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FACULTY OF SCIENCES
Master of Statistics: Biostatistics

Masterproef

Joint modelling of multiple outcomes on longitudinal behaviour of genetically modified mice

Promotor :
Prof. dr. Ziv SHKEDY

Promotor :
Dr. TOM JACOBS

Mohammad Romel Bhuia

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

Transnational University Limburg is a unique collaboration of two universities in two countries: the University of Hasselt and Maastricht University.

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Certification

I declare that this thesis was written by me under the guidance and counsel of my promoters.

…… …… Date……
Bhuia Mohammad Romel Student

We certify that this is the true thesis report written by **Bhuia Mohammad Romel** under our supervision and we thus permit its presentation for assessment.

…… …… Date……
Prof. dr. SHKEDY Ziv Internal Promoter

…… …… Date……
Dr. JACOBS Tom External Promoter

DEDICATED

TO

MY BELOVED MOTHER.

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September, 2013 *Bhuia Mohammad Romel*

Abstract

Alzheimer's disease (AD) is the most common form of dementia and characterized by progressive neuronal degeneration with depositions of amyloid plaques and neurofibrillary tangles. TauPS2APP mice are triple transgenic mice that express human mutated Amyloid Precursor Protein, Presenilin 2 and Tau. It is about as close as one can get to the Alzheimer pathology in rodents. The aim of the study is to investigate the differences in the evolution over time between TauPS2APP and wild-type mice for the behaviour data as well as to examine if the effects observed are only associated to learning effects. Moreover, to fit joint model combining outcomes in order to investigate if there is any benefit in such a joint modelling compared to a univariate analysis. Two datasets, extracted from a pre-clinical experiment on Alzheimer's disease, consisting information regarding 5 tests for different behaviours of young and old mice, are combined. Univariate generalized linear mixes models (GLMM) with random intercept is first fitted for each of the responses separately assuming different outcomes from same mouse are independent. Correlations among outcomes from same mouse are captured by modelling the responses jointly by fitting them pair-wise and combining the results using pseudo likelihood theory. Findings show significant evidences of difference in the evolution over time between genotypes for all the behaviors of young mice as well as combined data but not significant for old mice. There is apparent learning effect in the mice behaviors. Age and genotype effect are also found significant. Considering all the responses together in joint model, we observe significant differences between genotypes in the evolution of performance over time. Parameter estimates from joint model are more precise than univariate models and hence more reliable as they are obtained considering the association between pairs of behaviors of same mouse.

***Keywords**: Alzheimer's disease, TauPS2APP, Joint modelling, GLMM*

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1. Introduction

Alzheimer's disease (AD) is a widespread neurodegenerative disease leading to cognitive impairment, difficulty with memory and progressive brain atrophy. It is the most common form of dementia and is characterized by progressive neuronal degeneration with depositions of amyloid plaques and neurofibrillary tangles (Hölttä et al., 2013; Ozmen et al., 2009; Blennow et al., 2006). According to World Alzheimer Report (2012) there are more than 36 million people with disease worldwide and predicted to be 115 million by the year 2050. In 2010 the global cost of the disease was about 600 billion US dollars putting a huge economic burden for the society. The likelihood of having Alzheimer's disease increases substantially after the age of seventy and may affect around fifty percent of persons over the age of 85. Although the greatest known risk factor is increasing age, Alzheimer's is not just a disease of old age. Up to 5 percent of people with the disease have early onset Alzheimer's (also known as younger-onset), which often appears when someone is in their 40s or 50s. Although many advances in identifying the molecular mechanisms involved in AD have been made, there is still no cure or treatment available for this disease (Donmez, 2012).

Diagnosis of AD is difficult and the disease is not fully understood even there is no single theory available but may be related to beta-amyloid plaques or tau protein tangle formation within the brain. It is not clear when the disease starts. It may have developed over many years before symptoms first show, no cure but some symptomatic treatments are available. Clearance of abnormally phosphorylated Tau (pTau) may prevent neuronal cell death in Alzheimer's disease. The identification of the genetic factors in the familial forms of AD enabled the generation of transgenic animals which reproduce an essential part of its pathology. The triple-transgenic mice are the offspring of a new double-transgenic line bearing the human presenilin 2 gene N141I and amyloid precursor protein mutant genes (Ozmen et al., 2009). Amyloid beta peptides and microtubule-associated protein Tau are misfolded and form aggregates in brains of Alzheimer's disease patients. To examine their specific roles in the pathogenesis of Alzheimer's disease and their relevance in neurodegenerative processes, TauPS2APP triple transgenic mice was created that express human mutated Amyloid Precursor Protein, presenilin 2 and Tau. The TauPS2APP triple transgenic mouse model is very useful for studying the effect of new therapeutic paradigms

on amyloid deposition and downstream neurofibrillary tangle development (Grueninger et al., 2010).

TauPS2APP mice show cognitive impairment in their behaviors like lack of learning ability, memory deficiency and anxiety. In an attempt to model human pathological anxiety in rodents, a wide range of behavioral testing paradigms have been developed (Borsini et al., 1989; Hall, 1934). These tests are designed to explore the tendency of mice to engage in exploratory activity or social investigation in a structured or an open field. The premise that basic physiological mechanisms underlying response in rodents can be equated to similar mechanisms operating in humans provides a degree of face validity for these paradigms (Rodgers et al., 1997).

TauPS2APP and wild-type mice were examined for five different behaviors related to AD applying standardized behavioral tests. The goal of this study is to investigate the difference in behaviors between these two types of mice. The five outcomes of different nature on mice behavior are to be first modeled independently. It is obvious that different outcomes from same mouse are usually expected to be correlated. Evidently this phenomenon triggers the point to fit models that may capture these correlations which might change the direction of findings obtained from unvariate models. Hence, joint modeling could be a nice approach to model five behaviors combined as well as accommodating the association between pairs of outcomes from same subject.

1.1 Objectives of the Study

The main objective of the study is to investigate differences in the evolution of performance over time (age) between TauPS2APP and wild-type mice for five different behaviors. Moreover, to fit joint models combining five behaviors together for capturing the associations between pairs of behaviors of same mouse. The specific objectives are:

- To investigate the differences in the evolution over time (age) between genotypes for the behaviour data as well as to examine the learning effect;
- To investigate whether there is any benefit of joint modelling combining all outcomes compared to univariate modelling.

1.2 Organization of the study

The thesis is organized as section one for introduction followed by section two deals with description of data and variables used in this study. Section three presents detailed about materials and methods used to meet the objectives of the research. Results are presented in section four. Discussion along with some concluding remarks are presented in section five. Furthermore, content has been outline at the beginning and the list of the references is presented at the end of the study.

2. Data and Variables

Data for this study extracted from a pre-clinical experiment on Alzheimer's disease where different behaviours of TauPS2APP and wild type mice were assessed longitudinally. TauPS2APP mice were triple transgenic mice that express human mutated Amyloid Precursor Protein, Presenilin 2 and Tau. It is about as close as one can get to the Alzheimer pathology in rodents at this moment. Two datasets were combined consisting information on different activities of 5 tests for 31 young mice age between 4 to 9 months and 32 old mice age between 12 to 21 months. The response variables and covariates are presented in table 1.

Table 1: Description of the study variables

Variable	Description
Response	
LMA_DM	: Log distance moved within 60 minutes in locomotion activities
CMAT_TE	: Total number of entries in CMAT
CMAT_NA	: Number of alternations in CMAT
VMaze_DT	: Distance moved in test phase in VMaze
VMaze_DH	: Distance moved in habituation phase in VMaze
Covariate	
Genotype	: 1. TauPS2APP and 0. Wild-type
Age (Time)	: 4, 6 and 9 months for young mice; 12, 15, 18 and 21 months for old mice

