Stanford | MEDICINE

Identifying genetic variants associated with rare Mendelian diseases: case studies

Jingga Inlora
Stanford University
Genetics Department (Snyder lab)
inloraj@stanford.edu
5-3-2017

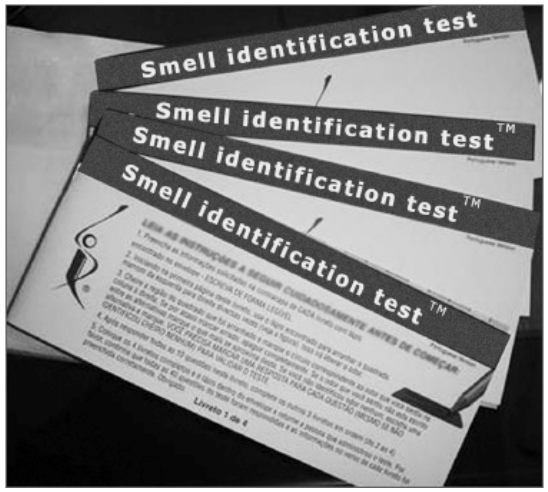
Outline

- Case #1: Isolated congenital anosmia (ICA) and *CNGA2* mutation
- Case #2: *APTX* mutation and hereditary oculomotor apraxia
- Case #3: DAVID Syndrome in patient with novel *NFKB2* mutation

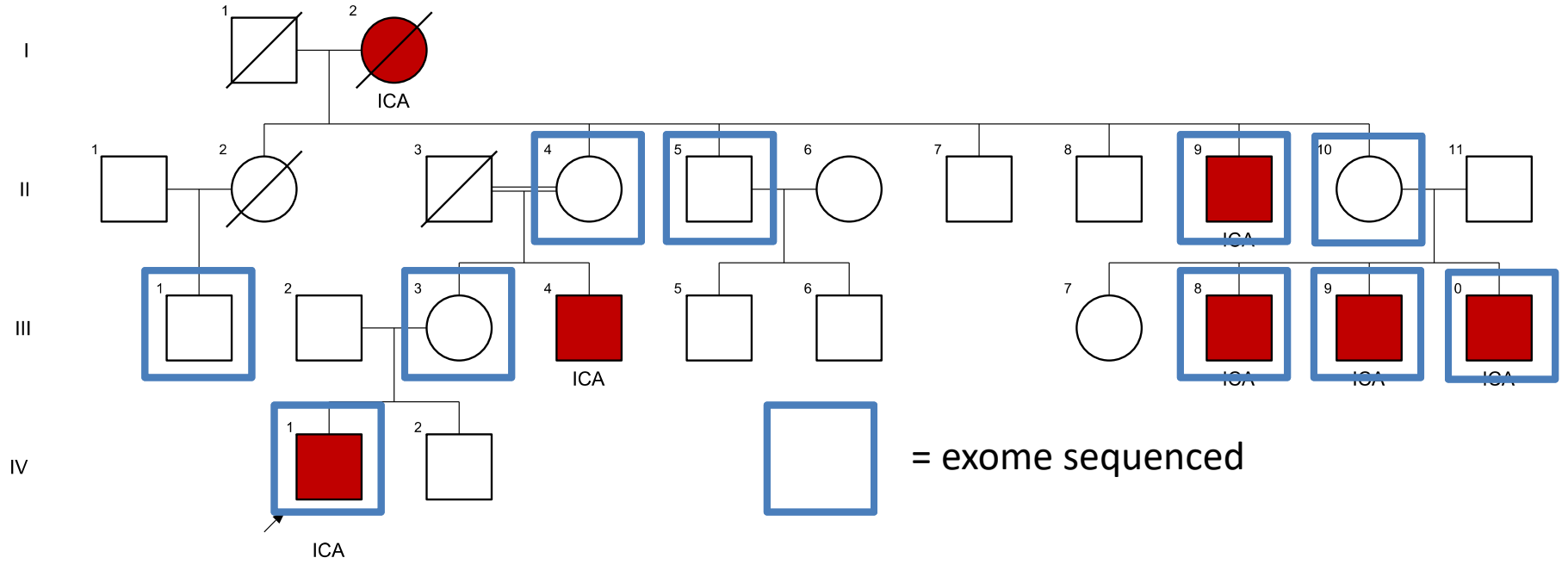
Isolated Congenital Anosmia (ICA)

- Rare condition where patients have no recollection of ever being able to smell (OMIM 107200)
  - no additional symptoms or other underlying disease-causing condition

- Smell Identification Test:
  - University of Pennsylvania Smell Identification Test (UPSIT)
  - Scale of 1 to 24
    - 1-9: anosmic;
    - 9-13: severe microsmia
    - 13-17: mild microsmic
    - 19-24 normosmia state



