AAAAI Winter

Teaching genetics to Allergy & Immunology fellows: the UCLA experience

Manish Butte, MD PhD

E. Richard Stiehm Endowed Chair
Professor & Division Chief

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UCLA

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Disclosures

- Conducting clinical trials for Pharming, X4
- Consulting / advisory board for Pharming, ADMA
- Founder / shareholder of Rarefied Biosciences

- *Nothing relevant*

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Outline

1. Goals
2. Curriculum
3. Hands on
   - Laboratory validation
   - Software
4. UCLA's experience
5. A demonstration case our fellows did

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Key points of Genetics of IEI

- IEI is (largely) caused by monogenic variants in the genome that alter the function of immune development, homeostasis or responses.
  - everyone with IEI should have a genetic diagnosis
- We do not believe that one gene ⇔ one disease anymore
  - multiple phenotypes are possible
  - Genetic and functional testing are necessary for IEI management
- We have a long way to go
  - We may know ~20% of the genes that cause IEIs
  - Only 20-30% of the work-ups are successful
  - Many new technologies and ideas (long-read, integrated 'omics)

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Scope of immunology practice

	Genetic testing helps
• Not just primary immunodeficiency	✓
• Autoimmunity	✓
• Autoinflammatory diseases (aka periodic fevers)	✓
• Immune dysregulation (HLH)	✓
• Severe atopy	✓
• Bone marrow failure	✓
• Autoantibody mimics of IEI	
• CAR-T cells for cancer and autoimmunity	
• Solid organ transplant immunosuppression management	

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Curriculum

- Why genetic testing is important
- Kinds of genetic testing (gene panels, exome, genome, microarray)
- IEIs in adults
- Genetic inheritance of IEIs
- Tables of IEIs (IUIS)
- *Phenotypes* and *biological pathways* are more important than pattern matching genes
- Variant classification (likely pathogenic / pathogenic)
- VUS resolution / software tools
  - Conservation tools
  - Pathogenicity prediction
  - SpliceAI
  - Literature searches (artificial intelligence fits in here)
- Gain-of-function diseases
- *Actionability* of genetic testing throughout (e.g., off label therapies)

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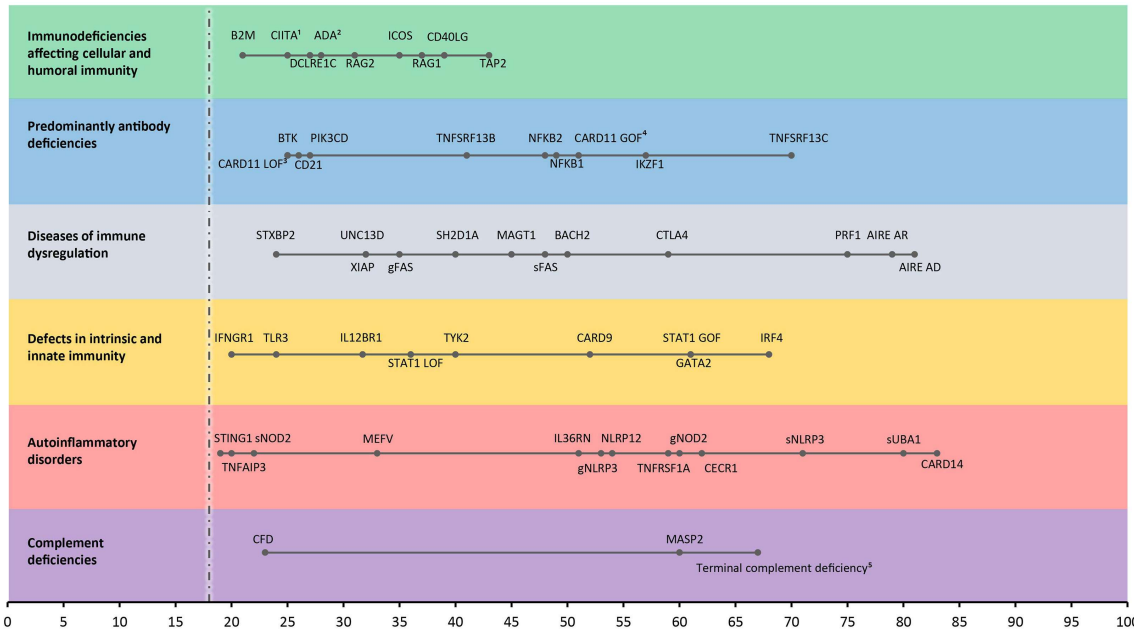
Inborn…but not only in children



Staels, …, Schrijvers. Front Imm 2021

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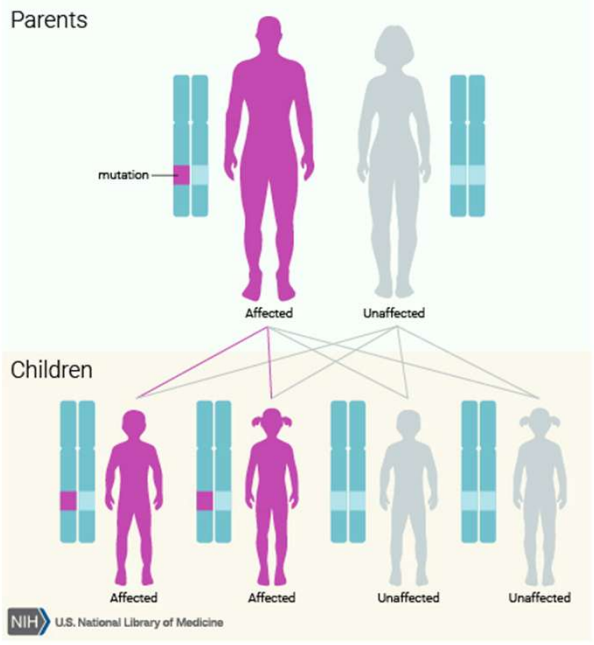
Autosomal Dominant



Example
"CVID" genes:
PIK3CD
TWEAK
NKFB1
NKFB2
IZKF1
IRFBP2
SEC61A1

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Clinical phenotypes and biochemical pathways are more important to learn than the genes

