Microarray data analysis of space grown *Arabidopsis* leaves for genes important in vascular patterning. A. J. Weitzel, 1,2 S. E. Wyatt 3, P. Parsons-Wingerter 1.

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Venation patterning in leaves is a major determinant of photosynthesis efficiency because of its dependency on vascular transport of photoassimilates, water, and minerals. *Arabidopsis thaliana* grown in microgravity show delayed growth and leaf maturation. Gene expression data from the roots, hypocotyl, and leaves of *A. thaliana* grown during spaceflight vs. ground control analyzed by Affymetrix microarray are available through NASA’s GeneLab (GLDS-7). We analyzed the data for differential expression of genes in leaves resulting from the effects of spaceflight on vascular patterning. Two genes were found by preliminary analysis to be upregulated during spaceflight that may be related to vascular formation. The genes are responsible for coding an ARGOS like protein (potentially affecting cell elongation in the leaves), and an F-box/kelch-repeat protein (possibly contributing to protoxylem specification). Further analysis that will focus on raw data quality assessment and a moderated t-test may further confirm upregulation of the two genes and/or identify other gene candidates. Plants defective in these genes will then be assessed for phenotype by the mapping and quantification of leaf vascular patterning by NASA’s VESSEL GENeration (VESGEN) software to model specific vascular differences of plants grown in spaceflight.

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NASA’S VESGEN Software

Research Discovery Tool
for Fundamental and Translational
Space Biology Research

Using NASA’s GeneLab for VESGEN Systems Analysis
of Vascular Phenotypes from Stress and
Other Signaling Pathways

Patricia Parsons-Wingerter
Space Biosciences Research Branch

VESGEN Patents Pending
Collaborators

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NASA’S VESsel GENeration Analysis [VESGEN] Software

Mapping and Quantification of Branching Vascular Patterns from Physiological Branching Rules

- Vascular trees, networks, and tree-network composites from set of weighted parameters for vessel connectivity, tapering and bifurcational branching

- Requirements of fluid dynamics for laminar flow
  Aqueous vascular transport by complex distributed system of fractal-based bifurcational branching

- Microvascular rules for fractal-based branching within humans, vertebrates, insects and dicot leaves therefore display many similarities

VESGEN Patents Pending
VESGEN Analysis for Fundamental Space Biology Research with Translational Applications to Astronaut Health and Countermeasures

Vascular Trees
Retinas of Astronauts and Human Bed Rest; Diabetic Retinopathy
Mouse/Avian Coronary Vessels, Chorioallantoic Membrane (CAM), Yolksac

Vascular Networks
Mouse Intestinal Inflammation, CAM Lymphatic Vessels, Abnormal Mouse Corneal Angiogenesis, Drosophila (Fruitfly) Wing

Vascular Tree-Network Composites
Mouse Postnatal Retina
Early Embryonic Coronary Vessels, Arabidopsis Leaf Venation
Research Hypothesis
Vascular patterning offers useful readout of molecular signaling that necessarily integrates crosstalk among complex signaling pathways

Fractal-Based Physiological Branching Rules
from fluid mechanics, anatomy, microscopic observations

Mapping and Quantification by Multiparametric Weighted Analysis
Fractal Dimension, $D_f$
Vessel Number Density, $N_v$
Vessel Length Density, $L_v$
Vessel Diameter, $D_v$
Branchpoint + Endpoint Densities, $Br_v + E_v$
Arabidopsis leaves from ISS: STS-130

Images of STS-130 leaves provided by A-L Paul & RJ Ferl, analyzed by P Parsons & M Vickerman
VESGEN mapping of Arabidopsis leaf venation with bioinformatic analysis


Differentiated Xylem

**a** D2

**f** D8

**b**

**g**

**c**

**d**

**e**

**c**

**d**

**e**

**Taxonomy & Phylogeny:** Grouping by Venous Branching Orders

**Integrative Bioinformatics:** AtHB8::GUS by Structural & Reticulate Vein Grouping

Venous Diameter by Distance Mapping

Day 2

Vein Order (*)