http://www.scielo.br/img/revistas/bjorl/v76n6/en_a04fig01.jpg



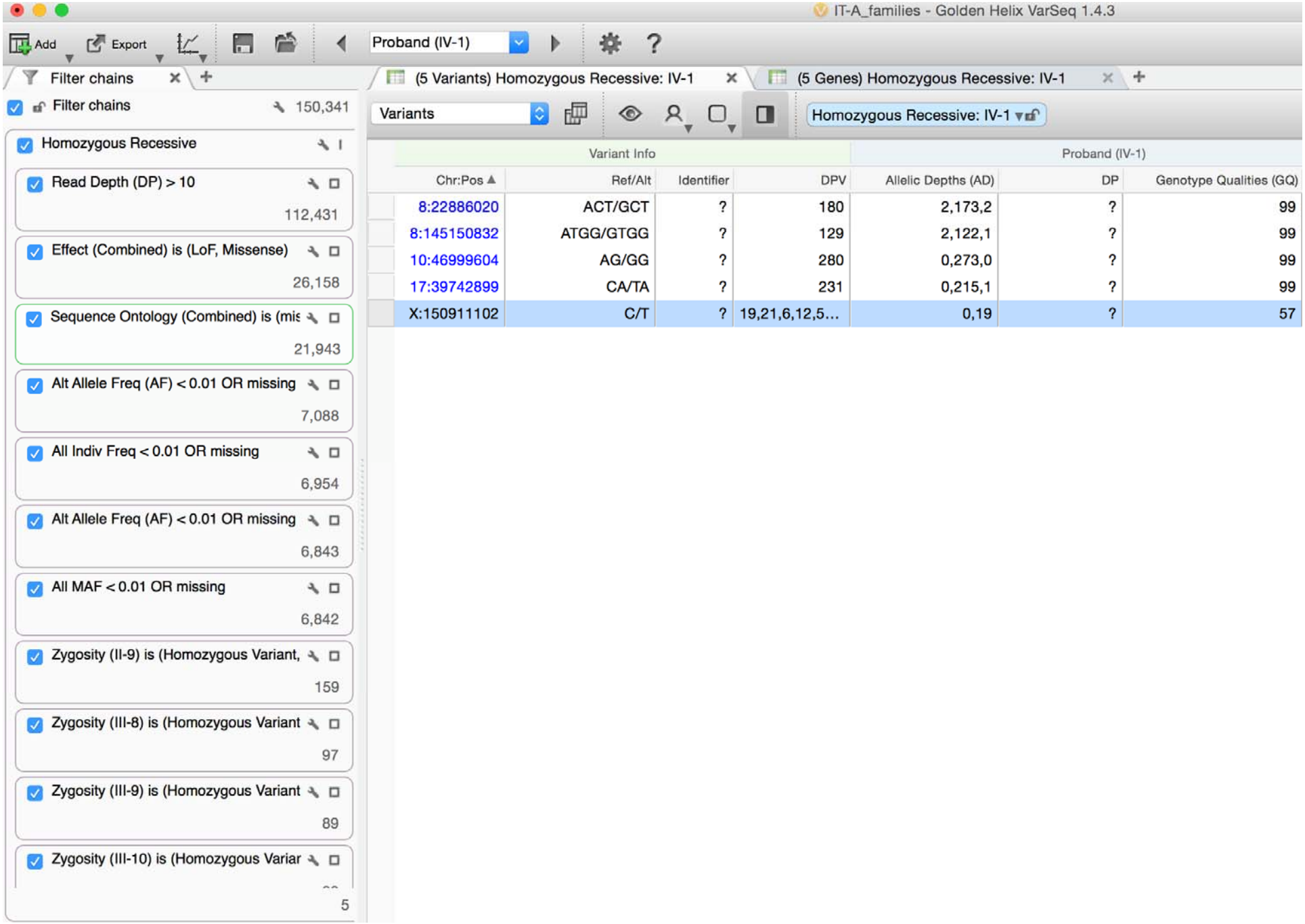
Iranian descent, consanguineous

Family ID	II-4	II-5	II-7	II-9	II-10	III-1	III-3	III-7	III-8	III-9	III-10	IV-1
Age	53Y	60Y	63Y	51Y	52Y	35Y	41Y	34Y	32Y	28Y	22Y	16Y
Sex	F	M	M	M	F	M	F	F	M	M	M	M
Smell test score	Normal	Normal	Normal	0/24	Normal	Normal	Normal	Normal	0/24	7/24	0/24	0/24
Disease status	Normal	Normal	Normal	ICA	Normal	Normal	Normal	Normal	ICA	ICA	ICA	ICA