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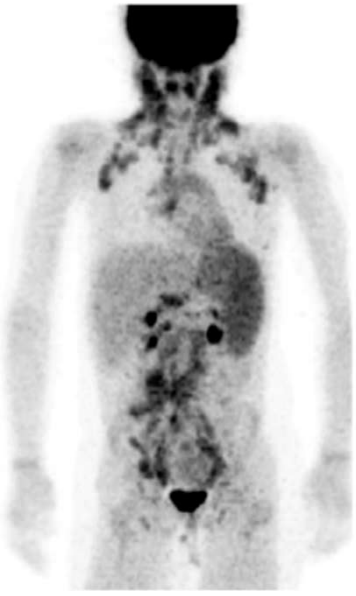
Lymphoproliferation

- Abnormal proliferation and survival of T and B cells
- It's cancer if they're clonal
  - Otherwise it's one of our IEIs



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Lymphoproliferation diagnosis

- Physical exam
  - Splenomegaly
  - Lymphadenopathy
- Lab studies
  - Elevated circulating lymphocytes (not always)
  - Elevated LDH (not always)
  - Elevated B12 (due to monocyte activation and haptocorrin)
  - Genetic testing
- Imaging
  - CT or FDG-PET CT

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Lymphoproliferation examples

- APDS (PI3K delta gain of function)
- ALPS (FAS-FAS ligand loss of function)
- BENTA (CARD11 gain of function)

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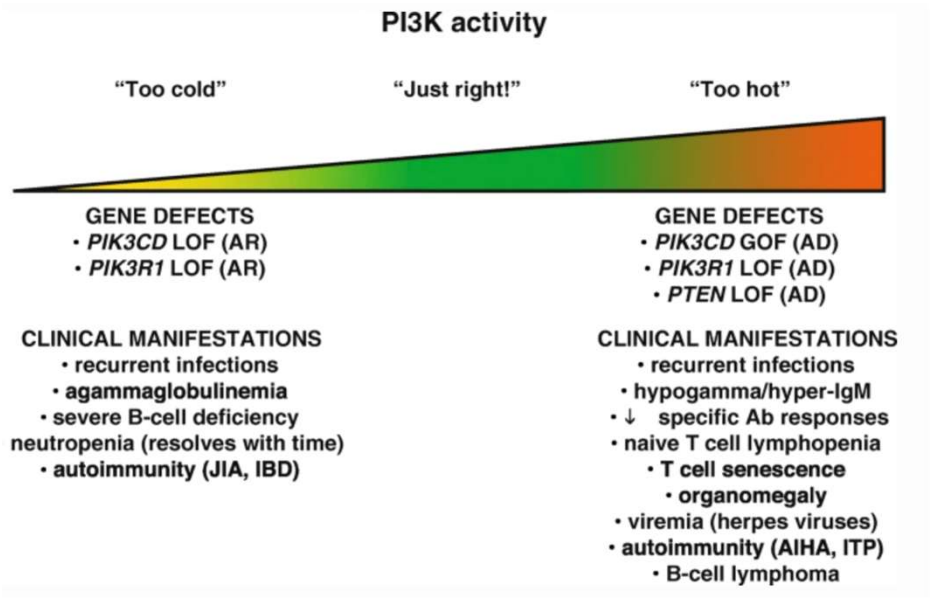
PI3K is Goldilocks



Tangye et al, JoCI 2019

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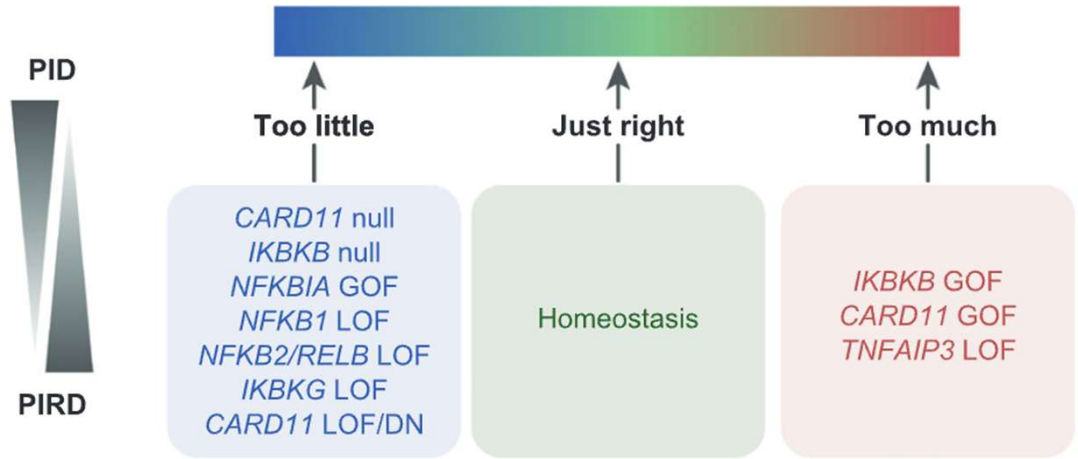
NFκB pathway is Goldilocks



Dabbah-Krancher G, Snow AL. Clin Exp Imm 2023

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Treatment ideas

- Slow down the pathway
  - mTOR inhibitor (sirolimus)
  - Leniolisib (for APDS)
- Deplete overactive cells
  - Rituximab
  - Daratumumab
- HSCT

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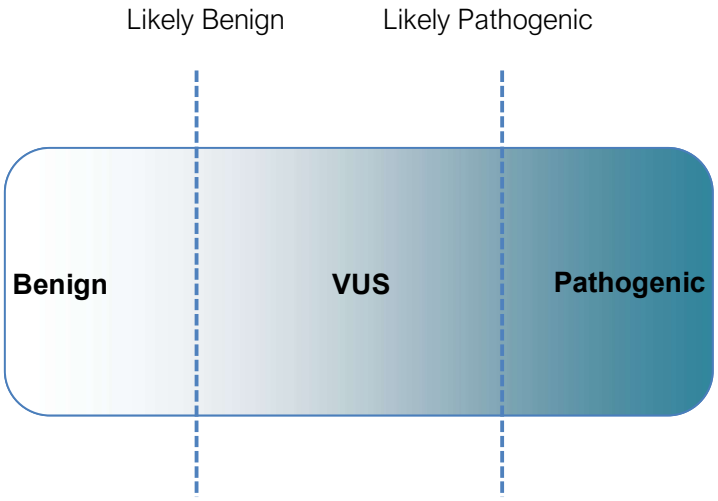
Variant classification



(Adapted from Richards et al, Genetics in Medicine, 2015)

