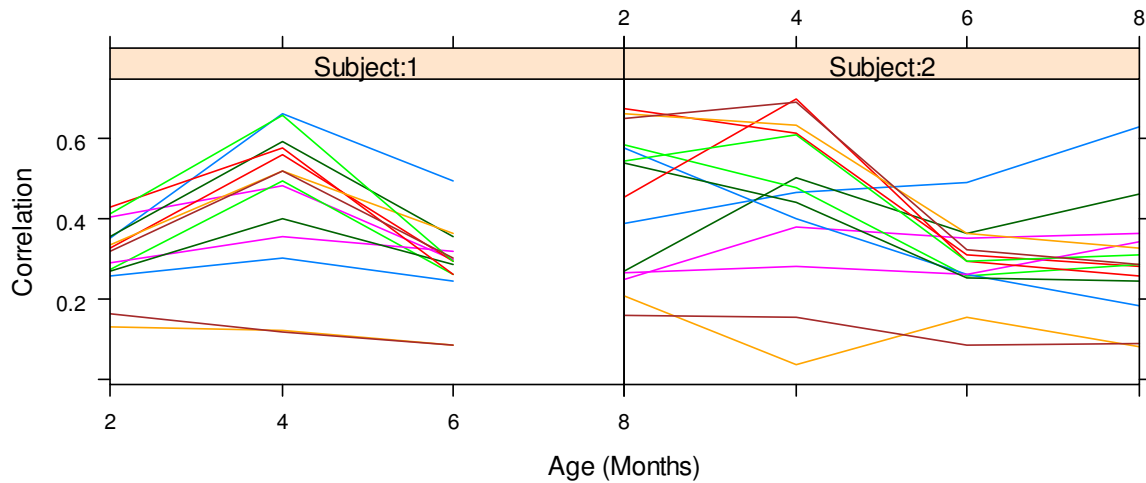
For the fixed effects parameters, the estimates and standard errors obtained for each outcome from joint modelling are quite similar to those obtained from the respective univariate models. If interest lies in inference for each outcome separately, there is clearly no gain from this multivariate analysis. Should inference be based on the joint responses, the parameter estimates can be averaged, while the standard errors for the joint response estimates can be computed using asymptotic theory (Fieuws & Verbeke, 2006).

Worth reflecting upon is the estimates of the variance components for this bivariate model especially the covariance matrix of random effects $D = \begin{pmatrix} 514.99 & 339.15 \\ 339.15 & 223.35 \end{pmatrix}$. The resulting correlation coefficient is $\rho = \dfrac{\sigma_{ab}}{\sigma_a \sigma_b} = \dfrac{339.15}{\sqrt{(514.99*223.35)}} = 1$, thus reducing the problem to a shared random effects model. Model (3) can therefore be implemented directly as a shared random effects model.

5.3.2   DKI_RD vs DTI_RD

As before, model (3) was implemented using UN, UNR and CS covariance structures. For UN, better convergence than in model (a) was obtained, with only models for regions 9, 13 and 14 not converging. For UN convergence was attained for all regions, while for CS the model

for region 5 (corpus_callosum_splenium) did not converge. The AIC values under the three covariance structures (Table 5) are quite similar. As before, the results for UNR are discussed further.

Table 5: AIC values for the bivariate model for MD using UN, UNR and CS

ROI	UN	UNR	CS
1	1245.4	1246.2	1251.3
2	1501.8	1511.1	1499.9
3	1405.9	1403.9	1405.8
4	1143.1	1145.5	1143.6
5	1461.3	1478.9	.
6	1146.9	1149.4	1149.2
7	1206.1	1204.1	1205.2
8	1342	1359.7	1358.1
9	.	1231.7	1229.7
10	1188	1188.7	1187
11	1179	1180.7	1178.7
12	1190.2	1196.9	1195
13	.	1144.5	1144.7
14	.	1214.9	1213.1
15	1181.7	1184.5	1183.8

Profiles for the correlations between the DKI and DTI pairs at each timepoint in selected WM and GM regions for the first two subjects are shown in Figure 15. The trend is similar to that earlier observed with most regions exhibiting the highest correlation between the biomarkers at four months while at six months, the correlation was quite low. There was however no correlation between the two biomarkers in corpus_callosum_body and the brainstem. There was a positive correlation between the biomarkers in moist regions apart from the corpus_callosum_splenium.

