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Bayesian analysis of mediation in Cell Transformation Assays for testing the carcinogenicity of chemicals

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ABSTRACT – Cell Transformation Assays (CTAs) are in-vitro tests of carcinogenicity where a minimum of 10 Petri dishes are treated with the same dose of a substance and 5 or more doses are considered in the same experiment. The number of fully transformed *foci* grown on each dish is the outcome collected after 5 weeks from treatment [1]. A Bayesian structural causal model for CTAs is proposed and exploited to estimate mediated effects of a known carcinogen on the outcome. Null estimates of direct effects at several doses suggest that some features of CTAs might be reconsidered.

1. INTRODUCTION

Fast and cheap assays for probing the carcinogenic potential of substances to be used by cosmetic, food and pharma companies are of wide interest to perform preliminary screenings without resorting to rodent-based long and expensive studies. A typical CTA corresponds to a one-way randomized experiment, nevertheless recommended statistical models from the literature [2] do not seem to cover the whole range of distributions that might be required by very different chemicals, e.g. those with a variance smaller than the mean.

A structural causal analysis [3,4] based on the Directed Acyclic Graph (DAG) shown in Figure 1 is here proposed to describe several causal relationships existing in this class of experiments. Node D refers to the dose level applied to a Petri dish in which a random number of cells will be alive after treatment (node A), and that will develop a total number of *foci* (node T) after 5 weeks, some of them being fully transformed (node F). When the same experiment is replicated using different batches of cells (node C) and of substratum-reagents (node S) then confounding of A→T and T→ F relationships may occur. In order to illustrate the approach, a case study on a known carcinogenic chemical with 9 increasing dose levels is considered. The whole experiment was not replicated, thus estimates are conditional to a specific batch.

2. METHODOLOGY

Let $M_{F:g,i,j}$ be the random variable "manifest number of fully transformed *foci*" at dose level i in Petri dish j with batch of reagents g. The random variable $M_{A:g,i,j}$ is the "manifest number of alive cells after treatment", while $M_{T:g,i,j}$ is the "manifest total number of *foci*". Non-parametric equations relating endogenous variables are derived according to the DAG (Figure 1):

$$m_{F:g,i,j} = h_F(m_{D:i}, m_{T:g,i,j}, m_{S:g}, m_{C:g}, u_F) \quad (1)$$

Dose levels are assigned at random, therefore two more similar equations for variables $M_{A:g,i,j}$ and $M_{T:g,i,j}$ are enough to complete the structural part of the specification. Furthermore, if batch does not change within the same experiment then the specialized functions required in our case study are:

$$m_{F:i,j} = h'_F(m_{D:i}, m_{T:i,j}, u_F) \quad (2)$$

$$m_{T:i,j} = h'_T(m_{D:i}, m_{A:i,j}, u_T) \quad (3)$$

$$m_{A:i,j} = h'_A(m_{D:i}, u_A) \quad (4)$$

We assume that exogenous variables are independent, thus the induced manifest random variables $M_{A:g,i,j}$, $M_{T:g,i,j}$ and $M_{F:g,i,j}$ may be considered without making functions in equations (1-4) explicit; modularity of relationships coded by the DAG is also assumed.

In a typical CTA, the sample size n is small, for example $n = 89$ in our case study. A parametric Bayesian model was elicited by exploiting constraints coded in the CTA protocol, e.g. that the lowest dose is close to the No-Observed-Adverse-Effect-Level (NOAEL), while the highest dose is close to the median lethal dose (DL50):

$$M_{A:i,j} \sim c_{ini} \cdot \text{Beta}(\phi_i \mid \alpha_{A:i}, \beta_{A:i}) \quad (5)$$

with c_{ini} the initial total number of seeded cells, ϕ the survival rate, and i=1,2,…,9. Some of the alive cells will later originate transformed foci according to rate η:

