

Toxicogenomic dose-response models for DNA chips data from rats treated by flutamide



Lyon 1

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Background and Objectives

- The use of genomic technology for assessing health risks associated which chemical exposure has great potential.
- Objectives:** To fully characterize testicular toxicity in adult Wistar rats induced by flutamide (FLU), to estimate the benchmark doses (BMD), and to estimate the BMD lower confidence limit (BMDL) modifying gene expression ¹.
- To achieve this objective, changes in toxicogenomic responses (gene behavior) in the testes, will be investigated on 43,379 genes (full-genome analysis) in rats exposed to FLU at different dose levels by oral gavage for 28 consecutive days.

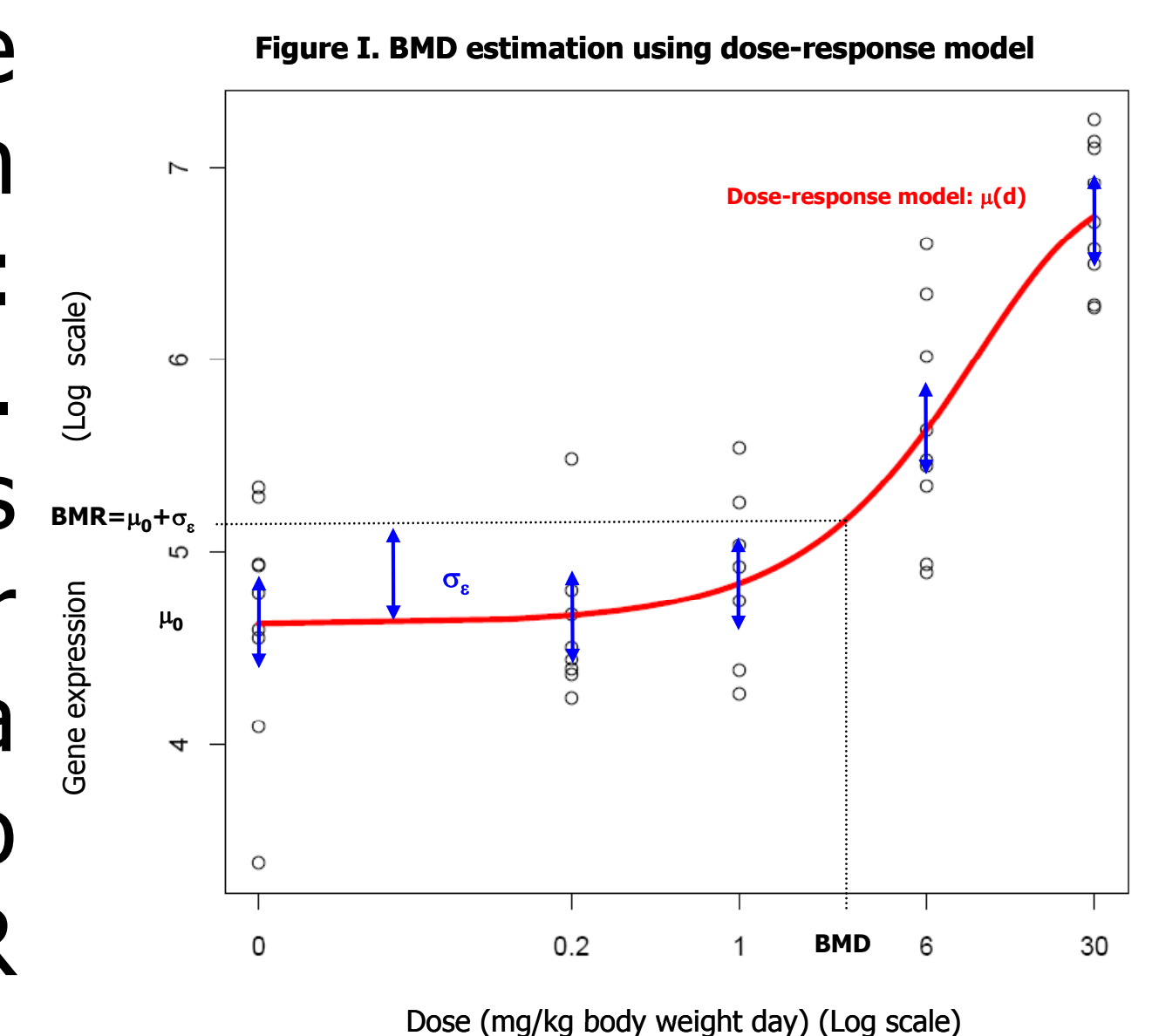
Methods

- 42** rats were randomized between 5 arms: **9** rats in vehicle (control group), **8** rats at 0.2, **7** rats at 1, **9** rats at 6 and **9** rats at 30 mg/kg body weight per day.
- For each rat, microarray (Agilent 4x44k) was made.
- Linear model (M0) was applied to detect** significant change of log(gene-expression) from baseline. False discovery rate was controlled during this step for the slope of linear model ($\alpha=20\%$). ²
- When significant, non-linear models were tested: **stimulation** or **inhibition** of gene expression (exp., Emax, logistic; see Table I). ³

