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The GARD assay for potency assessment of skin sensitizing chemicals

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Summary

The GARD assay is a cell-based transcriptional biomarker assay for the prediction of chemical sensitizers[1] targeting key event 3, dendritic cell activation, of the skin sensitization AOP. Here, we present a modified assay based on Random Forest modelling, which is capable of predicting CLP potency classes (1A - strong sensitizers, 1B - weak sensitizers, no category – non-sensitizers) as described by the European CLP regulation with an accuracy of 75 % (no cat), 75 % (1B) and 88 % (1A) based on a test set consisting of 18 chemicals previously unseen to the model.

We further can link the activation of distinct pathways to the chemical protein reactivity, showing that our transcriptomic approach can reveal information contributing to the understanding of underlying mechanisms in sensitization.

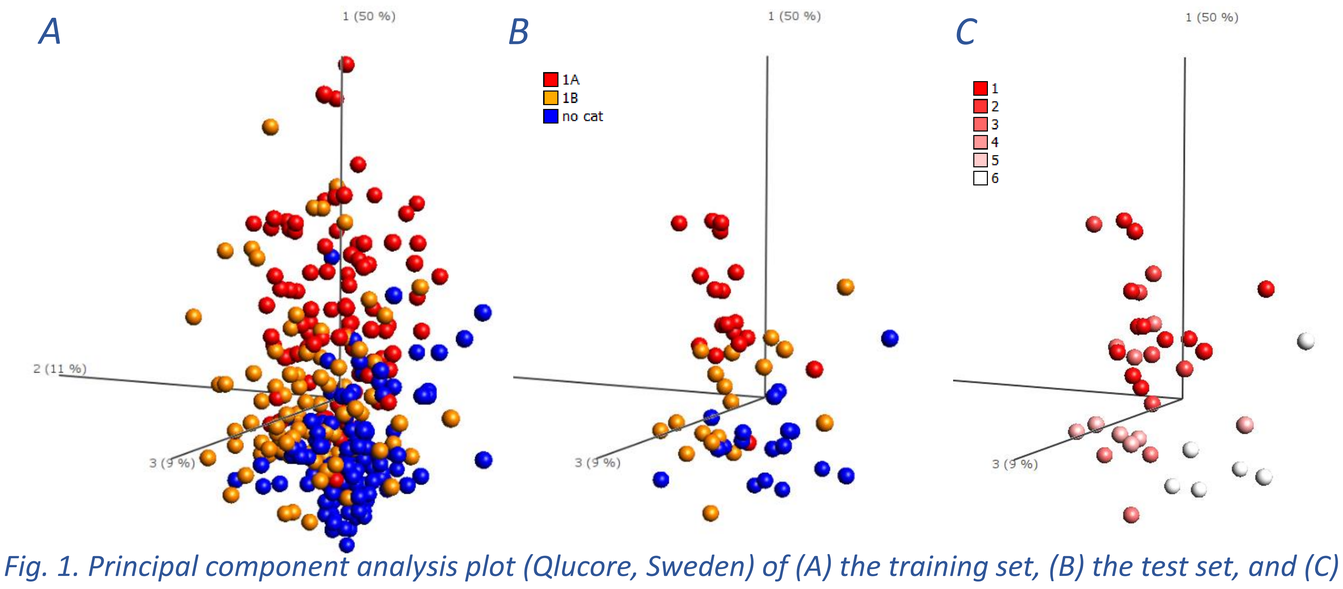

Fig. 1. Principal component analysis plot (Qlucore, Sweden) of (A) the training set, (B) the test set, and (C) test set samples with available human potency classifications[4] based on an input of 18 variabels identified by the Random Forest model.

Introduction and Aim

The Genomic Allergen Rapid Detection assay, in short GARD, is based on transcriptomic profiling of a derivative of the cell line MUTZ-3, resembling dendritic cells. It has been developed for hazard classifications of skin sensitizing chemicals and predicts those with an accuracy of 89 %[2]. However, risk assessment requires potency classification of sensitizing chemicals and based on earlier observations, where we have seen a) a correlation between GARD output and human potency, and b) a differential regulation of signaling pathways dependent on the individual potency of the investigated sensitizers[3], we hypothesized, that GARD can be developed into a tool for potency classification.

Table 3. Pathways unique for each of three protein reactivity groups.
Five pathways related to cell cycle regulation, apoptosis/survival and DNA damage were common to all reactivity groups (not shown).

