Some Alternatives to Likelihood Ratio and Wald Tests for Pharmacogenetic Studies using Nonlinear Mixed Effect Models

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8 June 2011

Context

Pharmacogenetics is the study of DNA variations on genes coding for proteins involved in drug absorption, distribution, metabolism, elimination and effect in relation to the inter-individual variability in drug response [1]

- Increasing availability of pharmacogenetic data
  - selection of metabolic pathways during drug development
  - individualized therapy
  - integration of diversity in population genetics
- Statistical analyses
  - ANOVA-based approach on derived PK parameters
    - loss of information provided by the complete time profile
    - does not account for additional effects or interactions
    - no direct predictions or dosing recommendations

  ↪ Nonlinear Mixed effect models (NLMEM)

[1] Licinio et Wong, 2002; Kalow et al., 2001

Asymptotic tests in NLMEM

- A biallelic single nucleotide polymorphism (SNP)
  - common, rare homozygotes and heterozygotes
  - effect on pharmacokinetic parameter ϕ_i
  - genotypic model

$$\phi_i = \mu + \beta_{G_i} + \eta_i \qquad \eta_i \sim N(0, \omega)$$

$$\beta_{G_i} = \begin{cases} 0 & \text{if } G_i = \text{common homozygote} \\ \beta_1 & \text{if } G_i = \text{heterozygote} \\ \beta_2 & \text{if } G_i = \text{rare homozygote} \end{cases}$$

$$M_{base} : \{\beta_1 = \beta_2 = 0\}$$
$$M_{full} : \{\beta_1 \neq \beta_2 \neq 0\}$$

- Likelihood ratio test (LRT)

$$S = -2 \times (L_{base} - L_{full}) \sim \chi^2_2$$

L_{base} et L_{full} the loglikelihoods of M_{base} and M_{full}

- Wald test

$$W = \begin{pmatrix} \beta_1 \\ \beta_2 \end{pmatrix}^T V^{-1} \begin{pmatrix} \beta_1 \\ \beta_2 \end{pmatrix} \sim \chi^2_2$$

V: block for β_1 and β_2 of the estimation variance matrix

↪ Type I error inflation in studies with small sample size and/or unbalanced genotypes [2,3]

[2] Bertrand et al. Journal of Biopharmaceutical Statistics, 2008

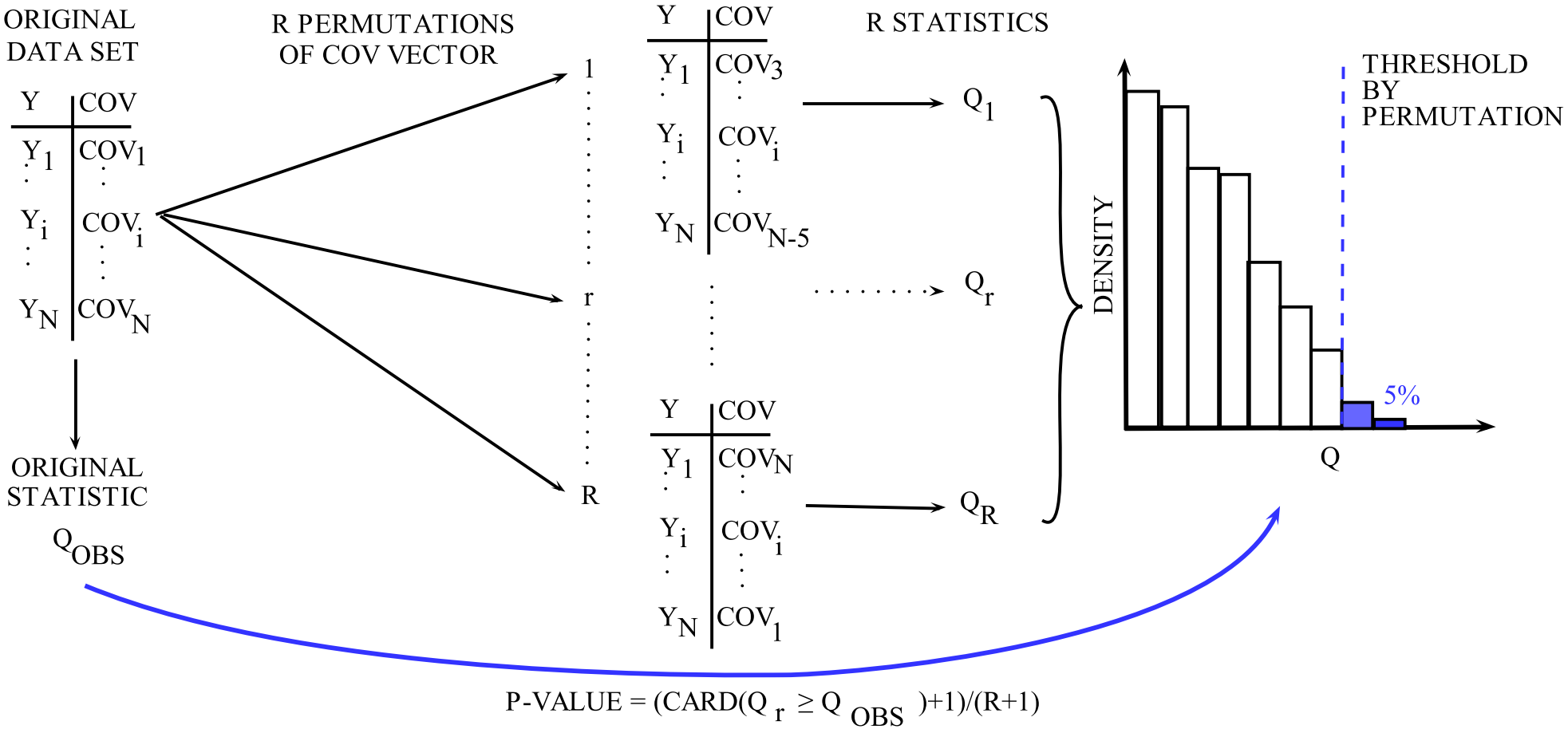
[3] Bertrand et al. Journal of Pharmacokinetics and Pharmacodynamics, 2009