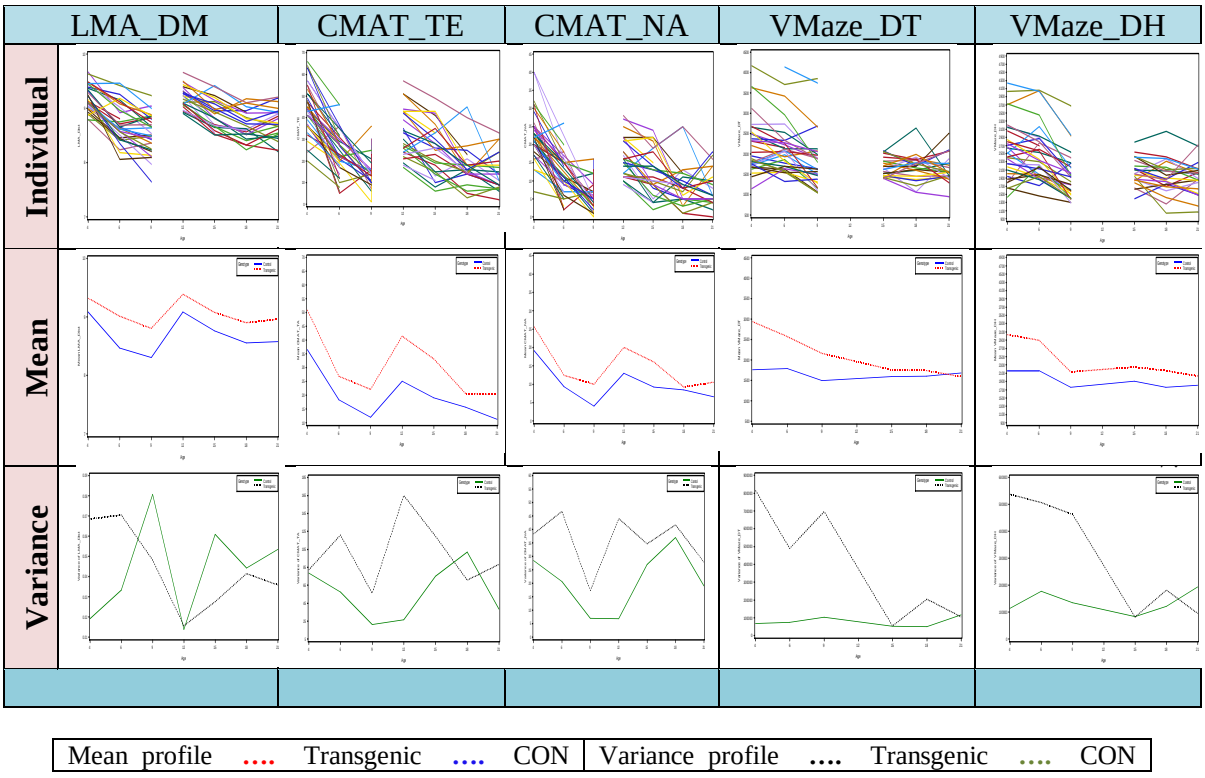
Although the scenario is different for different outcomes, there are 15 transgenic among 31 young mice and 16 transgenic among 32 old mice, from which 1 young and 10 old mice are dropped out throughout the entire period of study (Table 2). Even though the study is balanced by design, the data turned out to be unbalanced after combining two datasets of young and old mice since they are not measured at same time points.

Table 2: Distribution of mice by age and genotype

		Number of Mouse		
Mouse	**Age (Time point)**	**Control**	**Transgenic**	**Total**
Young	4	16	15	31
	6	16	15	31
	9	16	14	30
Old	12	16	16	32
	15	15	11	26
	18	14	10	24
	21	13	9	22

Besides baseline a high variability between mice could be observed, especially for the CMAT behaviours of old mice and VMaze behaviours of young mice. However, the within patient variability could be considered as moderate as shown in figure 1. Vmaze behaviours of old mice were measured only three time points except for age 12 months. In addition, one wild type mouse was missing at age 18 months. However the nature of the considered outcomes are not same which should be taken into account at modelling stage, e.g. Vmaze_DT and Vmaze_DH follow normal distribution, whereas LMA_DM is normal after logarithmic transformation, at the same time CMAT_TE and CMAT_NA are count data.

Figure 1: Individual, Mean and Variance Profile for Behavior Measures



It is noticeable that the mean performance is higher at baseline and declined steadily throughout subsequent ages. For all behaviors average performance of transgenic mice is evolving higher than those of wild type. Variance profiles are not constant over time and might be higher in wild-type than transgenic mouse though there are apparent mixing in some time points (figure 1).

3. Methodology

3.1 Univariate Generalized Linear Mixed Models

To investigate the differences in the evolution over time (age) between TauPS2APP and wild type mice for the behaviour data, first univariate Generalized Linear Mixes Models (GLMM) (Molenberghs and Verbeke, 2005; McCulloch, 1997; Breslow and Clayton, 1993; Wolfinger and Connell, 1993) with random intercept is fitted for each of the responses separately assuming different outcomes from same mouse are independent. The GLMM is chosen due to diverse nature of the outcome measures. The outcomes distance moved in LMA and VMaze are Gaussian while total entries and number of alternation in CMAT are count data.

Let Y_{ijk} be the outcome measured for mouse i at age (time) j and genotype k, where i = 1, 2,...., 31 for young mice and i = 1, 2,...., 32 for old mice; j = 4, 6, 9 for young and mice j = 12, 15, 18, 21 for old mice; k = '1' for TauPS2APP and '0' for wild type mouse. All the outcomes of each rat were measured longitudinally. This feature of the data was taken into account by fitting generalized linear mixed model with random intercept as follows.

$$\eta_{ijk} = X_{ijk}\beta_{jk} + b_i$$

Where, the random intercept b_i is assumed to be normally distributed with mean 0 and variance τ^2. β_{jk} is the vector of unknown fixed effect parameters for behavior k at time j. η_{ijk} is the corresponding link function (identity link for continuous and log link count outcomes respectively). X_{ijk} is the known design matrix for the fixed effects. The possible correlation among the observations at different age from the same mouse is captured by mouse-specific random intercepts. The fixed part of the model is defined as follows:

$$X_{ijk}\beta_{jk} = \mu + \beta_j + \gamma_k + \delta_{jk}$$

$=$ Overall effect + age effect + effect of genotype + interaction effect between age and genotype

Where μ is the overall effect, β_j is the effect of age j, γ_k is the effect of genotype k and δ_{jk} is the interaction effect between age j and genotype k. This interaction effect is used to answer the main research question, whether there are differences in the evolution over time between the genotypes for the behaviour data or not. If the interaction effect between age and genotype is significant then we can conclude that there are differences in the evolution of performance over time between TauPS2APP and wild-type mice. Otherwise the effects might

be associated to learning effects since the outcomes are related to the performances on locomotion and cognitive behaviours of mice measured at different time points. Learning is commonly defined as the difference between initial and final levels of performance on a cognitive task (Zhang et al., 2007; Glaser, 1967; McGeoch, 1942; Woodrow, 1946). Learning effect occurs in situations where response changes logically each time subjects take a test. Learning can also be assessed by examining improvement in performance during subsequent trials (Bromley-Brits et al., 2011). The idea is that a subject usually performs sensibly in subsequent tests having learned something or gathering experience from previous test.

3.2 Joint Modelling

A flexible joint model can be obtained by modeling each outcome separately using a mixed model, by assuming that, conditionally on these random effects, the different outcomes are independent, and by imposing a joint multivariate distribution on the vector of all random effects. This approach has many advantages and is applicable in a wide variety of situations. First, the data can be highly unbalanced. For example, it is not necessary that all outcomes are measured at the same time points. Moreover, the approach is applicable for combining linear mixed models, non-linear mixed models, or generalized linear mixed models. The procedure also allows the combination of different types of mixed models, such as a generalized linear mixed model for a discrete outcome and a linear mixed model for a continuous outcome (Molenberghs and Verbeke, 2005). In section 3.1 we assumed that different outcomes from same mouse were independent. In this section the correlation among different outcomes from same mouse are captured by modelling the responses jointly first by fitting pair-wise and then combined all the responses together.

3.2.1 Multivariate Generalized Linear Mixed Models

In multivariate GLMM, all the univariate models can be jointly modeled by specifying a joint distribution for the random effects. Let Θ^* be the vector containing all parameters (fixed effects parameters as well as covariance parameters). $l_i(\Theta^* | \mathrm{Y_{1i}}, \mathrm{Y_{2i}}, \ldots., \mathrm{Y_{mi}})$ then refers to the log-likelihood contribution of subject i to the full joint mixed model. Likelihood-based inference for fitting GLMMs can be used to obtain parameter estimates for this joint model (Fieuws et al., 2006). Using the same notation applied in section 3.1, a joint generalized linear mixed model for m responses $\mathrm{Y_{ijkm}}$ (m = 1, 2,...,5) can simultaneously be specified as

$$\begin{cases} \eta_{ijk1} = X_{ijk1}\beta_{jk1} + b_{i1}, & for\ response\ Y_{ijk1} \\ \eta_{ijk2} = X_{ijk2}\beta_{jk2} + b_{i2}, & for\ response\ Y_{ijk2} \\ \quad . & \quad . \\ \quad . & \quad . \\ \quad . & \quad . \\ \quad . & \quad . \\ \eta_{ijkm} = X_{ijkm}\beta_{jkm} + b_{im}, & for\ response\ Y_{ijkm} \end{cases}$$

The vector b_i of all random effects for mouse i is multivariate normal with mean 0 and covariance D, ie.,

$$b_i = \begin{pmatrix} b_{i1} \\ b_{i2} \\ \vdots \\ b_{im} \end{pmatrix} \sim N\left\{ \begin{pmatrix} 0 \\ 0 \\ \vdots \\ 0 \end{pmatrix}, D = \begin{pmatrix} \tau_1^2 & \rho_{12}\tau_1\tau_2 & \cdots & \rho_{1m}\tau_1\tau_m \\ \rho_{21}\tau_2\tau_1 & \tau_2^2 & \cdots & \rho_{2m}\tau_2\tau_m \\ \vdots & \vdots & \ddots & \vdots \\ \rho_{m1}\tau_m\tau_1 & \rho_{m2}\tau_m\tau_2 & \cdots & \tau_m^2 \end{pmatrix} \right\}$$

It is assumed that, conditionally on the random effects$b_{i1}, b_{i2}, \ldots, b_{im}$ the m outcomes $Y_{ijk1,}$ $Y_{ijk2,\ldots..}$, Y_{ijkm} are independent. The fixed part of the model is defined as follows:

$$X_{ijkm}\beta_{jkm} = \mu_m + \beta_{jm} + \gamma_{km} + \delta_{jkm}$$

Where μ_m is the overall effect, β_{jm} is the effect of age j, γ_{km} is the effect of genotype k and δ_{jkm} is the interaction effect between age j and genotype k on the behaviour m.

