Stanford MEDICINE

department of GENETICS

Genetic Determinants of Rare Diseases

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- **Introduction**
- **Our Current state of research**
- **An overview of two whole exome sequencing studies of mendelian diseases.**

  Case#1: Alopecia and Mental Retardation (APMR) Syndrome

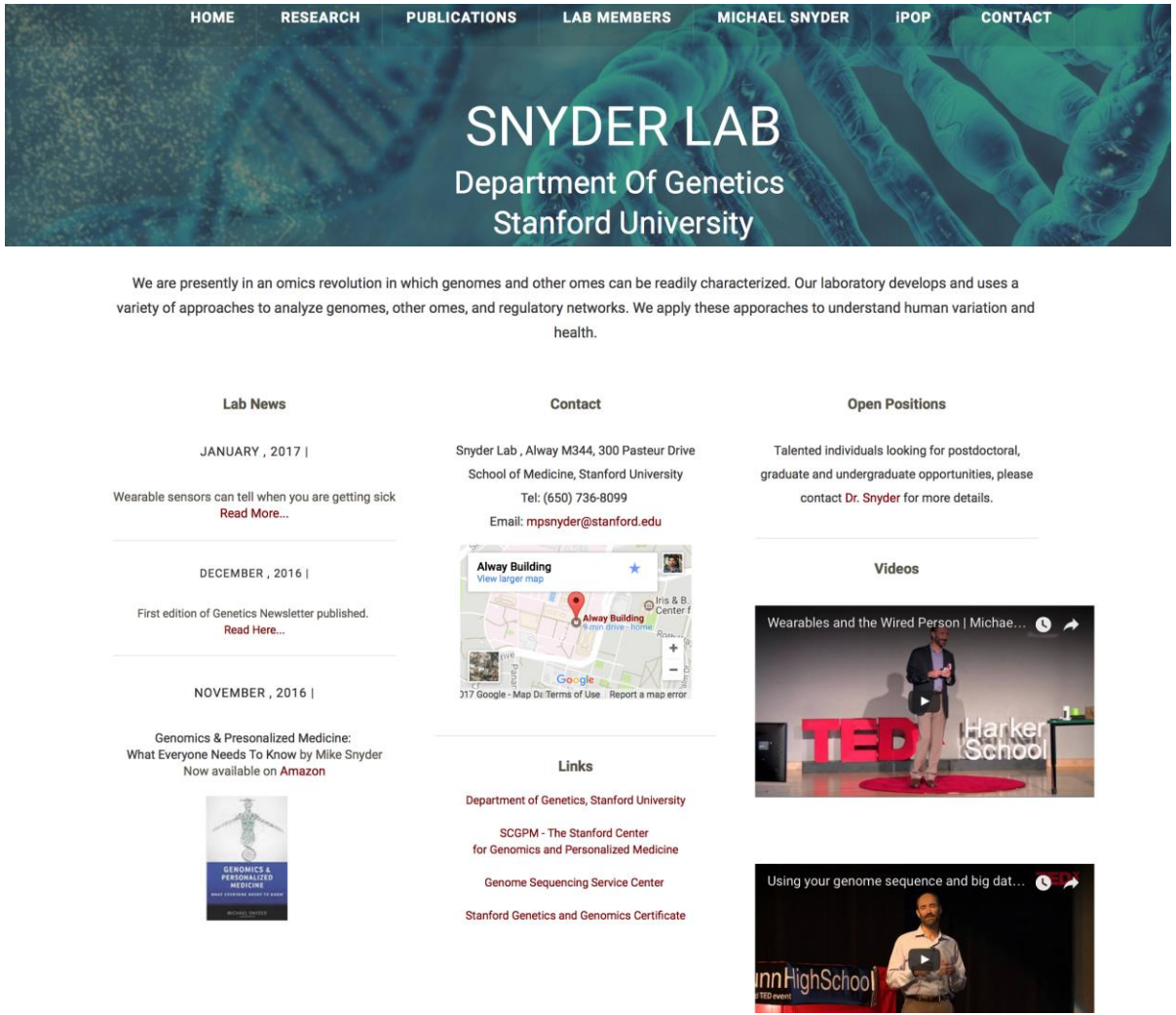
  Case#2: Pseudorheumatoid Dysplasia

- **Concluding remarks**

Current Research:

Our laboratory develops and uses a variety of approaches to analyze genomes, other omes, and regulatory networks. We apply these approaches to understand human variation and health.

http://snyderlab.stanford.edu/index.html



Genetic Determinants Of Rare And Complex Diseases:

Type 2 diabetes

Familial Tremor

Alopecia and Mental Retardation

Pseudorheumatoid Dysplasia

Isolated Congenital Anosmia

Deafness and infertility syndrome

Epilepsy

Leukodystrophies

Intellectual Disability

Hydroxyurea treatment responses in beta-thalassemia patients

Case #1

Hum Genet (2017) 136:287–296
DOI 10.1007/s00439-016-1756-5

ORIGINAL INVESTIGATION

Association of AHSG with alopecia and mental retardation (APMR) syndrome

M. Reza Sailani[1] · Fereshteh Jahanbani[1] · Jafar Nasiri[3] · Mahdiyeh Behnam[5] · Mansoor Salehi[4,6] · Maryam Sedghi[6] · Majid Hoseinzadeh[6] · Shinichi Takahashi[1] · Amin Zia[1] · Joshua Gruber[1] · Janet Linnea Lynch[1] · Daniel Lam[1] · Juliane Winkelmann[1] · Semira Amirkiai[1] · Baoxu Pang[1] · Shannon Rego[1] · Safoura Mazroui[7] · Jonathan A. Bernstein[2] · Michael P. Snyder[1]

Received: 18 August 2016 / Accepted: 25 December 2016 / Published online: 4 January 2017

Alopecia And Mental Retardation (APMR) Syndrome

- A very rare autosomal recessive condition
- Only a few families have been reported; most in the context of consanguinity.
- Affected individuals manifest with loss of hair on the scalp, the absence of eyebrows and eyelashes, and variable intellectual disability.
- However, there is high genetic heterogeneity in APMR cases as none of the families reported before shared the same aberrant locus associated with the disease.
- Linkage analyses in the past have identified three regions that are associated with APMR.

