Leveraging the ADC network to gain insights into how rare genetic variants alter risk for AD

A proposal to demonstrate the power of the ADCs working together

Guiding Principles

- Genetic variants that alter risk for AD can provide novel insights into the disease pathogenesis
- These insights in turn can potentially guide development of novel therapeutic approaches
- Uncommon variants (MAF 0.1-2%) can be identified in the active ADC cohorts and autopsy cases and studied collectively, *but* no single ADC will have a sufficient group size to make strong conclusions on how the genotypes alters the human phenotype
  - Other cohorts with well-annotated clinical and biomarker phenotype may also be leveraged
- Because of the the frequency of these variants, only by working together can we rapidly link genotype and phenotype
- We may also need to bring in outside expertise when genetic discoveries point to biology outside the brain!

Leveraging Recent Genetic Insights to Better Understand the Role of Innate Immune genes in AD.

- Genetic advances show uncommon variants in three innate immune genes alter risk for AD

Variant	OR	MAF cases	MAF control	p value
TREM2 R47H	2.46	0.004	0.002	5.38E-24
TREM2 R62H	1.67	0.014	0.009	1.55E-10
ABI3 S209F	1.43	0.011	0.008	4.56E-10
PLCG2 P522R	0.68	0.006	0.009	5.38E-10

- In ~14,000 active ADC participants we would expect from ~50 to ~150 individuals with these variants or 2-5 per center

nature genetics

Rare coding variants in *PLCG2*, *ABI3*, and *TREM2* implicate microglial-mediated innate immunity in Alzheimer's disease

Gene	MAF cases	MAF controls	hets/4,912 (controls)	hets/5,771 (cases)
TREM2	0.004	0.002	~20	~46
TREM2	0.014	0.008	~78	~159
PLCG2	0.006	0.009	~88	~69
ABI3	0.011	0.008	~78	~126