3.2.2 Pairwise Fitting of Joint Model

In high dimensional joint model computational problems due to the dimension of the joint covariance matrix of the random effects arise as soon as the number of outcomes increases. This problem can be solved by applying pairwise approach in which all possible bivariate models are fitted, and where inference follows from pseudo-likelihood arguments. The approach is applicable for linear, generalized linear, and nonlinear mixed models, or for combinations of these (Fieuws and Verbeke, 2006).

In the pairwise approach proposed by Fieuws et al. (2006), assuming that the multivariate GLMM is specified correctly, all bivariate GLMMs are correct. Within a maximum likelihood framework, each bivariate GLMM then yields consistent estimates with classical asymptotic properties. Some estimates from different bivariate GLMMs refer to the same parameter in Θ^*. For example, if a parameter is specific to only a set of items, then there will be m−1 estimates for this parameter. To obtain one single estimate for the parameters in Θ^*, averages will be taken over the estimates that are obtained from the different bivariate

GLMMs. These averages of maximum likelihood estimates are asymptotically normally distributed with the parameter value in Θ^* as the mean. Formally, in the first step log-likelihoods of the following form will be maximized separately:

$$\sum_{i=1}^{N} l_{rsi}(\Theta_{r,s}|Y_{ri}, Y_{si}),$$

where r=1, . . . ,m−1 and s=r+1, . . . ,m, and N denotes the total number of subjects. $\Theta_{r,s}$ represents the vector of all parameters in the bivariate GLMM corresponding to the specific pair (r, s). Let Θ be the stacked vector combining all pair-specific parameter vectors $\Theta_{r,s}$. Estimates for the elements in Θ are obtained by maximizing each of the m(m−1)/2 likelihoods separately. Fitting all possible pairwise models is equivalent to maximizing a function of the form

$$pl(\Theta) = l(\Theta_{1,2}|Y_1, Y_2) + l(\Theta_{1,3}|Y_1, Y_3) + \cdots + l(\Theta_{m-1,m}|Y_{m-1}, Y_m)$$

Although each part in equation above is maximized separately, its form (a joint log-likelihood replaced by a sum of log-likelihoods) is typically encountered within pseudo-likelihood theory (Arnold and Strauss, 1991; Geys *et al*., 1997). Therefore, results from pseudo-likelihood theory can be used for inference for Θ. The asymptotic multivariate normal distribution for $\widehat{\Theta}$ is given by

$$\sqrt{N}\,(\widehat{\Theta} - \Theta) \sim MNV(0, J^{-1}K\,J^{-1})$$

where $J^{-1}K\,J^{-1}$ is a 'sandwich-type' robust variance estimator. where J is a block diagonal matrix with diagonal blocks Jpp and K is a symmetric matrix containing blocks Kpq, with p, q=1, . . . , m(m−1)/2. These blocks are given by

$$J_{pp} = -\frac{1}{N}\sum_{i=1}^{N} E\left(\frac{\partial^2 l_{pi}}{\partial\theta_p\,\partial\theta_p'}\right) \text{ and } K_{pq} = \frac{1}{N}\sum_{i=1}^{N} E\left(\frac{\partial l_{pi}\,\partial l_{qi}}{\partial\theta_p\,\partial\theta_q'}\right)$$

Estimates are obtained by dropping the expectations and replacing the unknown parameters by their estimates. The specific expression for the first- and second-order derivatives in Kpq and Jpp respectively will depend on the link function and the covariates in the GLMM. At this stage we have an estimate and a distribution for the parameter vector Θ, but our interest lies in the parameter vector Θ^*. Note that these parameter vectors are not equivalent. Some parameters in Θ^* will have a single counterpart in Θ, whereas other elements in Θ^* will have multiple counterparts in Θ. Therefore estimates for the parameters in Θ^* are obtained by taking averages over all pairs. The estimate is therefore of the form $\widehat{\Theta}^* = A\widehat{\Theta}$, for an appropriate weight matrix A, from which it follows that Θ^* follows a multivariate normal

distribution with mean Θ^* and covariance matrix $A\Sigma(\widehat{\Theta})A'$, where $\Sigma(\widehat{\Theta})$ equals the covariance matrix for $\widehat{\Theta}$ obtained from distribution. Approximate Wald tests can then be constructed in a classical way to test for any linear combination of the parameters in Θ^* (Fieuws et al., 2006).

3.2.2.1 Poisson-Normal Joint Model

Let Y_{ijkm}, the mth count outcome from mouse i at time (age) j and genotype k, follows a Poisson distribution with mean μ_{ijkm} and $Y_{ijkm'}$, the m'th continuous outcome from mouse i at time (age) j and genotype k, follows a Normal distribution with mean $\mu_{ijkm'}$ and variance $\sigma^2_{m'}$ $(m \neq m')$. That is,

$Y_{ijkm}|b_{im} \sim ind.Poisson\left(\mu_{ijkm}\right) and\ Y_{ijkm'}|b_{im'} \sim ind.N(\mu_{ijkm'},\ \sigma^2_{km'})$.

Therefore, the correlated random intercept model is specified as

$\log(\mu_{ijkm}) = X_{ijkm}\beta_{jkm} + b_{im}$ …… for count outcome

$\mu_{ijkm'} = X_{ijkm'}\beta_{jkm'} + b_{im'}$ …….. for normal outcome

Where, $b_i = \begin{pmatrix} b_{im} \\ b_{im'} \end{pmatrix} \sim N\left\{\begin{pmatrix} 0 \\ 0 \end{pmatrix}, D = \begin{pmatrix} \tau^2_m & \rho\tau_m\tau_{m'} \\ \rho\tau_m\tau_{m'} & \tau^2_{m'} \end{pmatrix}\right\}$.

The random effects b_{im} and $b_{im'}$ are used to accommodate the longitudinal structure in the data.

3.2.2.2 Joint Models for Bivariate Normal Outcomes

Let Y_{ijkm} and $Y_{ijkm'}$ are two different outcomes from mouse i at time (age) j and genotype k; $(m \neq m')$. For capturing the longitudinal structure of the data it is assumed that given the corresponding random effects b_{im} and $b_{im'}$, the two outcomes Y_{ijkm} and $Y_{ijkm'}$ are independent and follow normal distribution with mean and variance μ_{ijkm} ; σ^2_m and $\mu_{ijkm'}$; $\sigma^2_{m'}$ respectively. That is,

$$Y_{ijkm}|b_{im} \sim ind.N\left(\mu_{ijkm},\ \sigma^2_m\right) and\ Y_{ijkm'}|b_{im'} \sim ind.N\left(\mu_{ijkm'}, \sigma^2_{m'}\right).$$

Therefore, the general linear mixed model with correlated random intercept is specified as

$$\begin{cases} Y_{ijkm} = X_{ijkm}\beta_{jkm} + b_{im} + \varepsilon_{ijkm} \\ Y_{ijkm'} = X_{ijkm'}\beta_{jkm'} + b_{im'} + \varepsilon_{ijkm'} \\ b_i = \begin{pmatrix} b_{im} \\ b_{im'} \end{pmatrix} \sim N\left\{ \begin{pmatrix} 0 \\ 0 \end{pmatrix}, D = \begin{pmatrix} \tau_m^2 & \rho\tau_m\tau_{m'} \\ \rho\tau_m\tau_{m'} & \tau_{m'}^2 \end{pmatrix} \right\} \\ \varepsilon_{ij} \sim N(0,\ \Sigma_{ij}) \end{cases}$$

3.2.2.3 Joint Models for Bivariate Poisson Outcomes

Let Y_{ijkm} and $Y_{ijkm'}$ are two different outcomes from mouse i at time (age) j and genotype k; $(m \neq m')$. For capturing the longitudinal structure of the data it is assumed that given the corresponding random effects b_{im} and $b_{im'}$, the two outcomes Y_{ijkm} and $Y_{ijkm'}$ are independent and follow Poisson distribution with mean μ_{ijkm} and $\mu_{ijkm'}$ respectively. That is,

$$Y_{ijkm}|b_{im} \sim ind.Poisson\ (\mu_{ijkm})\ and\ Y_{ijkm'}|b_{im'} \sim ind.Poisson\ (\mu_{ijkm'}).$$