Alopecia-mental retardation syndrome candidate regions: Linkage Analyses

3q26.3-q27.3 (chr3:170,900,000-187,900,000)
17Mbp region

Alopecia-mental retardation syndrome **1 (APMR1)**

Peter John · Ghazanfar Ali · Muhammad S. Chishti
Syed Muhammad S. Naqvi · Suzanne M. Leal
Wasim Ahmad

Localization of a novel locus for alopecia with mental retardation syndrome to chromosome 3q26.33–q27.3

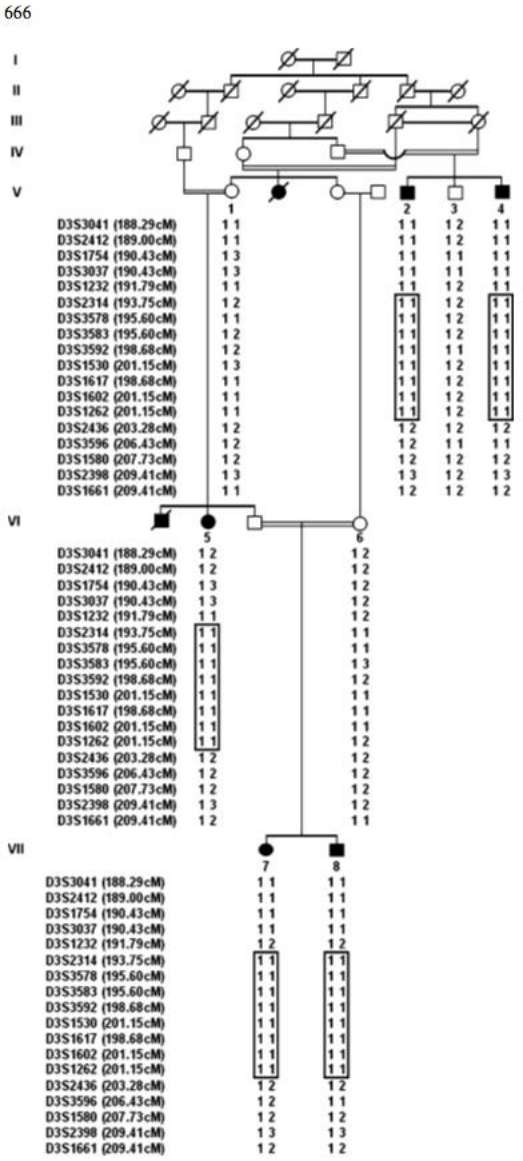


Fig. 1 Pedigree of family with APMR syndrome over seven generations. *Filled symbols* represent affected subjects. *Clear symbols* represent unaffected individuals. The disease-associated haplotypes are shown beneath each symbol. Haplotypes, generated by SIMWALK2, are displayed in *bars*

Alopecia-mental retardation syndrome candidate regions: Linkage Analyses

APMR2, on chromosome 3q26.2-q26.31
chr3:167,600,001-175,700,000

Clin Genet 2006: 70: 233–239
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CLINICAL GENETICS
doi: 10.1111/j.1399-0004.2006.00661.x

Short Report

A novel locus for alopecia with mental retardation syndrome (APMR2) maps to chromosome 3q26.2-q26.31

APMR3, Chr18: 18q11.2-q12.2

doi: 10.1111/j.1469-1809.2007.00362.x

Mapping of a Gene for Alopecia with Mental Retardation Syndrome (APMR3) on Chromosome 18q11.2-q12.2

A. Wali[1], G. Ali[1], P. John[1], K. Lee[2], M. S. Chishti[1], S. M. Leal[2] and W. Ahmad[1,*]
[1]*Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan*
[2]*Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Alkek Building, N1619.01, TX 77030 USA*

Alopecia–mental retardation syndrome: clinical and molecular characterization of four patients

A. Tzschach[1], B. Bozorgmehr[2], V. Hadavi[2], K. Kahrizi[3], M. Garshasbi[1,3], M.M. Motazacker[1,3], H.-H. Ropers[1], A.W. Kuss[1] and H. Najmabadi[2,3]

Article first published online: 4 JUL 2008

DOI: 10.1111/j.1365-2133.2008.08719.x

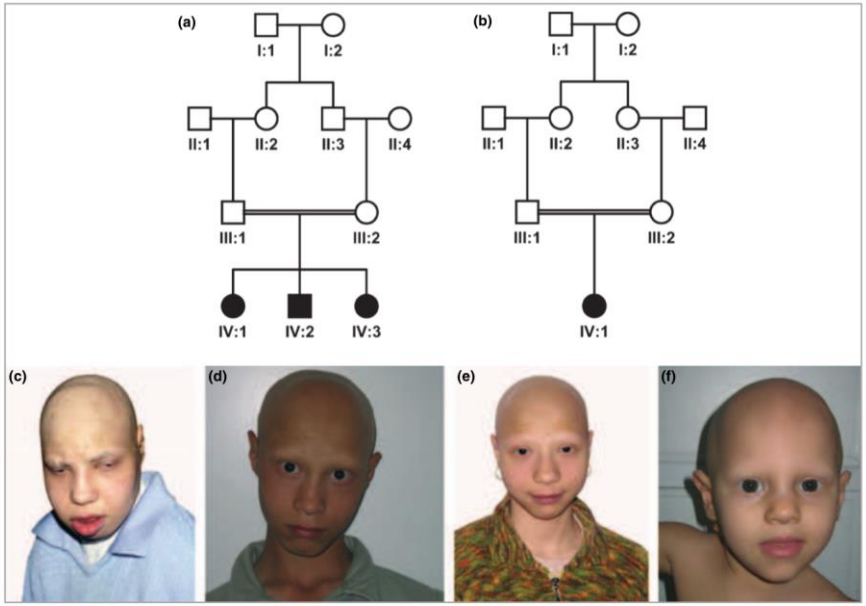
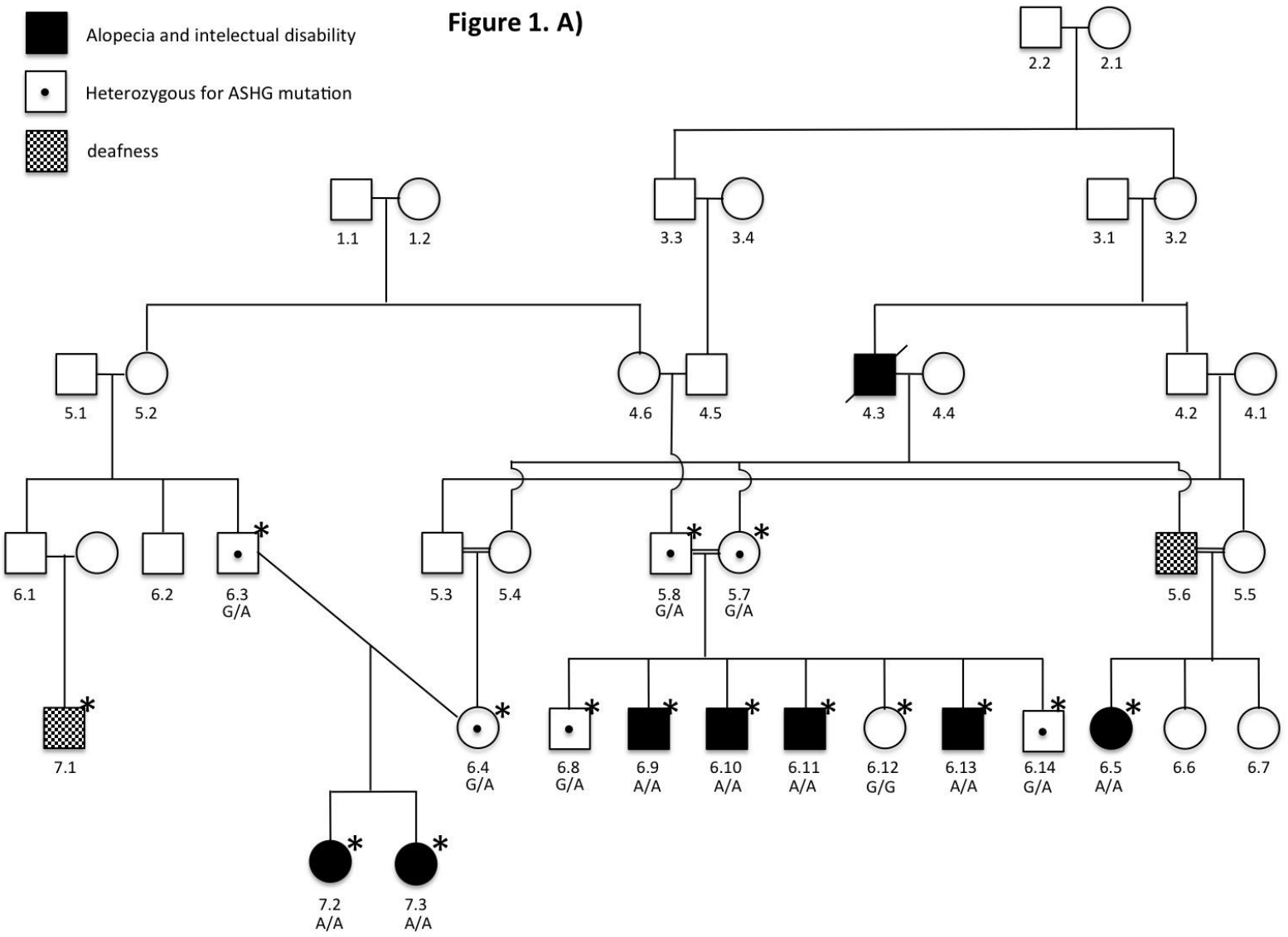