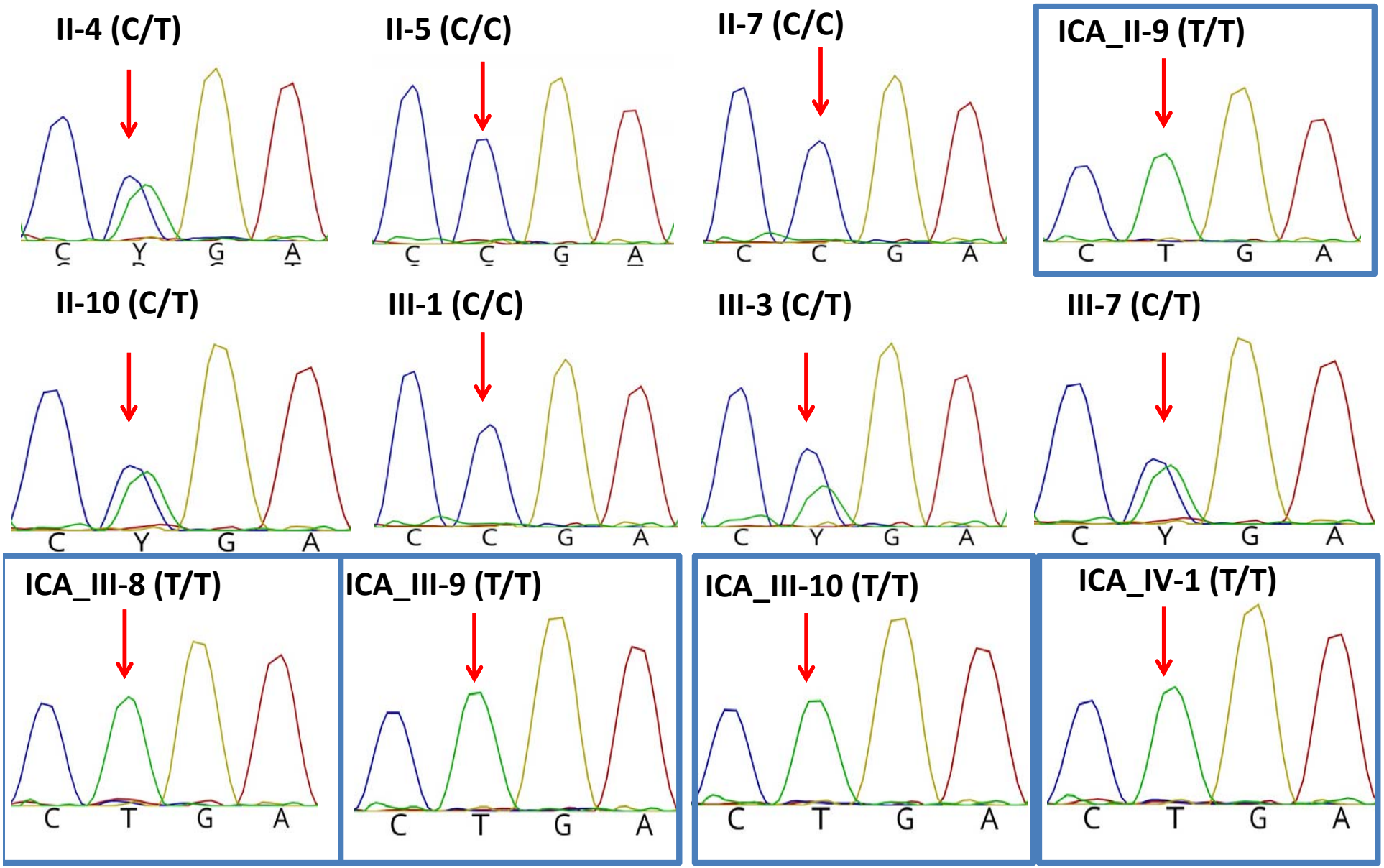
Exome sequencing analysis

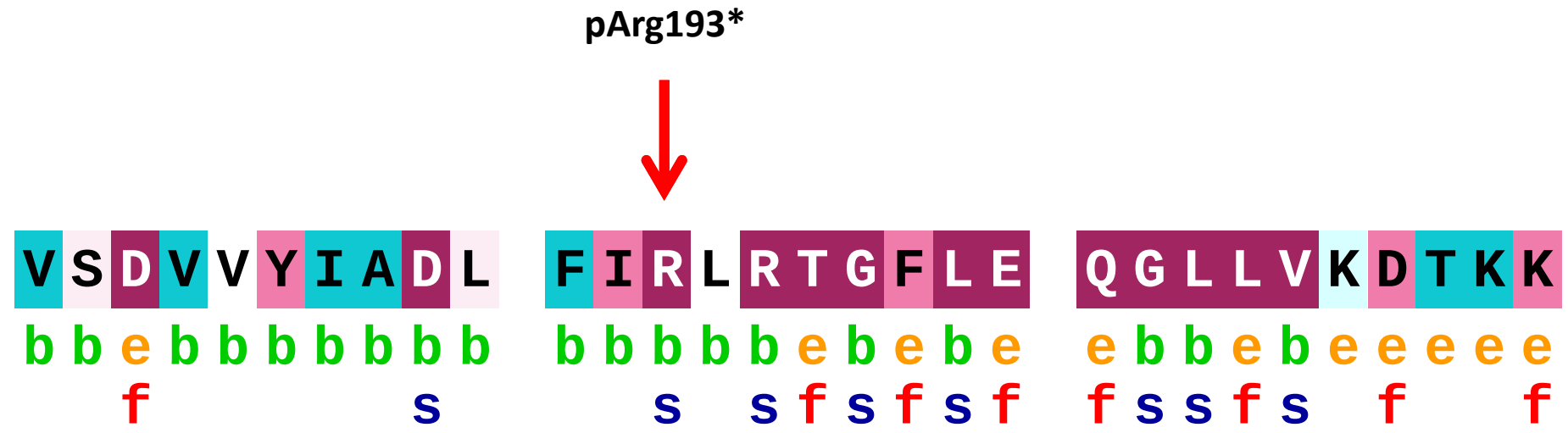
- KAPA library prep kit; IDT exome probes
- Roche Sequencing Solutions (Bina Technologies version 2.7.9) whole-exome analysis workflow
- Homozygous variants, allele freq < 0.01
- GoldenHelix Varseq (v1.1)

Individual ID	II-4	II-5	II-9	II-10	III-1	III-3	III-8	III-9	III-10	IV-1
Total Variations	114,760	108,192	113,826	112,106	108,115	111,921	112,019	113,508	111,772	112,236
Shared variants	88,042									
Homozygote variants	16 (Homozygote in affected members, but either heterozygote or homozygote for reference allele in controls)									
1KG MAF < 0.01	1									
EXaC MAF < 0.01	1									
dbSNP 144 MAF < 0.01	1									
NHLBI MAF < 0.01	1									
Exonic Variants	1									
Candidate	chrX:150,911,102; *CNGA2*.aAug10:c.577C>T; p.Arg193*									

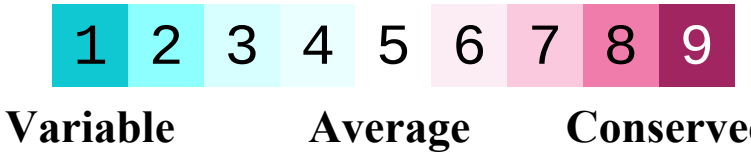

IT-A_families - Golden Helix VarSeq 1.4.3
Add
Export
Proband (IV-1)
Filter chains
Filter chains
150,341
Homozygous Recessive
Read Depth (DP) > 10
112,431
Effect (Combined) is (LoF, Missense)
26,158
Sequence Ontology (Combined) is (mis
21,943
Alt Allele Freq (AF) < 0.01 OR missing
7,088
All Indiv Freq < 0.01 OR missing
6,954
Alt Allele Freq (AF) < 0.01 OR missing
6,843
All MAF < 0.01 OR missing
6,842
Zygosity (II-9) is (Homozygous Variant,
159
Zygosity (III-8) is (Homozygous Variant
97
Zygosity (III-9) is (Homozygous Variant
89
Zygosity (III-10) is (Homozygous Variar
5
(5 Variants) Homozygous Recessive: IV-1
(5 Genes) Homozygous Recessive: IV-1
Variants
Homozygous Recessive: IV-1
Variant Info
Proband (IV-1)
Chr:Pos
Ref/Alt
Identifier
DPV
Allelic Depths (AD)
DP
Genotype Qualities (GQ)
8:22886020 ACT/GCT ? 180 2,173,2 ? 99
8:145150832 ATGG/GTGG ? 129 2,122,1 ? 99
10:46999604 AG/GG ? 280 0,273,0 ? 99
17:39742899 CA/TA ? 231 0,215,1 ? 99
X:150911102 C/T ? 19,21,6,12,5... 0,19 ? 57

Sanger sequencing





The conservation scale:



e - An exposed residue according to the neural-network algorithm.

b - A buried residue according to the neural-network algorithm.

f - A predicted functional residue (highly conserved and exposed).

s - A predicted structural residue (highly conserved and buried).

