Exploring Cardiovascular Disease in the Domestic Pig at RGD

https://rgd.mcw.edu

Mahima Vedi[1], Jennifer R Smith[1], G Thomas Hayman[1], Mary L. Kaldunski[1], Shur-Jen Wang[1], Stanley J Laulederkind[1], Morgan Hill[1], Wendy Demos[1], Monika Tutaj[1], Adam Gibson, Logan Lamers[1], Harika S Nalabolu[1], Ketaki Thorat[1], Jyothi Thota[1], Marek A Tutaj[1], Jeffrey L De Pons[1], Melinda R Dwinell[1,2] Anne E Kwitek[1,2]

[1]*Rat Genome Database, Department of Biomedical Engineering, Medical College of Wisconsin and Marquette University, Milwaukee, WI, 53226, USA*

[2]*Department of Physiology, Medical College of Wisconsin, Milwaukee, WI, 53226, USA*

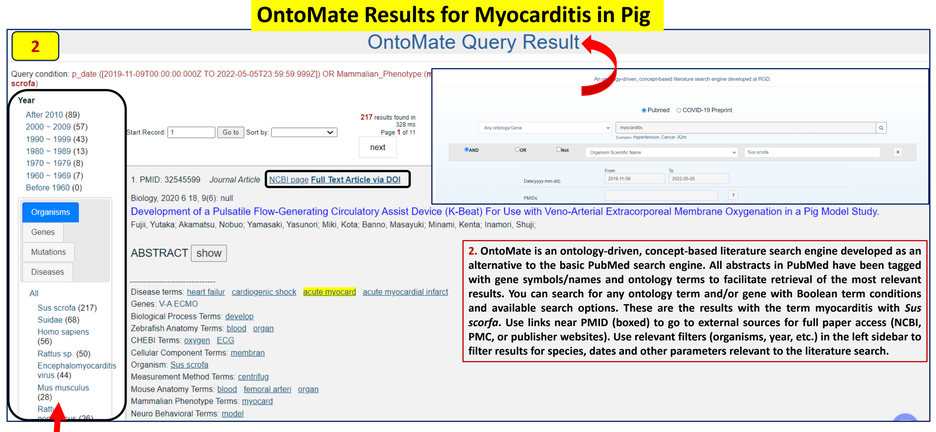
ABSTRACT

RGD (https://rgd.mcw.edu) has integrated data for rat and nine additional species (pig, human, mouse, chinchilla, dog, bonobo, 13-lined ground squirrel, vervet, and naked mole-rat) for cross-species analysis. RGD has a dedicated species portal for each species it houses that can be accessed directly from the RGD home page. Each species portal includes links to data and RGD-developed bioinformatics tools related to the species. Here, we introduce RGD's toolbox and datasets, focusing on cardiovascular disease as an example, for the pig research community interested in leveraging comparative genomics insights to understand human afflictions.

Cardiovascular disease is one of the fifteen disease portals at RGD, where users can explore relationships between diseases and genes in all RGD species, as well as available QTLs and strains. One can directly access the cardiovascular disease portal from the RGD main menu. A cardiovascular disease term-specific gene list for pig can be found by selecting the term in the ontology browser and selecting pig as the species in the cardiovascular disease portal. The user can download the gene list to analyze it with RGD tools available in the 'Analysis & Visualization' menu on the RGD home page or directly from within the pig species portal.

