DIFFERENTIAL GENE EXPRESSION IN DRUG HYPERSENSITIVITY REACTIONS: MOLECULAR BASIS FOR FEMALE PREDILECTION

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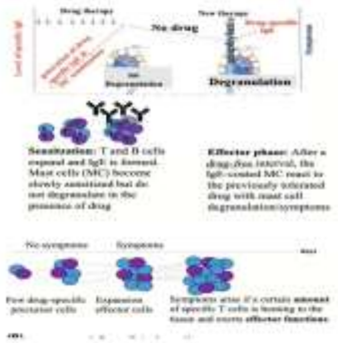
Introduction

Drug hypersensitivity reactions (DHRs) were defined by Pichler as immune mediated reactions characterized by exanthems, fever and internal organ involvement.
They account for one- third to one-sixths of all adverse drug reactions (ADRs) and represent the type B ADRs in which the reactions are neither dose dependent nor predictable. The hapten mechanism expounded by Landsteiner has been used to explain the hapten drug allergy mechanism. It states that small molecules like drugs or drug metabolites are too small to elicit a specific immune response but when bound covalently to proteins can generate a hapten protein complex and then elicit specific immune responses. Both antibody and T cell reactions can be highly specific and can discriminate chemically similar haptens

Drugs of various classes can elicit immune reactions elicit immune reactions and the risk to sensitization and clinical severity of symptoms depends on individual's immune status, immunogenetic predisposition and the female gender. Most studies show that women are more often affected than men (65–70 vs. 30–35%) with also a more cutaneous reaction rate (35% higher in females). In addition, a study reported hospitalized female patients were statistically significantly more likely to develop drug allergy than males. Estrogen has been hypothesized to play a role in this predilection, however a molecular mechanism for female susceptibility is not fully understood.
It has been hypothesized that sex-specific gene regulation underlies important phenotypic gender differences and may contribute to gender differential susceptibility to disease thus a female genetic susceptibility may play a role and the molecular mechanism need to be understood



Methods

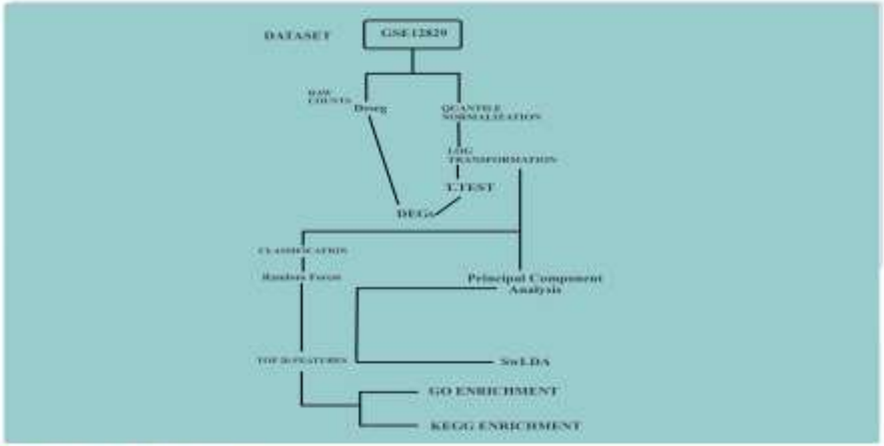
Dataset
We utilized the RNAseq dataset of Bellion et al (2010) where the authors characterized the gene expression profiles of peripheral blood mononuclear cells (PBMCs) isolated from patients during the acute phase of the reaction and upon resolution of clinical symptoms. 13 samples comprising 9 females and 4 males were utilized for this analysis with all samples taken from the gene profiles in the Acute phase of the DHRs

Differential Gene Expression
Deseq2 and Limma package were used to perform the differential expression. The Limma was performed on using the open source R software package (http://www. r-project.org) while the Deseq2 was performed using the T-bio-info (TBI) platform from Tauber Bioinformatics Research Centre. Significance was set for both at a p-value of < 0.05, p adjusted value (Benjamini -Hocberg) of < 0.05 and a log2FoldChange of equals or less than 2 for under-expression and greater than or equals to 2 for upregulation. A BaseMean of 10 across all the samples was also utilized for filtering across samples. Differential expression was performed on the raw counts.