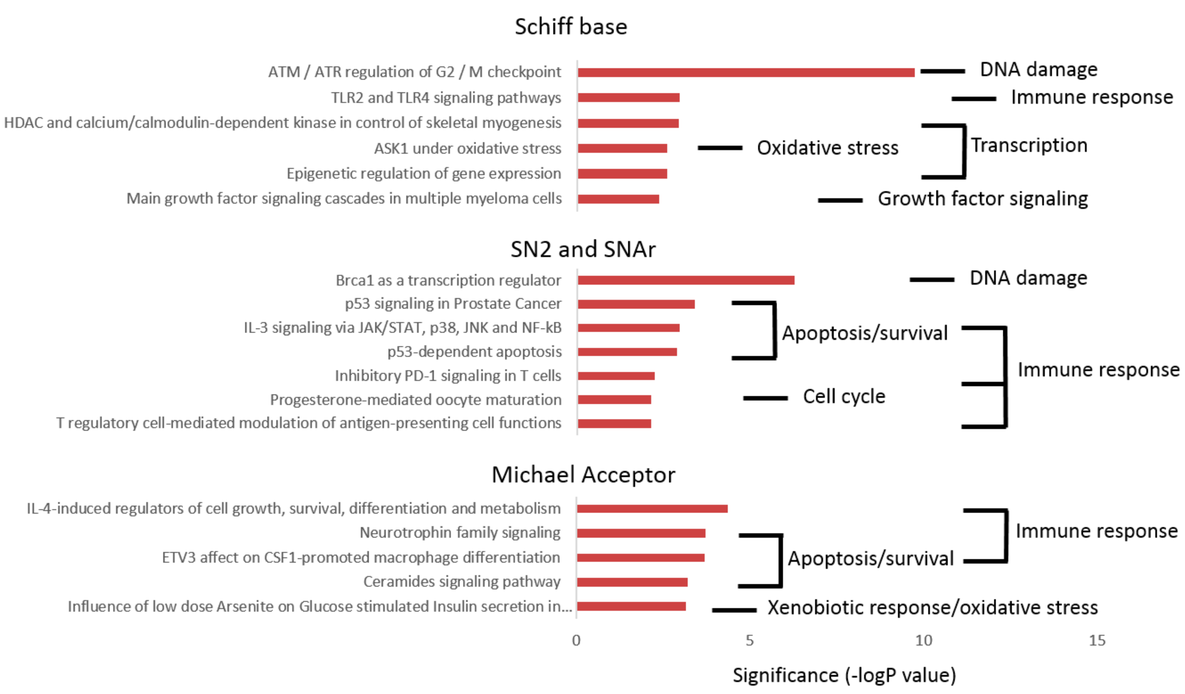

Table 1. Test set CLP and reactivity information.

Chemical	true CLP	predicted CLP	Protein reactivity
1-brombutane	no cat	***1B***	SN2
benzoic acid	no cat	***1B***	No binding
citric acid	no cat	***1B***	No binding
diethyl phthalate	no cat	no cat	No binding
ethyl vanillin	no cat	no cat	Schiff base formation
xylene	no cat	no cat	No binding
anethole	1B	1B	Michael acceptor
benzyl benzoate	1B	1B	Acyl transfer agent
linalool	1B	1B	No binding
lyral	1B	1B	Schiff base formation
butyl glycidyl ether	1B	1B	SN2
diethyl maleate	1B	***1A***	Michael acceptor
cyanuric chloride	1A	1A	SNAr
propyl gallate	1A	1A	Michael acceptor
bisphenol A-diglycidyl ether	1A	1A	SN2
glutaraldehyde	1A	1A	Schiff base formation
iodopropynyl butylcarbamate	1A	***1B***	Acyl transfer agent
p-benzochinone	1A	1A	Michael acceptor

Table 2.
Training and test set composition.

	Training set	Test set
total	70	18
CLP 1A	23	6
CLP 1B	25	6
CLP no cat	22	6

Table 4.
Prediction statistics.

	Sensi-tivity	Speci-ficity	Balanced accuracy
No cat	0.500	1.000	0.750
1A	0.833	0.917	0.875
1B	0.833	0.667	0.750

