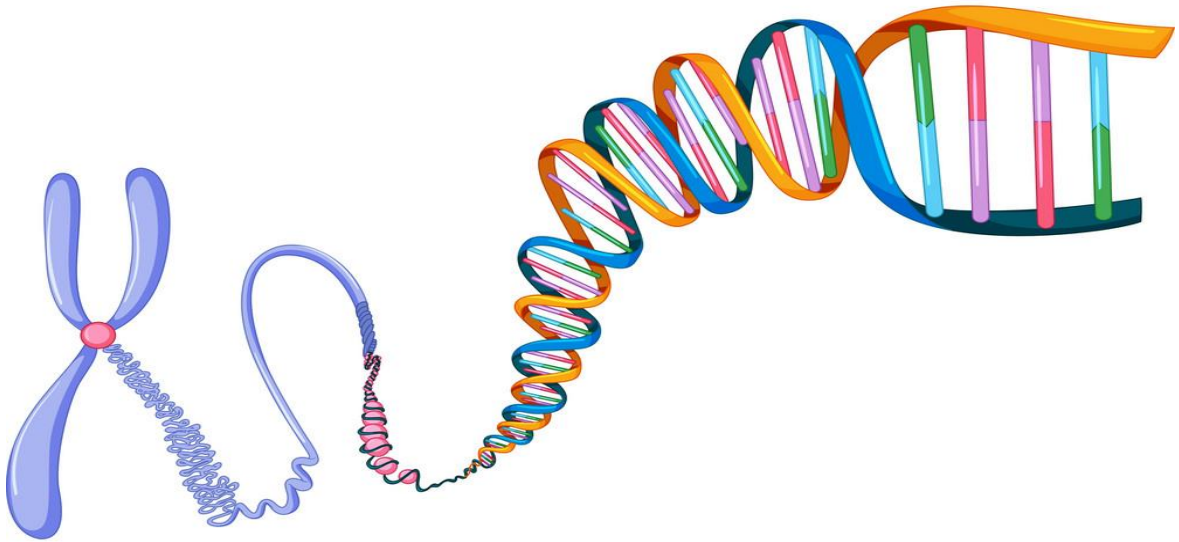
Mutation in ABCG8 and ABCG5 in correlation with Sitosterolemia



By : Casey Moyer, Bailey Orange and Alyssa Wheeler

Introduction

Plain Populations/ Old Order Amish

- Recessive disorders
- Limited gene flow and isolation

Clinic for Special Children - *Strasburg, PA*

- Large and developing public database for Plain Populations and Human population as a whole

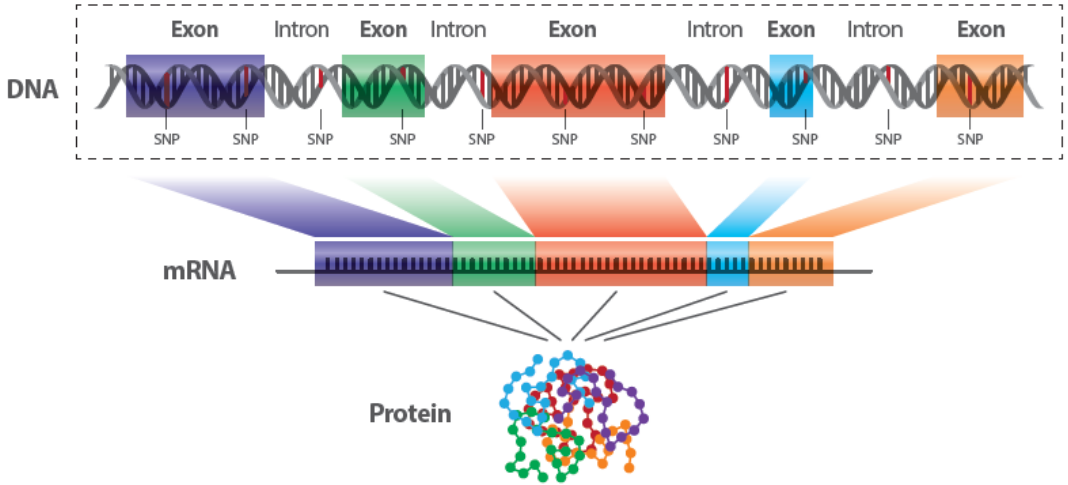
Crowley study

- 99 individuals from the Plain Population
- Development of a new NGS Assay

Clinic *for* Special Children®

Project Goal

- Analyze Variant Call Files (VCF) to look for gene mutations of a possible correlation in connection of a single disease.
- Exome sequencing from 99 patients in the Plains population
- Identify a previously identified variant and a new variant for one condition