Therefore, the generalized linear mixed model with correlated random intercept is specified as

$$\begin{cases} \log(\mu_{ijkm}) = X_{ijkm}\beta_{jkm} + b_{im} \\ \log\ (\mu_{ijkm'}) = X_{ijkm'}\beta_{jkm'} + b_{im'} \\ b_i = \begin{pmatrix} b_{im} \\ b_{im'} \end{pmatrix} \sim N\left\{ \begin{pmatrix} 0 \\ 0 \end{pmatrix}, D = \begin{pmatrix} \tau_m^2 & \rho\tau_m\tau_{m'} \\ \rho\tau_m\tau_{m'} & \tau_{m'}^2 \end{pmatrix} \right\} \end{cases}$$

3.2.2.4 Steps in combining results from pairwise models

As described in section 3.2.2, let Θ^* be the vector containing all parameters of full multivariate joint mixed model and Θ be the stacked vector combining all pair-specific parameter vectors Θ_P. Then asymptotic distribution of maximum pseudo likelihood estimator $\widehat{\Theta}$ is given by $\sqrt{N}\,(\widehat{\Theta} - \Theta) \sim MNV(0, J^{-1}K\,J^{-1})$. In convenient notation, $\hat{J} = \frac{1}{N}H$ and $\hat{K} = \frac{1}{N}G\,G^T$; Where $H = \begin{pmatrix} H_1 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & H_P \end{pmatrix}$, $G = \begin{pmatrix} G_1 \\ \vdots \\ G_P \end{pmatrix}$, N denotes the number of subjects, P denotes specific pairwise model, H_p is the hessian matrix and G_P be the accumulated subject specific gradient vectors obtained from pth pairwise model.

At the first stage of combining results, the block diagonal matrix H and symmetric matrix G as well as $\hat{\mathrm{J}}$ and $\widehat{\mathrm{K}}$ are calculated using $H_1, \ldots, H_P$ and $G_1, \ldots, G_P$ obtained from all the pairwise models. In second step, all the estimates of parameters obtained from pairwise model are stacked on the vector $\widehat{\Theta}$ and its covariance matrix is calculated using the formula $\Sigma(\widehat{\Theta}) = \frac{1}{N}(\mathrm{J}^{-1}\mathrm{K}\,\mathrm{J}^{-1})$. Appropriate coefficient matrix A is then constructed in such a format that one can get the average of the coefficients of pairwise model by using the formula $\widehat{\Theta}^{*}$=A$\widehat{\Theta}$. Hence A$\widehat{\Theta}$ and $A\Sigma(\widehat{\Theta})\mathrm{A}'$ respectively provided the desired estimates of the parameters of full multivariate joint mixed model and their robust standard errors. Finally covariance parameters were combined and the correlations between random intercepts were calculated. In SAS, the programs were written in IML to combine the results of pairwise models.

3.2.2.5 Pseudo-likelihood ratio statistics

To investigate the overall effect, that is the significance of fixed effect parameters on overall responses, pseudo-likelihood ratio statistics was used. As proposed by Faes et al. (2008), suppose we are interested in testing the null hypothesis $H_0: \gamma = \gamma_0$where γ is an *r*-dimensional subvector of the *p*-dimensional vector of regression parameters β and write β as $(\gamma^T, \delta^T)^T$. Then, the pseudo-likelihood ratio test statistic, given by

$$G_a^{*^2} = 2\left[PL(\widehat{\beta_{\mathrm{N}}}) - \mathrm{PL}\left(\gamma_0, \hat{\delta}(\gamma_0)\right)\right]/\bar{\lambda}$$

is approximately χ_r^2 distributed. $\widehat{\beta_{\mathrm{N}}}$ is the pseudo-likelihood parameter estimate of β and $\hat{\delta}(\gamma_0)$ denotes the maximum pseudo-likelihood estimator in the subspace where $\gamma = \gamma_0$. Furthermore, $\bar{\lambda}$ is the mean of the eigenvalues of $(J^{\gamma\gamma})^{-1}\Sigma_{\gamma\gamma}$, where $J^{\gamma\gamma}$ is the r×r submatrix of inverse of J and Σ_{rr} is the submatrix of $\Sigma = \mathrm{J}^{-1}\mathrm{K}\,\mathrm{J}^{-1}$.

3.3 Statistical Software

SAS version 9.2 and R version 2.15.2 were used to analyze the data.

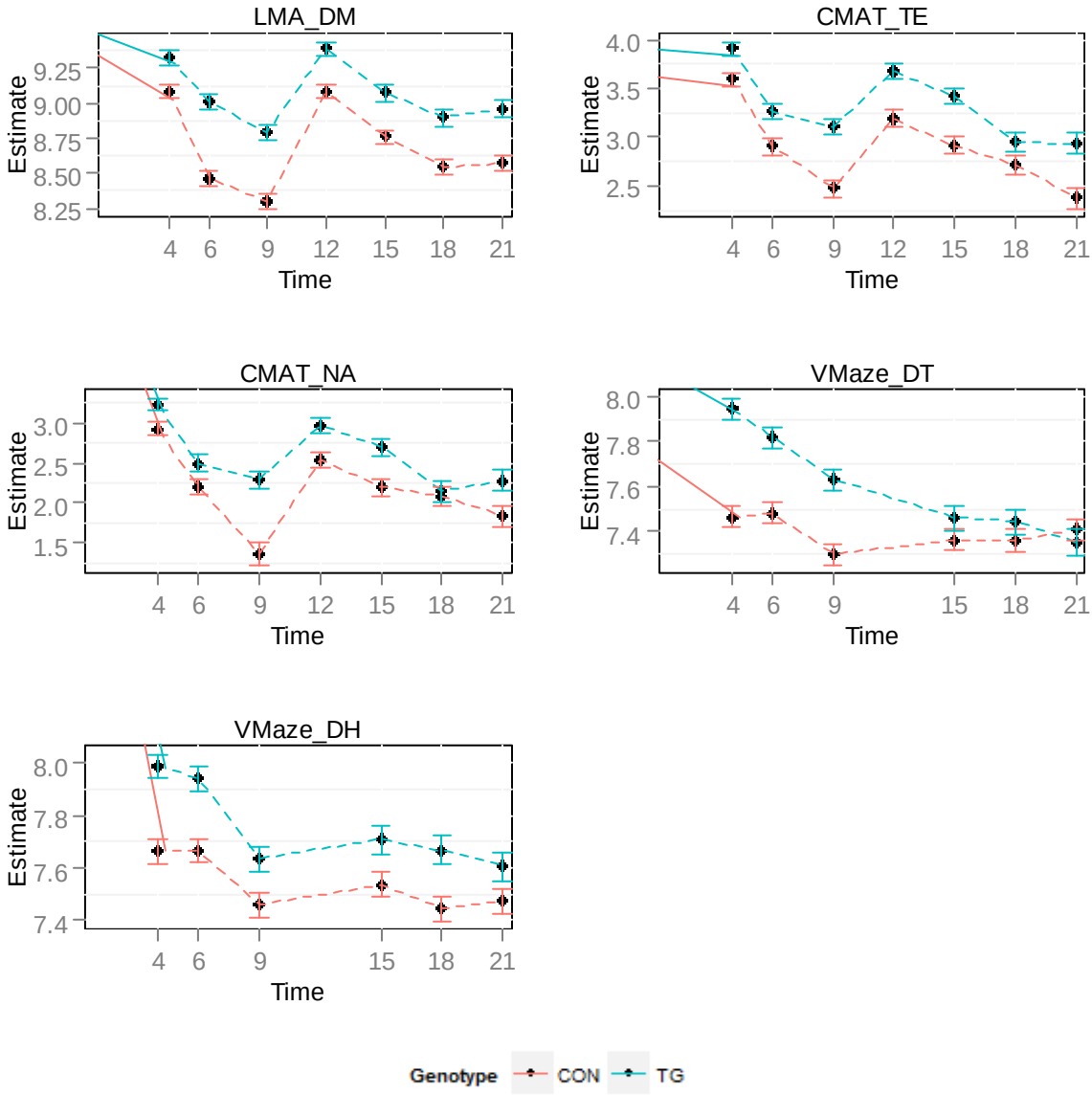
4. Results

4.1 Univariate Generalized Linear Mixed Models

In the first stage of modelling mice behaviours, generalized linear mixes models (GLMM) with unstructured fixed effect parameters are fitted for each of the five outcomes separately assuming them independent. The longitudinal structure of the data is taken into account by incorporating random intercept. Although random slope was plausible, it was not incorporated due to the problem of non-convergence. The reason for fitting models with unstructured fixed effect parameters is to get estimates for both genotypes at each time point which enables one to construct and test any particular type of contrast. The estimates with standard error bar of TauPS2APP and wild-type mice at each time point for all the five behaviors are plotted in figure 2.