Objectives

- To propose and evaluate by simulation some alternatives to the asymptotic tests to detect a SNP effect on a pharmacokinetic parameter using NLMEM
  1. a permutation test for both statistics
  2. the use of a F-distribution for the Wald test
     - four different values considered for the denominator degrees of freedom (DF)
- To apply these methods to the analysis of the pharmacogenetics of indinavir in the COPHAR2-ANRS 111 trial [4]

[4] Bertrand et al. European Journal of Clinical Pharmacology, 2009

Permutation test



F-distribution based alternative

- DF derived from balanced, multilevel ANOVA proposed by Pinheiro et Bates (2000)

$$DF_{PB} = \sum_{i=1}^{N} n_i - (N + p + k - 2)$$

p = number of pharmacokinetic parameters
k = number of effect coefficients

  - implemented in the nlme function in R

- DF proposed by Wolfinger (2000)

$$DF_W = N - q$$

q = number of random effects

  - implemented in the NLMIXED procedure in SAS

- DF adapted from a method developed by Gallant (1975) in multivariate nonlinear models

$$DF_G = N - p$$

with V multiplied by a factor N/DF_G

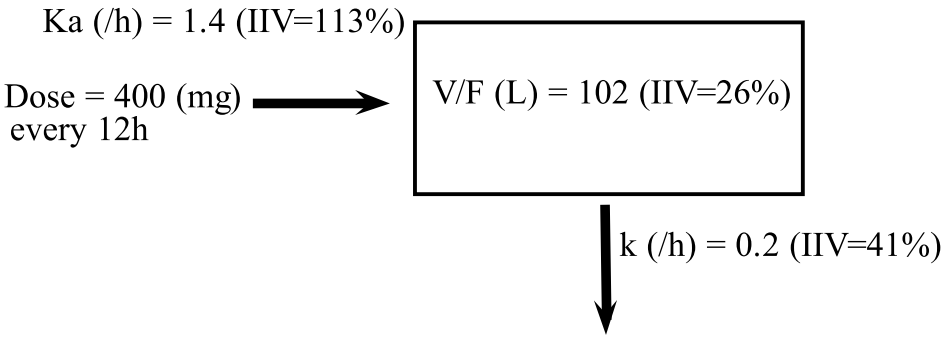
- DF from the Satterthwaite formula (1941) extended to NLMEM

$$DF_{FC} \approx 2V^2/\mathrm{Var}(V)$$

  - implemented in the MIXED procedure in SAS for LMEM
  - extension to NLMEM implemented in MONOLIX only