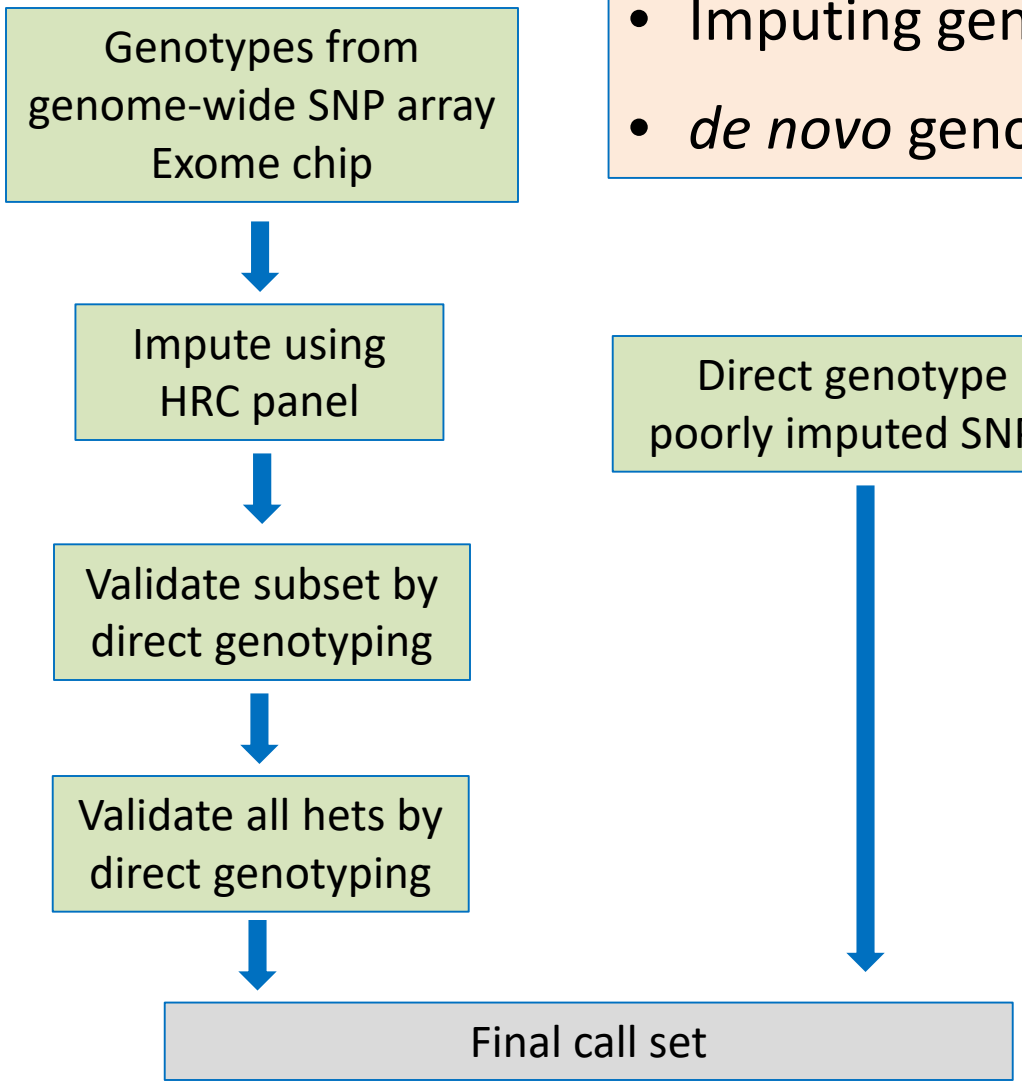
Innate immune system genes

- *HLA-DRB5*
- *MS4A cluster*
- *SORL1*
- *SPI1*
- *INPPSD*
- *CR1*
- *PLCG2*
- *ABI3*
- *TREM2*
- *APOE*

Polygenic “innate immunity” risk score

Genotyping

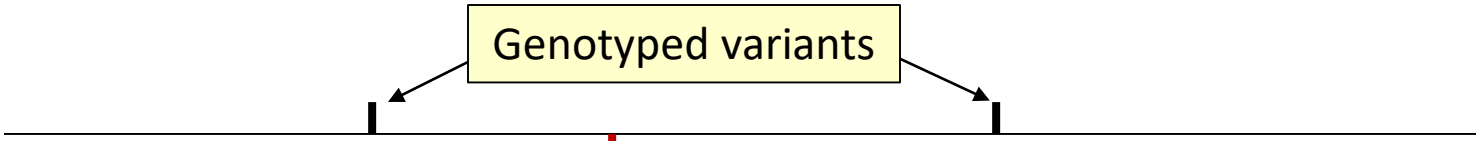
- Existing genotypes: common variants
- Imputing genotypes: Common and rare
- *de novo* genotypes: common and rare



MAF: minor allele frequency
Common: MAF ≥ 2%
Rare: MAF < 2%

Imputation

MAF: minor allele frequency



Imput genotype from external haplotype reference panel

Imputation panels: 1,000 - lower quality genomes (2010)
~32,284 mostly high quality genomes (HRC 2015)
39,235,157 SNPs
~65,000 mostly high quality genomes (2017)

Imputation R^2: 0.7 for MAF = 0.01%
0.8 for MAF = 0.1%

Imputed genotypes for ABI3, TREM2, and PLCG2 are **>88.5% concordant** with experimentally determined genotypes (*n* = 13,000 samples).

MAF 0.002 – 0.009

ADGC Cohorts

Cohort	Cases	Controls
ADC1	1,549	512
ADC2	727	156
ADC3	894	586
ADC4	304	377
ADC5	286	505
ADC6	213	338
ADC7	566	878
ADC8	517	664
ADC9	728	896