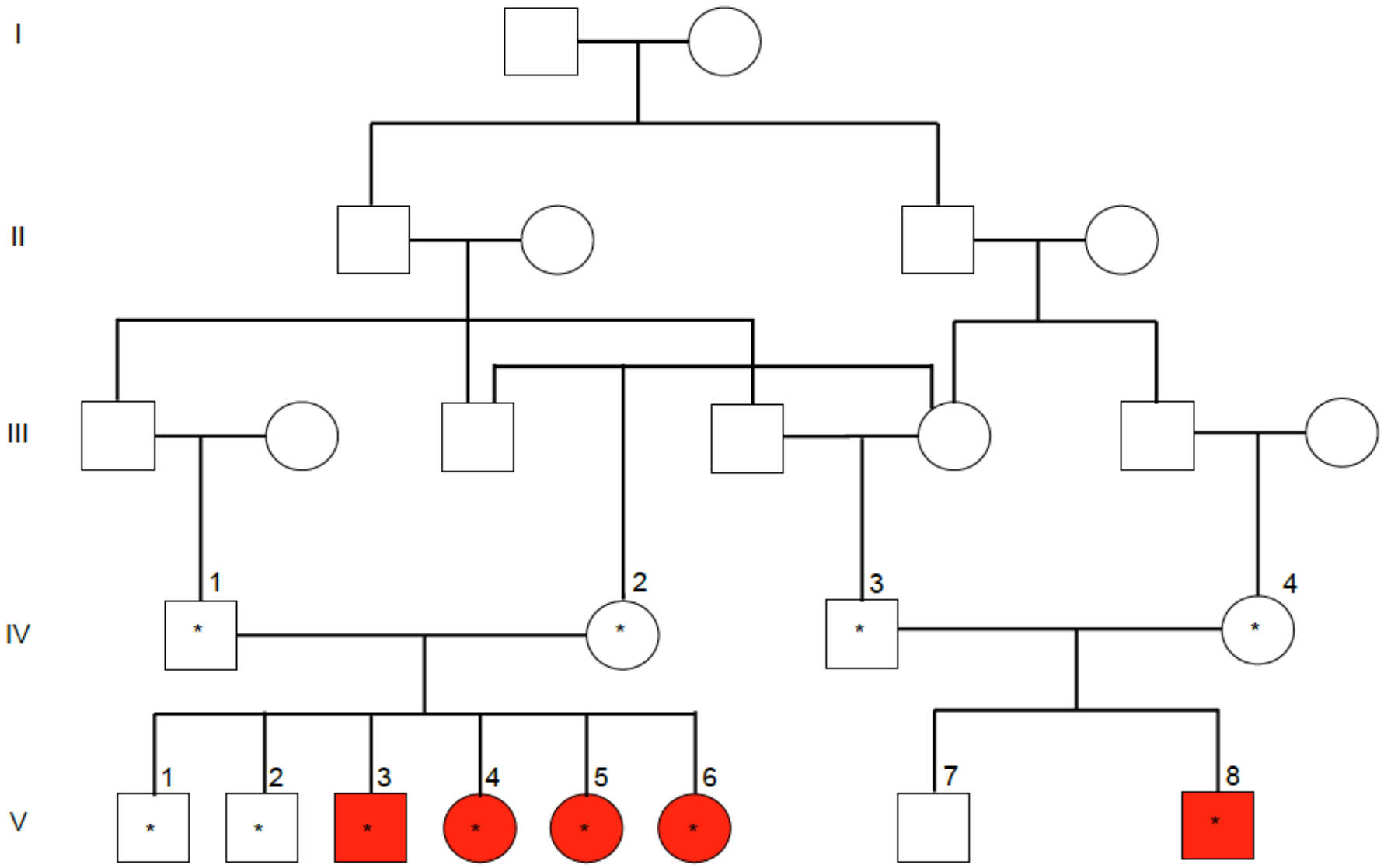
Summary

- Identified stop-gain variant within exon 6 of *CNGA2* gene.
- The variant segregates with the disease; all five affected individuals are hemizygous for this variant
  - Unaffected individuals are either heterozygous or homozygous for reference allele
- Previously reported variants associated with ICA includes *CNGA2* c.634C>T(p.R212*) (Karstensen *et al.* 2015) and *TENM1* c.4829C>T(p.P1610L) (Alkelai *et al.* 2016)
- Alpha subunit of CNG channel is critical for olfactory sensory neurons to generate odor-induced action potential
- *Cnga2* knockout mice are congenitally anosmic and have severely impaired olfactory function

Case#2 : Ataxia-oculomotor apraxia

- Hereditary ataxia: a group of disorders characterized by motor discoordination such as poor balance, abnormal eye and hand movements and dysarthria

  > 30 autosomal dominant forms and > 60 forms that are autosomal recessive or X-linked
- Overlapping presentations and there is a high degree of genetic heterogeneity
- Difficult to devise an efficient strategy for targeted molecular testing in many cases
- WES wad adopted to pursue a molecular genetic diagnosis



