Surfing the *PHOME* for Novel Anti-Platelet Agents:

Empirical Evaluation of a Bioinformatic Drug Re-Purposing Algorithm

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Introduction

- ***PHOME*** = ***PH***armacology + (defined) prote***OME***
- Drug discovery is challenging and often proceeds serendipitously
- 'New' drugs can emerge from 'old' drugs = 're-purposing' / 'hit-to-lead'
- Bioinformatics offers a unique but complex insight into pharmacology
- **Q. Can unknown pharmacological effects be predicted in known drugs?**

Study Objectives

- To develop a bioinformatic algorithm to predict novel pharmacological actions in platelets among known drugs
- To assign unknown pharmacological functions to known drugs
- To test these predictions under defined experimental conditions *in vitro* using human platelets

Design & Analysis

- Drugs and vehicle controls were randomised into experimental protocols and the operator was blinded
- Drug effect determined using 2-way ANOVA and Waller-Duncan post-hoc test (SPSS v.23). Data were transformed prior to analysis to minimise heteroscedasticity.

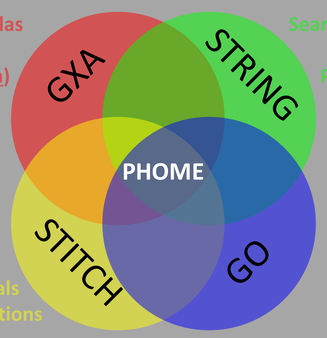
Identifying candidate drugs

1. The ***PHOME*** is a virtual drugome constructed from 4 public resources (GXA, STRING, STITCH, GO)
2. A knowledge graph comprising a protein-protein interaction network was constructed from entities and relations in GXA, STRING, STITCH, GO databases, taking drug targets from DrugBank and normalizing names against the STITCH database to identify known targets. A diffusion kernel (Random Walk with Restart) was applied over the set of drug targets and an enrichment analysis using FUNC was applied to identify the GO functions affected.
3. The PPI-network scored 553 well-known/clinically-used drugs for three GO effects: (i) collagen binding (GO:0005518), (ii) platelet activation (GO:0030168); (iii) platelet aggregation (GO:0070527)
4. Enrichment analysis assigned a *P* value (Wilcoxon rank-sum test) for each drug/GO effect combination
5. Low *P* values predict a biological effect and high *P* values predict a lack of effect
6. 10 drugs from those scoring both 3 × *P*=0 and 3 × *P*=1 were randomly selected for empirical testing

Constructing and Interrogating the *PHOME*

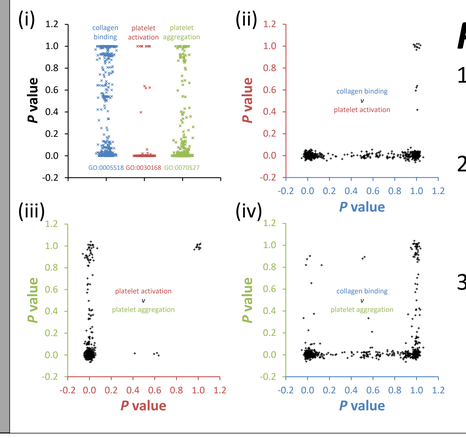
GXA
Gene eXpression Atlas
Platelet Proteins
(www.ebi.ac.uk/gxa)

STRING
Search Tool for the Retrieval of
INteracting Genes/Proteins
Protein-Protein Interactions
(string-db.org)



STITCH
Search Tool for
InTeracting CHemicals
Pharmacological Actions
(stitch.embl.de)

GO
Gene Ontology database
Drug Indications
& Gene Function
(geneontology.org)



PHOME output

1. 553 chemical entities were assigned 3 *P* values, one for each GO function
2. Values are shown in Panel (i) for collagen binding, platelet activation and platelet aggregation
3. Panels (ii) – (iv) show pairwise comparisons of the *P* values for each pairing of GO functions