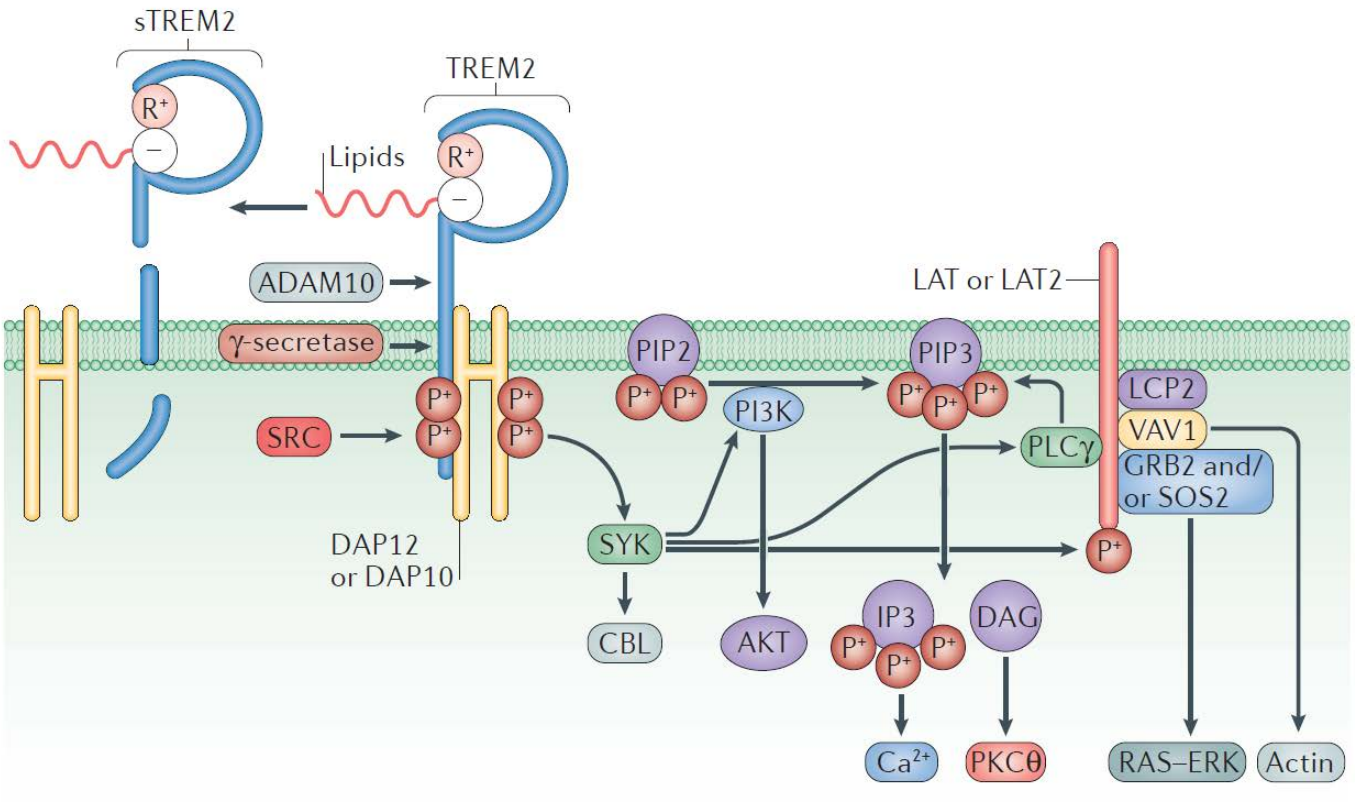
5,771 cases
4,912 controls
10,683 total

AD cases
MCI
other controls
prospective cohorts

TREM2, ABI3, PLCG2

- Selective expression on immune cells
  - Microglial cells in brain
  - Monocytes/Macrophages and other immune cells in periphery
- Other variants in TREM2 and PLCG2 cause immune related phenotypes
  - TREM2 PLOSL, Dementia with bone cysts, recessive loos of function variants
  - PLCG2 both loss of function and “hypermorphic” variants associated with immune syndromes
- Can peripheral immune phenotypes inform us of how these influence risk for AD?