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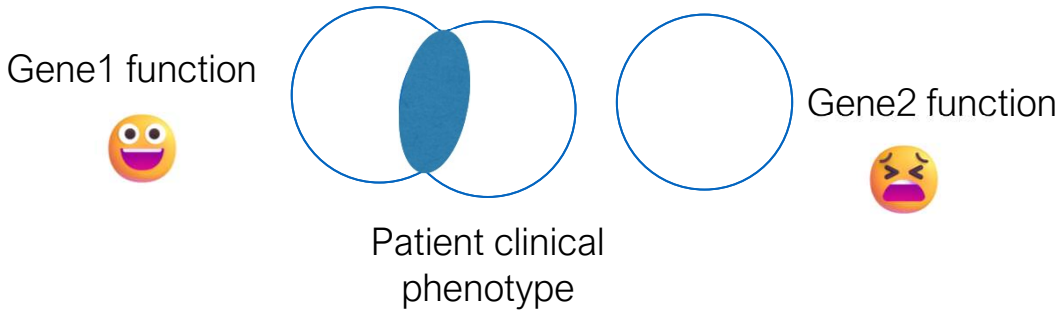
One person with multiple VUS

- Which variant do you focus on first?
- Prioritize those genes that
  - The clinical symptoms overlap with the gene function



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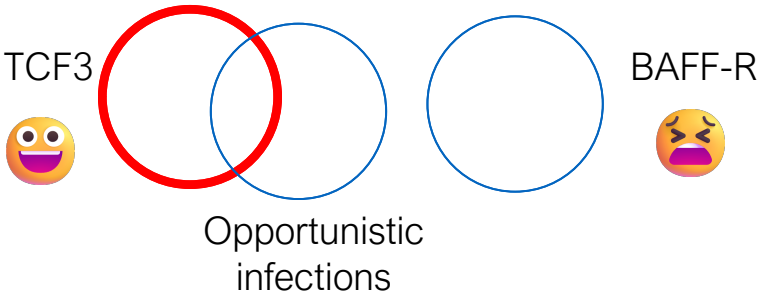
Multiple VUS

- For example



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Prioritizing VUS

- Is the **gene expressed in the immune system**?
- Is the **variant likely to affect the function** of the protein?

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Hints about which VUS to focus on

- **Candidate gene** approach
- **Predicted effects** on protein
- **Appropriate inheritance** pattern
  - CAVEAT: Mono-allelelic expression (Stewart…Bogunovic D, Nature 2025)

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How to *prove* a new pathogenic variant?

You'll need **functional assays** to prove it.

- Don't reinvent the wheel! Read articles.
- Phone a friend. Reach out to your local academic center

Fellows can learn these assays
Fellows can do these assays!

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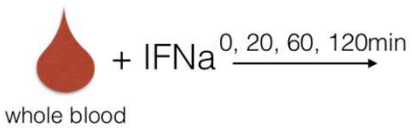
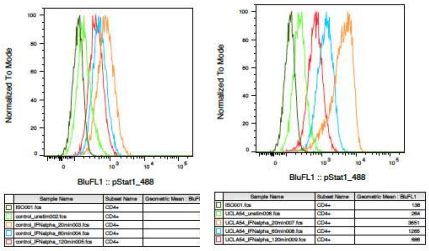
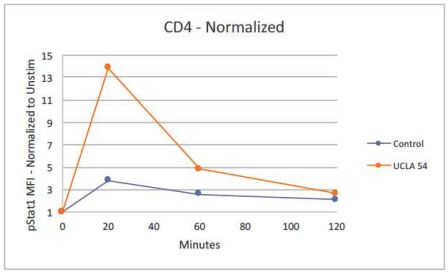
Example: STAT1 GOF

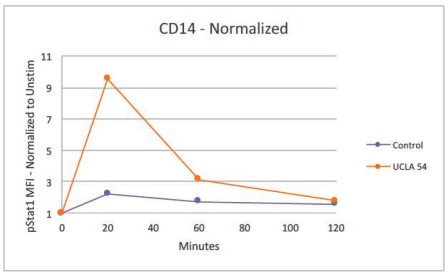


Fix, perm, stain
Flow cytometry



Height of peak





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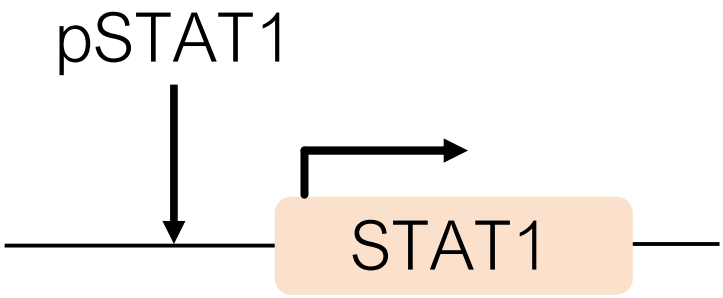
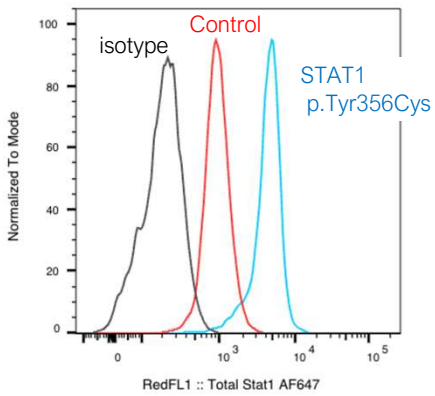
STAT1 GOF functional test

Based on Zimmerman O, …, Holland S. Frontiers Imm 2019



Elevated total STAT1



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Hands on teaching

Get your laptop or phone out

A **13-year-old female** presented with a history of **recurrent sinopulmonary infections, chronic diarrhea, low IgG and IgA levels, and splenomegaly**.

Her mother and sister have thyroid disease, and there is **no reported consanguinity**.

Genetic testing using the **Invitae panel** identified a **heterozygous variant of uncertain significance (VUS)** in CTLA4 (c.194A>G, p.Lys65Arg).