Iranian descent, consanguineous

Red label = affected; * = samples that are exome sequenced

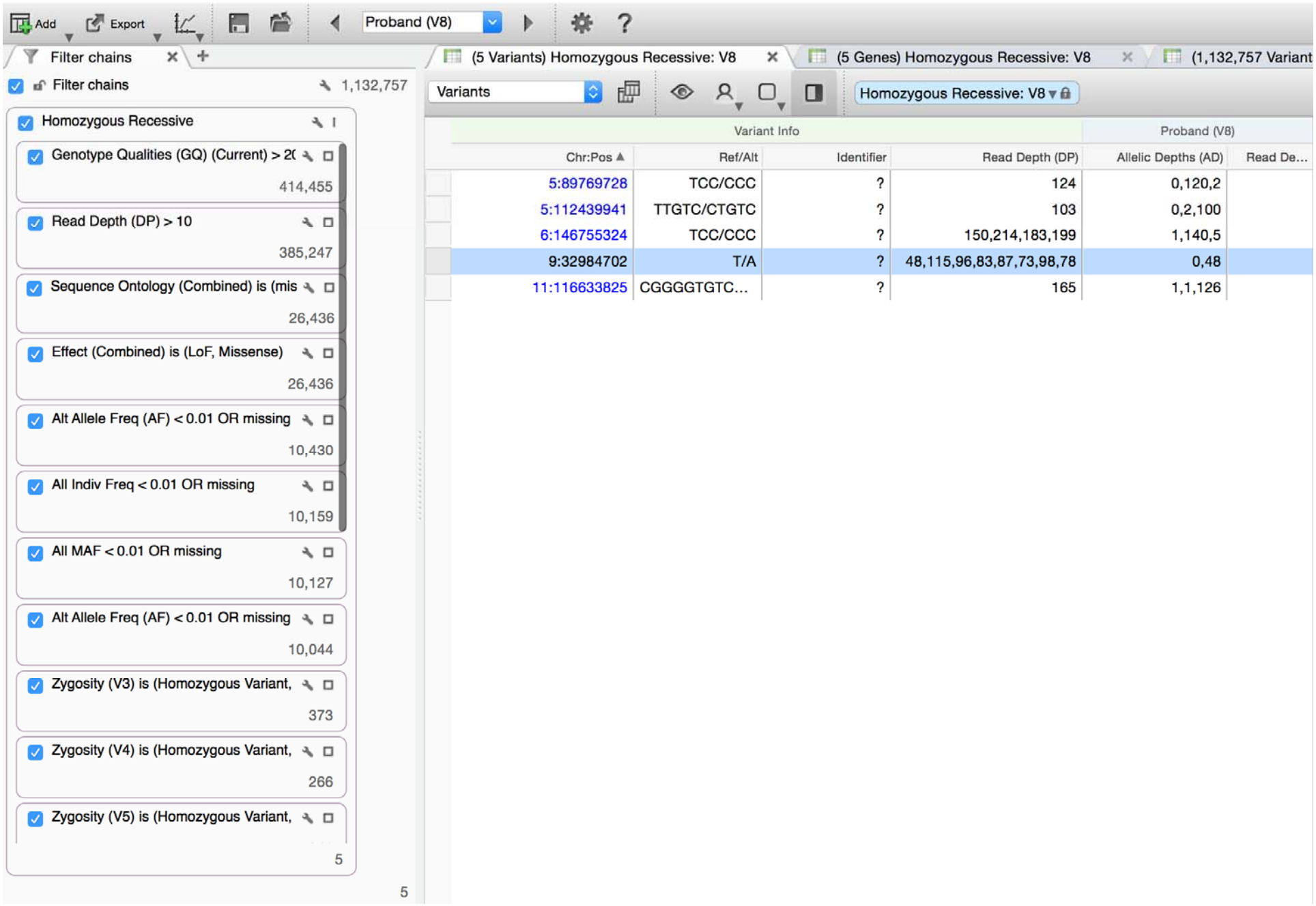
Disease status and clinical descriptions

Family member	IV-1	IV-2	IV-3	IV-4	V-1	V-2	V-3	V-4	V-5	V-6	V-7	V-8
Age	64	55	41	41	40	35	33	30	27	24	7	5
Sex	male	female	male	female	male	male	male	female	female	female	male	male
Disease status	Healthy	Healthy	Healthy	Healthy	Healthy	Healthy	affected	affected	affected	affected	Healthy	Affected
Clinical manifestation	Normal	Normal	Normal	Normal	Normal	Normal	Progressive ataxia, weak deep tendon reflex (DTR), lack of DTR in left side of the body, dysarthria, hand athetosis and gaze palsy	Progressive ataxia, weak deep tendon reflex (DTR), lack of DTR in left side of the body, dysarthria, hand athetosis and gaze palsy	Progressive ataxia, weak deep tendon reflex (DTR), lack of DTR in left side of the body, dysarthria, hand athetosis and gaze palsy	Progressive ataxia, weak deep tendon reflex (DTR), lack of DTR in left side of the body, dysarthria, hand athetosis and gaze palsy	Normal	Progressive ataxia, weak deep tendon reflex (DTR), lack of DTR in left side of the body, dysarthria, hand athetosis and gaze palsy