Fig 1. (a) Pedigree of family 1. (b) Pedigree of family 2. (c–e) Photos of the patients of family 1: IV:1 (c), IV:2 (d) and IV:3 (e). Note absence of scalp hair, eyebrows and eyelashes. (f) Patient IV:1 of family 2.

Chr8: 8p22–p21.3
Chr14:14q24.3–q31.3
Chr1:1p31.1–p22.3

Alopecia And Mental Retardation (APMR) Syndrome



These previous studies were based on linkage analyses and were able to identify regions of interest, but no specific causative genes or variants.

We employed whole-exome sequencing to identify the underlying genetic variant(s) associated with APMR syndrome in a large consanguineous family.

Methods:

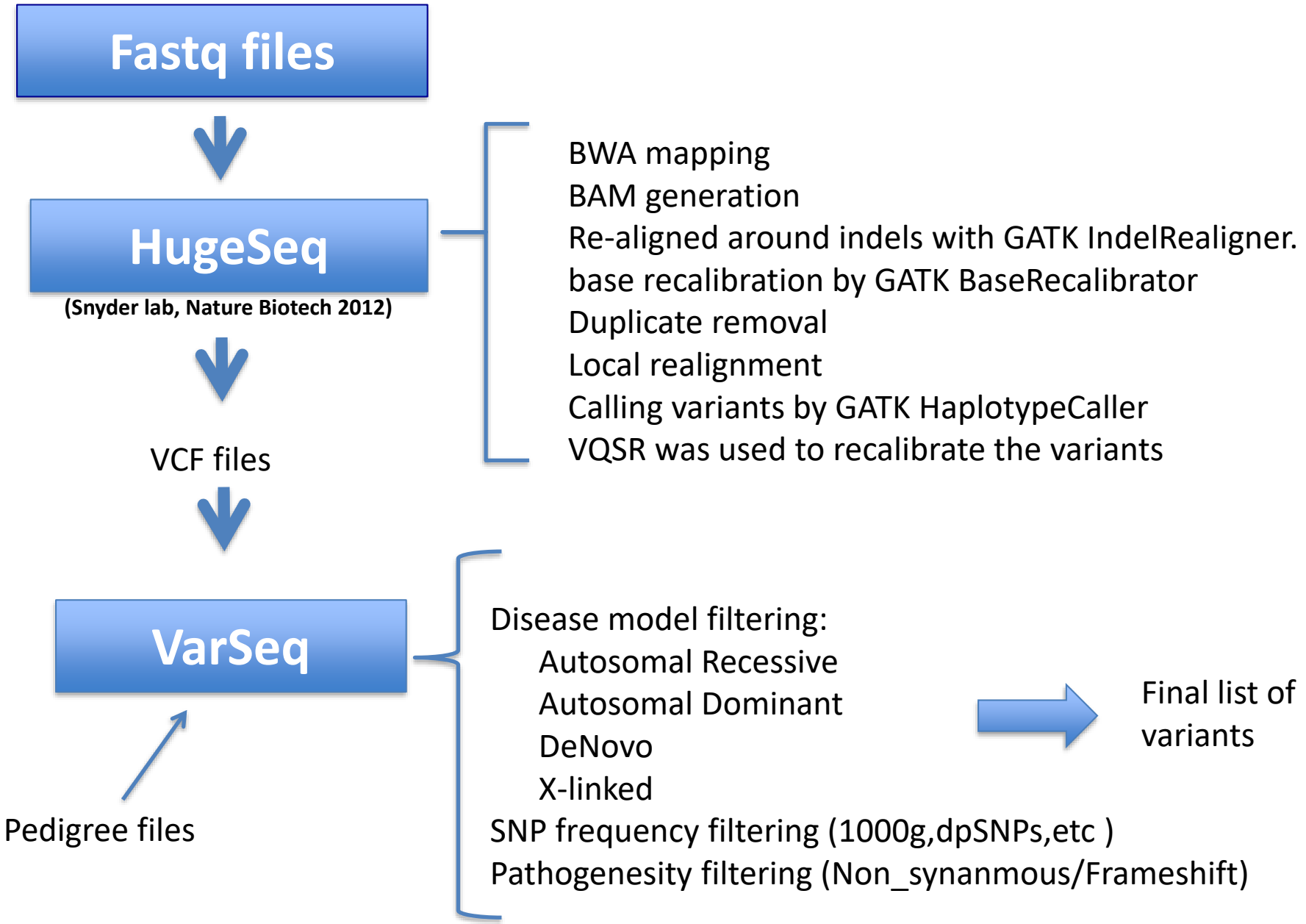
Capture Kit: Agilent SureSelectXT HumanAllExon V5