Aggregometry

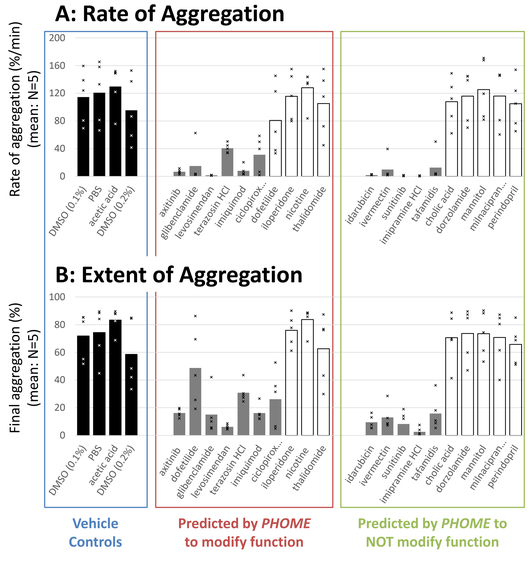


Fig. 1: Turbidimetric aggregometry[1] was performed in washed platelets[2] (200 × 10^6 ml^{-1}) using two Helena AggRAM aggregometers. Treatment groups included **4 vehicle controls**, **10 drugs predicted by the *PHOME* to modify function**, and **10 drugs predicted to have no effect**. Data are from five independent experiments. Data points are the mean of 4 or 5 replicate measurements made within each experiment. Aggregation was induced with Horm collagen **(1 µg.ml^{-1})**. Drugs were pre-incubated at **100 µM**. **(A)** Response variable is the initial rate of aggregation (%/min), (r_φ = -0.1; *P* = 1.0 (Fisher's Exact test). **(B)** response variable is the final extent of aggregation 6 min after addition of agonist (%), (r_φ = -0.2, *P* = 0.65 (FET)). Light grey bars indicate a statistical effect (Type I/Type II error ratio = 100).

Static Adhesion

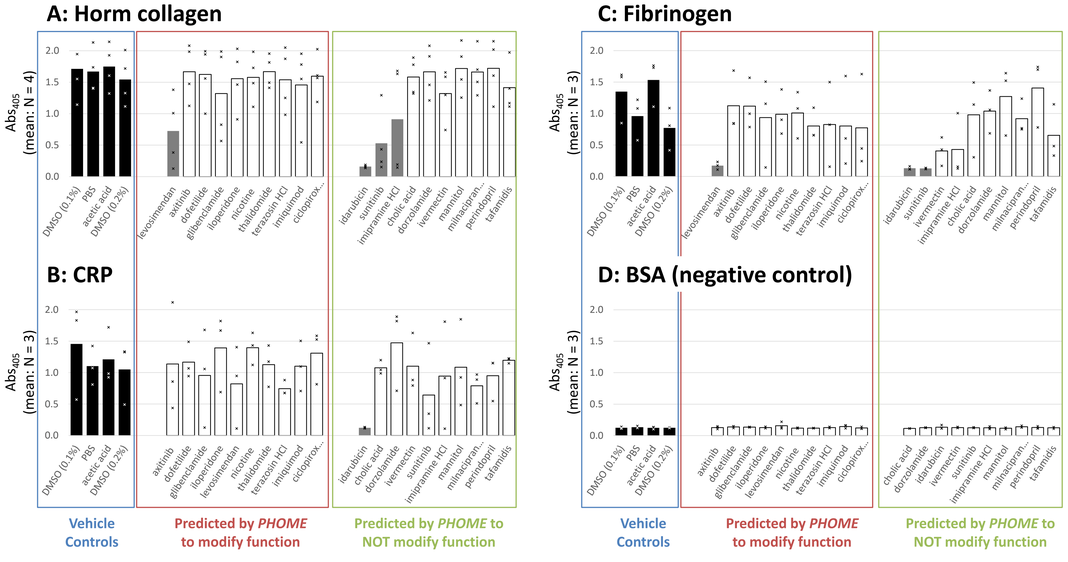


Fig. 2: Static Adhesion[3] of washed platelets[2] (125 × 10^6 ml^{-1}) to **(A)** Horm collagen, **(B)** collagen-related peptide (CRP), **(C)** fibrinogen and **(D)** BSA was measured. 100 µl of adhesive ligands (10 µg.ml^{-1}) were incubated overnight at 4°C in each well. Platelets were pre-treated as follows: **4 vehicle controls**, **10 drugs predicted by the *PHOME* to modify function**, and **10 drugs predicted to have no effect**. Drugs were used at 100 µM and platelets were incubated for 1 hour. Data are from four (collagen) or three (CRP, fibrinogen & BSA) independent experiments. Data points are the mean of 2 replicate measurements made within each experiment. (r_φ = -0.1; *P* = 1.0 (Fisher's Exact test). Light grey bars indicate a statistical effect (Type I/Type II error ratio = 100).

Conclusions

- The *PHOME* is a prototype algorithm for identifying previously unrecognised pharmacological effects of known drugs
- Quantitative functional data suggest that the *PHOME* is no more effective than a random selection of drugs
- Iterative targeted scrutiny can identify database errors leading to optimisation of the *PHOME*
- The study has identified drugs with clear functional effects on platelets that may be further investigated

References:

[1] Jarvis GE, *Meth Mol Biol* 2004;**272**:65-76 PMID: 15226534.
[2] Jarvis GE *et al., Brit J Pharmacol 2002;***137**:107-117 PMID: 12183336.
[3] Jarvis GE *et al., Blood 2008;***111:**4986-96 PMID: 18305222.

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