TREM2 PLOSL vs AD



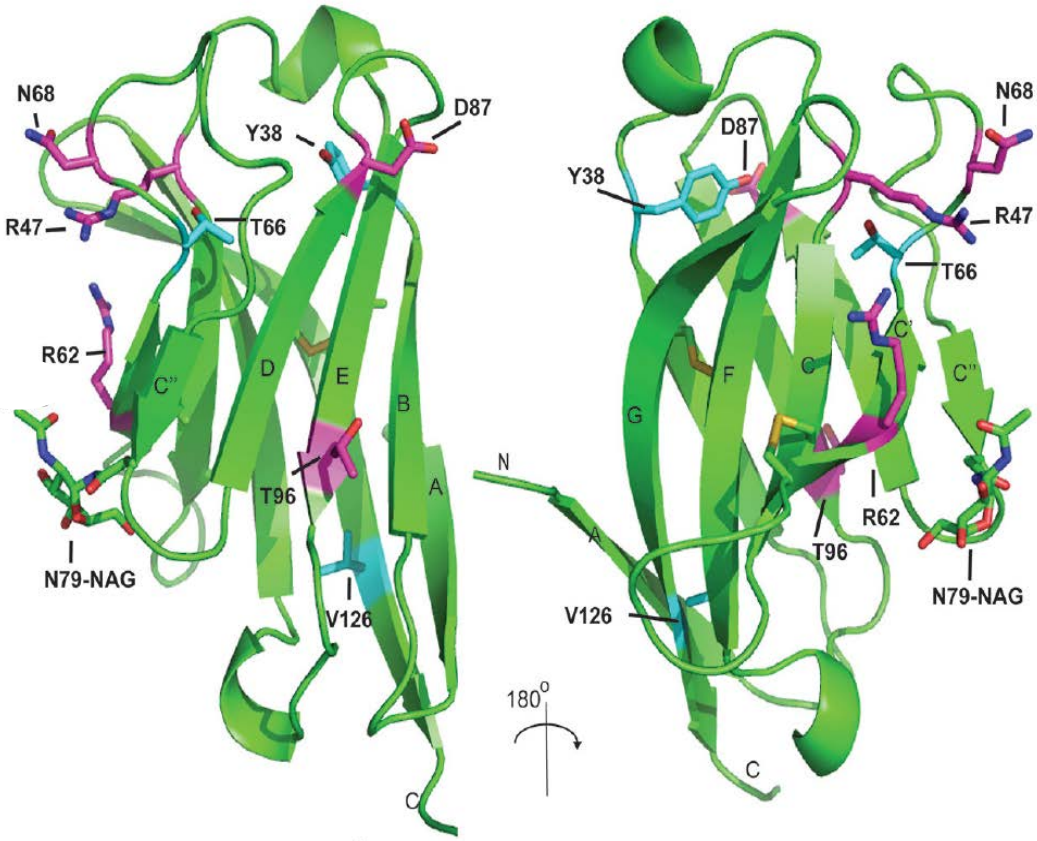
TREM2 variants: new keys to decipher Alzheimer disease pathogenesis

Marco Colonna and Yaming Wang

TREM2 PLOSL vs AD

- **Nasu-Hakola disease (polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy--PLOSL): a dementia associated with bone cystic lesions**
  - Recessive mutations in TREM2 or signaling partner TYROBP (loss of function)
  - Bone lesions due to osteoclast dysfunction
- AD variants, biology remains uncertain, partial loss of function?
  - Bone density alterations in TREM2 R47H, R62H carriers?
- TREM2 is expressed at low levels on circulating monocytes, but is expressed in tissue macrophages

TREM2 PLOSL vs AD

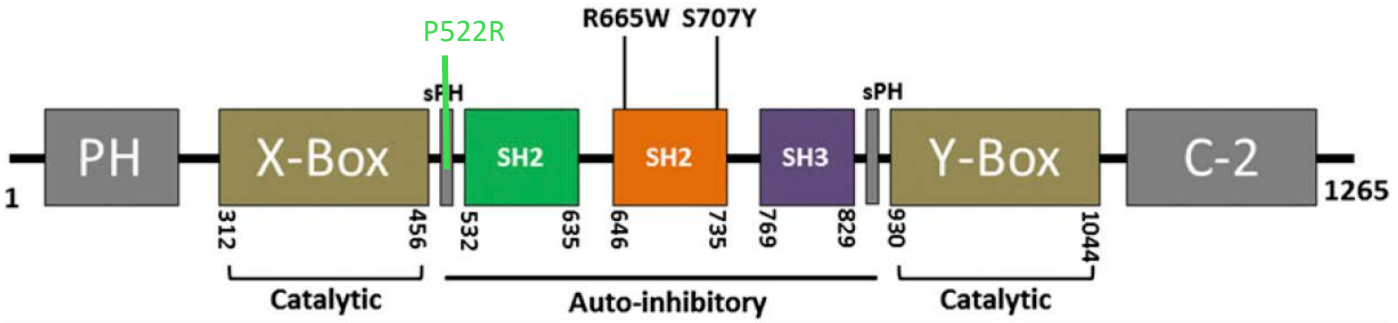


Neurodegenerative disease mutations in TREM2 reveal a functional surface and distinct loss-of-function mechanisms