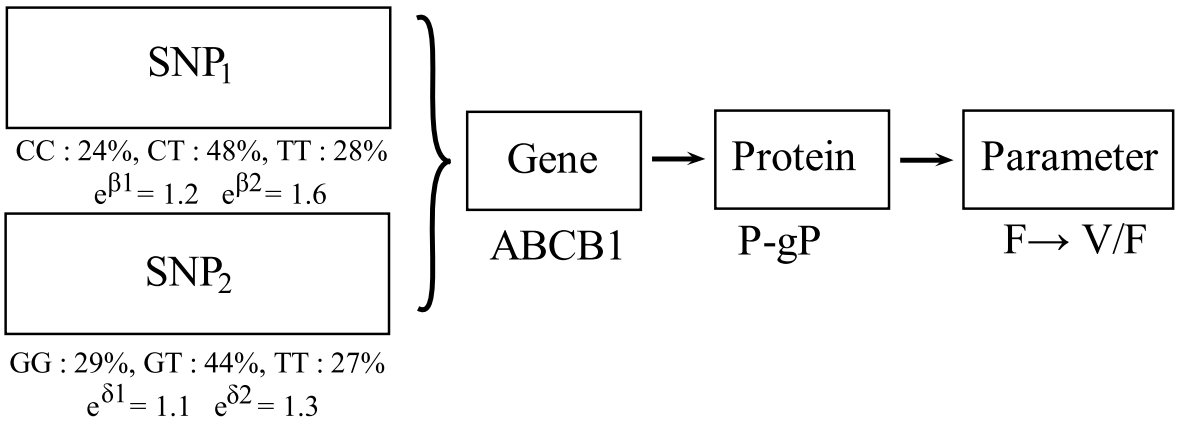
Simulation settings

- Pharmacokinetic data
  - model and parameters inspired from the COPHAR2 study

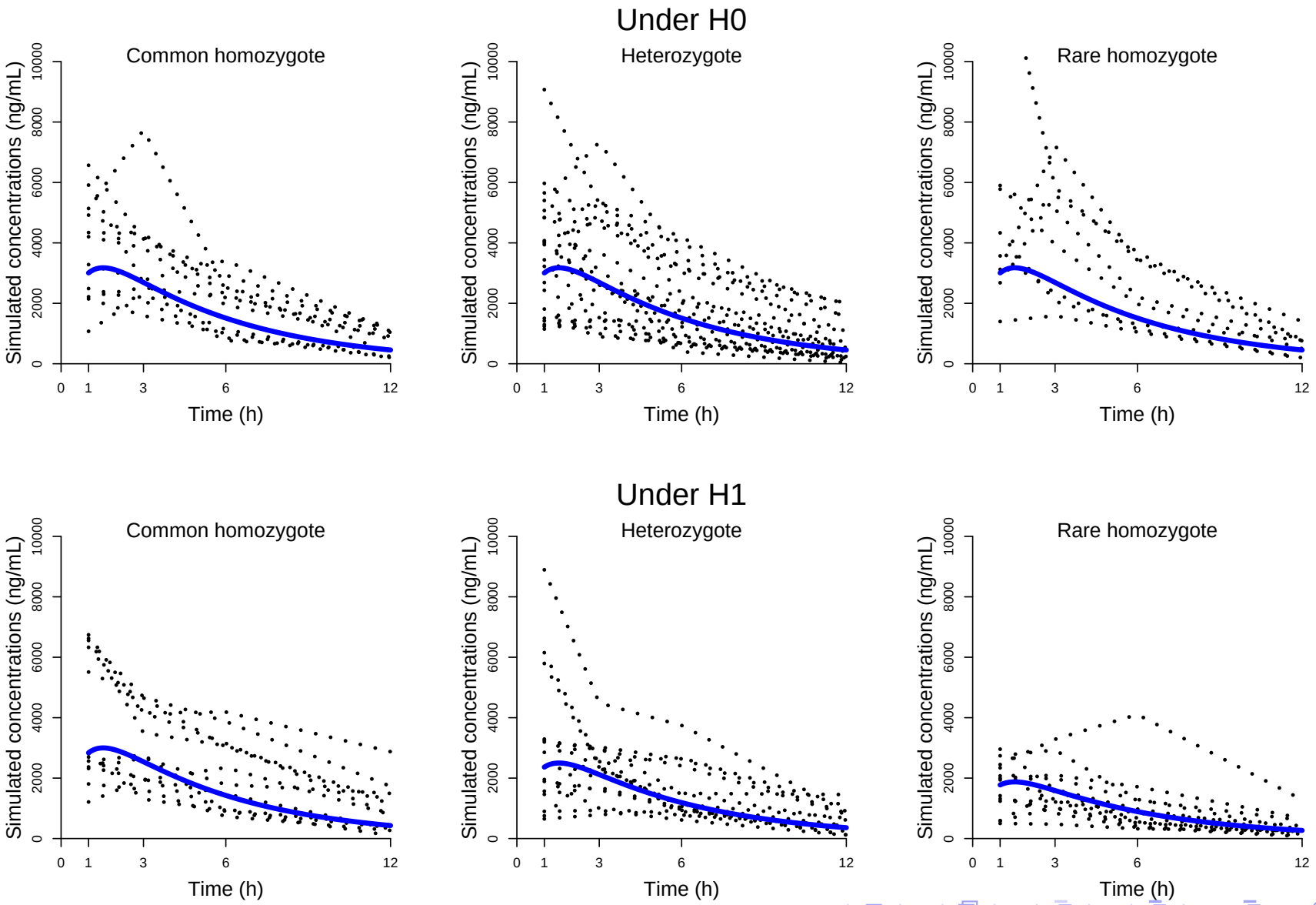


- exponential model for the inter-individual variability (IIV)
- proportional model for the residual error ($\sigma = 20\%$)

- Genetic effect under the alternative hypothesis (H_1)



Simulated Data (N=40/n=4)



Results from previous simulation studies [2,3]

- 1000 simulated data sets under H_0
- FOCE-I in NONMEM 5
- SAEM in MONOLIX 2.1

		N=40/n=4		N=80/n=2		N=100/n=4,1		N=200/n=4	
Test	Algorithm	K	α	K	α	K	α	K	α
LRT	FOCE-I	964	7.9					956	5.0
	SAEM	1000	8.9	1000	8.7	1000	8.4	1000	5.1
Wald	FOCE-I	924	11.7					860	6.5
	SAEM	1000	7.6	1000	7.8	1000	6.8	1000	5.9