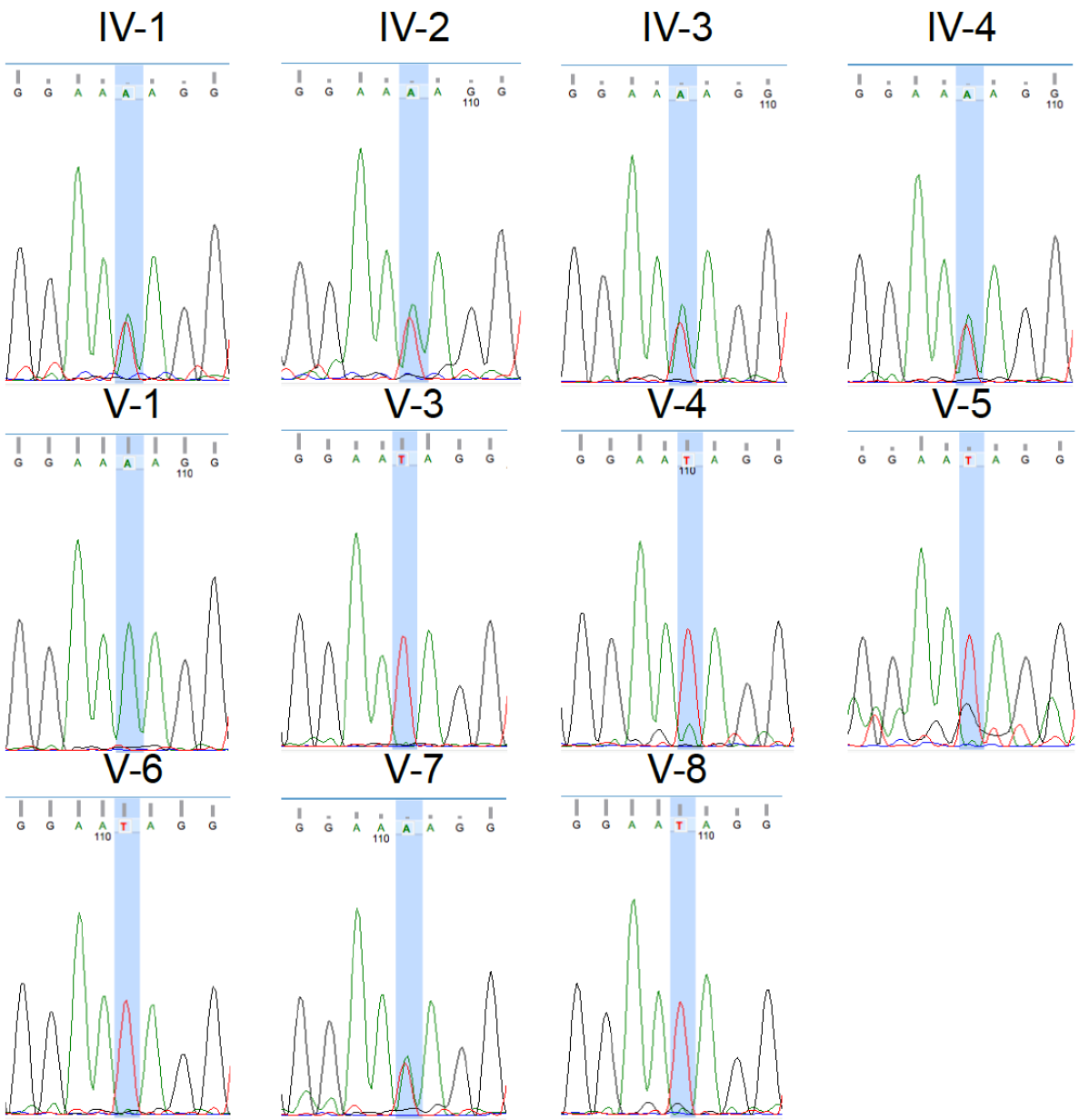
Exome sequencing analysis

- KAPA library prep kit, IDT exome probes
- Sentieon whole-exome analysis workflow (Version 201611.01)
- Homozygous variants, allele freq < 0.01
- GoldenHelix Varseq (v1.1)

Family member	**IV-1**	**IV-2**	**IV-3**	**IV-4**	**V-1**	**V-3**	**V-4**	**V-5**	**V-6**	**V-7**	**V-8**
Total variants	246619	258704	237096	246080	245318	276779	238190	264546	267455	258174	230905
Shared variants	87882										
GQ >20 and DP >10	56092										
Effect (loss-of-function or missense)	34675										
Homozygote variants	26 (Homozygote in affected members, but either heterozygote or homozygote for reference allele in controls)										
1KG MAF < 0.01	1										
EXaC MAF < 0.01	1										
dbSNP 144 MAF < 0.01	1										
NHLBI MAF < 0.01	1										
Exonic Variants	1										
Candidate	chr9:32984702; *APTX*; NM_001195248.1:c.739T>A;NP_001182177.1:p.Lys247Ter										


Add
Export
Proband (V8)
Filter chains
Filter chains
1,132,757
Homozygous Recessive
Genotype Qualities (GQ) (Current) > 2(
414,455
Read Depth (DP) > 10
385,247
Sequence Ontology (Combined) is (mis
26,436
Effect (Combined) is (LoF, Missense)
26,436
Alt Allele Freq (AF) < 0.01 OR missing
10,430
All Indiv Freq < 0.01 OR missing
10,159
All MAF < 0.01 OR missing
10,127
Alt Allele Freq (AF) < 0.01 OR missing
10,044
Zygosity (V3) is (Homozygous Variant,
373
Zygosity (V4) is (Homozygous Variant,
266
Zygosity (V5) is (Homozygous Variant,
5
5
(5 Variants) Homozygous Recessive: V8
(5 Genes) Homozygous Recessive: V8
(1,132,757 Variant
Variants
Homozygous Recessive: V8
Variant Info
Proband (V8)
Chr:Pos
Ref/Alt
Identifier
Read Depth (DP)
Allelic Depths (AD)
Read De...
5:89769728 TCC/CCC ? 124 0,120,2
5:112439941 TTGTC/CTGTC ? 103 0,2,100
6:146755324 TCC/CCC ? 150,214,183,199 1,140,5
9:32984702 T/A ? 48,115,96,83,87,73,98,78 0,48
11:116633825 CGGGGTGTC... ? 165 1,1,126