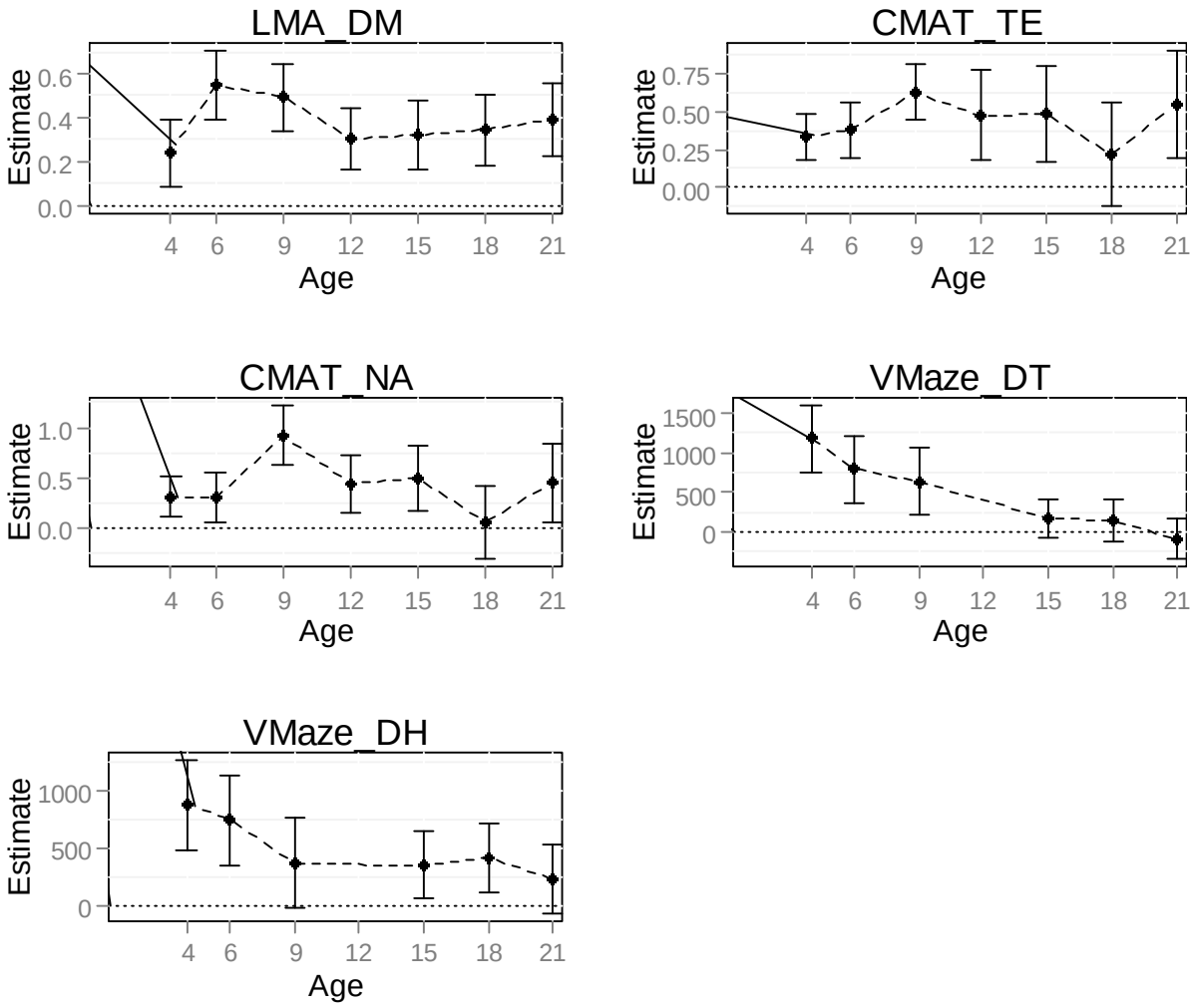
The figure shows that the average evolution is decreasing over time. TauPS2APP mice are evolving consistently higher than wild-type except age 21 for VMaze_DT and age 18 for CMAT_NA. If we draw separate trend line from age 4 to 9 months and from 12 to 21 months, we will observe two parallel lines for young and old mice. The performance of young mice is falling down more rapidly than old one. At subsequent trials, learning from their past behavior in addition to becoming more familiar with the environment, mice are reducing their movement and other cognitive activities. This indicates an apparent learning effect in the data.

Figure 2: Estimates with Standard Error Bar of Taups2app and Wild-Type Mice at Each Time Point



At this point it is not yet possible to decide on the significance of this difference. It is useful to explore the difference between genotype separately since even when both evolutions might be complicated, the difference in performance, which is of primary interest, could follow a simple model and vice versa (Verbeke and Molenberghs, 2002). The estimated difference (with 95% confidence interval) in behaviour measures between TauPS2APP and wild-type mice for five behaviors are plotted against time in figure 3.

Figure 3: Estimated Difference (with 95% CI) in Behaviors between TauPS2APP and Wild-type Mice



Significant differences between the estimates of TauPS2APP and wild-type mice are observed except the age 18 months for Cmat behaviors, 9 months for VMaze_DH and for old mice in case VMaze_DT . However, these confidence intervals are not adjusted for multiple testing; hence results might be misleading.

To have a valid inference about the significant differences between TauPS2APP and wild-type mice in the evolution of behaviours over time, we should see the interaction effect between age and genotypes from the fitted models. The P-values for the fixed effects obtained from fitted univariate GLMM for all the five behaviors are presented in table 3.

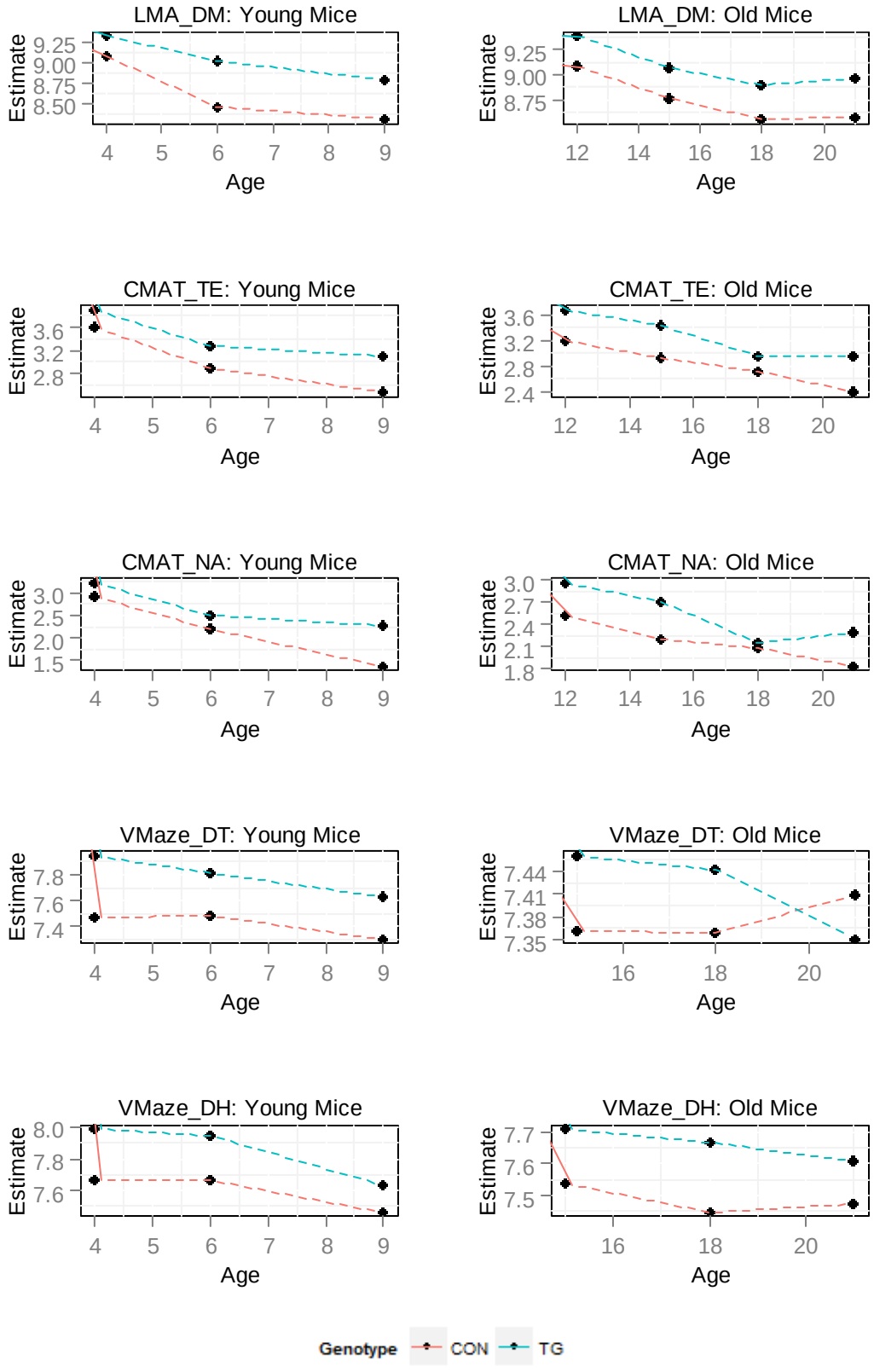
Table 3: P-values for the fixed effects obtained from fitted univariate GLMM