Figure 15: Correlation Profiles for the bivariate model for RD
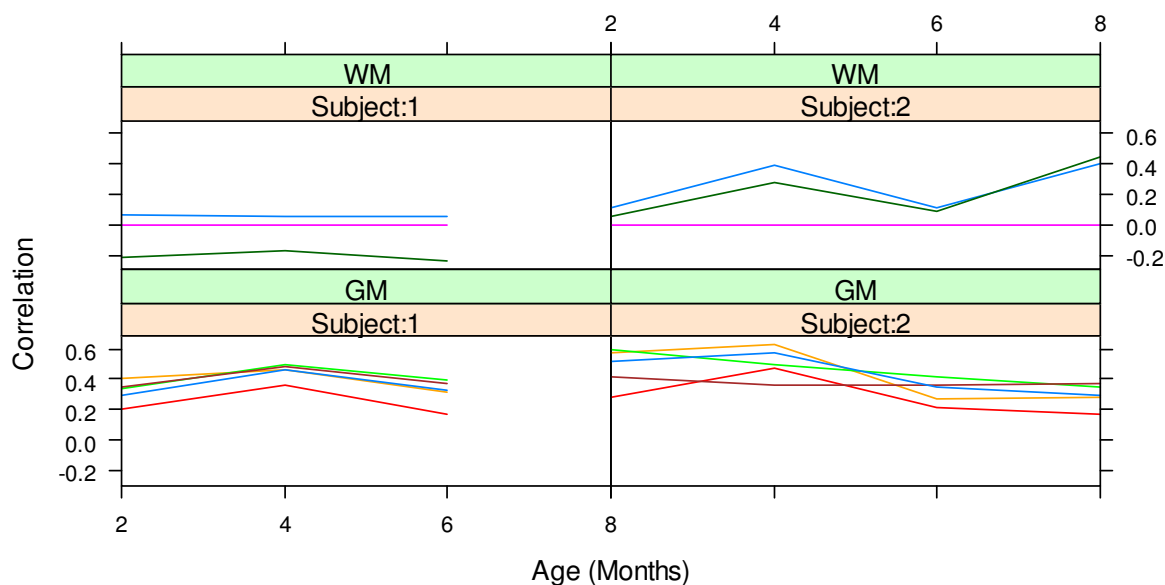
5.3.3 DKI_AD vs DTI_AD

As before, convergence was a challenge for UN with models for regions 1, 4, 6, 7, 8 and 13

not attaining convergence. With CS, only model for region 5 (corpus_callosum_splenium)

didn't converge, while for UNR models for all regions converged. As before, there was no

much difference in the AIC values amongst the models with UN, UNR and CS. The

correlation plots in Figure 16 had a similar trend as those in section 5.3.1 and 5.3.2 with the

correlation being highest at 4 months. There were also regions with negative correlations

indicating that an increase in one response resulted in a decrease in the other.