Day 8

©P Parsons, M Vickerman, A-L Paul, R Ferl, ASGSR 2012  *Grav and Space Research* 2(1) 2014
The Arabidopsis spaceflight transcriptome: a comparison of whole plants to discrete root, hypocotyl and shoot responses to the orbital environment

2 datasets available for download here:
ISA-TAB Metadata file
Raw Data File

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Space Grown *Arabidopsis* with Microarray Data from GeneLab: Identification of Genes Important in Vascular Patterning

(A Weitzel, P Parsons, S Wyatt; ASGSR 2016)

- Analysis of transcriptomic data from space flight and ground control leaves identified differential expression of 22 genes, of which seven may be related to plant vasculature.

- Two gene clusters suggest there may be phenotypic changes in leaf venation resulting from development in microgravity.

  - **KISS ME DEADLY** [KMD] coding F-box genes
  - **NAM**, **ATAF1/2**, and **CUC2** [NAC] related genes

- Vascular-related changes in leaf gene expression can potentially be phenocopied by mutants in ground-based experiments and corroborated by VESGEN analysis.

- Genetic, transcriptional and other molecular changes reported by GeneLab can be mapped to vascular phenotypes by VESGEN by bioinformatic co-localization of single molecular expression.
VESGEN mapping of vascular networks for progressive GI inflammation progression with mouse model

Parsons, Reinecker, Chen et al. *Gravitational Space Biology* 25(1):69 2012; *PlosONE* 2013
Vascular Networks in Transgenic Mouse Retina

Fluorescence Microscopy

VESGEN Network Output

Distance Mapping

Colorized Skeleton

Mouse Retina

Control (P15)

TA-Treated

with J Sears & Q Ebrahem (Cole Eye Institute), from Vickerman et al, Anat Rec 292(3), 2009
VESGEN Hypothesis  ‘Fingerprint’ or ‘Signature’ Vascular Pattern as Useful Integrative Readout of Complex Molecular Signaling Pathways

bFGF as Simple Stimulator

VEGF as Vascular Complexity Factor: Phenotypic Readout with eNOS Signaling
Microvascular Research 72 (2006)

TGF-β1 as Simple Inhibitor but Complex Potentiator
Microvascular Research 59 (2000)

The form of an object is a ‘diagram of forces’
D’Arcy Thompson
Fruitfly (*Drosophila melanogaster*): Major Genetic Model Organism

Mapping by VESGEN: Hairless (H-C2) overexpression induces phenotypic series of increasing ectopic wing venation

6 Longitudinal Veins (LV) with anterior and posterior Cross Veins (CV)

Wildtype, no heat shock

* where veinlets may arise after heat shock in wt or H-C2

LV1
LV2
LV3
LV4
LV5
LV6

**1** Ectopic veins distal between LV1 and LV2

**2** Ectopic veins distal between LV1 and LV2 and below LV5

**3** Increased branching of ectopic veins with vein dots between LV4 and LV5

**4** Increased branching and detachment of posterior CV from LV5

**5** Massive network of ectopic veins in above fields

Expression of Genes Involved in Drosophila Wing Morphogenesis and Vein Patterning Are Altered by Spaceflight

Parsons, Hosamani, Vickerman, Bhattacharya
Grav Space Res 3(2):54-64 2015, ASGSR 2012
Insights into progression of visually impairing disease

VESGEN Analysis of Vascular Patterning for Fundamental Space Biology with Translational Applications to Astronaut and Terrestrial Health

PIV with NASA Aeronautics

**VESGEN Patents Pending**


**IOVS 51(1):498 2011**

**GSR 2(1):68 2014**

**GSR 3(2):54-64 2015**
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Lerner Research Institute— Paul DiCorleto, Unni Chandrasekharan

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