MODEL	MODEL DESCRIPTION: $\mu_i(D)$	BEHAVIOR	NAME
Exponential	$a_i \times (b_i + (1 - b_i) \times e^{(-k_i \cdot D)}) + \epsilon_i$	Stimulation or Inhibition	M1
Emax	$a_i + \frac{G_{max} \times D}{\sqrt{D50^2 + D}} + \epsilon_i$	Stimulation	M2
	$a_i - \frac{G_{max} \times D}{\sqrt{D50^2 + D}} + \epsilon_i$	Inhibition	M3
Logistic	$\frac{G_{max}}{1 + e^{-\frac{D - D50}{k_i}}} + \epsilon_i$	Stimulation	M4
	$\frac{a_i}{1 + b_i \times e^{-k_i \cdot D}} + \epsilon_i$	Inhibition	M5

- Model selection:** For each gene, choice between linear and non-linear models was based on Schwarz criterion (BIC).

- BMD estimation:** defined as the dose level leading to a change in predicted baseline ± 1 residual SD: **BMR** = $\mu_0 \pm \sigma \rightarrow \mathbf{BMD} = \mu^{-1}(\mathbf{BMR})$. ¹ When normality of residual was rejected (Kolmogorov-Smirnov or Shapiro-Wilk test, $\alpha=5\%$), a bootstrap method was used to estimate the quantile of the BMR distribution.



- BMDL** : intersection with the BMR and the upper (or lower for inhibition) 90% CI on the model. BMDL was estimate by **BMD - z_{95%} x SD** with $z \sim N(0,1)$; ⁴ we used the *delta-method* to compute SD and to evaluate CI on the model.
- All analysis were performed using R 2.10® software, for non-linear models with *nls()* function; for the delta-method we used *deltamethod()* function.

Results

- Genes data were log normalized (quantile normalisation⁵) and after a QC control 32,944 genes were retained.

Linear dose-response relationship

- A significant linear change from baseline in gene behavior was detected for **6,343** genes (19.2%): **3,304** stimulations (52%) and **3,039** inhibitions (48%).

Non-Linear dose-response relationship

- Non linear model for stimulation behavior:

MODEL	NAME	CONVERGENCE	# STIMULATION
Exponential	M1	2,352 (71.2%)	3,304
Emax	M2	2,706 (81.9%)	
Logistic	M4	2,697 (81.6%)	

For 2,325 genes, it was possible to achieve convergence for the 3 non-linear models and at least one model for 2,732 genes.

- Non linear model for inhibition behavior:

MODEL	NAME	CONVERGENCE	# INHIBITION
Exponential	M1	2,336 (76.9%)	3,039
Emax	M3	2,451 (80.7%)	
Logistic	M5	2,440 (80.3%)	

For 2,180 genes, it was possible to achieve convergence for the 3 non-linear model and at least one model for 2,598 genes.

Model selection:

	Table III. CHOICE OF THE BEST MODEL (Shwarz criterion)						
	Linear	Exponential	Emax		Logistic	TOTAL	
	M0	M1	M2	M3	M4	M5	
STIMULATION	2,159	80	532	-	533	-	3,304
	65.4%	2.4%	16.1%	-	16.1%	-	
INHIBITION	2,484	182	-	303	-	70	3,039
	81.7%	6%	-	10%	-	2.3%	
TOTAL	4,643	262	835		603		6,343
	73.2%	4.1%	13.2%		9.5%		

- Linear and Emax models were the main preferred significant models, over exponential and logistic shape.