Molecular Methods

- Blood collection from subjects
- New assay development for Next Generation Sequencing
    - 168 uniquely targeted genes
    - 162 syndromes
    - 202 alleles
    - Anchored multiplex PCR
        - Archer Variant Plex for illumina

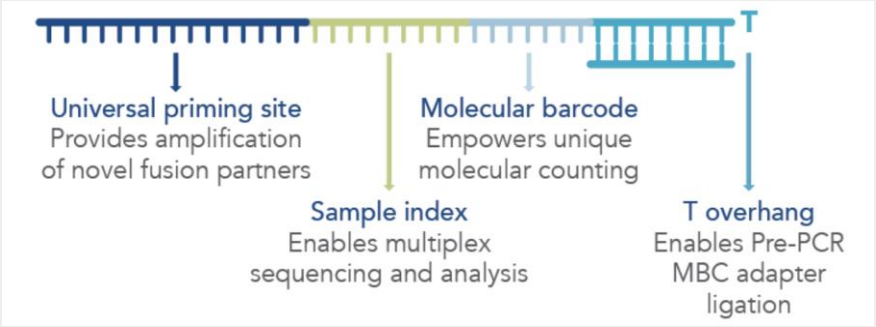
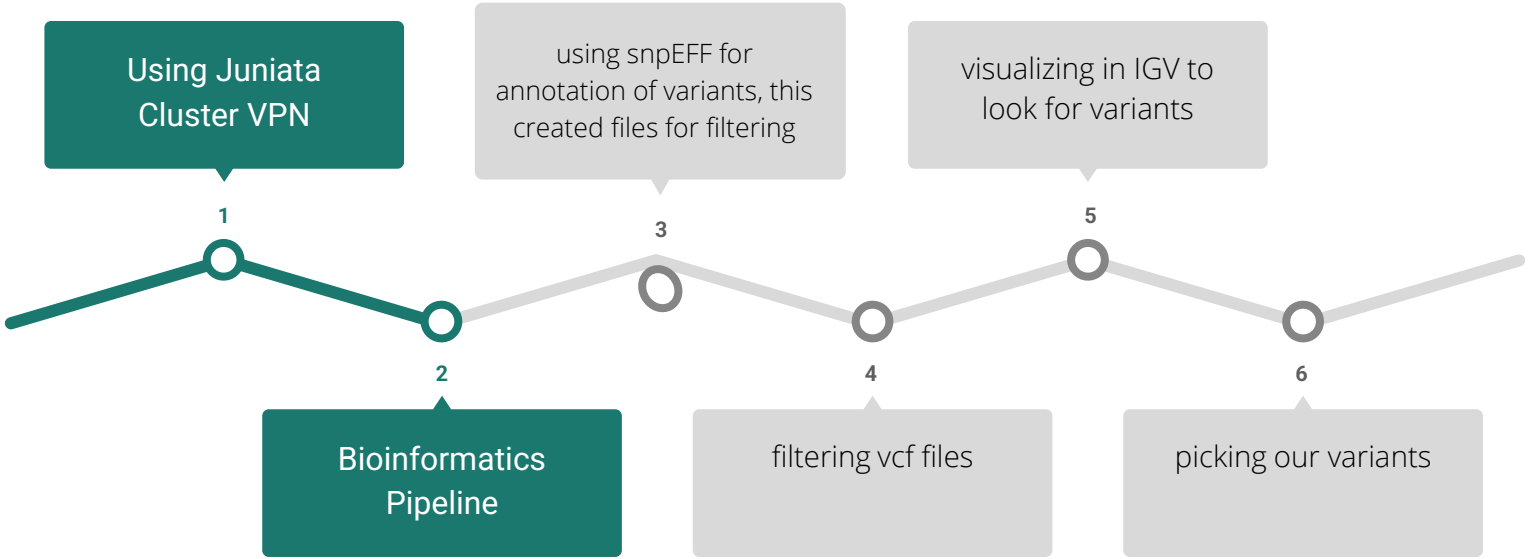