Mouse type	Effect	P-value				
		LMA_DM	**CMAT_TE**	**CMAT_NA**	**VMaze_DT**	**VMaze_DH**
Young	Age	<.0001	<.0001	<.0001	<.0001	<.0001
	Genotype	<.0001	<.0001	<.0001	<.0001	0.0006
	Age*Genotype	0.0055	0.0546	0.0042	0.0012	0.0041
Old	Age	<.0001	<.0001	<.0001	0.8175	0.0568
	Genotype	<.0001	0.0038	0.0109	0.4263	0.0078
	Age*Genotype	0.7592	0.1005	0.0846	0.1704	0.4288
Overall	Age	<.0001	<.0001	<.0001	<.0001	<.0001
	Genotype	<.0001	<.0001	<.0001	<.0001	<.0001
	Age*Genotype	0.0131	0.0656	0.0075	<.0001	0.0056

Table 3 show significant evidences of difference in the evolution over time between genotypes for all the behaviors except border line significant for total number of entries in CMAT. Similar conclusions can be drawn from the combined data of young and old mice since the interaction between age and genotypes is significant for all the behaviours except total number of entries in Cmat. The effect of age and genotype are found significant for all the behaviors for young mice as well as combined data of young and old mice; while these effects are significant for locomotion activity and Cmat behaviors of old mice.

Further investigation is done to see whether there is learning effect in the mice behaviors data. The estimates of performances at each time point are plotted separately for young and old mice in figure 4.

Figure 4: Estimates of performances at Each Time Point for Young and Old Mice



From figure 4 it is observed that for both the genotypes and both young and old mice performances in behaviour measure are decreasing rapidly with increase in time. For young mice performances are decreasing quickly compared to old mice. Moreover, the starting points are more or less similar for both old and young mice. This is a clear indication of learning effect. If there would not have learning effect in the data then obviously the starting point of old mice would be at the end point of young regarding performance. Moreover, parallel evolution of performances of both young and old mice also rendering the present of learning effect in the data.

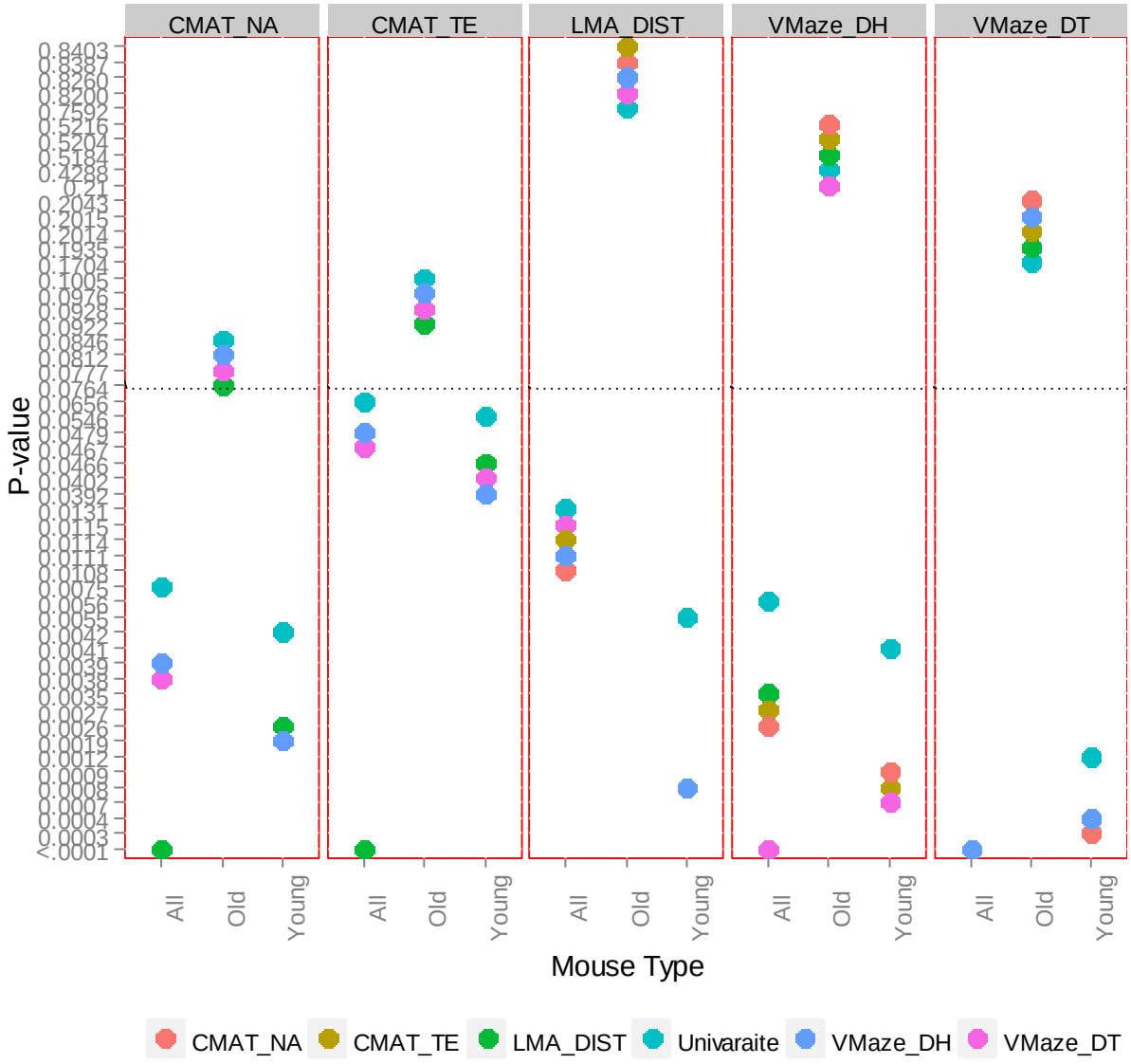
4.2 Joint Modelling

Although estimates of the parameters of a joint models can be obtained by using maximum likelihood estimation, due to the computational complexity of five dimensional random intercepts, 10 pairwise models are fitted and the pseudo-likelihood theory is used to combined the all pairwise estimates as well as to obtain their corresponding sandwich-type robust standards errors. The steps followed to combine the estimates and standard errors from all the pair wise models to make one set of estimates are described in section 3.2.2.5.

4.2.1 Pairwise fitting of joint models

In this section the correlation among different outcomes from same mouse are captured by modelling the responses jointly by fitting them pair-wise to avoid computational problems due to non-convergence of more than two dimensional joint models. For each of the fitted pair-wise models, differences in the evolution of responses over time between the genotypes of a specific behaviour, is investigated through the interaction effect between age and genotypes using same model as univariate analysis. The difference of pairwise models from univariate is that here the correlation between two outcomes is captured through the correlation between their random intercepts. The p-values for the interaction effects between age and genotypes obtained from all the pair-wise models fitted for each of the behaviours are plotted in figure 5. The p-values for all, young and old mice are plotted in three different columns respectively. P-values obtained from univariate modelling are also plotted for the purpose of comparison.