Case courtesy of Sebastian Ochoa, MD

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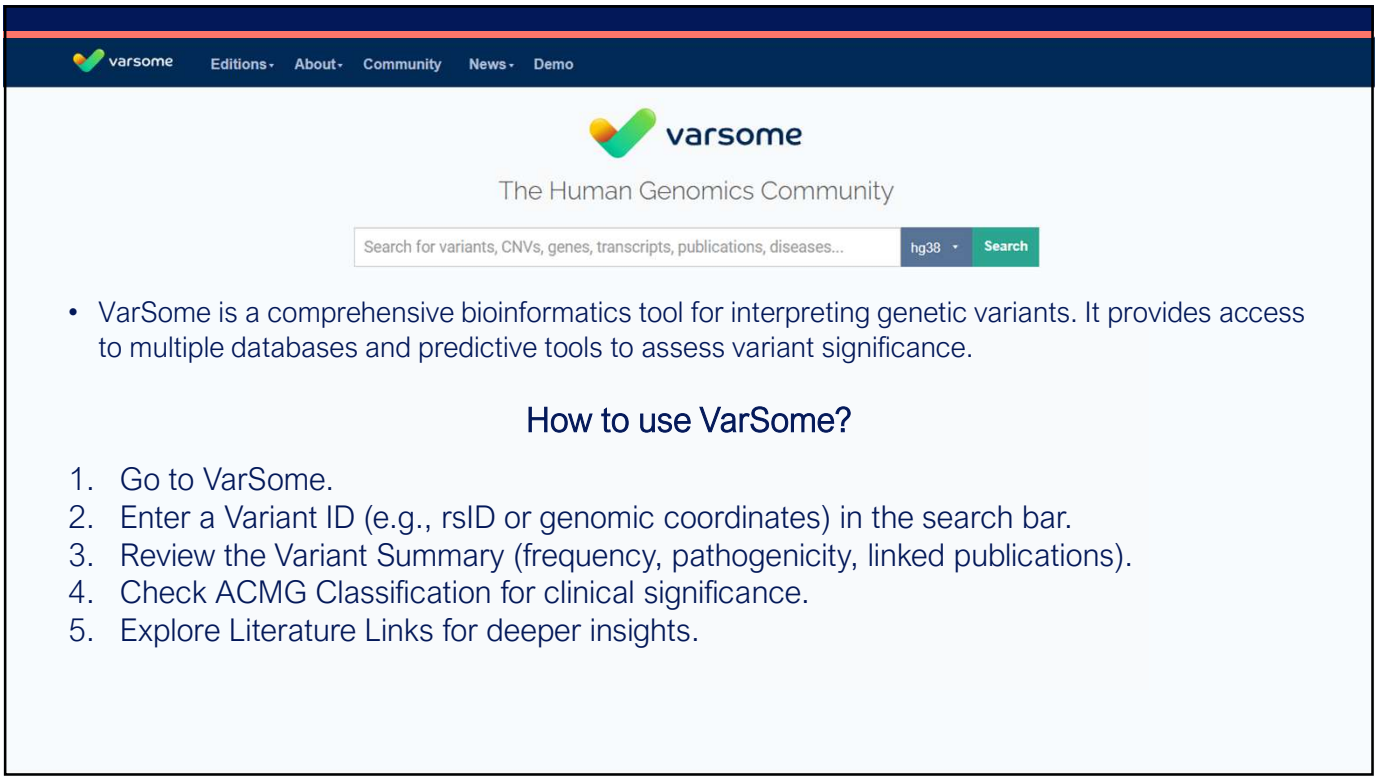

- VarSome is a comprehensive bioinformatics tool for interpreting genetic variants. It provides access to multiple databases and predictive tools to assess variant significance.

How to use VarSome?

1. Go to VarSome.
2. Enter a Variant ID (e.g., rsID or genomic coordinates) in the search bar.
3. Review the Variant Summary (frequency, pathogenicity, linked publications).
4. Check ACMG Classification for clinical significance.
5. Explore Literature Links for deeper insights.

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Varsome

1. Go to https://varsome.com/
2. Type in the variant

CTLA4:p.Lys65Arg

use the gene name and either the cDNA coordinates or protein change

- Find the MAF in gnomAD
- Obtain in silico predictor scores for CADD, alphamissense and spliceAI scores
- Determine variant classification in ClinVar and any associated publications

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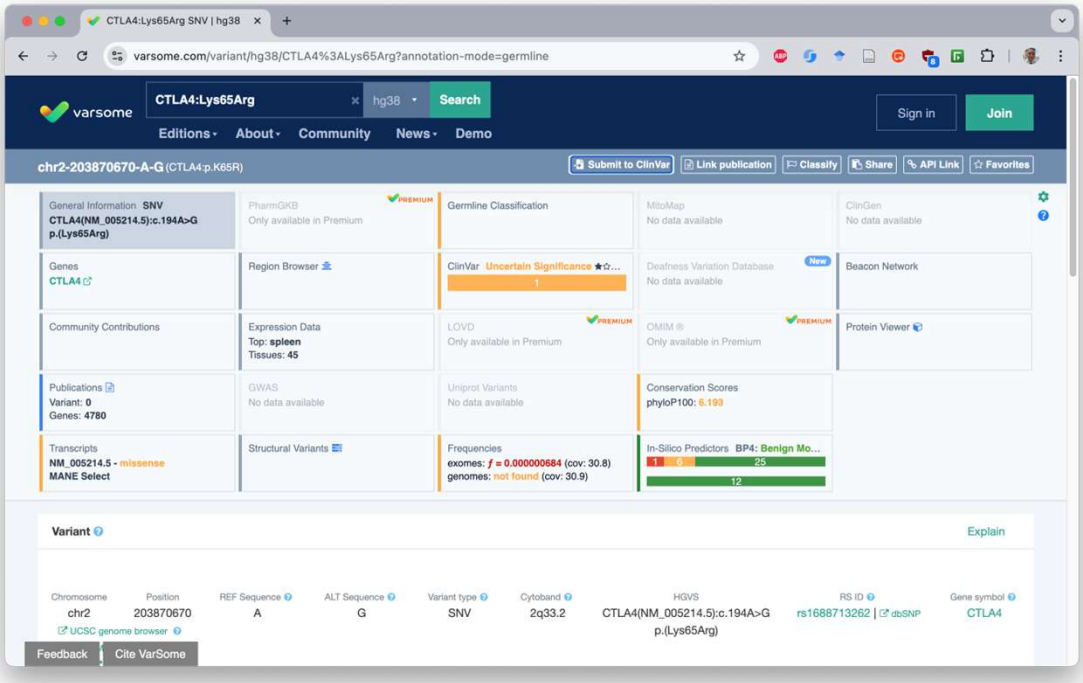