Figure 16: Correlation Profiles for the bivariate model for AD
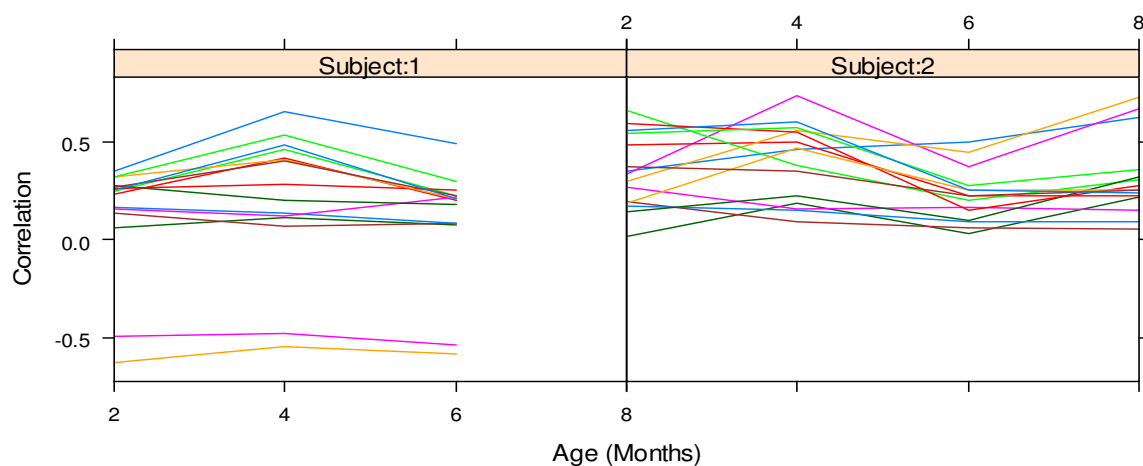
5.4   Computational challenges in modelling

In fitting both the univariate and multivariate linear mixed models, one of the challenges encountered was in attaining model convergence. For the univariate models, models with an unstructured covariance failed to converge for most regions. An attempt to increasing the maximum number of iterations for the estimation algorithm was unsuccessful in resolving the convergence problems. However, simplifying the covariance structure by reducing the number of variance components to estimate resulted to convergence in all the univariate models as well as for the bivariate models.

Additionally for the bivariate joint modelling, two variants of unstructured covariance were implemented in Proc Mixed: UN and UNR. While both options results in the estimation of the same number of parameters, they have some subtle differences, which we illustrate with output from the second subject for model (a) in section 5.3.1.

According to the SAS documentation, UN constrains the variances to be non-negative while the covariance parameters are unconstrained. Further, the structure is not constrained to be positive definite hence can often cause convergence problems. In this setting, the correlation between the random effects may exceed one as is evident from Table 6. The covariance matrix in this case is parameterized in terms of variances and covariance.

UNR on the other hand parameterizes the covariance matrix with variances and correlations, hence a slightly different parameterization from UN. The correlations in this case are constrained to satisfy the condition $|\rho_{ij}| < 1$. The choice between UN and UNR when the covariance matrix is not near any boundary constraint is purely for simplicity in computing the correlation between the random effects as was demonstrated in Molenberghs & Verbeke (2005). For such cases, the parameter estimates are invariant of the choice made. In our case, though, the estimated corelations fall within the boundary hence some variations in the parameter estimates, as well as on the fit statistics was expected.

Table 6: Comparison of SAS UN and UNR covariance structure options

Covariance Parameter Estimates (UNR)						
Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Lower	Upper
Var(1)	954.12	351.94	2.71	0.0034	517.94	2310.41
Var(2)	498.2	180.13	2.77	0.0028	273.23	1180.99
Corr(2,1)	1	0	.	.	.	.
Covariance Parameter Estimates (UN)						
Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Lower	Upper
UN(1,1)	889.61	360.77	2.47	0.0068	459.13	2404.11
UN(2,1)	687.07	248.54	2.76	0.0057	199.95	1174.19
UN(2,2)	459.07	180.83	2.54	0.0056	240.72	1197.5

UNR directly provides the correlation output which in this case is one due to rounding off as can be seen from the computations using values in the G matrix ;

$$\rho = \frac{\sigma_{ab}}{\sigma_a \sigma_b} = \frac{689.45}{\sqrt{(954.12*489.2)}} = 0.999998$$. For UN the correlation estimate given by

$$\rho = \frac{\sigma_{ab}}{\sigma_a \sigma_b} = \frac{687.07}{\sqrt{(889.61*459.07)}} = 1.075$$ which is beyond the correlation boundaries.