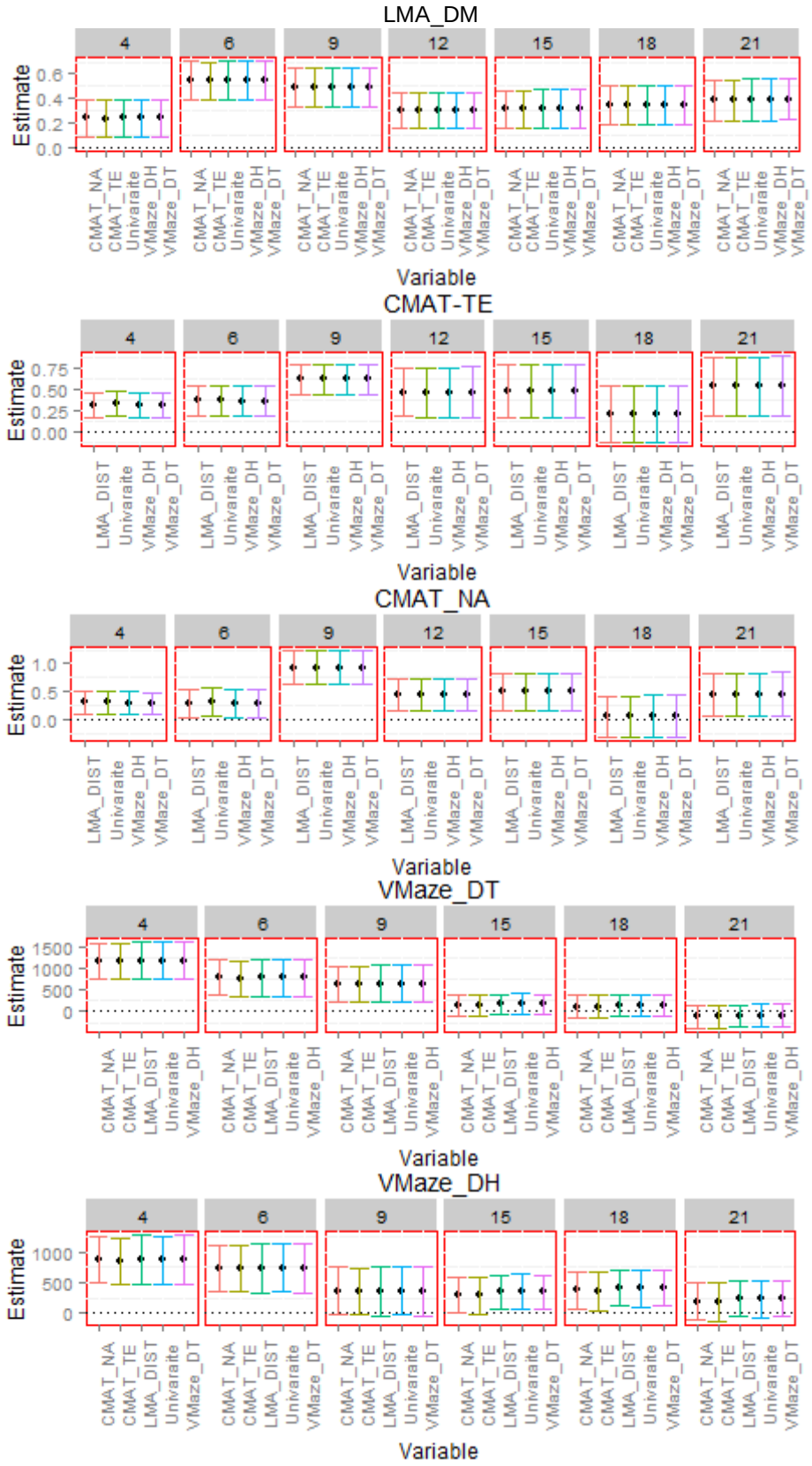
Figure 5: P Values from Joint Models (Pair-Wise) for Differences in the Evolution between Genotypes



In most of the cases p values obtained from pairwise models are lower than those of univarite models, while for some of the cases they are more or less similar which is a clear signal of the benefit of joint modelling. In line with univariate analysis significant differences are observed in the evolutions of almost all behaviours between TauPS2APP and wild-type mice for combined data. For young mice differences in the evolution of performance between genotype over time are found significant for all the five behaviors. Joint models also show no evidence of difference in the evolutions for old mice. Inference from joint modelling might be more reliable for the reason that it gives precise estimates correcting for correlation among different outcomes from same subject.

Estimated difference in performances (with 95% confidence intervals) between genotypes obtained from pair-wise models for each of the behaviors are plotted in figure 6 along with

Figure 6: Estimated Difference in Performance between Genotype for Joint (Pair-wise) Models



independent estimates to see if there is mentionable difference between two types of modelling techniques. There is no mentionable difference observed among the estimates of pairwise models.

Applying the theory of pseudo likelihood estimation, the parameter estimates and their standard errors obtained from pairwise fitted models were combined in single estimate for each. The estimates were simply averaged. The combined estimates of the fixed effect parameters of multivariate joint mixed model and their robust standard errors using combined data of young and old mice for the behavior VMaze_DH is presented in table 4 and table 8 in appendix for other behaviors.

Table 4: Parameter estimates and standard errors of joint model for VMaze_DH

Effect	Joint Model		Univariate Model	
	Estimate	SE	Estimate	SE
Intercept	1182.19	77.379	1790.55	128.22
Age 4	367.52	141.828	361.76	175.20
Age 6	368.90	156.056	363.14	175.20
Age 9	-30.24	145.603	-35.9993	175.20
Age 15	112.15	114.664	107.67	103.62
Age 18	-50.34	99.092	-50.3415	104.72
Genotype	184.82	156.204	237.73	195.62
Age 4* Genotype	675.12	257.350	635.45	260.25
Age 6* Genotype	539.22	260.502	499.56	260.25
Age 9* Genotype	168.54	243.071	131.85	261.16
Age 15* Genotype	106.50	121.774	111.04	157.45
Age 18* Genotype	179.64	127.029	179.64	158.81

From the results of multivariate joint model it is observed that the parameter estimates are more precise than the univariate estimates. In terms of inference there is no mentionable difference but in terms of precision joint model is better than independent models.

The significance of fixed effect parameters on overall responses is tested by using pseudo-likelihood ratio statistics. Results are presented in table 5.

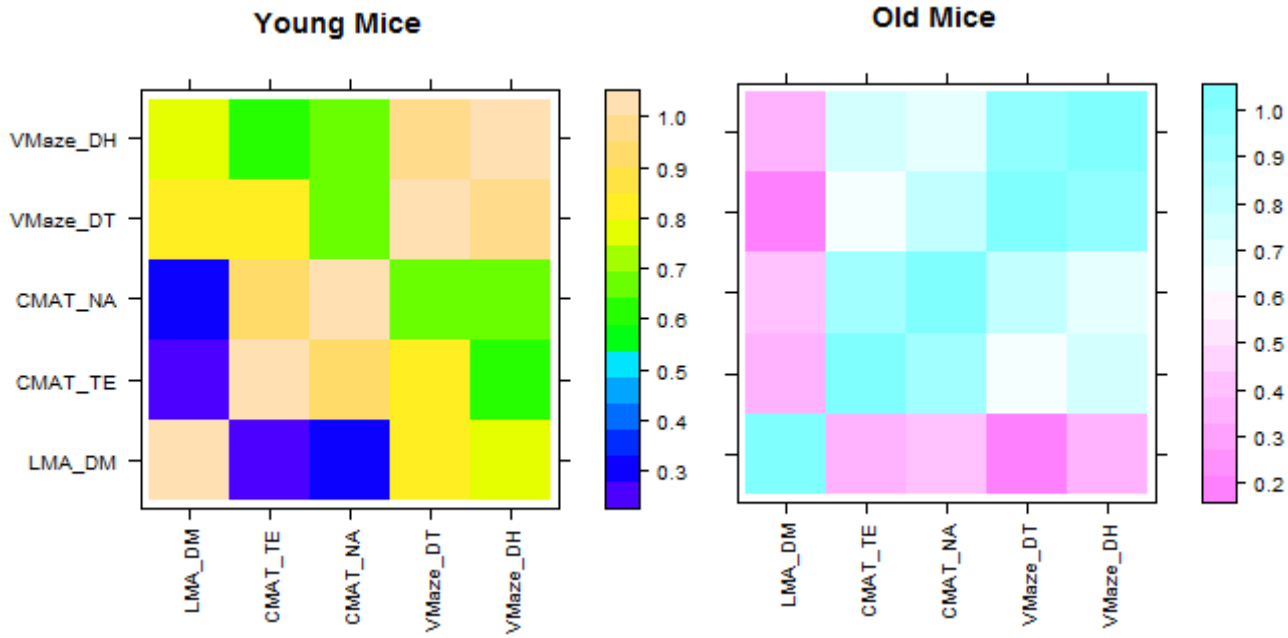
Table 5 : Pseudo Likelihood Ratio Statistic and P-value for overall effect

Effect	Pseudo Likelihood Ratio Statistic (G_a^{*2})	P-value
Age	1569.261	<0.0001
Genotype	319.387	<0.0001
Age*Genotype	125.545	0.0007

Considering all the responses together, we observe significant differences in the evolution of performance over time between TauPS2APP and wild-type mice. The age and genotype effects are also found to have significant on overall behaviors.

The correlations between random intercepts incorporated for modeling different behaviors are presented through the following image plots (figure 7).

Figure 7: Image Plot for Correlation between Random Intercepts in Pair-wise Modelling



These correlations can be interpreted as the association between the individual deviations from the overall profile. There is almost perfect correlation between the intercepts of distance moved in habituation and test phase in VMaze for both young and old mice which lead to a shared random intercept model. Moreover, the intercepts of VMaze behaviors are highly correlated with other behaviors except LMA_DM for old mice. For young mice except locomotion activity and Cmat behaviors, correlations among random intercepts for all other

behaviors are significant. While, for old mice correlation between random intercepts of all behaviors, except the correlation between random intercept of LMA_DM and VMaze_DT, are significant. Based on the estimated variance-covariance matrix of the random effects, one can estimate the correlation between the different outcomes. These correlations are much smaller when compared with the correlation between the random effects (Faes et al., 2008).