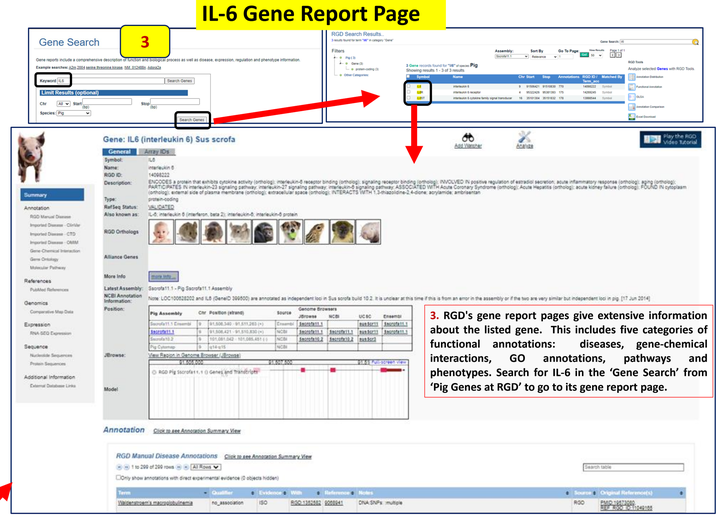
RGD's domestic pig (*Sus scrofa)* species portal also provides access to tools such as OntoMate to find publications using ontology tags, JBrowse to view genes in their genomic context, the Object List Generator and Analyzer tool (OLGA) and Gene Annotator (GA) tools to explore functional annotations for a list of genes, Multi-Ontology Enrichment Tool (MOET) to find over-represented terms within a list of genes/proteins and InterViewer for visualizing protein-protein interactions. Each of the tools provides links for navigation between these tools to simplify the analysis workflows. Also, links to RGD's gene report pages for specific gene search, Gene Ontology annotations, interactive pathway annotations and diagrams, and RGD's Disease annotations, which present consolidated data for disease categories, can be accessed directly from the pig species page. Thus, RGD has a wealth of information and tools for the pig research community.

OntoMate Results for Myocarditis in Pig



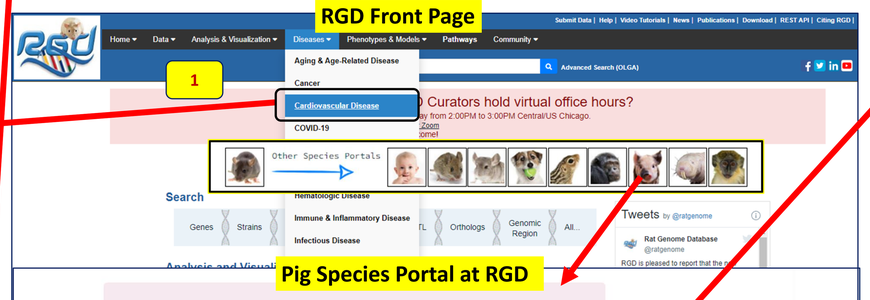
2. OntoMate is an ontology-driven, concept-based literature search engine developed as an alternative to the basic PubMed search engine. All abstracts in PubMed have been tagged with gene symbols/names and ontology terms to facilitate retrieval of the most relevant results. You can search for any ontology term and/or gene with Boolean term conditions and available search options. These are the results with the term myocarditis with *Sus scrofa*. Use links near PMID (boxed) to go to external sources for full paper access (NCBI, PMC, or publisher websites). Use relevant filters (organisms, year, etc.) in the left sidebar to filter results for species, dates and other parameters relevant to the literature search.