K = number of data sets on which the test could be performed
α = type I error
Prediction interval for 5% = [3.6 – 6.4]

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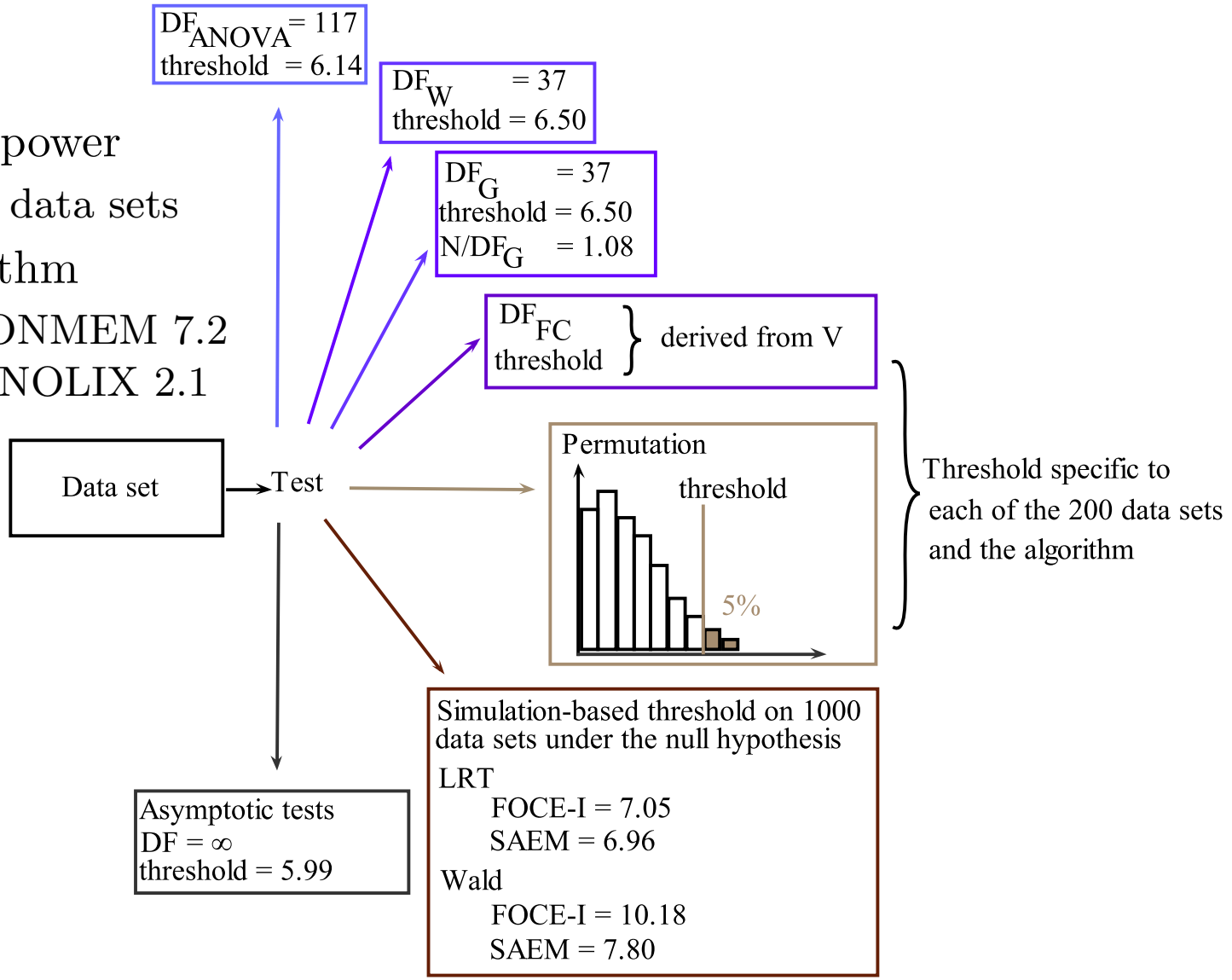
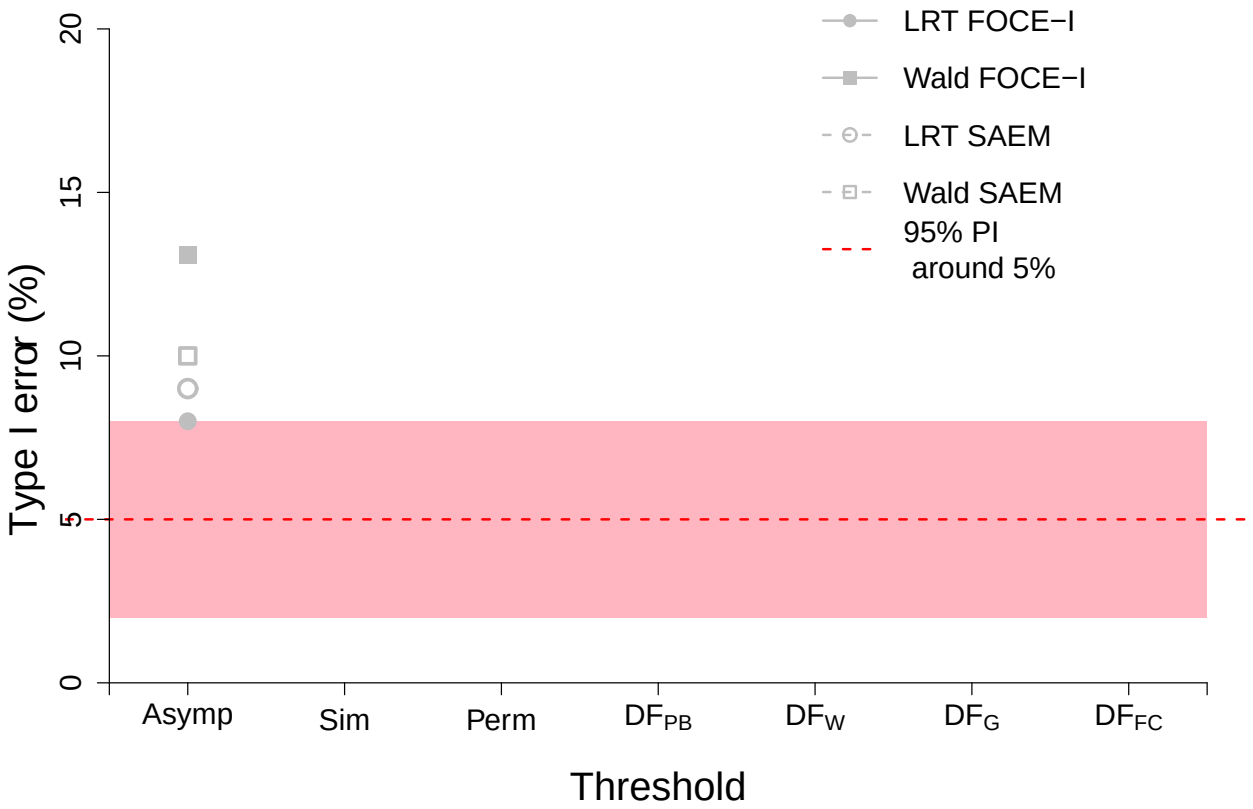
Evaluation