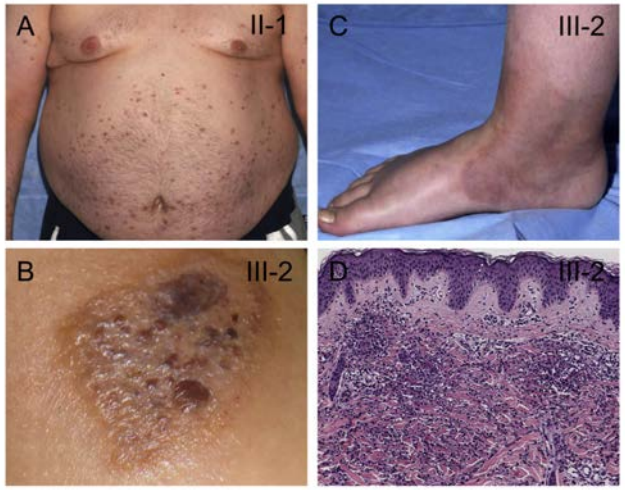
Daniel L Kober[1,2], Jennifer M Alexander-Brett[2], Celeste M Karch[3], Carlos Cruchaga[3], Marco Colonna[4], Michael J Holtzman[2,5,6], Thomas J Brett[2]*

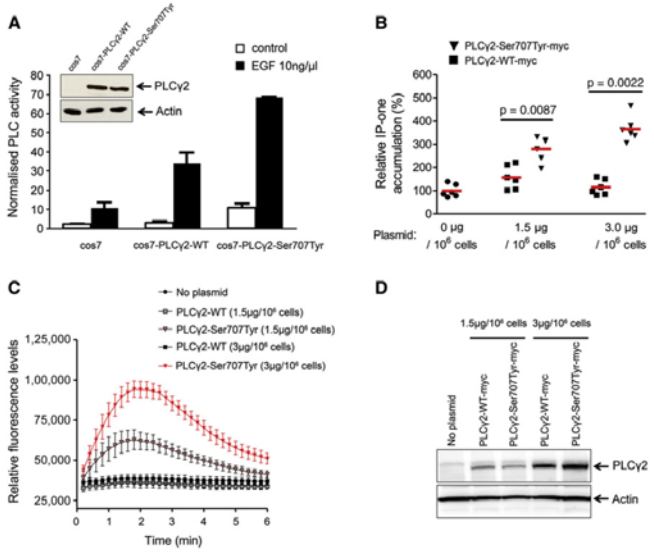
PLCG2 and Innate Immunity

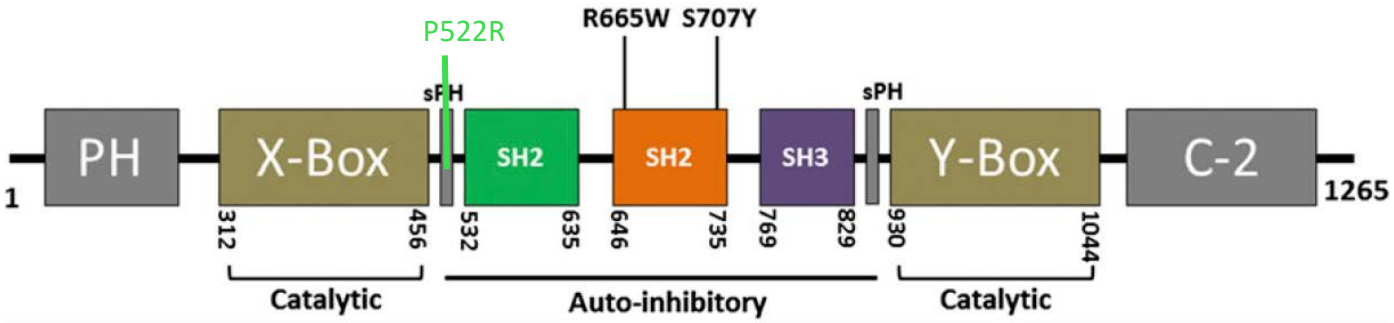
- PLCG2 is a phsopholipase expressed on microglia in the brain, and many immune cells in the periphery
  - A classically druggable target and probably straightforward if one needs to antagonize its activity
- Hypermorphic mutations (S707Y) cause a rare autoimmune disease and increase PLCG2 actvitiy
- Autosomal dominantly inherited inflammatory disease characterized by recurrent blistering skin lesions, bronchiolitis, arthralgia, ocular inflammation, enterocolitis, absence of auto-antibodies, and mild immunodeficiency