Unsupervised Analysis
Normalization was performed through log fold transformation and variance stabilization, zero and values less than the median expression across the samples were also filtered before a Principal Component Analysis was performed.

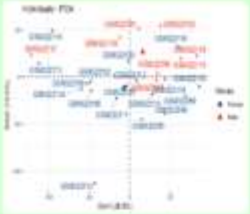
Supervised analysis
Supervised analysis was done with Random forest with the number of trees set at 200, this was then used to classify samples based on gender, the top 20 features were selected. PCA was repeated based on the top 20 selected genes to validate the classification. Random forest was equally repeated with the selected features likewise step wise linear discriminant analysis.

Annotation
The genes highlighted in the differential gene expression were compared and then annotated via the Annotation package of the TBI and further validated by the AnnotationDbi and Org.Hs.Eg.Db R packages. Selected outputs used were for ENSEMBL IDs

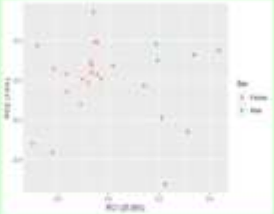


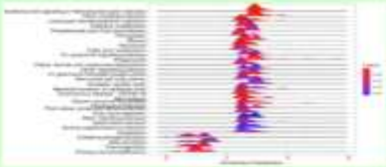
Results

PCA before classification



After classification

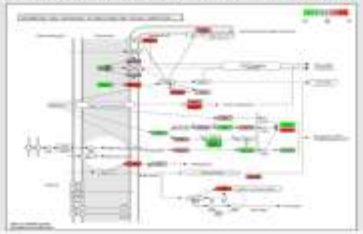




Top 20 genes

GENENAME	SYMBOL
ENSG00000102738	mitochondrial ribosomal protein S31
ENSG00000163914	rhodopsin
ENSG00000006059	keratin 33A
ENSG00000100599	Ras and Rab interactor 3
ENSG00000012817	lysine demethylase 5D
ENSG00000115641	four and a half LIM domains 2
ENSG00000129824	ribosomal protein S4 Y-linked 1
ENSG00000169021	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1
ENSG00000108433	golgi SNAP receptor complex member 2
ENSG00000156052	G protein subunit alpha q
ENSG00000204634	TBC1 domain family member 8
ENSG00000160310	protein arginine methyltransferase 2
ENSG00000167996	ferritin heavy chain 1
ENSG00000172500	FGF1 intracellular binding protein
ENSG00000076662	intercellular adhesion molecule 3
ENSG00000159674	spondin 2
ENSG00000164008	chromosome 1 open reading frame 50
ENSG00000088726	transmembrane protein 40
ENSG00000152214	Ras like without CAAX 2

Ridgeplot, dotplot and the Epithelial signaling pathway



Conclusions

From the classification of the datasets from Bellion et al, a list of genes was seen to be distinctly differentially expressed between the sexes in DHRs. One of the genes is the Gs Alpha subunit of the G coupled receptor which by activating its downstream second messenger system has been reported to be important in maintaining the epithelial integrity and also play a critical role in functions of the melanosomes which equally protect the skin from ultraviolet damage. Protein arginine methyltransferases have been implicated in carcinogenesis and their function in inflammatory conditions have been fully elucidated by Kim et al, as they have been shown to induce T lymphocyte activation in tandem with chemokine signaling. Additionally, the role of Spondin 2 (Mindin) in maintenance of the extracellular matrix and its differential expression between cancerous and non-cancerous lung cancers has also been documented. These distinct gene expression profiles in PMBCs can be used to understand the female predilection in these clinical entities.

