# Prediction of chemical respiratory sensitizers using GARD

## a novel in vitro assay based on a genomic biomarker signature

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## INTRODUCTION

Exposure to chemicals may induce allergic hypersensitivity reactions in skin or respiratory tract. To minimize exposure, chemicals are routinely screened for their sensitizing potential. Proactive identification has historically been performed using animal models, but the use of animals for safety assessment of cosmetics was recently banned within EU. To identify chemicals capable of inducing respiratory sensitization, methods are greatly underdeveloped, with no validated assay. For both endpoints, there is an urgent need for development of non-animal based methods for hazard classification of chemicals.

Genomic Allergen Rapid Detection (**GARD**) is an in house-developed method for identification and hazard classification of skin sensitizing chemicals. The test is based on a gene expression profile of a human myeloid cell line, using transcriptome-wide microarray technology (**Fig. 1**). The aims of the current project are **(1)** to expand the applicability domain of the GARD assay to classify also respiratory sensitizers through the identification of a separate biomarker signature, and **(2)** to perform an in-house validation in order to determine the predictive performance of the identified biomarker signature.

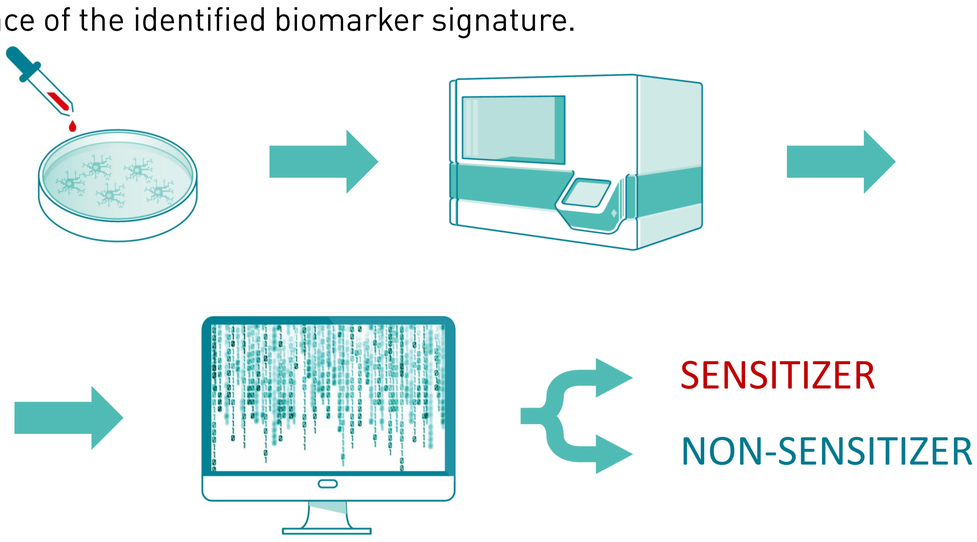


**Figure 1. GARD – Genomic Allergen Rapid Detection.** Chemically-induced changes in transcriptional levels in cells exposed to unknown testing compounds are compared to predictive genomic biomarker signatures using supervised machine learning. Unknown samples are classified as either skin sensitizers, respiratory sensitizers or non-sensitizers based on output from the machine learning algorithm.

## RESULTS – BIOMARKER SIGNATURE IDENTIFICATION

The transcriptome of the cells was analysed upon 24 h of stimulation using a panel of reference chemicals comprising 10 well known respiratory sensitizers and 22 non-respiratory sensitizers (**Tab. 1**). Establishment of the predictive biomarker signature was performed in two consecutive steps, visualized using Principal component Analysis (PCA, **Fig. 2**). First, differentially regulated transcripts were identified using ANOVA p-value filtering between respiratory sensitizers and non-respiratory sensitizers, resulting in 999 transcripts (out of 29.141 transcripts) with p≤0.024 (**Fig. 2A**).

**Table 1**. Compounds used for Biomarker signature identification.

| ***Respiratory sensitizers*** |
|---|
| Ammonium hexachloroplatinate |
| Ammonium persulfate |
| Ethylenediamine |
| Glutaraldehyde |
| Hexamethylen diisocyanate |
| Maleic Anhydride |
| Methylene diphenol diisocyanate |
| Phtalic Anhydride |
| Toluendiisocyanate |
| Trimellitic anhydride |
| ***Non-respiratory sensitizers*** |
| 1-Butanol |
| 2-Aminophenol |
| 2-Hydroxyethyl acrylate |
| 2-Nitro-1,4-Phenylenediamine |
| 4-Aminobenzoic acid |
| Chlorobenzene |
| Dimethyl formamide |
| Ethyl vanillin |
| Formaldehyde |
| Geraniol |
| Hexylcinnamic aldehyde |
| Isopropanol |
| Kathon CG |
| Methyl salicylate |
| Penicillin G |
| Propylene glycol |
| Potassium Dichromate |
| Potassium permanganate |
| Tween 80 |
| Zinc sulphate |