5. Discussion and Conclusion

Alzheimer's disease is the most common form of dementia and is characterized by progressive neuronal degeneration with depositions of amyloid plaques and neurofibrillary tangles (Hölttä et al., 2013; Ozmen et al., 2009; Blennow et al., 2006). The likelihood of having Alzheimer's disease increases substantially after the age eighty. TauPS2APP triple transgenic mice was created that express human mutated Amyloid Precursor Protein, presenilin 2 and Tau. The TauPS2APP triple transgenic mouse model is very useful for studying the effect of new therapeutic paradigms on amyloid deposition and downstream neurofibrillary tangle development (Grueninger et al., 2010).

The aim of the study was to investigate the differences in the evolution over time between genotypes for the behaviour data as well as to examine if the effects observed only associated to learning effects. Moreover, to fit joint model combining outcomes for investigating if there is any benefit in such a joint modeling compared to a univariate analysis. Data for this study was extracted from an experiment on alzheimer's disease where different behaviours of TauPS2APP and wild type mice were assessed longitudinally. Two datasets consisting information on different activities of 5 tests for young and old mice were combined. To investigate the differences in the evolution over time between the TauPS2APP and wild type mice for the behaviour data, univariate generalized linear mixes models (GLMM) with random intercept was first fitted for each of the responses separately assuming different outcomes from same mouse are independent. The longitudinal feature was taken into account by incorporating random intercept in the model. The unstructured fixed effect parameters at each age for both genotypes separately were estimated to notice the evolution of performance of different behaviors mice over time. The correlations among different outcomes from same mouse are captured by modelling the responses jointly. Mixed models are widely used in the literature for the analysis of single outcome variable, measured repeatedly over time. We can denote this standard situation as the analysis of univariate longitudinal data. Multivariate longitudinal data arise when a set of different responses on the same unit are measured repeatedly over time. In such a case a mixed model can be used for each response variable, separately. However, this strategy is not useful to answer the research questions whether the evolution of one response is related to the evolution of another response. Even to see how the association between responses evolves over time, a joint modeling strategy is needed (Kundu, 2011).

Five outcomes of different nature which might lead to several difficulties to model them jointly; the most important one is the issue of convergence. It arises computational problems due to the dimension of the joint covariance matrix of the random effects. It is very difficult to model more than three variables jointly. The solution is to fitting them pairwise and then combining the estimates using the method proposed by Fieuws et al. (2006), where inference follows from pseudo likelihood approach and interested hypotheses could be tested using approximate Wald type test. The principal idea of the pseudo-likelihood methodology is to replace the computationally cumbersome likelihood by a function set up as the product of easy-to-compute functions, but which is no longer equal to the full likelihood. The method can be used for arbitrary combinations of outcome types (Faes et al. 2008). The application of pairwise modelling strategy to obtain parameter estimates of high dimensional GLMMs has many advantages. First, the strengths of the random-effects approach for joint modelling are kept. For example, insight can be gained in the association structure of the latent traits. Also, discarding subjects from the analysis due to missing observation or considering questionable imputation techniques is not needed. Second, no strong *a priori* (unidimensionality) assumption about the covariance structure of the random effects needs to be made, thereby avoiding potential biases in the fixed effects estimates. Finally, high dimensional integrational problems are avoided. As such, the complicated five-dimensional integration problem in the application has been reduced to a set of feasible two-dimensional integrations. the pairwise approach will be valid as soon as missingness at random holds in each bivariate GLMM. (Fieuws et al., 2006). The pairwise approach is more than an valuable alternative for the Bayesian Markov chain Monte Carlo methods that have been proposed for multidimensional item response theory models (Bolt and Lall, 2003; Beguin and Glass, 2001).

Findings show significant evidences of difference in the evolution over time between genotypes for almost all the behaviors of young mice as well as for combined data. Difference in the evolution is not significant for old mice might be symptoms of Alzheimer's disease in genetically modified mice. As AD is age related the old mice start suffering from cognitive impairment which leads to disability of performing as like as they did in young age. The effect of age and genotype are found significant except Vmaze behavior of old mice. There is apparent learning effect in the data. For all the behaviors the starting points are more or less equal for both old and young mice but performance of youg mice is decreasing rapidly

with age as compared to old mice. This is the indication of learning effect. Considering all the responses together in multivariate joint model, significant differences are found in the evolution of performance over time between TauPS2APP and wild-type mice. Age and genotype have significant effect on overall behaviors. Parameter estimates from joint model are also more precise than the univariate estimates. The estimates of Cmat behaviors sometimes demonstrated strange result since for some of the mouse IDs of young mice at age 9 months, there are measurements for both the genotypes which is not realistic. Nevertheless this problem could not be solved throughout the short interval of this study period. In the presence of missing observations, it is sensible to incorporate the techniques dealing with missing data. However this issue is beyond the scope of this thesis.

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7. Appendix

Figure 8: parameter estimates and standard errors from univariate models and joint model

Behaviour	Effect	Joint Model		Univariate Model	
		Estimate	SE	Estimate	SE
LMA_DM	Intercept	8.48	0.060	8.5719	0.05701
	Age 4	0.51	0.069	0.5111	0.07732
	Age 6	-0.11	0.074	-0.1087	0.07732
	Age 9	-0.26	0.090	-0.2686	0.07732
	Age 12	0.51	0.057	0.5117	0.06416
	Age 15	0.19	0.062	0.1852	0.06477
	Age 18	-0.03	0.061	-0.02189	0.06555
	Genotype	0.39	0.084	0.3897	0.08824
	Age 4*genotype	-0.15	0.112	-0.1522	0.1159
	Age 6*genotype	0.15	0.116	0.1580	0.1159
	Age 9*genotype	0.09	0.121	0.1023	0.1166
	Age 12*genotype	-0.09	0.085	-0.08741	0.09756
	Age 15*genotype	-0.08	0.094	-0.07232	0.1007
	Age 18*genotype	-0.05	0.075	-0.04436	0.1021
CMAT_TE	Intercept	2.35	0.145	2.3790	0.1072
	Age 4	1.20	0.156	1.2111	0.1285
	Age 6	0.52	0.177	0.5230	0.1350
	Age 9	0.10	0.174	0.09387	0.1392
	Age 12	0.81	0.133	0.8131	0.09794
	Age 15	0.53	0.144	0.5369	0.1025
	Age 18	0.32	0.187	0.3282	0.1072
	Genotype	0.56	0.206	0.5597	0.1487
	Age 4*genotype	-0.25	0.226	-0.2410	0.1727
	Age 6*genotype	-0.20	0.255	-0.1951	0.1808
	Age 9*genotype	0.06	0.242	0.06611	0.1752
	Age 12*genotype	-0.07	0.176	-0.07199	0.1315
	Age 15*genotype	-0.05	0.199	-0.05518	0.1378
	Age 18*genotype	-0.32	0.224	-0.3216	0.1483
CMAT_NA	Intercept	1.81	0.177	1.8343	0.1289
	Age 4	1.08	0.189	1.0906	0.1539
	Age 6	0.37	0.214	0.3675	0.1648
	Age 9	-0.45	0.242	-0.4638	0.1882
	Age 12	0.69	0.159	0.6980	0.1303
	Age 15	0.36	0.199	0.3612	0.1386
	Age18	0.24	0.216	0.2478	0.1429
	Genotype	0.45	0.238	0.4528	0.1813
	Age 4*genotype	-0.16	0.257	-0.1452	0.2128
	Age 6*genotype	-0.17	0.299	-0.1524	0.2271

Behaviour	Effect	Joint Model		Univariate Model	
		Estimate	SE	Estimate	SE
	Age 9*genotype	0.46	0.313	0.4657	0.2369
	Age 12*genotype	-0.02	0.222	-0.01721	0.1784
	Age 15*genotype	0.05	0.263	0.05083	0.1895
	Age 18*genotype	-0.39	0.291	-0.3911	0.2053
Vmaze_DT	Intercept	1651.63	90.829	1668.74	131.38
	Age 4	90.05	108.999	87.1132	179.67
	Age 6	121.04	111.247	118.41	179.67
	Age 9	-171.90	118.900	-177.48	179.67
	Age 15	-75.68	90.191	-80.0351	103.37
	Age 18	-78.86	83.894	-79.6615	104.40
	Genotype	-123.45	137.011	-92.4447	200.52
	Age 4*genotype	1278.21	265.839	1265.93	266.93
	Age 6*genotype	894.17	228.161	878.01	266.93
	Age 9*genotype	748.51	258.720	733.19	267.82
	Age 15*genotype	253.63	135.228	259.32	157.04
	Age 18*genotype	230.73	178.485	233.06	158.33

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