Figure 1: demonstration of molecular barcode in Anchored Multiplex PCR.https://www.idtdna.com/pages/technology/next-generation-sequencing/archer-next-generation-sequencing-technology#amp-chemistry

Bioinformatics Methods



Identified Variant

Gene: ABCG5

Location: Chromosome 2

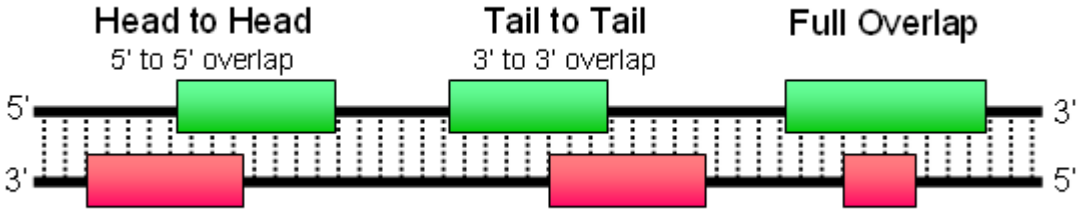
Mutation G -> A

Role

- Encodes for ABC protein transporters
- Functions as half transporter to limit intestinal absorption and promote biliary excretion of sterols

Previously Identified Variant						
sample ID	**Mutation (Gene_variant)**	**type**	**position**	**total sequencing coverage at this position**	**total number of reads of supporting alternative allele**	**variant allele frequency**
34587	ABCG8_c.1720G>A	snp	44102516	429	218	0.5082

New Variant



Gene: ABCG8

Location: chromosome 2

Mutation A -> G

Head-to-head orientation with ABCG5

Role

- Encodes for ABC protein transporters
- Functions
  - Exclude non-cholesterol sterol entry at intestine
  - Promote excretion of cholesterol and sterols into bile
  - Facilitate transport of sterols back into the intestinal lumen

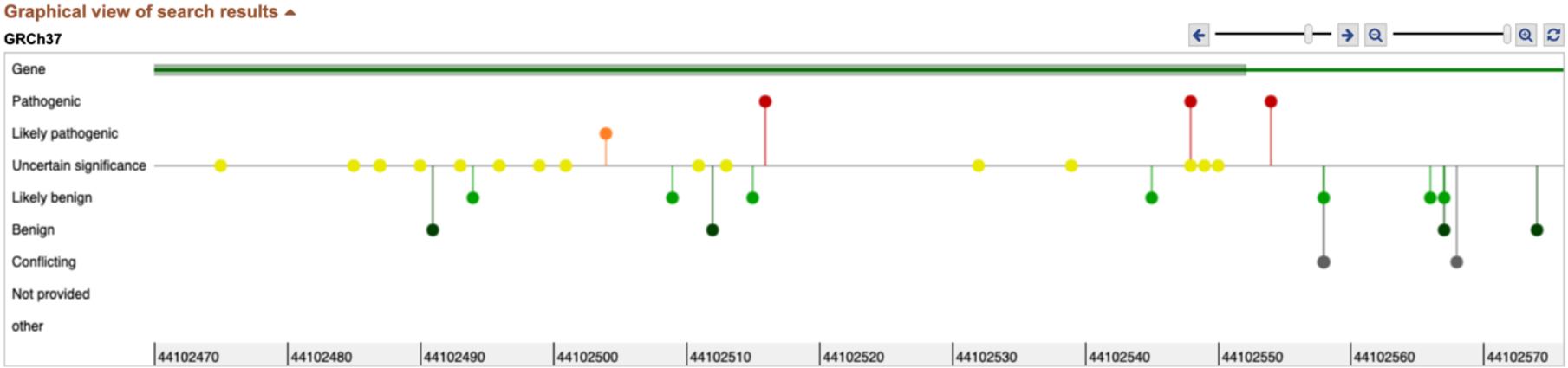


Figure 2: Clinvar pathogenic impact for ABCG8 gene
https://www.ncbi.nlm.nih.gov/clinvar/?term=abcg8%5BGENE%5D%20AND%20GRCh37%3A2%3A44102472-44102574