$$M_{T:i,j} \sim \text{Poisson}(\eta_i \cdot m_{A:i,j}) \quad (6)$$

$$\eta_i \sim \text{Gamma}(\alpha_{T:i}, \beta_{T:i}) \quad (7)$$

Finally, the number of fully transformed *foci* are present

as a proportion π of the number of total *foci*, that is:

$$M_{F:i,j} \sim \text{Binomial}(\pi_i, m_{T:i,j}) \quad (8)$$

$$\pi_i \sim \text{Beta}(\alpha_{F:i}, \beta_{F:i}) \quad (9)$$

After eliciting the initial distributions of model parameters (results not shown), total effects (TE), natural direct and natural indirect effects (NDE, NIE) [3,4] were estimated by Markov Chain Monte Carlo imputation of counterfactuals using Bayesian predictive distributions. Sufficient conditions for identifying all effects [3] are satisfied within stratum g (one batch-experiment).

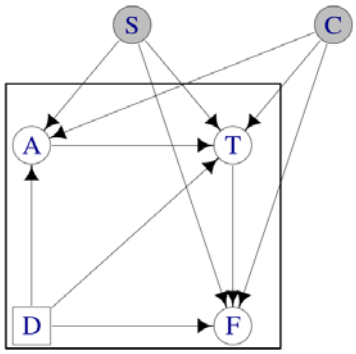

Figure 1 DAG with dish-specific variables inside plate

3. RESULTS AND DISCUSSION

Estimates of TE, NDE, NIE were obtained taking water as common reference value (Figure 2) and then taking dose level i as reference for dose level $i+1$, thus in this last case the effect captures changes due to an increase of dose and is useful to detect turning points (results not shown).

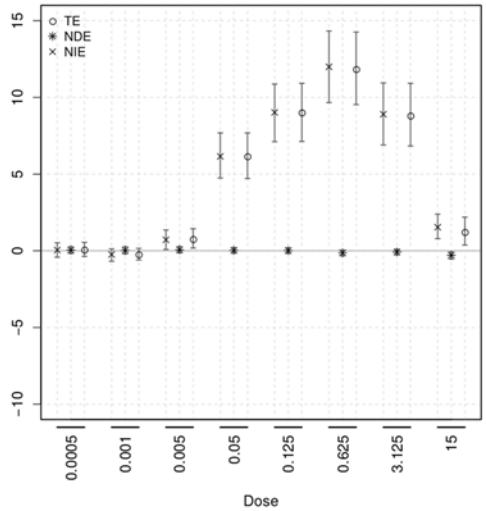

Figure 2 TE, NDE and NIE with water as reference

In Figure 2, Bayesian point estimates and 95% credibility intervals are shown: TE is almost entirely due to NIE at all dose levels but the highest. The number of fully transformed *foci* seems mainly determined by a change in the total number of *foci* instead of a change in the probability of transition to the fully transformed state. We conjecture that the role of M_T should be reconsidered, for example by modifying the CTA protocol to have high values of M_T even at low doses but with a small M_F.

At the highest dose, NDE is negative, a result that could be due to the increase of toxicity of the tested chemical. The relationship between log-dose and the expected value of parameter π in the posterior distribution was found almost constant for all but the 3 highest doses where a linear decrease was observed.

Model adequacy was checked by calculating values of discrepancy variables "number of distinct counts" and "range of the number of *foci*", both for M_T and M_F (not shown): 2 of the 9 dose levels might benefit from model improvement.

A large number of other different carcinogenic chemicals should be tested to assess whether the no-NDE pattern is a general feature in CTAs or it depends on the tested chemical. In all of these experiments the number of alive cells should be also measured, a practice not yet fully implemented in routine work.

4. CONCLUSIONS

Estimates obtained in our case study are conditional to a fixed batch g, but the natural extension to several batches is not trivial in practice. Current CTAs are replicated at most in 3 times, furthermore heterogeneity among cell batches might be due to dozens different genes and involve complex intercellular signaling. This is an important area to address in future research, for example by including molecular information and/or foci image descriptors [5] into the model.

5. REFERENCES

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