IL-6 Gene Report Page

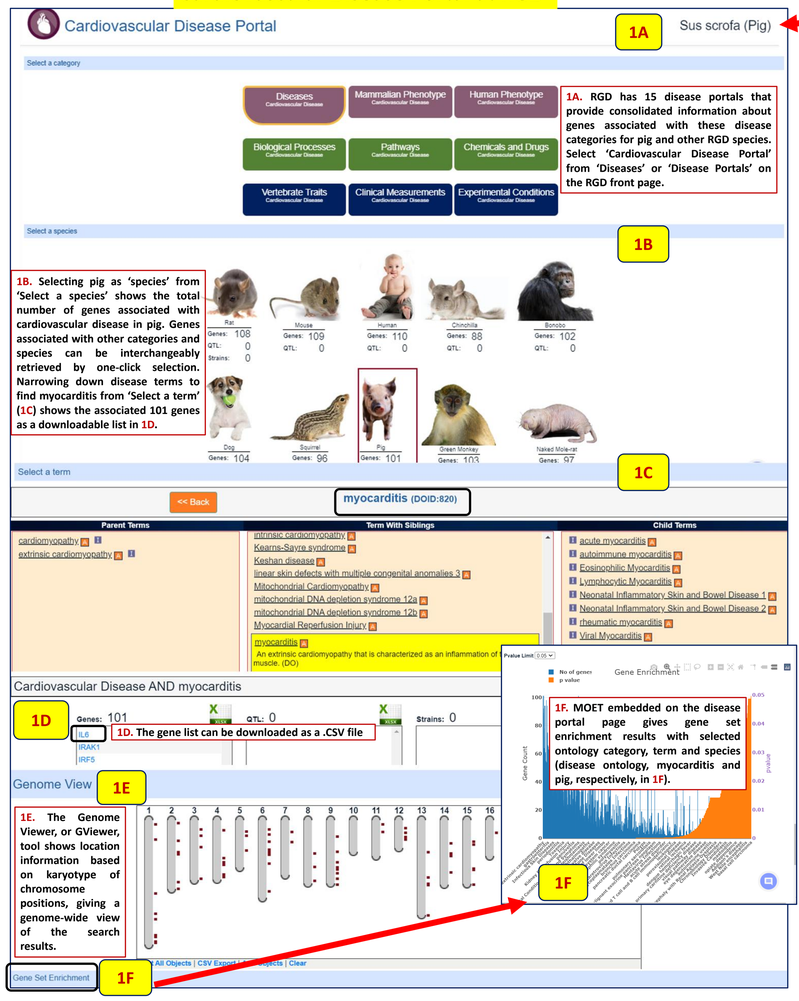


3. RGD's gene report pages give extensive information about the listed gene. This includes five categories of functional annotations: diseases, gene-chemical interactions, GO annotations, pathways and phenotypes. Search for IL-6 in the 'Gene Search' from 'Pig Genes at RGD' to go to its gene report page.

RGD Front Page



Cardiovascular Disease Portal at RGD

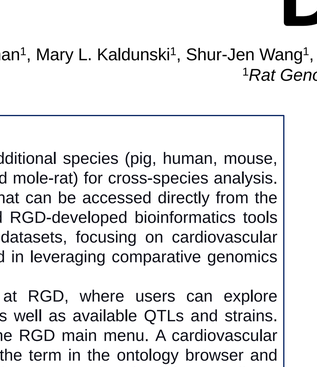


1A. RGD has 15 disease portals that provide consolidated information about genes associated with these disease categories for pig and other RGD species. Select 'Cardiovascular Disease Portal' from 'Diseases' or 'Disease Portals' on the RGD front page.

1B. Selecting pig as 'species' from 'Select a species' shows the total number of genes associated with cardiovascular disease in pig. Genes associated with other categories and species can be interchangeably retrieved by one-click selection. Narrowing down disease terms to find myocarditis from 'Select a term' (1C) shows the associated 101 genes as a downloadable list in 1D.

1D. The gene list can be downloaded as a .CSV file

1E. The Genome Viewer, or GViewer, tool shows location information based on karyotype of chromosome positions, giving a genome-wide view of the search results.

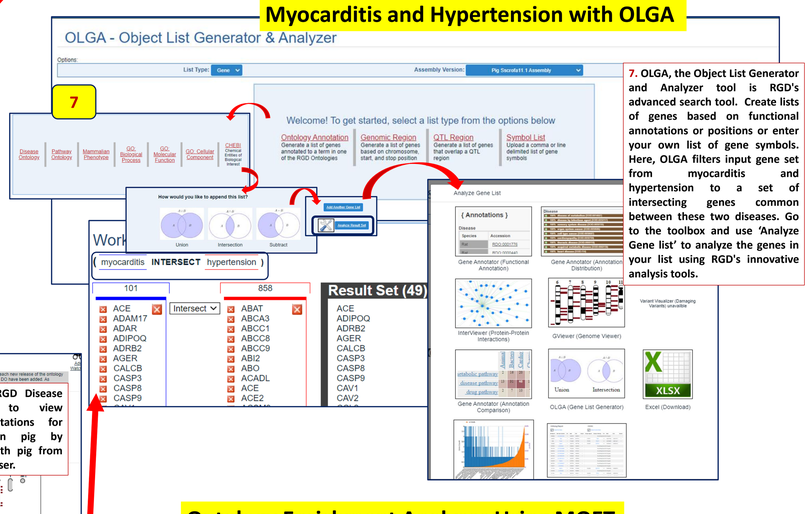


1F. MOET embedded on the disease portal page gives gene set enrichment results with selected ontology category, term and species (disease ontology, myocarditis and pig, respectively, in 1F).