Four libraries per one lane of HiSeq2500

Hugeseq and BINA pipeline

VarSeq for variant filtering

Variant Calling Pipeline



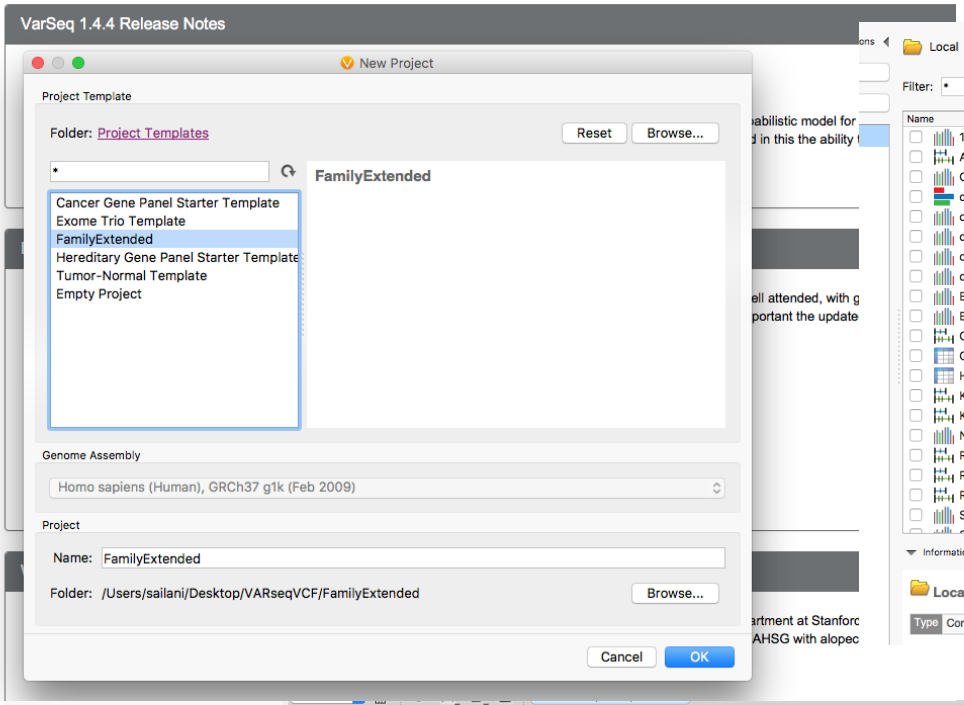
VarSeq filtering Steps:

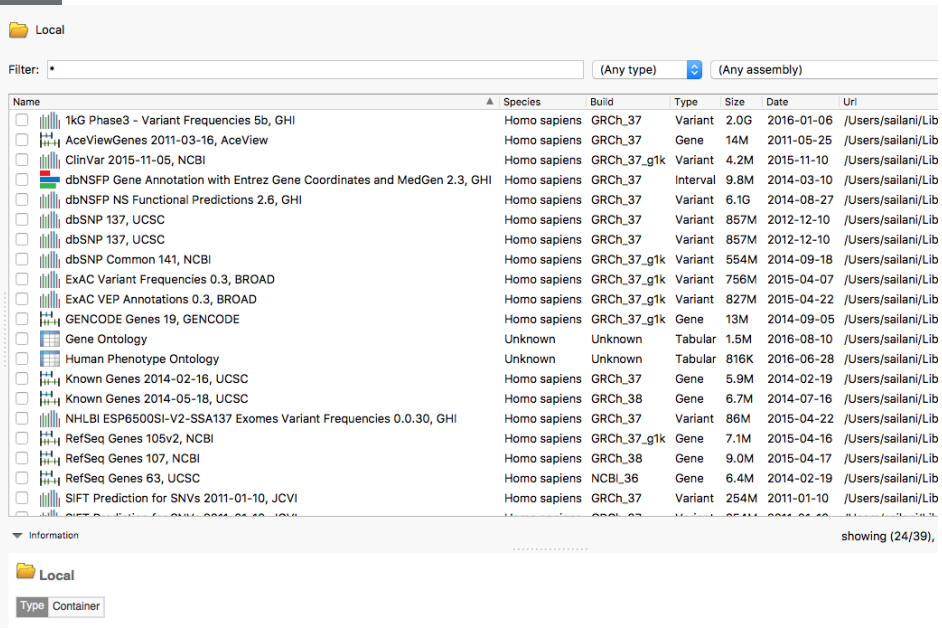
As an **autosomal recessive mode of inheritance** is indicated both by the pattern of segregation of APMR syndrome in this family and in previous reported families, we began by searching for **homozygous variants**.

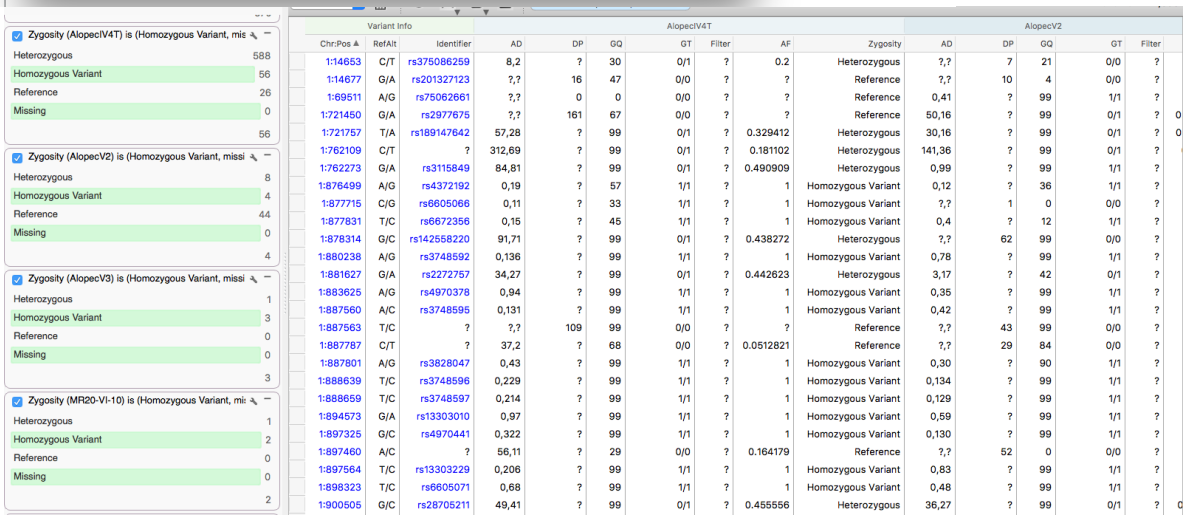
We selected variants with minor allele frequency (MAF) less than 0.01 in public databases:

- dbSNP Common 144 (Database of Single Nucleotide Polymorphism, NCBI).
- 1000 Genome project phase 3 (http://www.1000genomes.org).
- Exome Aggregation Consortium (ExAC).
- Exome Variant Server-NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (http://evs.gs.washington.edu/EVS/).