- N=40/n=4
- Type I error and power
  - 200 simulated data sets
- Estimation algorithm
  - FOCE-I in NONMEM 7.2
  - SAEM in MONOLIX 2.1

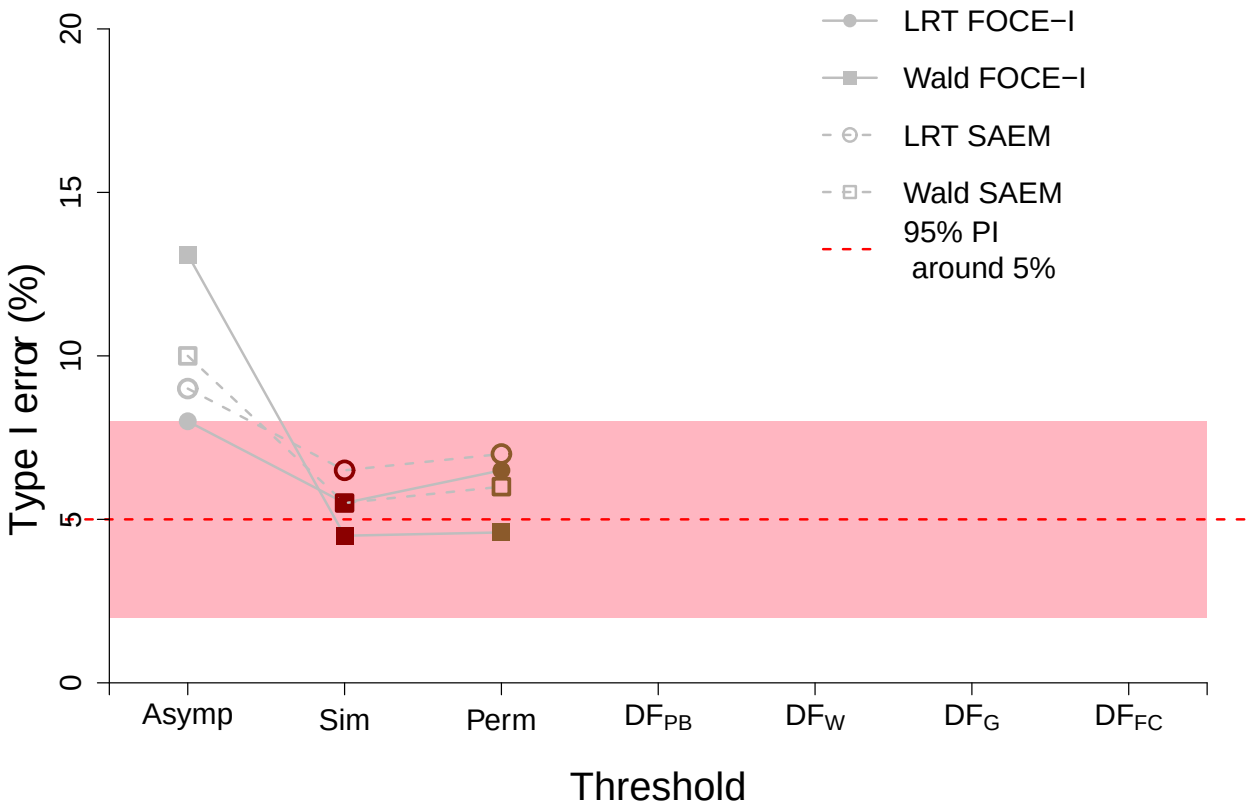


Type I error



- Improvement in FOCE-I stability in NONMEM 7.2 with K $\geq$ 195

Type I error



