The GARD assay for potency assessment of skin sensitizing chemicals

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Introduction

Although Allergic Contact Dermatitis (ACD) is widespread, few *in vitro* tests are available to identify chemicals, sensitizers, with the ability to cause the disease. So far, there are no assay for prediction of sensitizing potency.

The Genomic Allergen Rapid Detection (GARD) platform is an *in vitro* platform for the assessment of sensitizers. The platform is based on gene expression analysis of a SenzaCells, a human myeloid cell line, after stimulation by the test substance.

Here, we demonstrate an expansion of the GARD assay, GARDpotency, for the assessment of sensitizing potency.

Conclusions

We present a novel biomarker signature consisting of 52 genes. It can be used within current GARD protocols to predict skin sensitizing potency according to CLP (accuracies: 90% (no cat), 82% (1A) and 74% (1B). As more clinical data becomes available, the concept can easily be modified to cover also additional human potency categories. Meanwhile, in the absence of validated methods for assessment of sensitizing potency, we believe that our assays fill an important gap towards a complete risk assessment of chemicals.

Material and methods

The original GARD assay is developed for binary prediction of sensitizers (Y/N). Here, It was hypothesized that the GARD platform could be used to classify sensitizing potency as well. The categories were classified according to the CLP regulation; strong sensitizer (1A), weak sensitizer (1B) and non-sensitizer (non cat).

The GARD protocols is based on gene expression analysis of SenzaCells after stimulation by test substances. SenzaCells are human myeloid cell line similar to dendritic cells, which are immunologically active during sensitization.

Seventy separate cell cultures were stimulated by unique chemicals (reference set) according to GARD protocols. Array data from the 70 samples generated was used to build a model to categorize the samples according to CLP. The model built was based on random forest and backward elimination.

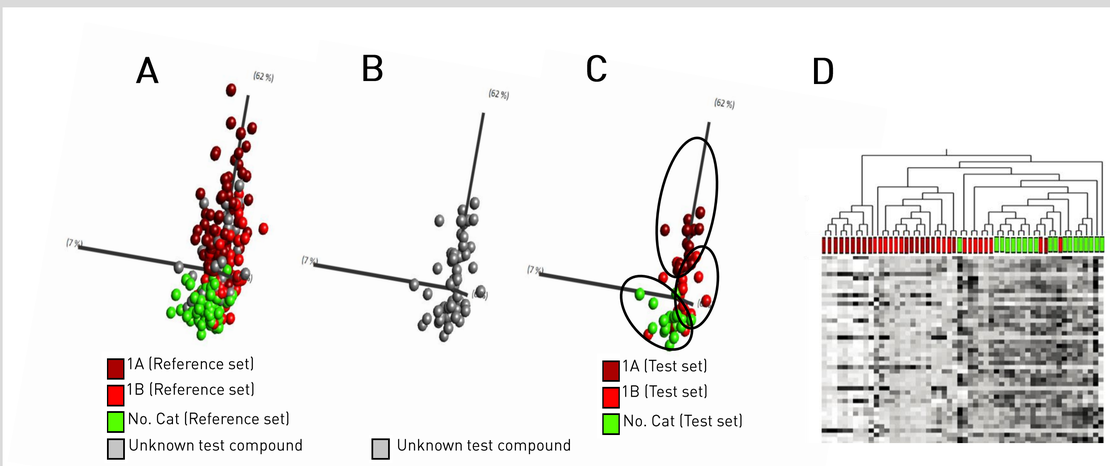

Figure 2. Visualization of the classifications by the final model containing the 52 signature genes. **A)** PCA plot with the reference set (n=70 chemicals) and the test set (n=18 chemicals) **B)** The reference set removed. **C)** The test set colour coded according to CLP category. D) Heat map of gene expression in the test set. Colour coded according to CLP category.

Table 2. Statistics by CLP class. Six chemicals per class. Three biological replicates each.

	No Cat	1A	1B
Sensitivity	89%	83%	56%
Specificity	92%	81%	92%
Accuracy	90%	82%	74%

Result

By internal training of the dataset it was found that the model with the minimum OOB value, *i.e.* the best model, was generated when 52 specific transcripts were left.

The model signature was validated by 18 novel chemical stimulations in triplicate. The test set chemicals were not included in the feature selection and were balanced to six chemicals in each CLP category (**table 1**).

Table 1. Test set (18 chemicals) used to validate the model based on the gene signature comprising 52 genes. GARD misclassifications are shown in italics.

Chemical	True CLP	GARD predicted CLP
1-brombutane	no cat	no cat
benzoic acid	no cat	no cat
citric acid	no cat	no cat
diethyl phthalate	no cat	no cat
ethyl vanillin	no cat	no cat
xylene	no cat	no cat
anethole	1B	1B
benzyl benzoate	1B	1B
linalool	1B	1B
lyral	1B	*1A*
butyl glycidyl ether	1B	*1A*
diethyl maleate	1B	*1A*
cyanuric chloride	1A	*no cat*
propyl gallate	1A	1A
bisphenol A-diglycidyl ether	1A	1A
glutaraldehyde	1A	1A
iodopropynyl butylcarbamate	1A	1A
p-benzochinone	1A	1A

A PCA plot of the reference and test set are visualized in **figure 2A**. The reference data set was removed from **figure 2B** and the remaining test set coloured in **figure 2C** to show that there is a clear separation of the CLP categories in the external test set as well. Also, the gene expression of the 52 genes group according to CLP categories in a heat map analysis (figure 2D).

The visual analysis was validated by the generated Random forest model and the statistics can be found in **table 2**.

References

Zeller *et al.*, ALTEX, 2017, Johansson *et al.*, ALTEX 2017, Forreryd *et al.*, Tox In Vitro 2016, Johansson *et al.*, Tox In vitro 2013

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