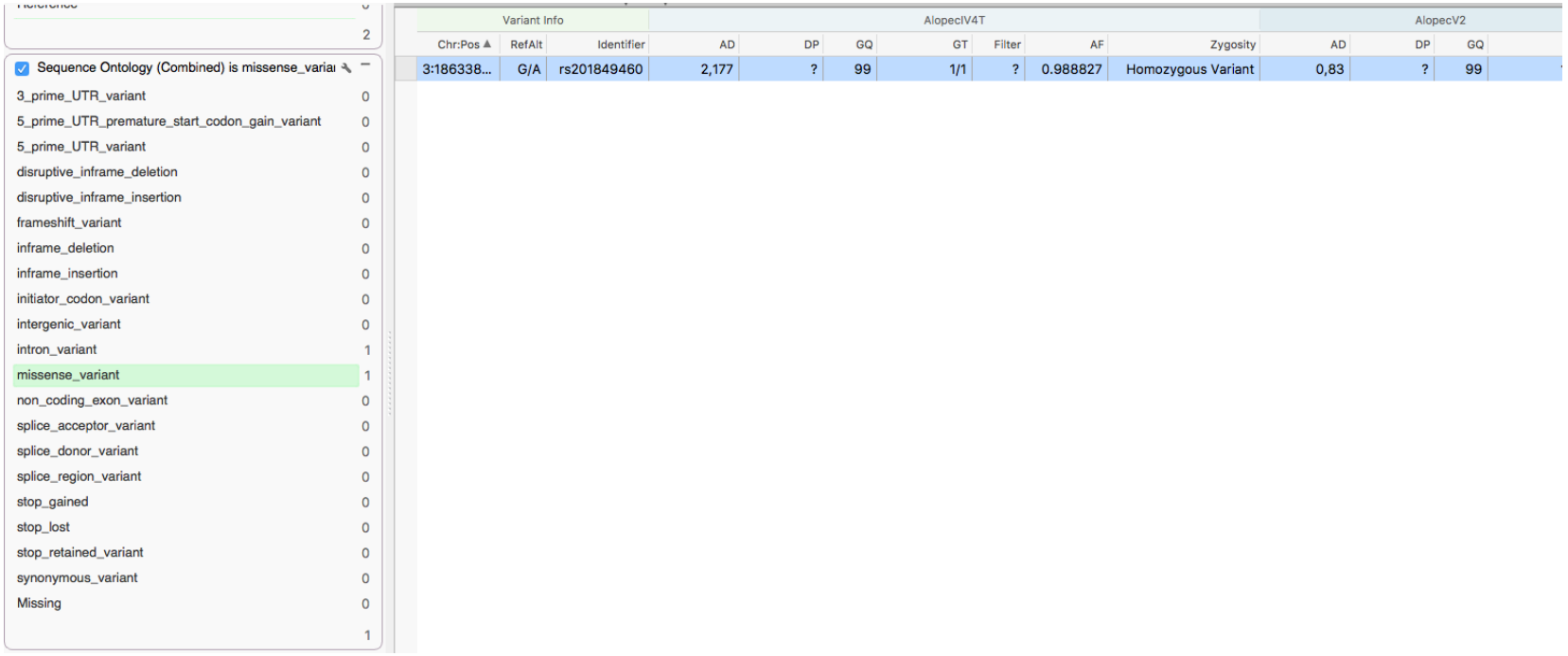
VarSeq filtering Steps:







VarSeq filtering Steps:



Variant filtering steps using VarSeq software

Individual ID	7.2	7.3	6.10	6.05
Total Variations	135,267	135,766	138,352	137,386
Shared variants	126,637			
Homozygote variants	13,156			
1KG MAF 0.01	282			
EXaC MAF 0.01	152			
UK 10K Twin MAF 0.01	66			
NHLBI MAF 0.01	2			
Exonic Variants	1			
Candidate	Chr3: 186338565 ; rs201849460 ; AHSG: c.950G>A, p.Arg317His			

MAF minor allele frequency. 1 kg, 1000 Genome project phase 3,. EXaC, Exome Aggregation Consortium version 0.3. dbSNP 144, Database of Single Nucleotide Polymorphism, NCBI. NHLBI, Exome Variant Server; NHLBI GO Exome Sequencing Project (ESP)

The identified variant (rs201849460) is rare, predicted to be deleterious and falls within exon 7 of AHSG gene.

(MAF 0.0008% in EXaC, 0.04% in dbSNP 144; no homozygotes)

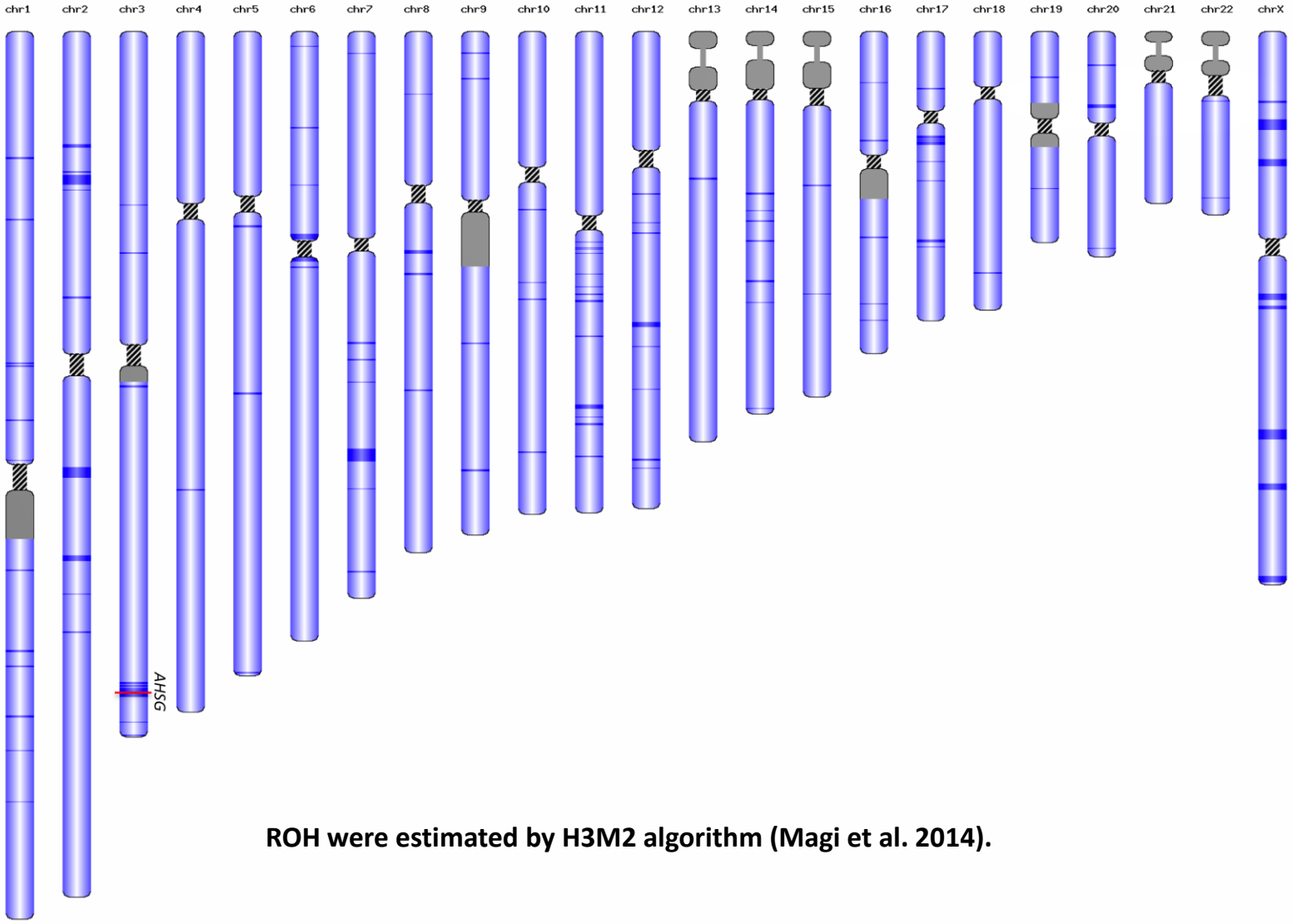
rs201849460

Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
South Asian	4	16508	0	0.0002423
African	1	10348	0	9.664e-05
Latino	1	11566	0	8.646e-05
European (Non-Finnish)	4	66630	0	6.003e-05
East Asian	0	8646	0	0
European (Finnish)	0	6614	0	0
Other	0	908	0	0
Total	**10**	**121220**	**0**	**8.249e-05**