Sanger sequencing



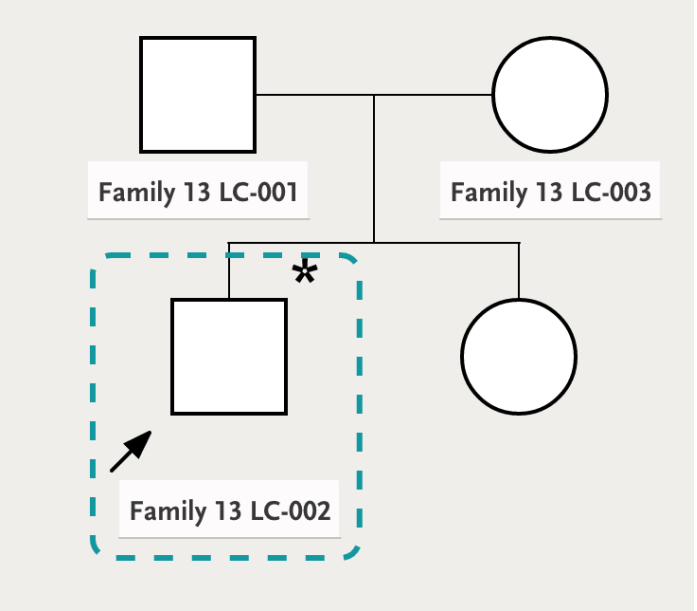
Sample	Affected	Genotype
IV-1	No	T/A
IV-2	No	T/A
IV-3	No	T/A
IV-4	No	T/A
V-1	No	A/A
V-3	Yes	T/T
V-4	Yes	T/T
V-5	Yes	T/T
V-6	Yes	T/T
V-7	No	T/A
V-8	Yes	T/T

Summary

- We identified a novel homozygous stop-gain mutation (c.739T>A; p.274Lys>Ter) in the *APTX* gene, leading to a diagnosis of ataxia with oculomotor apraxia type 1 (AOA1)
- The variant segregates with the disease; all five affected individuals are homozygous recessive for this variant
- Numerous pathogenic variants of *APTX* have been identified
- APTX is a ubiquitous nuclear protein that is involved in single-stranded DNA break repair pathway
  - Fibroblasts from patients with AOA1 are hypersensitive to oxidative damage
  - Increased oxidative DNA damage was found in the cerebellum of AOA1 patient (Harris et al., 2009)
- Our study expands the spectrum of pathogenic APTX mutations associated with AOA1

DAVID Syndrome and *NFKB2* gene

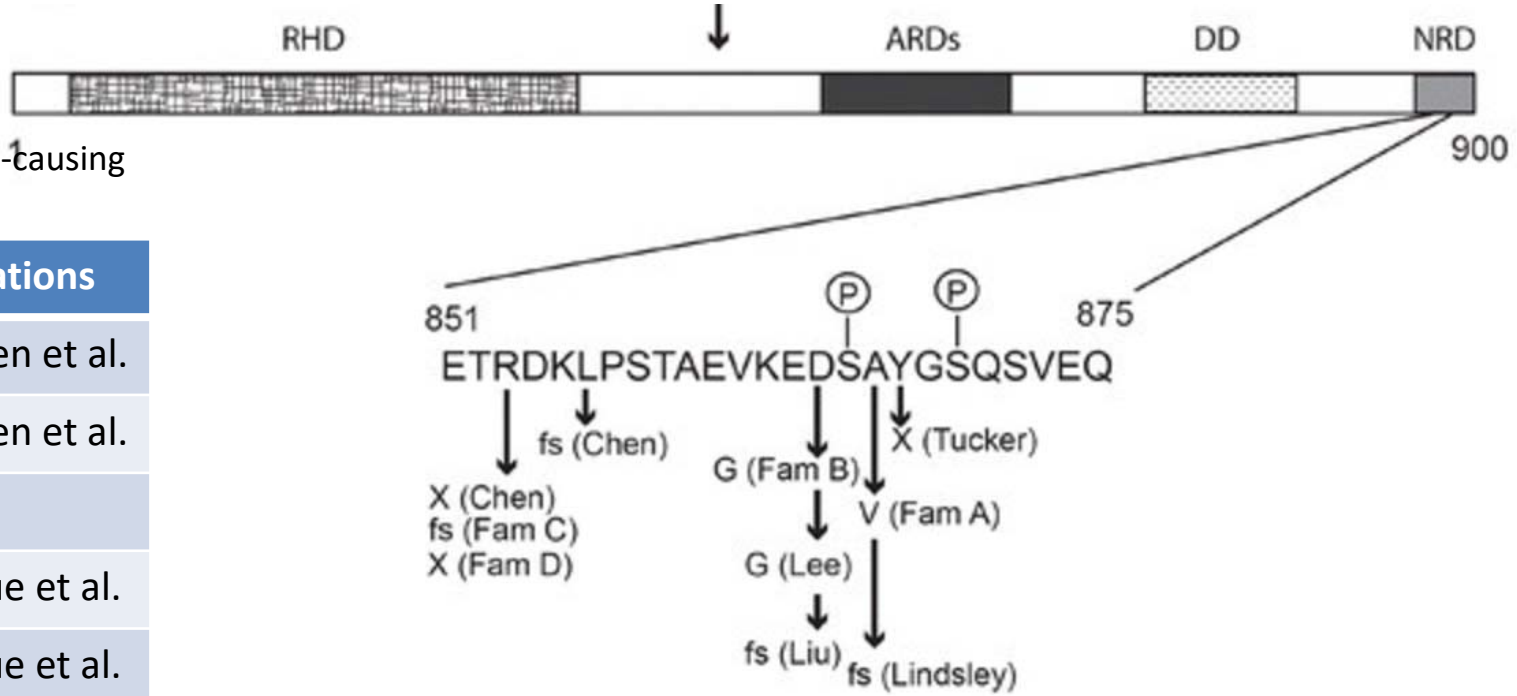
- Proband: 14 y.o. Causasian diagnosed with ACTH (adenocorticotropic hormone deficiency) and has consistently low immunoglobulins
  - Neither parents nor proband's sister is affected
  - No consanguinity



- DAVID syndrome: Deficient Anterior pituitary with Variable Immune Deficiency (Quentin *et al.* JCEM 2011)
  - symptomatic hypoglycemia
  - combined variable immunodeficiency (CVID)
  - negative pituitary autoantibodies