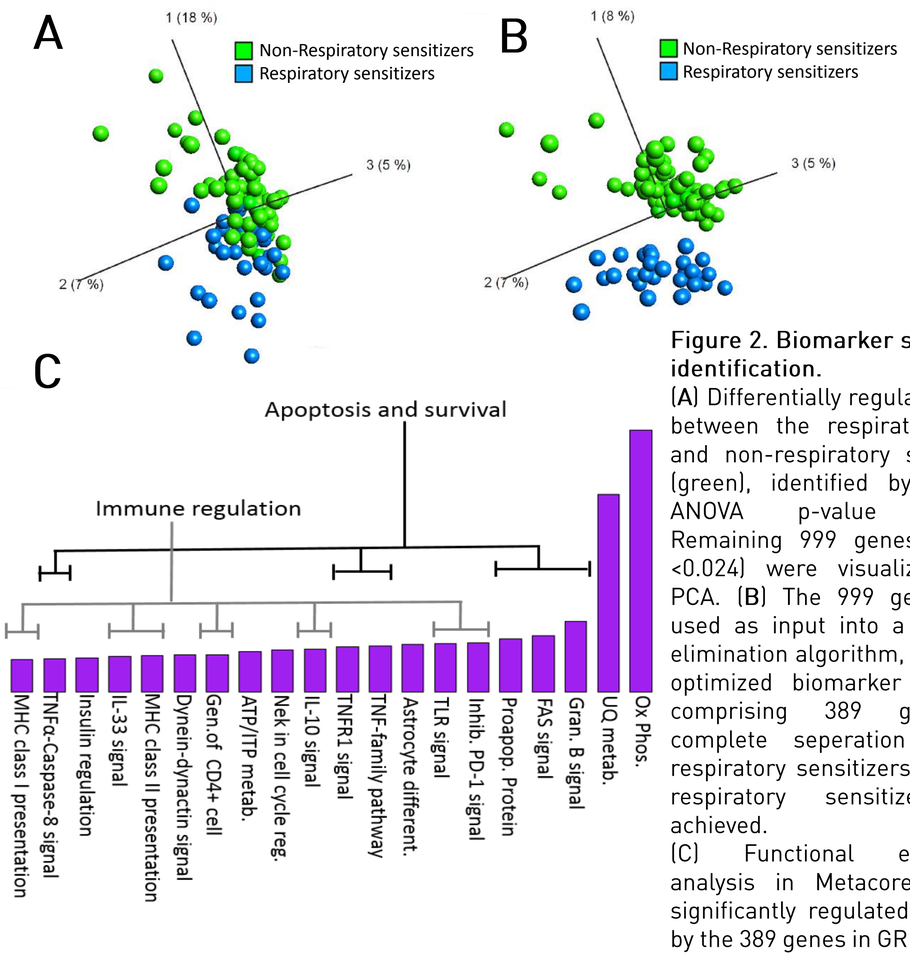


**Figure 2. Biomarker signature identification.**
(**A**) Differentially regulated genes between the respiratory (blue) and non-respiratory sensitizers (green), identified by one-way ANOVA p-value filtering. Remaining 999 genes (p-value <0.024) were visualized using PCA. (**B**) The 999 genes were used as input into a backward elimination algorithm, leaving an optimized biomarker signature comprising 389 genes. A complete seperation between respiratory sensitizers and non-respiratory sensitizers was achieved.
(C) Functional enrichment analysis in Metacore showing significantly regulated pathways by the 389 genes in GRPS.

Second, we applied an in house developed algorithm for backward elimination on the filtered genes in order to further optimize the signature by iteratively removing genes contributing with least information. Highest predictive capacity was observed after elimination of 610 analytes, leaving 389 denoted GARD Respiratory Prediction Signature (GRPS). GPRS demonstrated a potent ability to distinguish respiratory sensitizers from non-respiratory (**Fig. 2B**). Enrichment analysis indicated that the most highly populated pathways activated by respiratory sensitizers belonged to oxidative phosphorylation, ubiquinone metabolism, apoptosis/survival, or being associated with immune regulation **(Fig. 2C)**.

## CONCLUSIONS

Here, we present GRPS, a novel predictive biomarker signature for classification of respiratory sensitizers with a potent ability to predict sensitization.

Combining GRPS with our previously identified biomarker signature for classification of skin sensitizers, the GARD testing strategy provides a hitherto unique method for safety assessment of both skin and respiratory sensitization in the same sample.

## RESULTS – IN HOUSE VALIDATION

The predictive capacity of GRPS was validated using an external test set comprising 70 chemical stimulations (including triplicate or duplicate replications of 25 chemicals) and visualized using PCA plots. A model was trained on the panel of reference chemicals used to identify GRPS (training dataset). The test set was plotted into the PCA space without influencing PCA components (**Fig. 3A**). Samples were colored according to sensitizing properties (**Fig. 3B**) and the training dataset was removed to facilitate interpretation (**Fig. 3C**). Visual classifications were verified using a Support Vector Machine (SVM): the output is illustrated in **Fig. 3D**. Chemicals were classified as respiratory sensitizers if any of the replicate stimulations had an SVM decision value > 0. Accuracy, sensitivity and specificity of GRPS was estimated to 84%, 67%, and 89%, respectively.