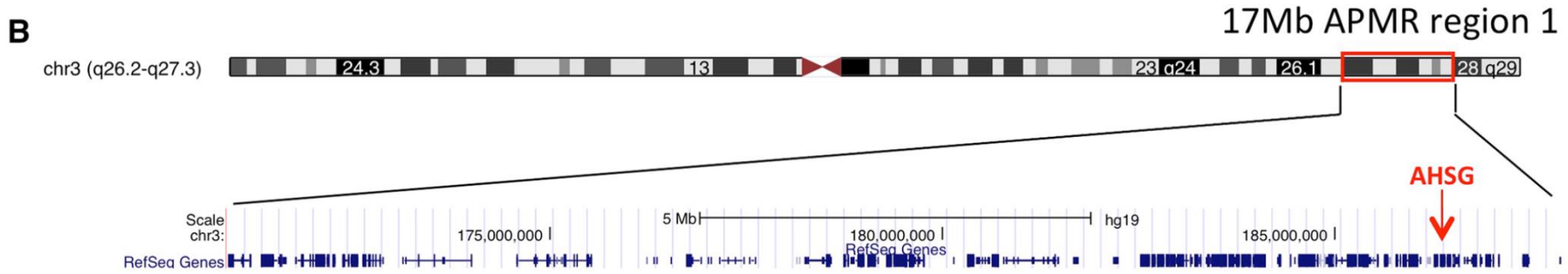
Runs of Homozygosity and AHSG location on chromosome 3.

ROH are long stretches of consecutive homozygous genotypes reflecting segments shared identically by descent (Broman and Weber 1999) and known to harbor mutations for recessive diseases.



ROH were estimated by H3M2 algorithm (Magi et al. 2014).

AHSG resides within a 17 Mb linkage region previously reported to be associated with APMR (APMR1)



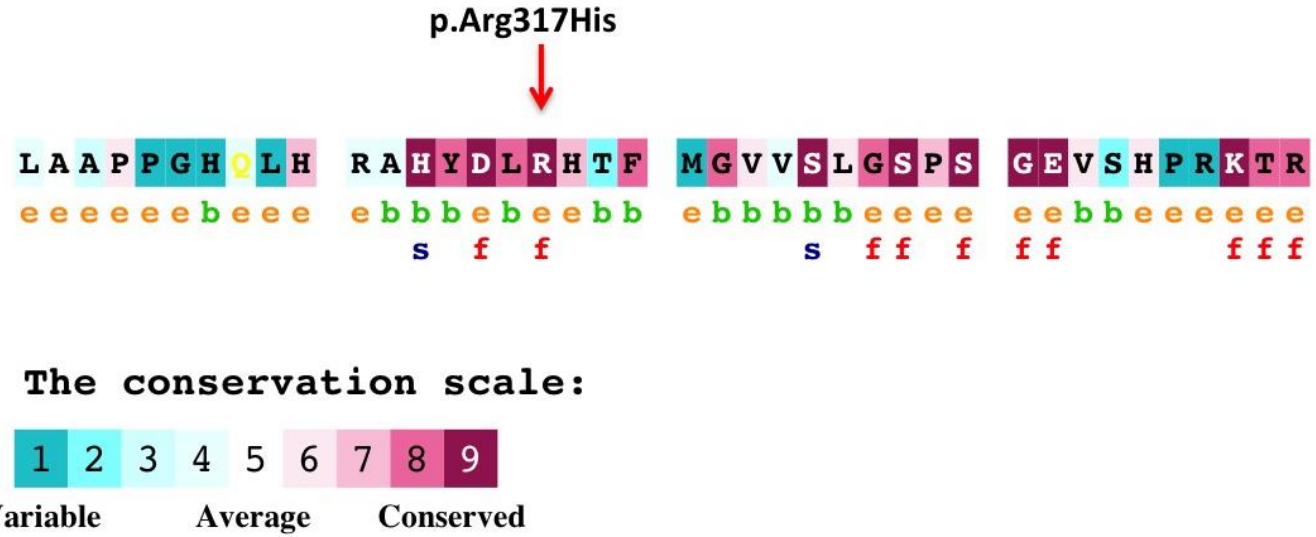
Alopecia-mental retardation syndrome **1 (APMR1)**

3q26.3-q27.3 (chr3:170,900,000-187,900,000)
17Mbp region

Peter John · Ghazanfar Ali · Muhammad S. Chishti
Syed Muhammad S. Naqvi · Suzanne M. Leal
Wasim Ahmad

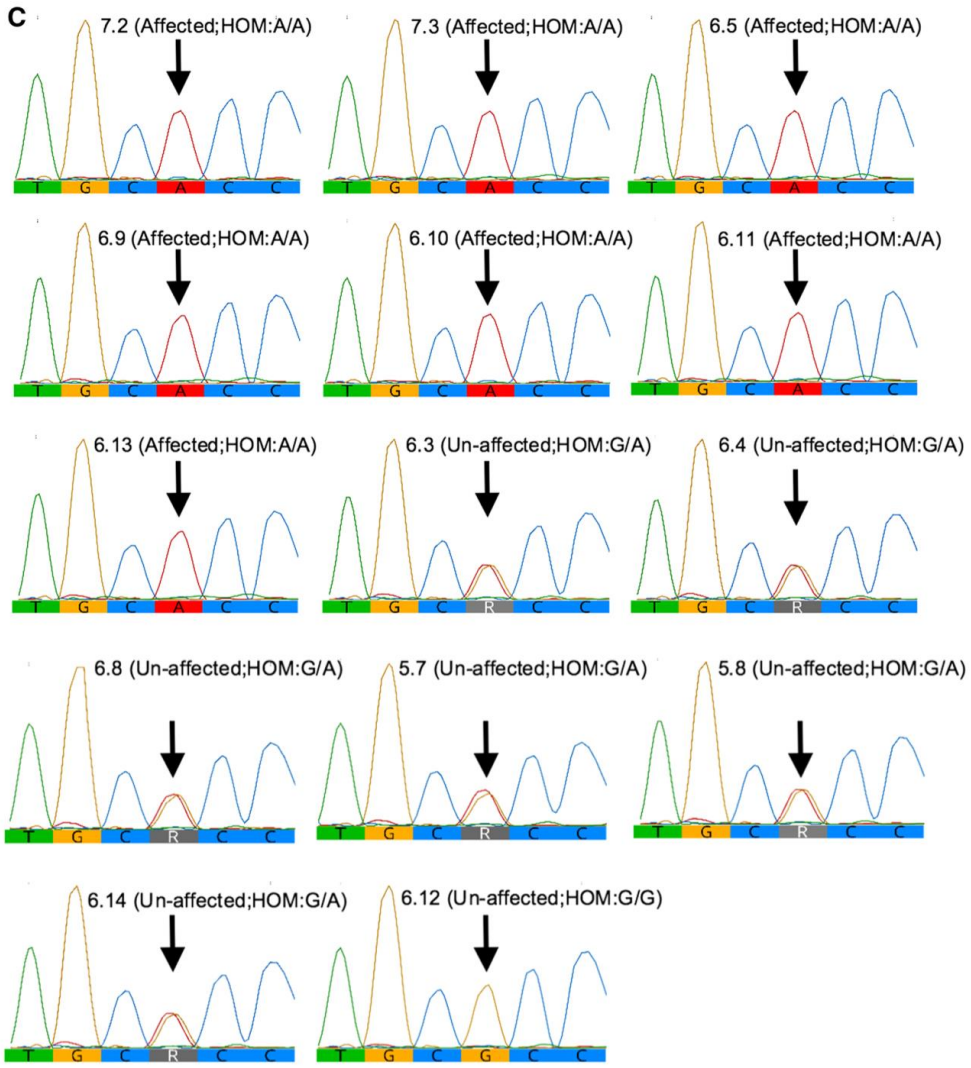
Localization of a novel locus for alopecia with mental retardation syndrome to chromosome 3q26.33–q27.3