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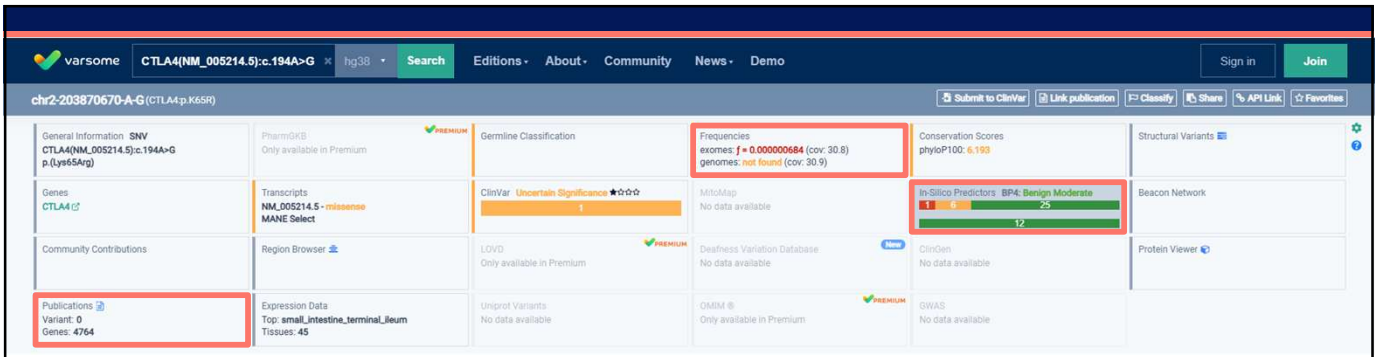

How to Find Variant Information in Varsome?

- Frequency in gnomAD: Check how common the variant is in the general population using the Genome Aggregation Database (gnomAD).
- In Silico Predictors: Utilize computational tools that predict the impact of a variant on gene function (e.g., SIFT, PolyPhen, CADD).
- Links to Publications: Access scientific research and case studies related to the variant through integrated literature databases (e.g., ClinVar, PubMed).

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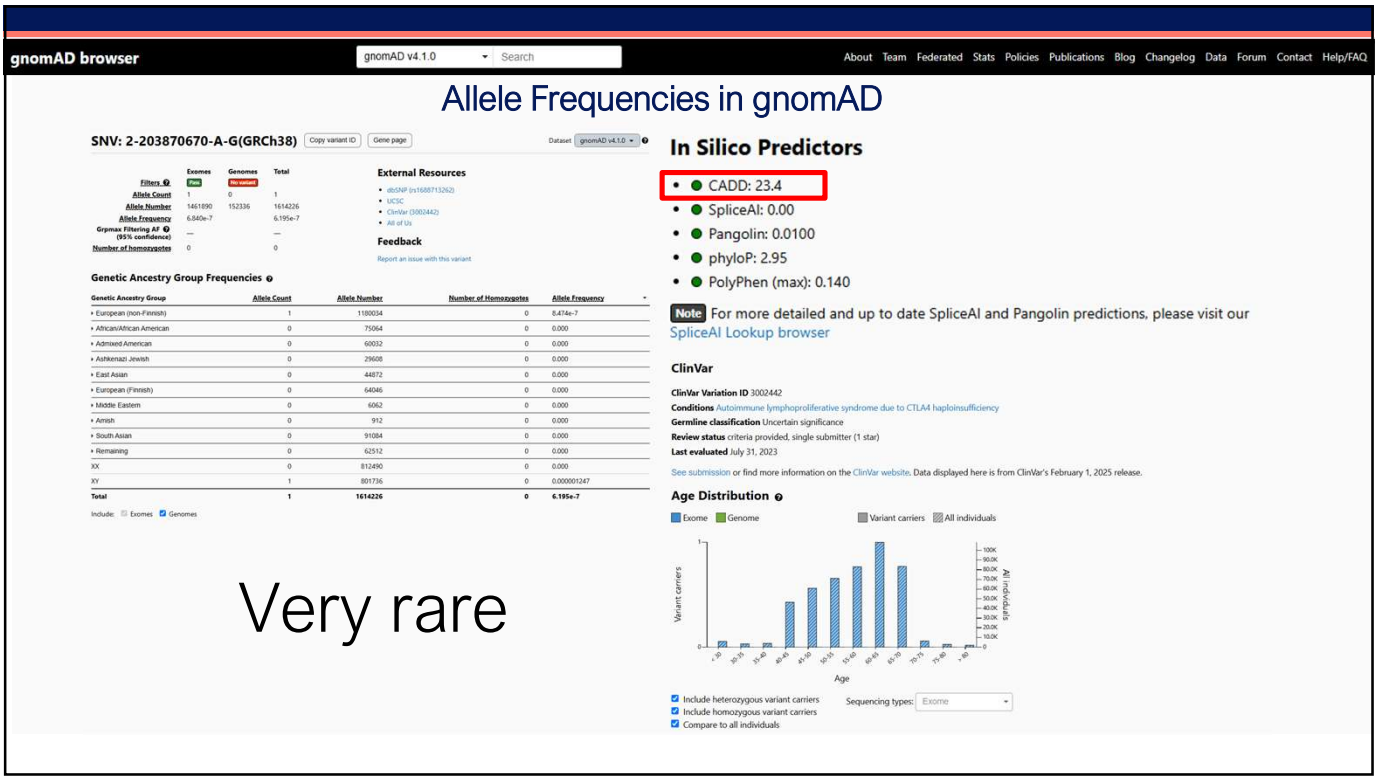
Allele Frequencies in gnomAD