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Abel Sousa et al. Gender differential transcriptome in gastric and thyroid cancers. Frontiers in Biology, July 2020 | Volume 11 | Article 808

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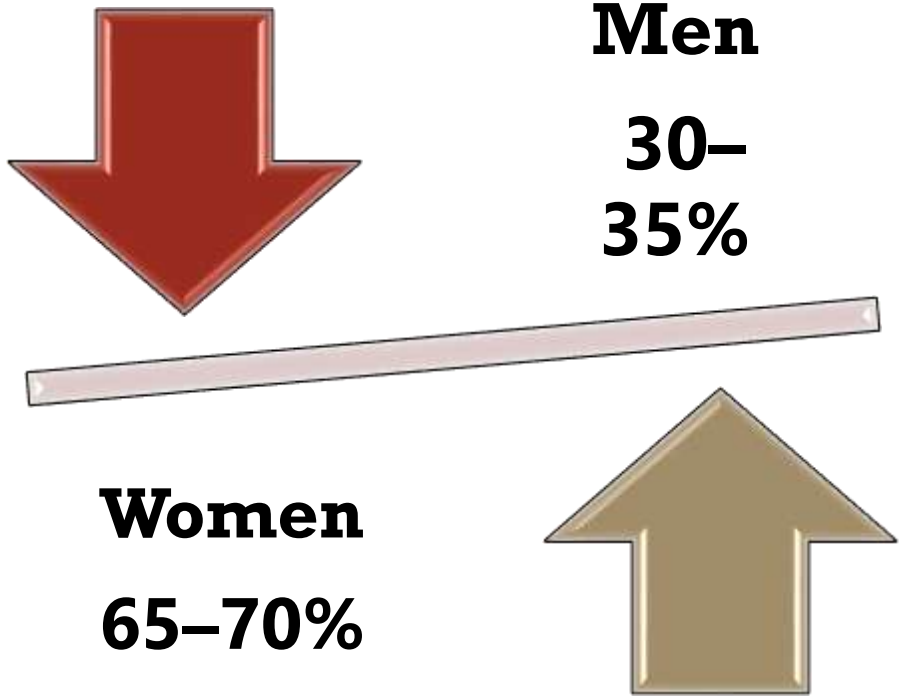

OBJECTIVES

- Identify differential gene expression in the female gender and their male counterparts in DHRs
- Apply a supervised machine learning techniques to retrieve top features and use the features to for classification
- Annotate the said features and their gene ontologies

METHODOLOGY

- We utilized the RNAseq dataset of Bellion et al (2010) where the authors characterized the gene expression profiles of peripheral blood mononuclear cells (PBMCs) isolated from patients during the acute phase of the reaction and upon resolution of clinical symptoms. 13 samples comprising 9 females and 4 males were utilized for this analysis with all samples taken from the gene profiles in the Acute phase of the DHRs