NFKB2 gene

- Mutations in *NFKB2* gene are known to cause common variable immunodeficiency (CVID) is a heterogeneous disorder characterized by antibody deficiency, poor humoral response to antigens, and recurrent infections
- Known to affect 1:10,000-1:15,000 people



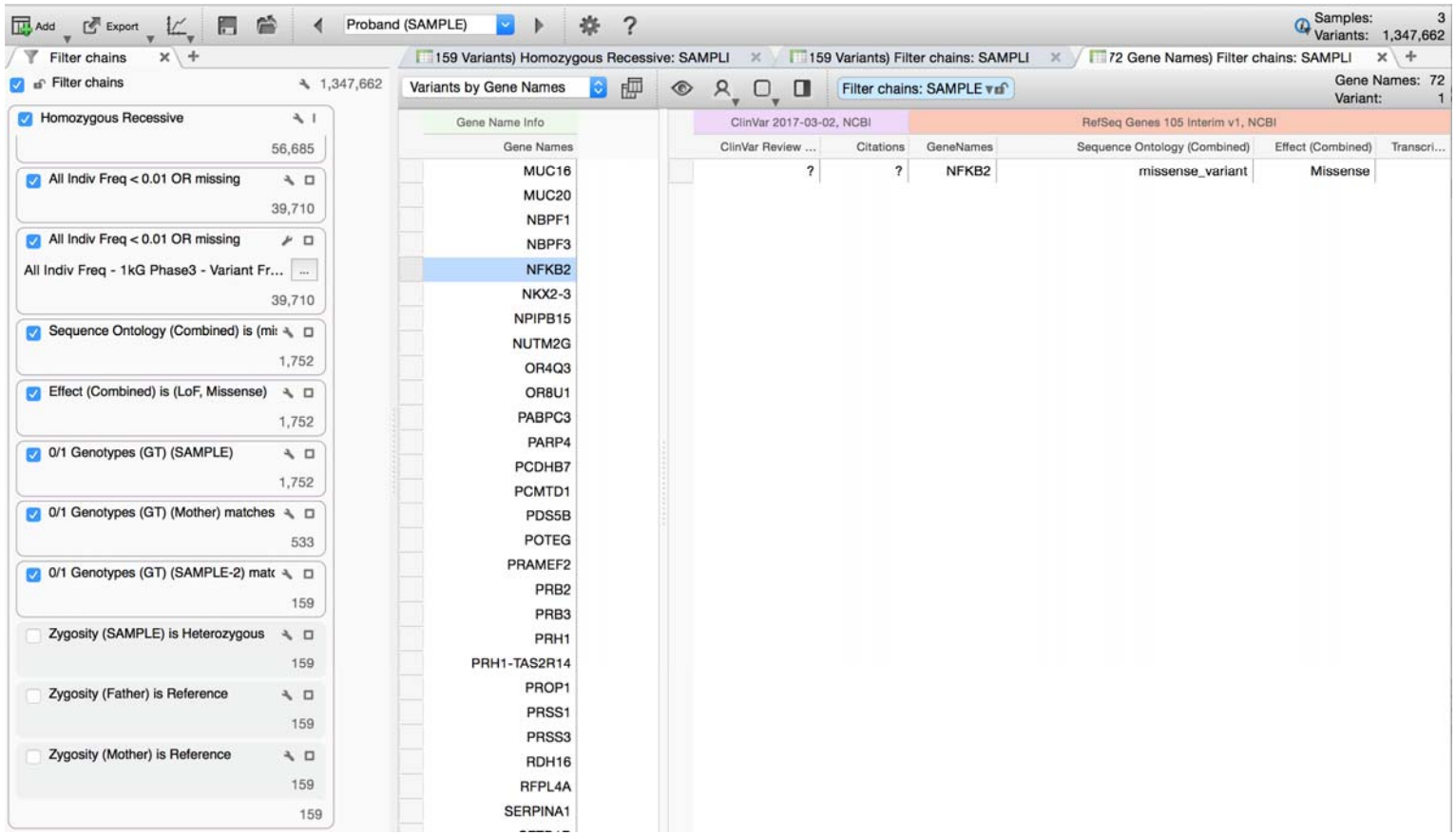
Previously reported CVID-causing NFKB2 mutations:

mutations	citations
p.Lys855Serfs*7	Chen et al.
p.Arg853*	Chen et al.
p.A867V	Brue et al.
p.D865G	Brue et al.
p.R853Afs*29	Brue et al.
p.R853*	Brue et al.

http://www.sciencedirect.com/science/article/pii/S0002929713004229; Brue et al. BMC Med Genet 2014

Exome sequencing analysis

- WES Sequencing and analysis were performed by Personalis Inc.
- De novo variants



Summary

- We identified a novel de novo mutation in the *NKFB2* gene (c.2596A>C ; p.S866R)
- Testing at Cincinnati Children's Hospital confirmed the presence of the mutation
- *NFKB2* encodes p100 protein that is processed to produce the active p52 NF-kappa-B subunit in the non-canonical NFKB pathway
  - Process involves Ser866 and Ser870 phosphorylation by IKK1 (inhibitor of nuclear factor kappa-B kinase subunit alpha) to trigger processing of p100 to p52
- The variant likely prevents phosphorylation and inhibit p52 production

Conclusion

- NGS (WES) will enable us to identify all variants in the human genome, especially the clinically relevant alleles
- These efforts will facilitate precision medicine by tailoring diagnosis and disease treatments based on one's genome
- Literature search, communications with genetic counselors, doctors help tremendously in diagnosis

Acknowledgments

- Snyder lab
  - Reza Sailani
  - Shannon Rego
  - Orit Dagen-Rosenfeld
  - Mike Snyder
- Jon Bernstein
- Collaborators for providing the DNA samples
- Stanford Center for Genomics and Personalized Medicine
- NIH