PLCG2 and Innate Immunity







PLCG2 and Innate Immunity

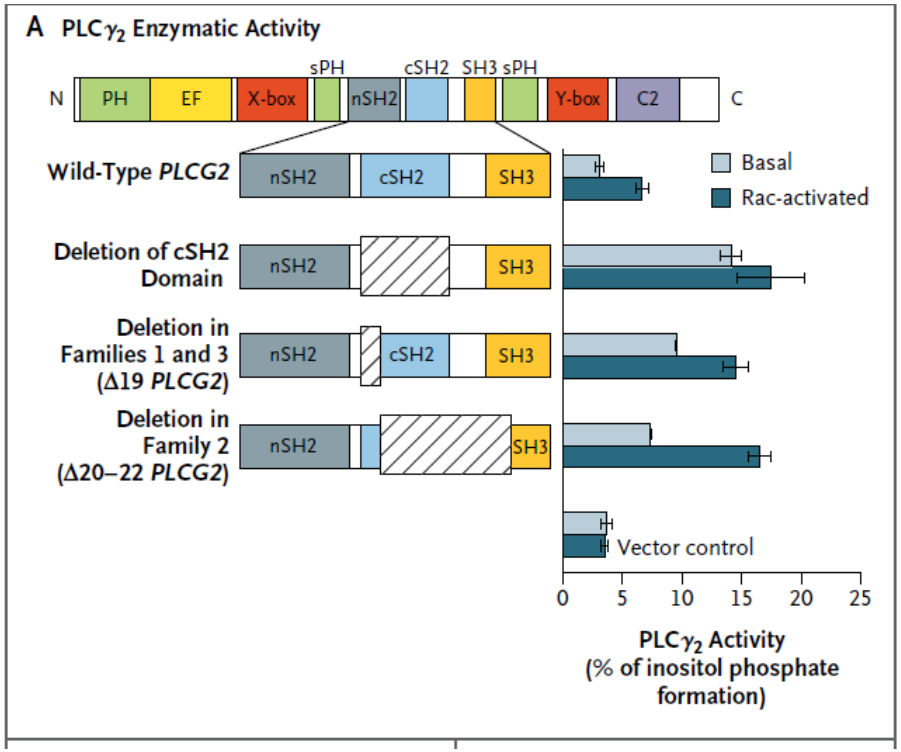
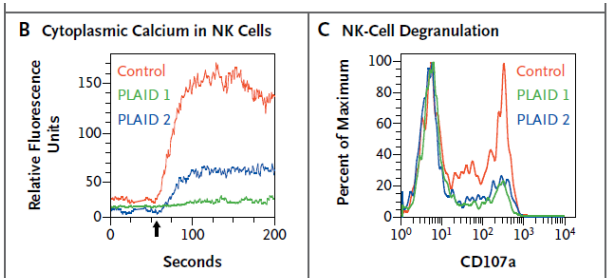


Table 1. Summary of the Clinical Manifestations of Phospholipase Cγ2–Associated Antibody Deficiency and Immune Dysregulation in the Subjects.

Clinical Manifestation	Frequency
	no./total no. (%)
Cold urticaria	27/27 (100)
Recurrent sinopulmonary infection	12/27 (44)
Antibody deficiency*	15/20 (75)
Common variable immunodeficiency	3/27 (11)
Symptomatic autoimmune disease†	7/27 (26)
Positive test for antinuclear antibodies‡	13/21 (62)
Symptomatic allergic disease	15/27 (56)



PLCG2 exon-skipping mutations result in protein products with constitutive phospholipase activity but with reduced intracellular signaling at physiological temperatures causing cold-induced urticaria and immune dysregulation