Pig Species Portal at RGD

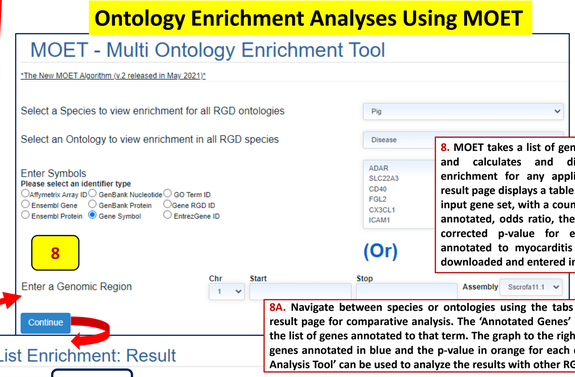
4. Click on 'RGD Disease Annotations' to view disease annotations for myocarditis in pig by selecting it with pig from Ontology Browser.

Myocarditis and Hypertension with OLGA

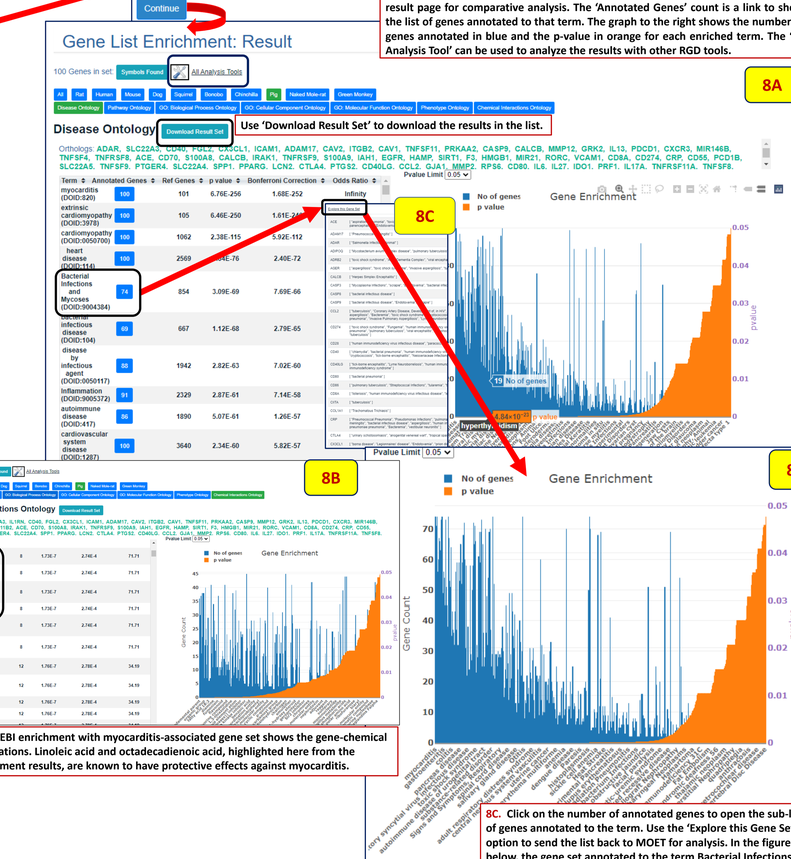


7. OLGA, the Object List Generator and Analyzer tool is RGD's advanced search tool. Create lists of genes based on functional annotations or positions or enter your own list of gene symbols. Here, OLGA filters input gene set from myocarditis and hypertension to a set of intersecting genes common between these two diseases. Go to the toolbox and use 'Analyze Gene list' to analyze the genes in your list using RGD's innovative analysis tools.

Ontology Enrichment Analyses Using MOET



8. MOET takes a list of genes from any RGD species and calculates and displays ontology term enrichment for any applicable ontology. MOET's result page displays a table of terms enriched for the input gene set, with a count of the number of genes annotated, odds ratio, the p-value and Bonferroni-corrected p-value for each term. Gene list annotated to myocarditis term in pig in 1D was downloaded and entered in MOET.