Very rare

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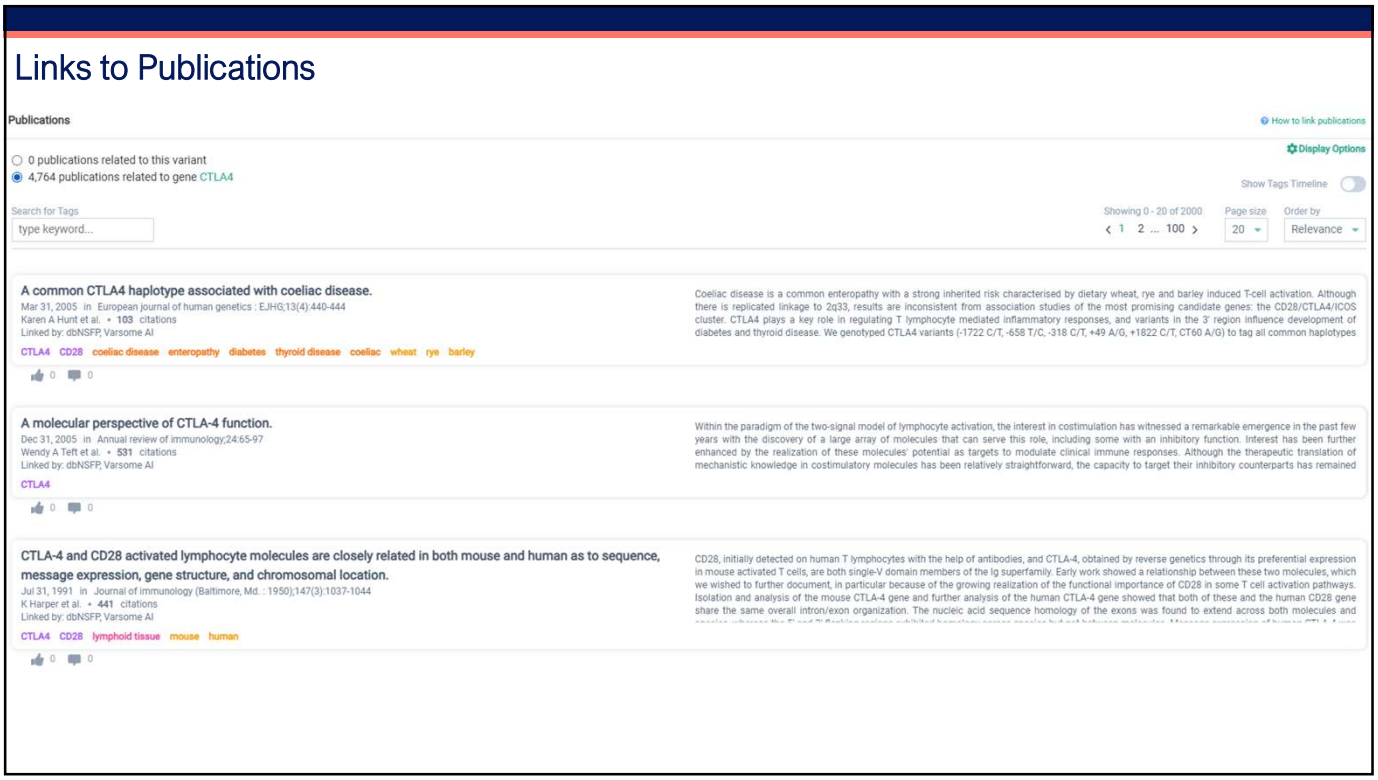
Links to Publications



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UCLA's experience

- Wait for a few months into fellowship before starting
- Make sure the fellows are getting weekly immunology reading

- Set aside 1-2 hours every other week for genomics
  – Regular time

- Make a list of undiagnosed cases from clinic / inpatient
  – It's more fun when you're working on cases they know well

- Faculty mentorship is important
- Teach to make genetic differential diagnoses

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UCLA Lessons learned

- The work is hard, fellows get discouraged
  – Work in pairs / teams

- Most of the cases go unsolved
  – Focus on learning the software tools (Varsome, etc)
  – Ruling out putative genes on a list of VUSes is still a win

- Publish case reports – expanding the clinical phenotype is key

- The larger community at your university is a great resource
  – Human genetics department
  – Undiagnosed Disease Network
  – Other rare disease groups (neuro, cardiac, etc)

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Take home points

- Immunology training means learning genetics
- This is a key part of fellowship training
- Don't turn away from VUS
- Curriculum should be straightforward
- Don't focus only on solving cases
- Focus on actionability
- Validation assays may be needed-- teach this too!
- Leverage your larger university environment

mbutte@mednet.ucla.edu

UCLA

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EM

Exome meeting 9/29/21
Utku + Gloria

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Clinical history

17 yo female with polyglandular autoimmune disease and recurrent serositis who presents to Dr. Kuo's clinic for immune evaluation.

Hashimoto's thyroiditis
Type 1 diabetes
Pancreatitis (presumed autoimmune) with persistently elevated TGs
Pericarditis s/p window
Pleural effusions currently with nighttime O2 requirement

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Clinical history

17 yo female with polyglandular autoimmune disease and recurrent serositis who presents to Dr. Kuo's clinic for immune evaluation.

Childhood history:
Normal pregnancy
Cesarean delivery for failure to progress after induction
No infections or autoimmune manifestations during early childhood
Normal growth and development until age 3

Relevant family history:
Mother – mild allergic rhinitis, otherwise healthy, no miscarriages
Father – psoriasis, psoriatic arthritis (on ixekizumab), MSH6 variant
Younger sister – OAS, otherwise healthy
Paternal aunt – alopecia, MSH6 variant
Paternal grandfather – colorectal/testicular cancer in 30s, MSH6 variant
Paternal grandmother – breast cancer in 30s s/p radiation
Paternal great aunt – alopecia, thyroid disorder

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Clinical history

17 yo female with polyglandular autoimmune disease and recurrent serositis who presents to Dr. Kuo's clinic for immune evaluation.

Age 3 – fatigue, drop in growth curve
Age 5 – facial swelling with periorbital edema, diagnosed with Hashimoto's, symptoms and growth improved with synthroid
Age 5 and age 9 – elevated LFTs (incidental), underwent liver biopsy x2 (normal)
Age 10 – weight loss, polyuria, polydipsia, hospitalized for T1DM, diagnosed with T1DM
Age 12 – emesis, abdominal pain, hospitalized for pancreaitits (presumed autoimmune etiology) and continues to have elevated TGs (on HD omegas)
Age 15 – facial puffiness, dyspnea on exertion, isolated episode of syncope, hospitalized for pericarditis requiring emergent pericardiocentesis, followed by pericardial window, discharged on colchicine, ibuprofen, steroids
Age 17 – facial puffiness, dyspnea on exertion, hospitalized for bilateral pleural effusions s/p thoracentesis x2, started on tadalafil for pulmonary HTN, unremarkable workup including ID/rheum/onc, discharged on lasix and 1L oxygen at night

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Clinical history

17 yo female with polyglandular autoimmune disease and recurrent serosiits who presents to Dr. Kuo's clinic for immune evaluation.

No food allergies or other evidence of atopic disease
No GI complaints

Normal growth (50%ile for weight/height)
Normal appetite, activity, sleep patterns
Normal dental health
Never started menarche, but had normal hormone workup per endo

Peds rheum evaluation with Dr. Hoftman: symptoms gradually improving on celebrex, prednisone, colchicine

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Results from CHLA

IgG 643, IgM 159, IgA 74
Total CD3 1452, CD3CD8 672, CD3CD4 591, CD4/CD8 0.88; Tregs 7% per report
NK 36, B cells 1132
Report of "a mild deficiency in memory B cells, class switch, antibody production"

Beta 2 glycoprotein IgA/IgG/IgM, cardiolipin IgA/IgG/IgM, RNP IgG, Sm IgG negative
C3 36, C4 4
IL-6 29.3 (high)

Myocardial biopsy: 6/2021
Myocardium showing rare hypertrophic myocytes and scattered T lymphocytes and histiocytes by immunostaining. No definitive evidence of myocarditis. Focal mild interstitial fibrosis. No evidence of viral inclusion, eosinophils, infarct, granuloma or malignancy. CD3 - scattered T lymphocytes. CD20 - rare B lymphocytes. CD68 - scattered histiocytes.