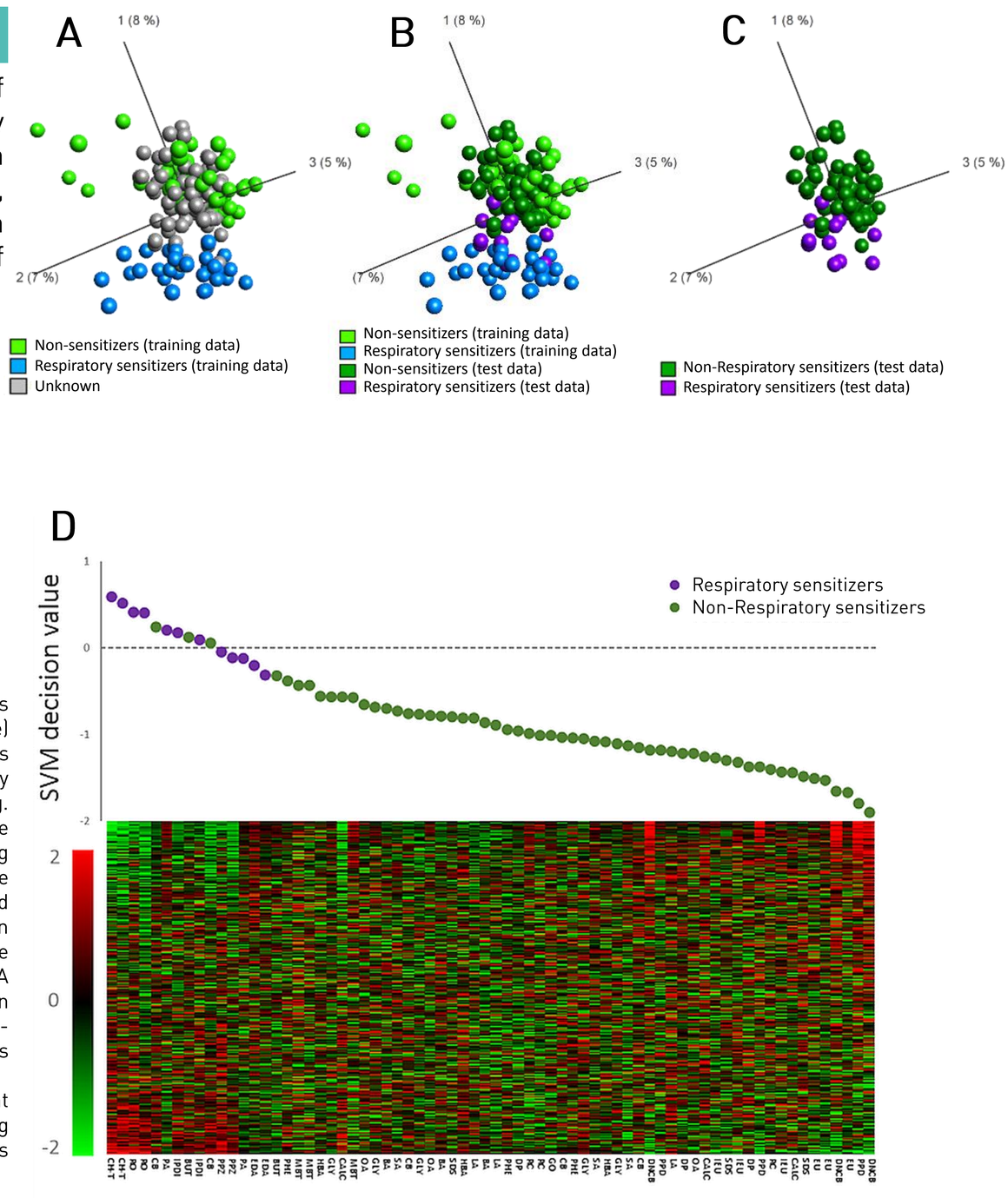


**Figure 3. Validation of biomarker signature.** (**A**) The panel of reference chemicals used to identify GRPS (training dataset) was used to generate the PCA space, using the 389 genes as variable input. The test set was plotted into space, without contributing to the components. (**B**) Samples in test set are colored according to sensitizing properties. (**C**) The training dataset is removed to facilitate interpretation. (**D**) An SVM model was applied to predict samples in the test dataset. SVM decision values are plotted for each compound. The cutoff used for classification as respiratory sensitizers (SVM decision value > 0) is illustrated with a dashed line.

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**Additional reading**

Forreryd A and Johansson H et al. ***Prediction of Chemical Respiratory Sensitizers Using GARD, a Novel In vitro Assay Based on a Genomic Biomarker Signature.*** PLOS ONE. 2015
Forreryd A et al. ***Evaluation of high throughput gene expression platforms using a genomic biomarker signature for prediction of skin sensitization.*** BMC Genomics. 2014
Johansson H et al. ***The GARD assay for assessment of chemical skin sensitizers.*** Toxicology *in vitro.* 2013

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