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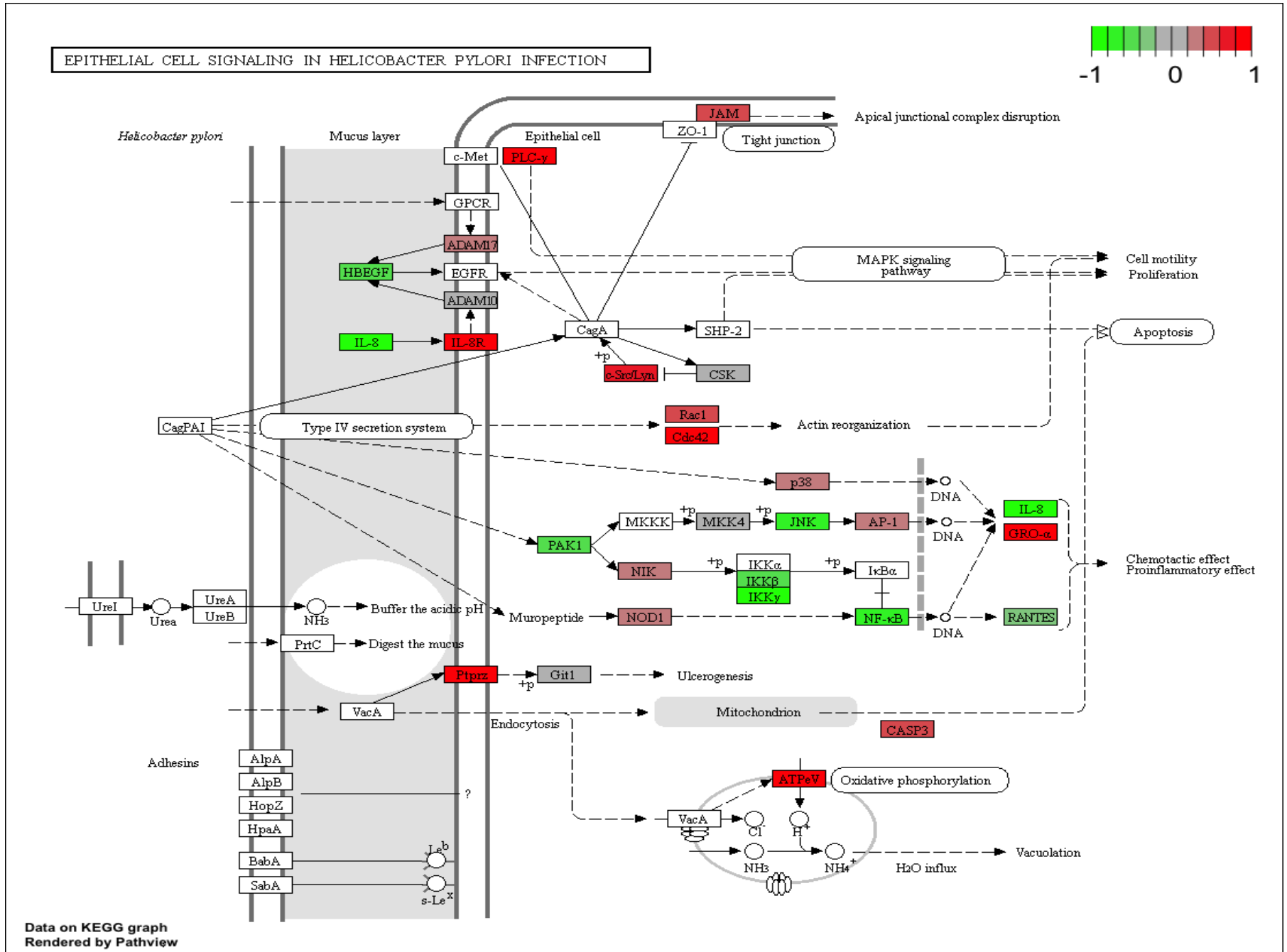
EPITHELIAL CELL SIGNALING IN HELICOBACTER PYLORI INFECTION
-1
0
1
Helicobacter pylori
Mucus layer
Epithelial cell
JAM
ZO-1
Tight junction
Apical junctional complex disruption
c-Met
PLC-γ
GPCR
ADAM17
HBEGF
EGFR
ADAM10
MAPK signaling pathway
Cell motility
Proliferation
IL-8
IL-8R
CagA
SHP-2
Apoptosis
+p
c-Src/Lyn
CSK
CagPAI
Type IV secretion system
Rac1
Cdc42
Actin reorganization
p38
DNA
MKKK
+p
MKK4
+p
JNK
AP-1
DNA
IL-8
GRO-α
PAK1
NIK
+p
IKKα
IKKβ
IKKγ
+p
IκBα
Chemotactic effect
Proinflammatory effect
UreI
Urea
UreA
UreB
NH3
Buffer the acidic pH
Muropeptide
NOD1
NF-κB
DNA
RANTES
PrtC
Digest the mucus
Ptprz
+p
Git1
Ulcerogenesis
VacA
Endocytosis
Mitochondrion
CASP3
Adhesins
AlpA
AlpB
HopZ
HpaA
BabA
SabA
?
Le^b
s-Le^x
ATPeV
Oxidative phosphorylation
VacA
Cl⁻
H⁺
NH3
NH4⁺
H2O influx
Vacuolation
Data on KEGG graph
Rendered by Pathview

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- One of the genes is the Gs Alpha subunit of the G coupled receptor which by activating its downstream second messenger system has been reported to be important in maintaining the epithelial integrity and also play a critical role in functions of the melanosomes which equally protect the skin from ultraviolet damage.
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