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Results from UCLA

CVID B cell panel:
- Normal B cell count (525), however, extremely elevated immature B cells (222) and low switched memory B cells (0.5); for comparison, unswitched memory B cells (16)
- Depressed NK cells (9)

T cell memory panel:
- Normal T cell counts and CD4/CD8 ratio; however, notable abundance of CD8 naïve T cells (1093) compared to CD8 memory T cells (45); mitogen/antigen assays not sent

Immunoglobulins:
- IgG 703 (IgG2 116, IgG4 1), IgM 166, IgA 134, IgE 3

Titers:
- Strep pneumo 2/23 (never got pneumovax), tetanus protective, measles negative
- EBV and CMV IgG negative, COVID-19 spike IgG >10

Borderline eosinophilia (540 prior to steroids)

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Results from UCLA

Cytokine panel with elevated IL-10 (41) and elevated IL-18 (1415)
Complement with low C3 (5), low C4 (5), low CH50 (20)

Autoantibody panel:

	7/1/2021 1040
AUTOIMMUNE	
Antinuclear Ab	<1:40 *
nDNA (Crithidia) Ab IFA	<1:10 *
Histone Antibody,IgG	1.6 * ▲
Smooth Muscle Ab	Positive
Smooth Muscle Ab Titer	1:40 * !
Mitochondrial Ab	<1:20 *
SSA Antibody	<20 *
SSB Antibody	<20 *
Immune Complex-C1q Binding	5.3 * ▲
Endomysial IgA Antibody	<1:10
Transglutaminase IgA	<20.0 *
Gliadin (Deamidated) Ab, IgA	48.8 * ▲
Gliadin (Deamidated) Ab, IgG	<20.0 *

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Results from UCLA

Lymph node biopsy 7/8/2021:
Interfollicular hyperplasia with eosinophils, rare plasma cells and numerous histiocytes. No evidence of a monotypic B-cell population by immunostains. Flow cytometric studies show no monotypic B-cells. Negative for EBV-EBER by ISH.

Regressed germinal centers per direct communication with pathologist.

FNA: Smears show a polymorphous population of lymphocytes with predominantly small mature lymphocytes with clumped chromatin and scant cytoplasm, occasional intermediate and larger lymphoid cells.

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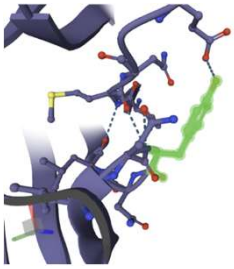
Invitae/GDB findings

STAT1 c.1067A>G p.Tyr356Cys

- Not in gnomad, but predicted to be damaging per 3/6 in silico algorithms
- Tyr is conserved, located in DNA binding domain; hydroxyl forms critical interaction with Glu393 and also has contacts with Asn355 and Asn357



- Patient history lacking in chronic mucocutaneous candidiasis, recurrent infections, but she does exhibit autoimmunity and non-immunologic features (poor growth, delayed menstruation) associated with heterozygous gain-of-function mutations in STAT1 (IMD31C)

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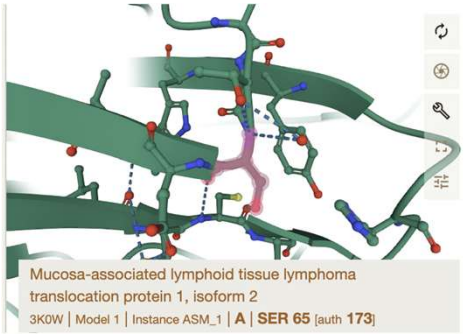
Invitae/GDB findings

MALT1 c.517T>C p.Ser173Pro

- 6 hets on gnomad (4 Ashkenazi, 2 non-Finnish), no homozygotes, AF <0.1%
- Located in Ig-like C2 domain; forms critical contact with distal residues per PDB; however, not predicted to be damaging per in silico predictions
- Coverage 93% of coding sequencing, so cannot rule out additional variant vs. deletion/insertion



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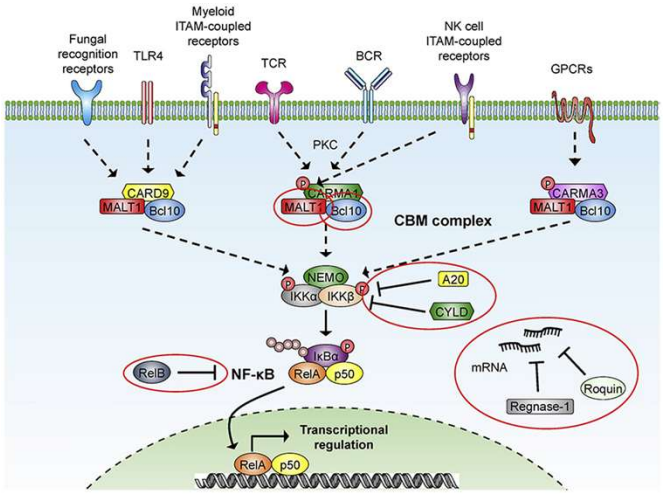
Invitae/GDB findings

MALT1 c.517T>C p.Ser173Pro



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Invitae/GDB findings

MALT1 c.517T>C p.Ser173Pro

- MALT1 has both proteolytic activity (caspase homolog) and scaffolding function
- MALT1 proteolytic activity suppresses autoimmunity in a T cell intrinsic manner
  - Malt1-/- combined immunodeficiency phenotype
  - Malt1PD mice develop a spontaneous and lethal IPEX-like disease associated with lymphadenopathy, elevated IgG1 and IgE, expansion of effector T cells, as well as lymphocytic infiltrates in various tissues
- Hallmark of MALT1 deficiency in humans includes recurrent infections (bacterial, viral, fungal) periodontal disease, enteropathy, dermatitis, failure of thrive
  - Per Lu & Turvey, 70% of patients experience S. aureus, and Candida (impaired Th17 immunity), CMV infection (NK dysfunction)
  - No description of polyendocrinopathy manifestations