6 Implementation of the models in SAS

6.1 Univariate longitudinal models

SAS Proc Mixed was used to fit the 210 univariate models for each response (Mean_ROI and

SD_ROI). SAS macro programming was used for ease of looping through the models and

results output as datasets for further processing. The basic model code used is presented

hereunder:

```
/*Univariate Linear mixed model*/
proc mixed data=dki  method=reml;
class genotype timepoint;

*MODEL statement specifies an UNstructured mean:Estimate at each timepoint
and outputs the results to a file;

model Mean__ROI_=genotype*timepoint/noint s outp=predicted
outpm=meanprediction cl;

*REPEATED statement specifies the within sibject correlation Structure;

repeated timepoint/subject=Animalnr_unique type=un ;

*ESTIMATE statements used to compute an estimate of the difference between
genotypes at each timepoint;

estimate "Treat diff at 2 months"  genotype*timepoint 1 0 0 0 –1  0  0
0/cl ;
estimate "Treat diff at 4 months"  genotype*timepoint   0 1 0 0  0 –1  0
0/cl;
estimate "Treat diff at 6 months"  genotype*timepoint   0 0 1 0  0   0 –1
0/cl;
estimate "Treat diff at 8 months"  genotype*timepoint   0 0 0 1  0   0  0 –
1/cl;

*Output Estimates and Contrasts to a file;
ods output estimates=est;
run;quit;

/*Proc Multtest Multiple comparisons adjustment*/
data est;
set est;
rename probt=RAW_P;
run;
proc multtest pdata=est holm hoc bon fdr out=est;
run;
```

6.2 Bivariate longitudinal models

Having adopted the data for joint modelling as suggested in various literature ((Gao ,

Thompson, Xiong, & Miller, 2006), (Fieuws & Verbeke, 2006), (Molenberghs & Verbeke,

2005)) the three bivariate models of section 4.3 were implemented for each region of interest

through a SAS macro using SAS Proc Mixed. The core syntax was as shown hereunder:

```
proc mixed data=joint  method=reml covtest noclprint cl;
class genotype timepoint parameter Animalnr_unique;
model Mean__ROI_=genotype*timepoint*parameter /noint s outp=predicted
outpm=meanprediction cl;

*Residual covariance structure specified using REPEATED;

repeated timepoint/subject=Animalnr_unique(timepoint) type=simple  r
group=parameter*timepoint;

*Covariance between the random effects of each response captured using the
RANDOM statement;

random parameter/subject=Animalnr_unique type=unr   v=2 vcorr=2 g gcorr;

*Files output for further processing;
*Output final variance-covariance matrix for the two parameters and RE
covariance matrix to a file;

ods output covparms=oneparcovest;
ods output vcorr=variancepars;
ods output fitstatistics=aicval;
run;quit;
```

7   Discussion and conclusions

While the mental well –being of the population cannot be over emphasized, it's generally expected that the mental capacity deteriorates as we age. The volume of both white and grey matter increases during the youthful age and declines as we age, hence affecting the neuro-cognitive status. Alzheimer's disease is one popular age related irreversible dementia whose early diagnosis could have benefits to the patient. Using mice of two genotypes (APP/PS1 and WT), MRI for their brains was done using two techniques (DKI and DTI) resulting into the dataset used in this report. We sought for potential differences in between the two genotypes of mice aged 2 to 8 months.

Data exploration suggested lack of a significant difference in the evolution profiles in most regions of interest, although DKI parameters seemed more sensitive to differences within regions 1-5 as compared to DTI parameters. In particular, DKI_RK, DKI_MK, DKI_AD and DKI_K23 profiles suggested differences in regions 1-5, while only DT_RD and DTI_L3 had signs of differences in the profiles of the two genotypes. Variability was further explored from which there were minimal differences in evolution of variability between the two genotypes. From Figure 5, it was evident that although there was minimal variability in APP/PS1 profiles, WT mice exhibited much variability in the profiles over time.

Linear mixed effects models for the univariate longitudinal data were fitted under different covariance structures using an unstructured mean. An unstructured mean model provided estimates at each timepoint thus making it possible to evaluate differences across the timepoints. A compound symmetry covariance structure (which assumes constant variability and constant correlation within a subject) was deemed the most appropriate since it assured us of convergence in all regions of interest. Results indicated that DKI was able to detect differences (Most of the detectable differences were in the grey matter) especially at later timepoints as compared to DTI.