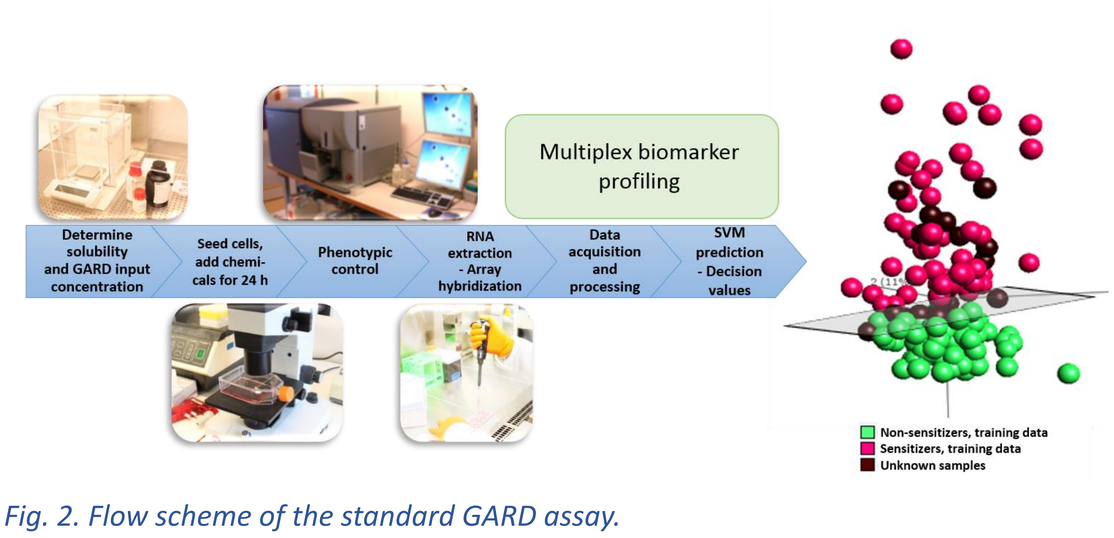

Fig. 2. Flow scheme of the standard GARD assay.

Results and Discussion

We here present a potency prediction approach based on a Random Forest model and 18 transcripts. 18 chemicals previously unseen to the model were classified as shown in Tables 1, 4 and Fig. 1. Interestingly, diethyl maleate, misclassified as 1A instead of 1B, is a human potency class 2 according to[4], and iodopropynyl butylcarbamate, wrongly predicted as 1B instead of 1A, is classified as human potency class 4[4]. Thus, the model seems to show more agreement with human data than CLP classifications (mainly derived from animal data) based on this limited dataset. Also Fig. 1C supports the hypothesis, that both data and model contain information allowing the prediction of human potency.
Furthermore, Key Pathway Advisor analysis reveals that these data can be used to investigate the cellular response in more detail (Table 3). In conclusion, we show that the modified GARD assay is capable of providing potency information, which is imperative for quantitative risk assessment of chemical sensitizers.

Material and Methods

Cells were handled and exposed to chemicals as previously described[1] and summarized in Fig. 2 until microarray data was obtained. Training set (n = 70) and test set (n = 18) was defined previous to model construction to represent the three CLP classes and different chemical reactivity groups in a balanced way (Table 2). Microarray data were normalized using the SCANfast algorithm[5] and arithmetic means of the transcript intensities from replicate stimulations in the training set were used to develop Random Forest models[6] , using the VarSelRF package[7] in R statistical environment. The predictive biomarker signature comprising 18 transcripts was identified by minimizing the OOB error rate using bootstrapping, and applied to predict replicate samples in the test set, i.e chemicals previously unseen to the model. Majority votes of replicate stimulations defined the predicted class. In order to ensure that the training and test set choice were not biased, alternative models were built with randomly shuffled compositions of training and test set. The number of chemicals in each set and their CLP distribution were kept constant. Pathway analysis was performed with the Key Pathway Advisor Tool (Thomson Reuters) with lists of 500 most significant genes for sensitizers in each protein reactivity group compared with non-protein binding non-sensitizing chemicals after initial variance filtration. The lowest p-value was reached when comparing bi-molecular nucleophilic substitution/nucleophilic aromatic substitution (SN) to “no binding” (p=0.0019), followed by Schiff base chemicals (SB, p=0.0055) and Michael acceptor (MA) samples (p=0.0169).

References: [1]Johansson H *et al*. BMC Genomics. 2011; [2]Johansson H *et al*. Toxicological Sciences 2014; [3]Albrekt A.S. *et al*. BMC Pharmacology and Toxicology 2014; [4]Baskettter D. *et al.*, Dermatitis 2014; [5]Piccolo S.R. *et al*. Genomics 2012; [6]Breiman, L. Machine Learning 2001. [7]Diaz-Uriarte, R. BMC Bioinformatics 2007. **Acknowledgements:** This work was supported by grants from The Swedish Foundation for Strategic Research, The Swedish Research Council, AFA Försäkring and Wenner-Gren Foundations (travel grant K.Zeller). The authors thank Cosmetics Europe for providing the chemicals for this study.