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- Inflation corrected using the permutation and simulation-based approaches for both estimation algorithms

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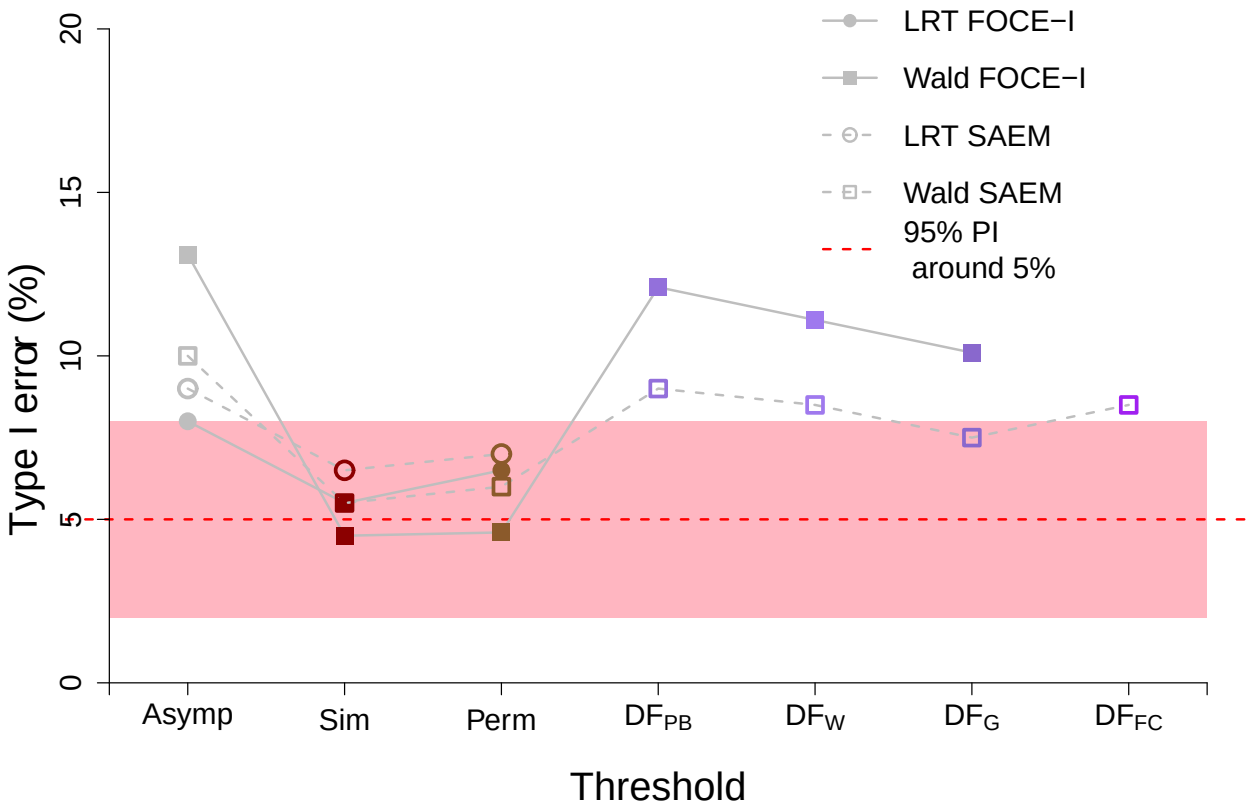
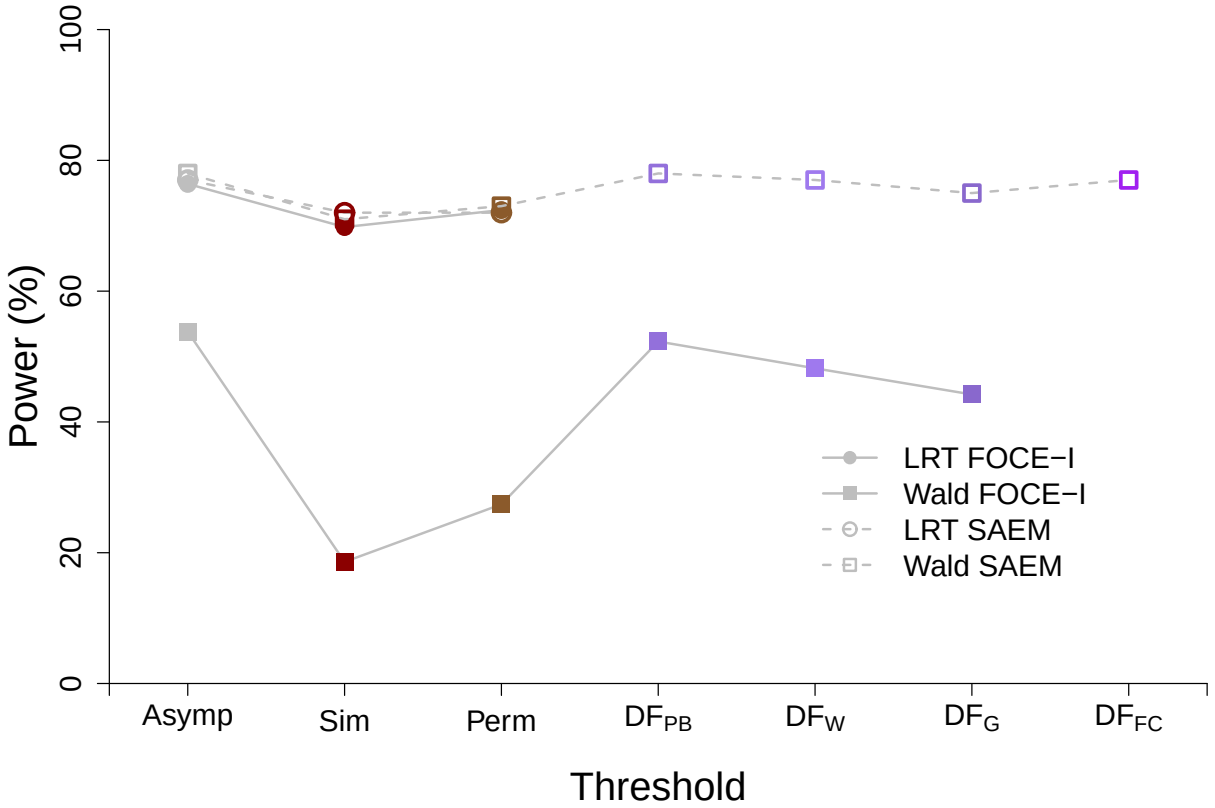