Similarly, the variability of the responses was modelled using linear mixed effects model, with the results indicating little differences in the variability across the genotypes. There were however detectable differences in the variability in majorly grey matter sub-regions.

To ensure validity of inference, correction for multiple tests within the univariate models was done using FDR. While most multiple comparison corrections adjust for the familywise error rate, FDR controls the false discovery rate- the proportion of tests that are falsely claimed to be significant, while they are not. The impact of correcting for multiplicity was evident from the results of unadjusted estimates in section 5.2.1 and 5.2.2, whose confidence intervals mostly included zero, hence not detecting any significant differences in all regions.

Joint modelling of multivariate longitudinal data was also implemented with an aim of seeking the correlation between corresponding DTI and DKI parameters. While there was a positive correlation between DKI and DTI MD responses, there were regions with negative correlations for AD and RD bivariate models. Moreover, DKI and DTI RD parameters were independent of each other in the corpus_callosum_body and the brainstem, indicating that in these regions, DKI cannot act as a biomarker for DTI.

In conclusion, this study provided some evidence of the possibility of using DKI as a biomarker for DTI in different regions of the brain. However, treating the different brain sections as independent entities may be misleading considering that the grey and white matter regions have a particular spatial structure, which may introduce particular dependencies. Future developments should therefore seek to account for these dependencies. The sample size used should also be explored further regarding its appropriateness, and an optimal sample size computed.

Works Cited

Agronin, M. E. (2007). *Alzheimer Disease and Other Dementias: A Practical Guide.*

    Philadelphia: Lippincott Williams & Wilkins.

Braak, H., & Braak, E. (1997). Frequency of stages of alzheimer-related lesions in different age

    categories. *Neurobiology od ageing, 18*(4), 351-357. Retrieved 2012

Cheung, M. M., Hui, E. S., Chan, K. C., Helpern, J. A., Qi, L., & Wu, E. X. (2009). Does

    diffusion kurtosis imaging lead to better neural tissue characterization? A rodent brain

    maturation study. *NeuroImage, 45*, 386–392.

Duff, K., & Suleman, F. (2004). Transgenic mouse models of Alzheimer's disease: How useful

    have they been for therapeutic development? *BRIEFINGS IN FUNCTIONAL*

    GENOMICS AND PROTEOMICS, 3(1), 47-59.

Faes, C., Aerts, M., Molenberghs, G., Geys, H., Teuns, G., & Bijnens, L. (2008, September

    30). A High-Dimensional Joint Model for Longitudinal Outcomes of Different Nature.

    Stat Med, 27(22), 4408-27.

Falangola, M. F., Jensen, J. H., Babb, J. S., Hu, C., Castellanos, F. X., Di Martino, A., . . .

    Helpern, J. A. (2008, December). Age-Related Non-Gaussian Diffusion Patterns in the

    Prefrontal Brain. *J Magn Reson Imaging., 28*(6), 1345-1350. doi:10.1002/jmri.21604

Fieuws, & Verbeke, G. (2004). Joint modelling of multivariate longitudinal profles: pitfalls of

    the random-effects approach. *Statist. Med.*, 3093–3104.

Fieuws, S., & Verbeke, G. (2006, June). Pairwise Fitting of Mixed Models for the Joint

    Modeling of Multivariate Longitudinal Profiles. *Biometrics, 62*, 424–431.

Gao , F., Thompson, P., Xiong, C., & Miller, J. P. (2006). Analyzing Multivariate Longitudinal

    Data Using SAS. *SAS Users Group International Conference.*

Higbee, K. L., & Higbee, K. L. (2001). *Your Memory: How It Works and How to Improve It.* Cambridge, Massachusetts: Da Capo Press.