Conditions - Germline

Condition	Classification (# of submissions)	Review status	Last evaluated	Variation/condition record
Sitosterolemia	Pathogenic/Likely pathogenic (3)	★★☆☆	May 31, 2018	RCV000005256.13
not provided	Pathogenic (2)	★★☆☆	Jan 11, 2024	RCV000726168.6
Sitosterolemia 1	Pathogenic/Likely pathogenic (3)	★★☆☆	May 10, 2023	RCV000993692.7
Cardiovascular phenotype	Pathogenic (1)	★☆☆☆	Oct 5, 2020	RCV002408452.1
ABCG8-related condition	Likely pathogenic (1)	★☆☆☆	Feb 14, 2023	RCV003407278.4

Disease

- Mutation in both genes leads to sterol accumulation and atherosclerosis
- Untreated leads to premature coronary artery disease and death

Sitosterolemia

- Excess accumulation of plant sterols in the blood
- Rare lipid disorder
  - Lesions in the skin of lipid and fat build up
- Usually shows in early childhood

Mechanism

- Defective ABC transporter
- Plant sterols not effectively transported into the gut lumen
- Liver cannot excrete sterols into the bile as effectively

Diagnosis/Treatment

Tests

- Standard lipid profiles do not check for plant sterol in the blood
  - Might have higher total cholesterol
- Serum plant sterol level
  - Measures levels of sitosterol, campesterol, and stigmasterol
  - 30x level of plant sterol than healthy patients
- Genetic test for confirmation

Treatment

- Diet
  - Reduce intake of plant sterols and shellfish sterols
- Medications
  - Ezetimibe
  - cholestyramine (with Ezetimibe)

Conclusion and Recommendations

- If patient has either variant:
  - Serum plant sterol level panel
    - Affected individuals: 10 to 65 mg/dL plant sterol in blood
    - Normal individuals: 1.7 - 3.0 mg/L plant sterol in blood
- Side effects to look for:
  - Partial loss of function
  - Cardiovascular symptoms
  - Total cholesterol fluctuation

Future Directions

- Study effects of plant sterol levels in Plains Population due to amount/variety of vegetables in diet
- Study possibility of editing gene using CRISPR or other gene therapy

Plant	Amount of Sterols(per 100g)
Broccoli	39mg
Cauliflower	18-40mg
Carrot	12-16mg
Lettuce	9-17mg
Potato	7mg
Tomato	7mg
Avocado	75mg
Apple	12-18mg
Banana	12-16mg

Table 1: Amount of plant sterols in common fruits and vegetables
https://www.ncbi.nlm.nih.gov/books/NBK570127/table/lipid_diet_cardiov.T.plant_sterol_and_st/

Bibliography

“ABCG5 ATP Binding Cassette Subfamily G Member 5 [Homo Sapiens (Human)] - Gene - NCBI.” *National Center for Biotechnology Information*, U.S. National Library of Medicine, www.ncbi.nlm.nih.gov/gene/?term=NM_022436.3.

“ABCG8 ATP Binding Cassette Subfamily G Member 8 [Homo Sapiens (Human)] - Gene - NCBI.” *National Center for Biotechnology Information*, U.S. National Library of Medicine, www.ncbi.nlm.nih.gov/gene/?term=NM_022437.3

“Cis-Natural Antisense Transcript.” Cis-Natural Antisense Transcript, 24 Apr. 2009, mcmanuslab.ucsf.edu/node/247.

Farzam, Khashayar. “Sitosterolemia (Phytosterolemia).” *StatPearls [Internet].*, U.S. National Library of Medicine, 25 July 2023, www.ncbi.nlm.nih.gov/books/NBK572142/.

“Sitosterolemia - Symptoms, Causes, Treatment: Nord.” *National Organization for Rare Disorders*, 20 Nov. 2023, rarediseases.org/rare-diseases/sitosterolemia/.

Hooper, Amanda J, et al. “Clinical Utility Gene Card for: Sitosterolaemia.” *Nature News*, Nature Publishing Group, 28 Dec. 2016, www.nature.com/articles/ejhg2016187.