- Improvement in FOCE-I stability in NONMEM 7.2 with K $\geq$ 195
- Inflation corrected using the permutation and simulation-based approaches for both estimation algorithms
- Inflation corrected using the DF_G with SAEM only
  - DF_{PB}=117 close to asymptotic estimate
  - DF_W=37 and DF_{FC}=39.8 [36.3-43.8] close to N

Power

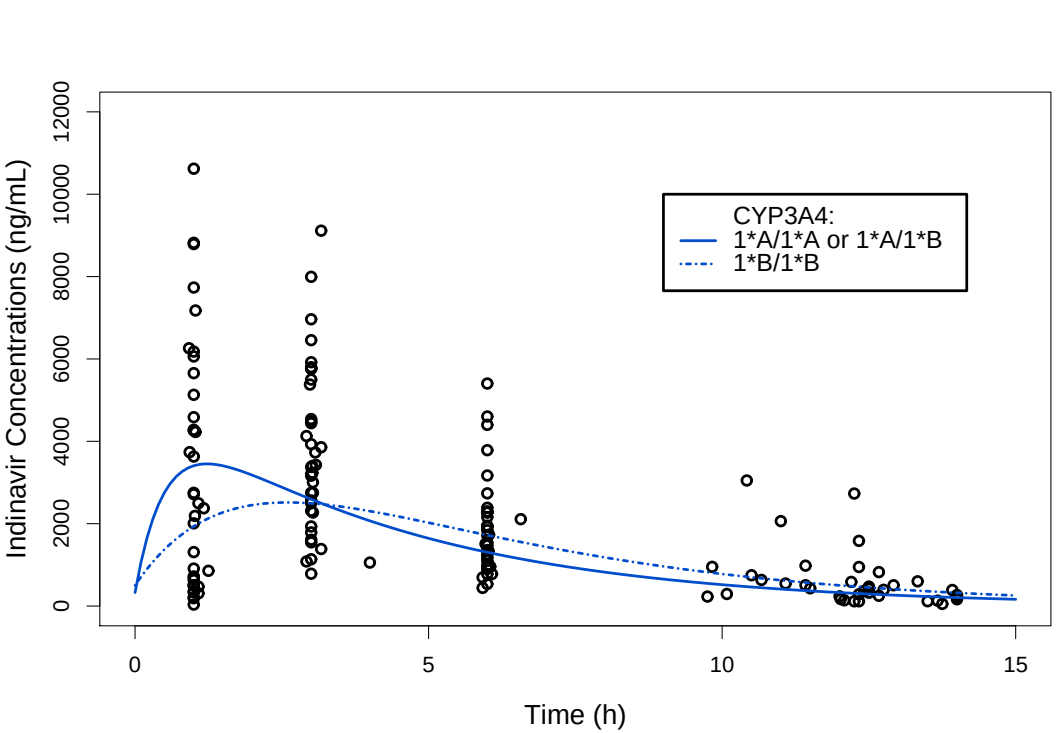


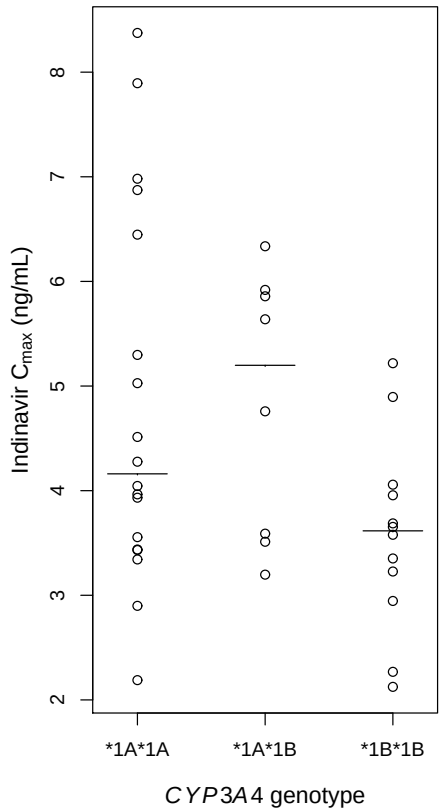
- Similar Power estimates for both tests, about 70% after correction using SAEM
- Loss of power for the Wald test with FOCE-I after correction based on permutations or simulations
  - strong correlation of the genetic effect coefficients with their estimation error

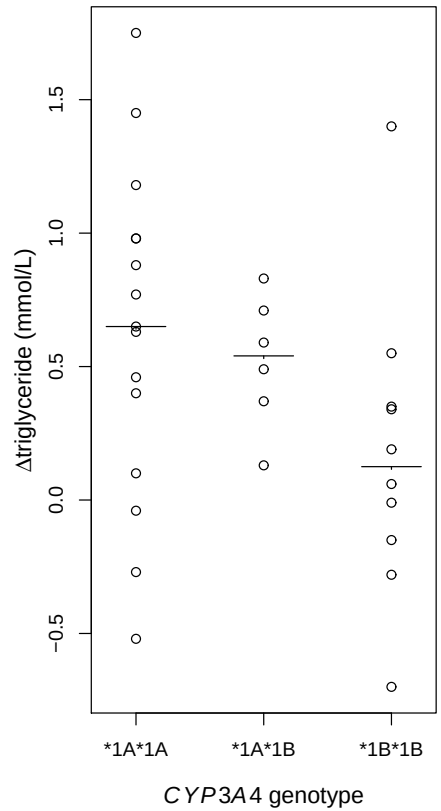
COPHAR2-ANRS 111 study

- Multicentre noncomparative pilot trial
  - to evaluate the impact of therapeutic drug monitoring of protease inhibitors in HIV-positive patients naïve of treatment
- Indinavir pharmacogenetic substudy
  - 40 pharmacokinetic profiles at steady state
  - short term efficacy and toxicity outcomes
  - ABCB1 gene exons 21 and 26, *CYP3A4*1B*, *CYP3A5*3* and **6*
- Covariate model building
  - modelling performed using SAEM in MONOLIX 2.1
  - screening on individual parameter estimates using nonparametric tests
  - forward selection based on LRT
  - covariates in the final model assessed with all methods

Covariate model







- Asymptotic tests = Age on Cl/F and *CYP3A4*1B1B* on k_a
  - ↪ age effect discarded based on Permutation test and DF_G

⇒ 70% decrease in indinavir k_a in *CYP3A4*1B1B* patients

⇒ lower C_{max} and short term triglyceride toxicity in *CYP3A4*1B1B* patients

Conclusions

- Type I error inflation of asymptotic tests in pharmacogenetic studies with small sample size and/or unbalanced genotypes
- Permutation based approach
  - feasible in pharmacogenetic studies for both LRT and Wald test
  - comes with substantial computational burden
- F-distribution based approach DF_G
  - easy to implement
  - validated on real data and other simulated designs (N=80/n=2 and N=100/n=4,1)
  - further studies with more complex variability model required
  - effective due to inflation factor N/DF_G for the under-evaluation of the estimation variance
    - restricted maximum likelihood [5] ?

$\Rightarrow$ First use asymptotic test plus DF_G and in case of discrepancy perform permutations

[5] Meza et al. Biometrical Journal, 2007