Hui, E. S., Cheung, M. M., Qi, L., & Wu, E. X. (Eds.). (2008). Towards better MR characterization of neural tissues using directional diffusion kurtosis analysis. *NeuroImage, 42*, 122–134.

Kießling, F. (2011). *Small Animal Imaging: Basics and Practical Guide.* Springer.

Kutner, M. H., Nachtsheim, C. J., Neter, J., & Li, W. (2005). *Applied Linear Statistical Models.* Singapore: Mc Graw Hill.

Molenberghs, G., & Verbeke, G. (2005). *Models for discrete longitudinal data.* New York: Springer.

Prince, M., Bryce, R., & Ferri, C. (2011). *World Alzheimer Report 2011: the benefits of early diagnosis and intervention.* King's College London, Institute of Psychiatry. London: Alzheimer's Disease International (ADI). Retrieved 07 27, 2012, from http://www.alz.co.uk/research/WorldAlzheimerReport2011.pdf

Rizopoulos, D., Verbeke, G., & Molenberghs, G. (2010, August 3). *Recent and Selected Short Courses.* Retrieved July 07, 2012, from Geert Verbeke: https://perswww.kuleuven.be/~u0018341/shortcourses.htm

Verbeke, G., & Molenberghs, G. (2000). *Linear mixed models for longitudinal data.* New York: Springer.

Verhoeven, K. J., Simonsen , K. L., & McIntyre, L. M. (2005). Implementing false discovery rate control: increasing your power. *OIKOS, 108*, 643-647.

Appendix

Appendix 1: Coding scheme for parameters and regions of interest in the brain

Value	Region of Interest	Value	Parameter
1	bulbus	1	DKI_MK
2	corpus_callosum_genu	2	DKI_RK
3	corpus_callosum_body	3	DKI_K23
4	Hippocampus_dorsal	4	DKI_AK
5	corpus_callosum_splenium	5	DKI_KA
6	Hippocampus_CA_layers	6	DKI_MD
7	brainstem	7	DKI_RD
8	cerebellum	8	DKI_AD
9	hypothalamus	9	DTI_FA
10	Caudate_Putamen	10	DTI_MD
11	Thalamus	11	DTI_RD
12	cortex_motor	12	DTI_AD
13	cortex_ss	13	DTI_L2
14	cortex_aud	14	DTI_L3
15	cortex_vis		

Auteursrechtelijke overeenkomst

Ik/wij verlenen het wereldwijde auteursrecht voor de ingediende eindverhandeling:
Longitudinal analysis of differences in white and grey matter brain regions of APP/PS1 and wild type mice, using diffusion kurtosis imaging and diffusion tensor imaging

Richting: **Master of Statistics-Biostatistics**
Jaar: **2012**

in alle mogelijke mediaformaten, - bestaande en in de toekomst te ontwikkelen - , aan de Universiteit Hasselt.

Niet tegenstaand deze toekenning van het auteursrecht aan de Universiteit Hasselt
behoud ik als auteur het recht om de eindverhandeling, - in zijn geheel of gedeeltelijk -,
vrij te reproduceren, (her)publiceren of distribueren zonder de toelating te moeten
verkrijgen van de Universiteit Hasselt.

Ik bevestig dat de eindverhandeling mijn origineel werk is, en dat ik het recht heb om de rechten te verlenen die in deze overeenkomst worden beschreven. Ik verklaar tevens dat de eindverhandeling, naar mijn weten, het auteursrecht van anderen niet overtreedt.

Ik verklaar tevens dat ik voor het materiaal in de eindverhandeling dat beschermd wordt door het auteursrecht, de nodige toelatingen heb verkregen zodat ik deze ook aan de Universiteit Hasselt kan overdragen en dat dit duidelijk in de tekst en inhoud van de eindverhandeling werd genotificeerd.

Universiteit Hasselt zal mij als auteur(s) van de eindverhandeling identificeren en zal geen wijzigingen aanbrengen aan de eindverhandeling, uitgezonderd deze toegelaten door deze overeenkomst.

Voor akkoord,

Muchene, Leacky

Datum: **14/09/2012**