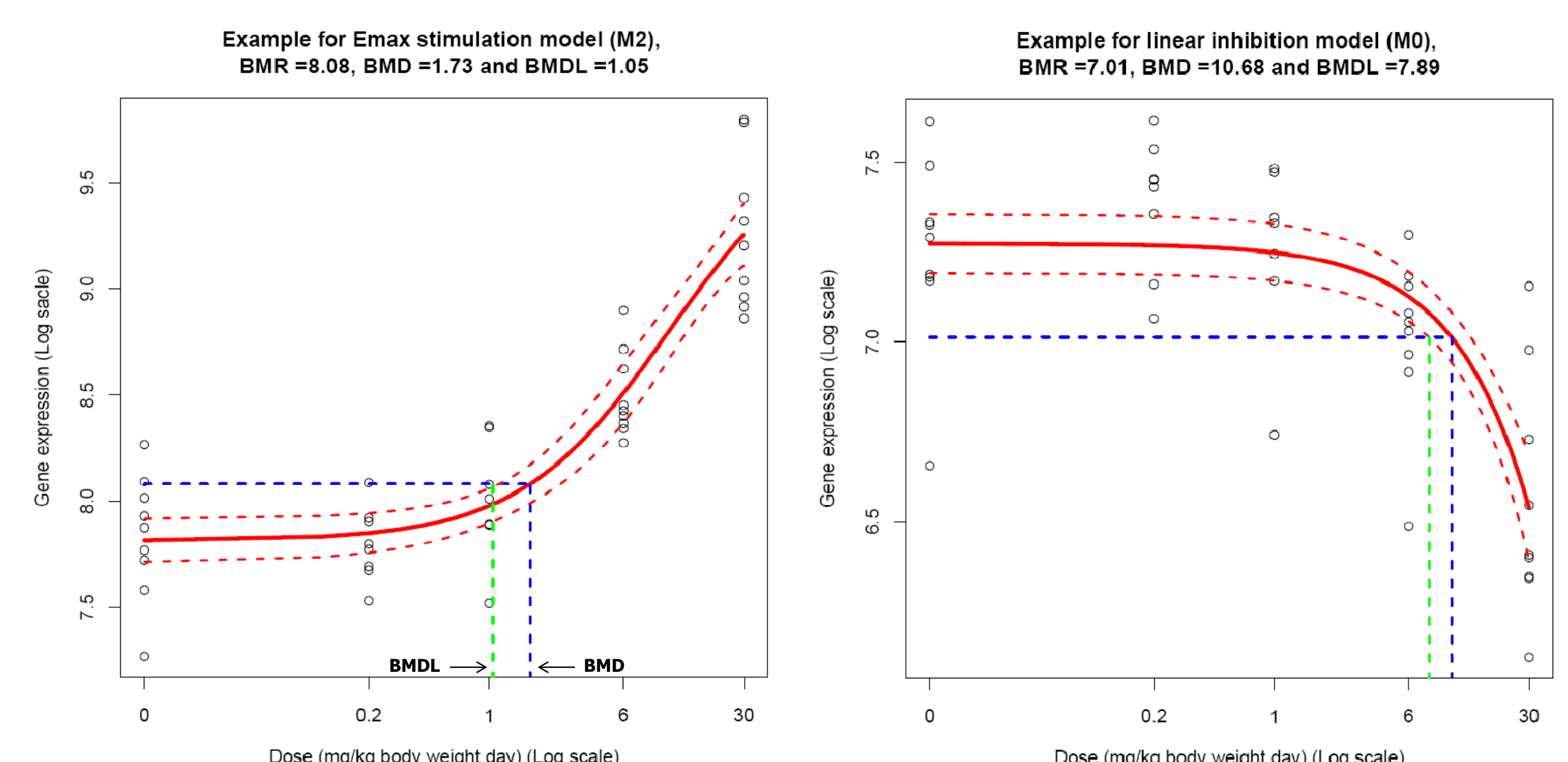
References

(1) Russel S. Toxicological Sciences, 98: 240-248; 2007; (2) Benjamini Y, Hochberg Y: Crit Rev Toxicol 57: 289-300; 2003. (3) Benchmark Dose Software (BMDS) Version 2.1, User's Manuel; 2009. (4) Moerbeek M. Risk Analysis, 24: 2004. (5) B. M. Bolstad, BIOINFORMATICS; 2003.

BMD and BMDL estimation:

- For 96.8% of estimated models, residuals were normal and for 3.2% bootstrap was used to estimate the quantile.
- Example for 2 genes:

Figure II. BMD analysis for stimulation and inhibition behavior



- Good estimation of the BMD at the intersection but not all the time.

Conclusion and perspectives

- An algorithm has been created to model dose-effect relationship toxicity expressed by DNA chips.
- This algorithm allows to characterizing the benchmark doses for a large set of genes.
- Running time : ~ 6 hours.