The NEW ENGLAND JOURNAL of MEDICINE

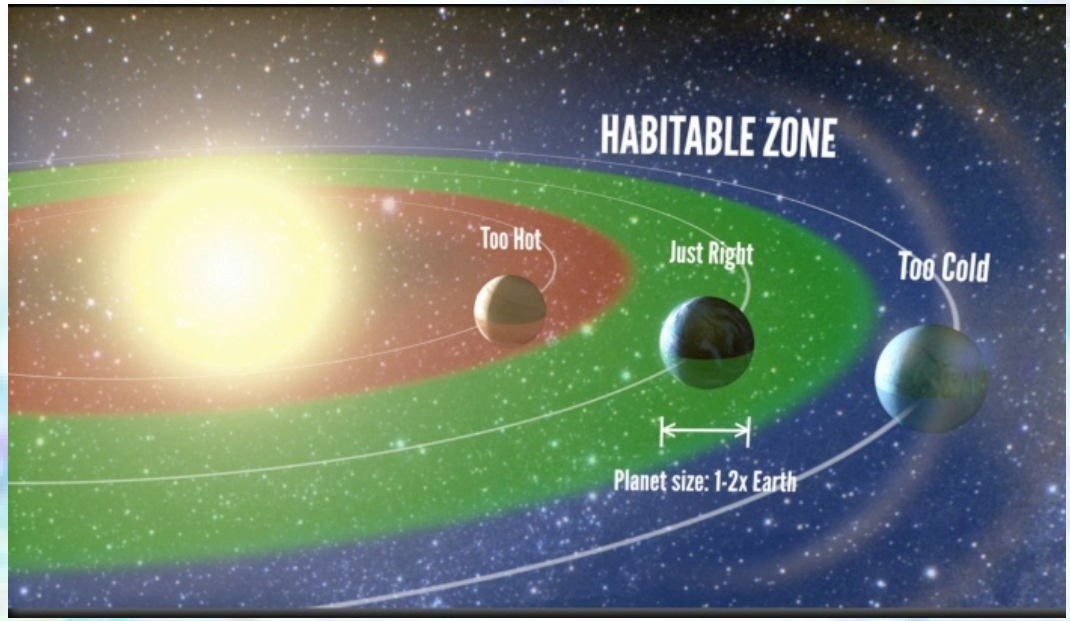
ORIGINAL ARTICLE

Cold Urticaria, Immunodeficiency, and Autoimmunity Related to *PLCG2* Deletions

ABI3 is a member of Abelson-interactor family of adaptor proteins

- Sparse Literature (handfulof pubs) most of the fucnction inferred by
- Facilitate signal transduction as components of several multiprotein complexes
- Regulate actin polymerization and cytoskeletal remodeling through interactions with Abelson tyrosine kinases
- ABI1 plays a role in macropinocytosis as a component of the WAVE2 complex and forms a complex with EPS8 and SOS1 that mediates signal transduction from Ras to Rac.
- ABI2: Part of the WAVE complex that regulates lamellipodia formation. The WAVE complex regulates actin filament reorganization via its interaction with the Arp2/3 complex. Regulates ABL1/c-Abl-mediated phosphorylation of MENA. As component of the WAVE1 complex, required for BDNF-NTRK2 endocytic trafficking and signaling from early endosomes
- ABI3 may inhibits ectopic metastasis of tumor cells as well as cell migration. This may be accomplished through interaction with p21-activated kinase.
  - 3 known phsophoserines 213,216,342
  - SH3 domain (C terminus 303-366)
  - Coiled coil (33-61)
  - Proline Rich 224-306

The Goldelocks Principle and Immunoproteostasis in AD



Innate Immune activity in the brain probably needs to be tuned appropriately to achieve therapeutic benefit without causing harm either by being too active (hot) or to inactive (cold)

We need the human genetic data to guide us…

So if we decide to identify ADC participants with these variants what could/should we do

- More Extensive Autoimmune Histories/Evaluations
- Deep Peripheral Immunophenotyping
- Focused Hypothesis driven assays
  - E.g. PLCG2 activity and expression levels
- Profiling of blood/CSF/imaging immune biomarkers?
- Assess Bone Lesions in TREM2 variant carriers?
- Blood cells or fibroblasts for iPSC studies
- Other?

Moving Forward

- Establish a working group of ADC investigators and additional basic/clinical immunologists to quickly execute a plan for
  - Identifying active ADC participants with these variants
    - Should we do the same for the autopsy brains?
  - Formulate an assessment plan with respect to
    - Clinical histories and sings of autoimmune dysfunction
    - Obtaining immune biomarkers
    - Obtaining additional biospecimens
  - Develop plans and guidelines for work flow, data sharing, future publication, etc…
  - Consider Development of antibodies for multi-site autopsy studies
- Work with NIA and other stakeholders to figure out funding mechanisms
- Impact will be increased if we can create the plan quickly and execute it quickly.