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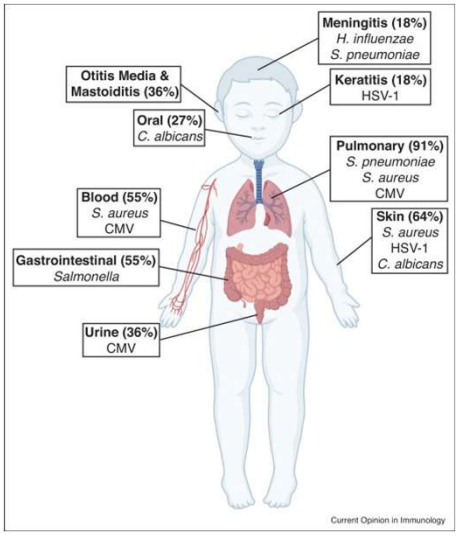
Invitae/GDB findings

MALT1 c.517T>C p.Ser173Pro



Lu & Turvey 2021

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Invitae/GDB findings

IL12B c.301C>T p.Leu101Phe

- 28 hets on gnomad, no homozygotes; 1 clinvar submission - unclear significance
- Located in Ig-like C2 domain as first of 4 leucines
- Gene is generally tolerant to missense variations in the general population; variant is predicted to be benign 6/6 and no significant contributions to protein structure

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GDB findings

MSH6 c.3743_3744insT, paternally inherited variant

- The heterozygous c.3743_3744insT variant in *MSH6* has not been previously observed in the general population (using the gnomAD Exome database), though this gene is generally tolerant to loss of function variation in the general population. This variant is classified as pathogenic in the ClinVar database (Variation ID: 183794). This variant was confirmed using Sanger sequencing as an alternate methodology.

A heterozygous c.3743_3744insT variant in the *MSH6* gene was identified. Variants in this gene are associated with several conditions including autosomal dominant hereditary nonpolyposis colorectal cancer type 5 [MIM: 614350] [see Interpretation].

Gene	Genomic Change (hg19)	Zygosity	Coding Change	Protein Change	Classification	Inherited From
MSH6	chr2:g.48033439_48033440insT	Heterozygous	NM_000179.2: c.3743_3744insT	p.Tyr1249Leufs*26	Pathogenic	Unknown

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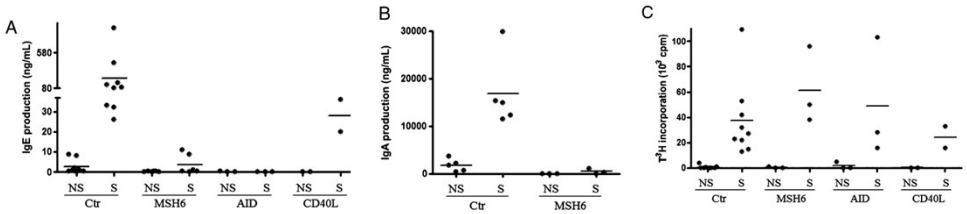
GDB findings

MSH6 c.3743_3744insT, paternally inherited variant

- MSH6/MSH2 form a heterodimer to recognize single base errors to initiate mismatch repair during SMH and/or CSR
- Evaluation of 8 patients with MSH6 deficiency consistent with partial CSR defect (low circulating switched memory B cells, low IgG2 and IgG4, normal/elevated IgM, inability to undergo CSR *in vitro*), but does not have a significant impact on B cell function *in vivo*



Gardes et al., J. Immunol. 2012

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GDB findings

MSH6 c.3743_3744insT, paternally inherited variant

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Expanded gene list

- FAS – intronic variant, not predicted to alter splicing on hg38 (not able to find construct on hg19)
- FASLG – no rare variants
- FADD – no rare variants
- CASP10 – no rare variants
- CASP8 – no rare variants
- CTLA4 – no rare variants
- LRBA – no rare variants
- ITK – no rare variants
- MAGT1 – no variants
- PIK3CD – causes AD activated pi3k delta syndrome, has a variant 4% in Ashkenazi 2% in general, reported as benign previously
- PRKCD – no rare variants
- STAT3 – no rare variants
- KRAS – no rare variants
- NRAS – no rare variants
- ADA2 – homozygous intronic variant, 6% in population, not predicted to alter splicing
- CECR1 – no rare variants
- SH2D1A – no rare variants
- TNFRSF6 – no rare variants
- XIAP – no rare variants
- NFKB1, NFKB2 – no rare variants
- TNFRSF13B – no rare variants
- Also negative for NK deficiency genes – GATA2, IRF8, RTEL1, GINS1, MCM4, MCM10

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JAK1

- Causes AD Autoinflammation, immune dysregulation, and eosinophilia
- 194 het, 1 homozygous on Gnomad, good age distribution including >80
- Not in a conserved/functional domain

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JAK3

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5770691/

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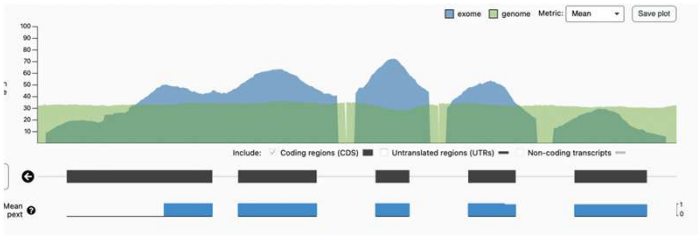
TNFRSF18

- Nonclinical TNF receptor superfamily gene, also known as GITR/AITR (glucocorticoid-induced/activation-induced tumor necrosis factor receptor)
- Cell surface receptor constitutively expressed at high levels on Tregs and at low levels on naïve and memory T cells
- Synonymous variant in splice region, SpliceAI score 0.38-0.39
- 3% in Ashkenazi, 0.1 in total population
- 494 hets, 3 homozygotes in Gnomad



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- 494 hets, 3 homozygotes in Gnomad
- Exome coverage is not ideal – question whether there can be a second hit in the gene in addition to this variant causing AR disease
  - This variant alone is unlikely to cause AD or dominant-negative disease as it's pretty common in Ashkenazi
  - Would need RNA seq + Sanger to confirm

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Upcoming studies

Lymphocyte mitogen/antigen proliferation
NK counts and cytotoxicity
FOXP3 T regulatory cells (MCW)

Del/dup vs. NF-kB signaling for MALT1?
Sanger + RNA sequencing – single site for TNFRS18 variant

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