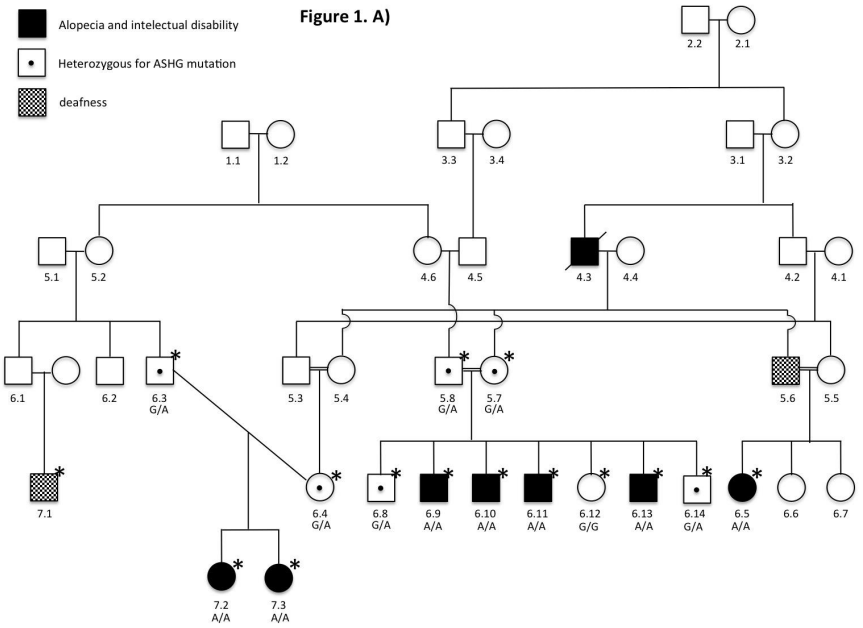
The amino acid sequence of AHSG colored according to the conservation scores.



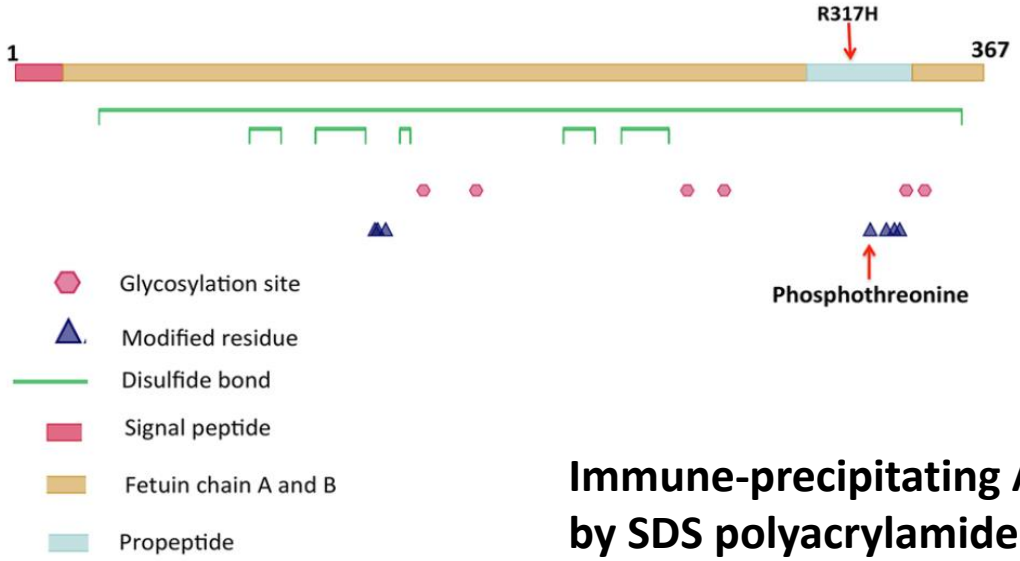
p.Arg317His occurs in a predicted functional residue, highly conserved and exposed (generated by ConSurf method).