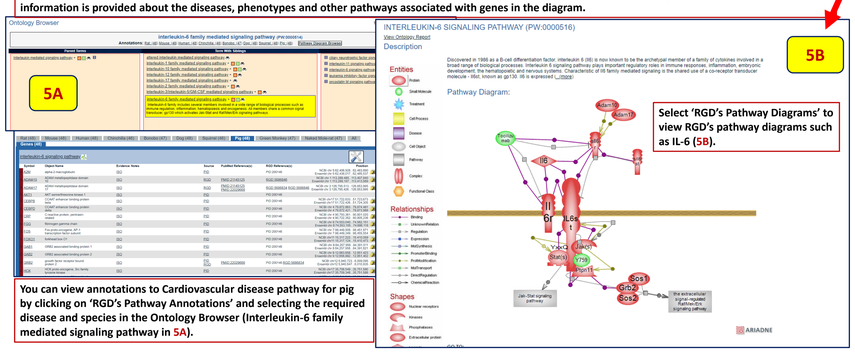
8A. Navigate between species or ontologies using the tabs across the top of the result page for comparative analysis. The 'Annotated Genes' count is a link to show the list of genes annotated to that term. The graph to the right shows the number of genes annotated in blue and the p-value in orange for each enriched term. The 'All Analysis Tool' can be used to analyze the results with other RGD tools.

8B. ChEBI enrichment with myocarditis-associated gene set shows the gene-chemical associations. Linoleic acid and octadecadienoic acid, highlighted here from the enrichment results, are known to have protective effects against myocarditis.

8C. Click on the number of annotated genes to open the sub-list of genes annotated to the term. Use the 'Explore this Gene Set' option to send the list back to MOET for analysis. In the figure below, the gene set annotated to the term Bacterial Infections and Mycoses is analyzed with MOET.

IL-6 Signaling Pathway at RGD

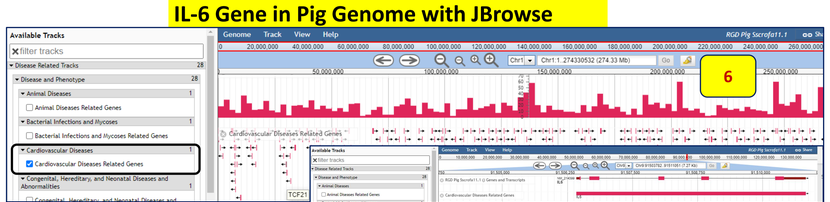
5A and 5B. RGD's interactive pathway diagrams allow users to explore a variety of metabolic, signaling, regulatory, disease and drug pathways. Additional information is provided about the diseases, phenotypes and other pathways associated with genes in the diagram.



Select 'RGD's Pathway Diagrams' to view RGD's pathway diagrams such as IL-6 (5B).

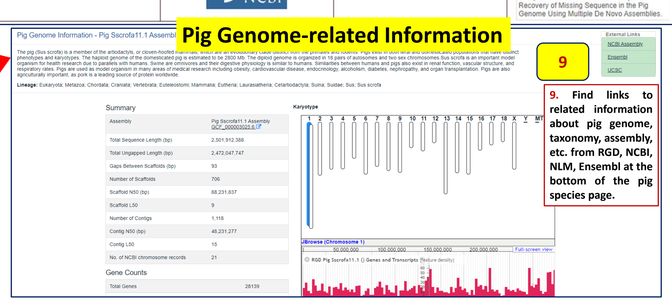
You can view annotations to Cardiovascular disease pathway for pig by clicking on 'RGD's Pathway Annotations' and selecting the required disease and species in the Ontology Browser (Interleukin-6 family mediated signaling pathway in 5A).

IL-6 Gene in Pig Genome with JBrowse



6. The JBrowse genome browser shows genes and other data objects in their genomic context. In addition to the standard gene/transcript tracks, RGD's pig JBrowse has tracks for disease- and chemical-associated genes. IL-6 is one of the genes associated with myocarditis from the gene list in 1D. Click on 'Cardiovascular Disease Related Genes' in the left JBrowse menu to view other cardiovascular disease-related genes in JBrowse.

Pig Genome-related Information



9. Find links to related information about pig genome, taxonomy, assembly, etc. from RGD, NCBI, NLM, Ensembl at the bottom of the pig species page.

RGD is grateful for funding support from the National Heart, Lung, and Blood Institute (NHLBI; R01HL064541) and from the National Human Genome Research Institute (NHGRI) as part of the Alliance of Genome Resources (U24HG010859).