We confirmed the homozygosity of this variant by Sanger sequencing

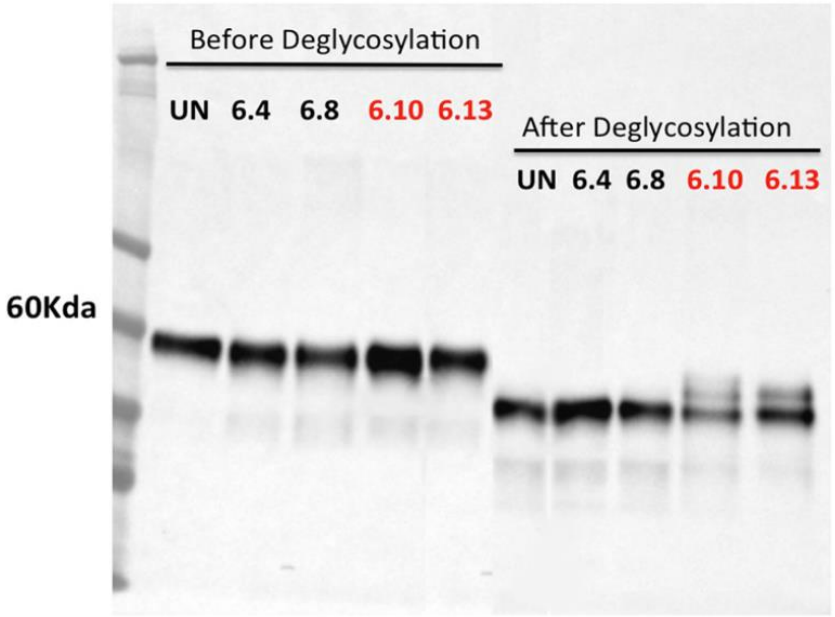




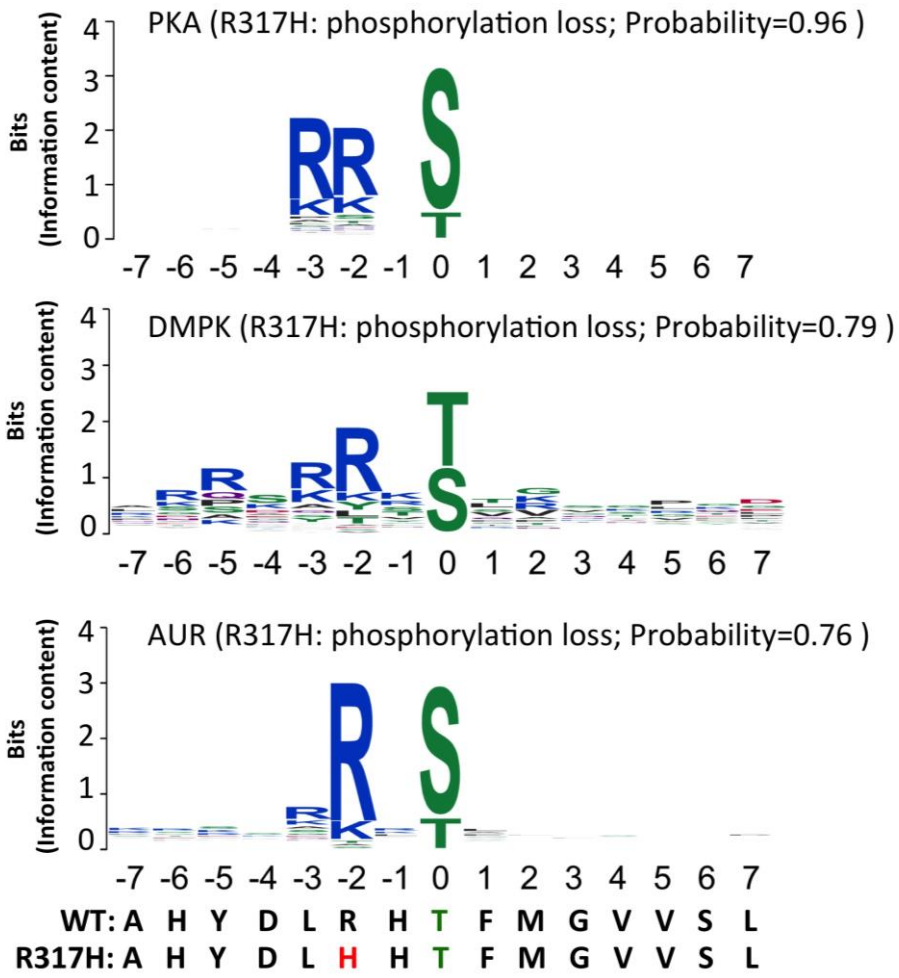
Post-translational modifications (PTMs) are common in AHSG protein
The identified variant is predicted to affect the pro-peptide



Immune-precipitating AHSG from serum followed by SDS polyacrylamide gel electrophoresis



Arg317His disrupts a phosphorylation motif



p.Arg317His disrupts this phosphorylation motif, potentially leading to a loss of phosphorylation at p.Thr319.

We used MIMP algorithm to evaluate the potential impact of p.Arg317His on this motif.

- **AHSG as a phosphorylated glycoprotein is a multifunctional extracellular calcium-regulatory protein.**
- **Is an important participant of diverse normal and pathological processes, including production of growth hormone (GH), nerve growth factor (NGF), transforming growth factor II (TGFb).**

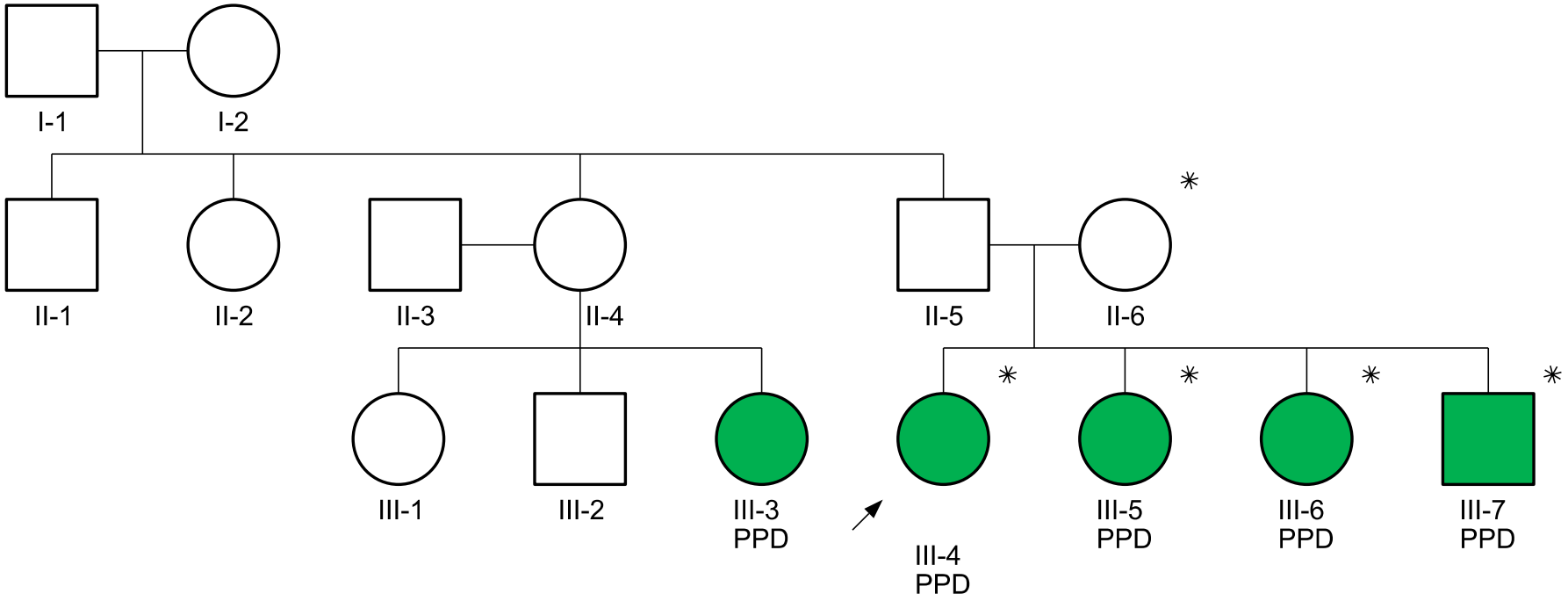
- **Promotes primary keratinocyte migration.**
- **Is particularly abundant at sites where basal keratinocytes are reorganizing to form hair follicle placodes.**

We therefore suggest AHSG gene as a potential Candidate for APMR in this family.

Case #2

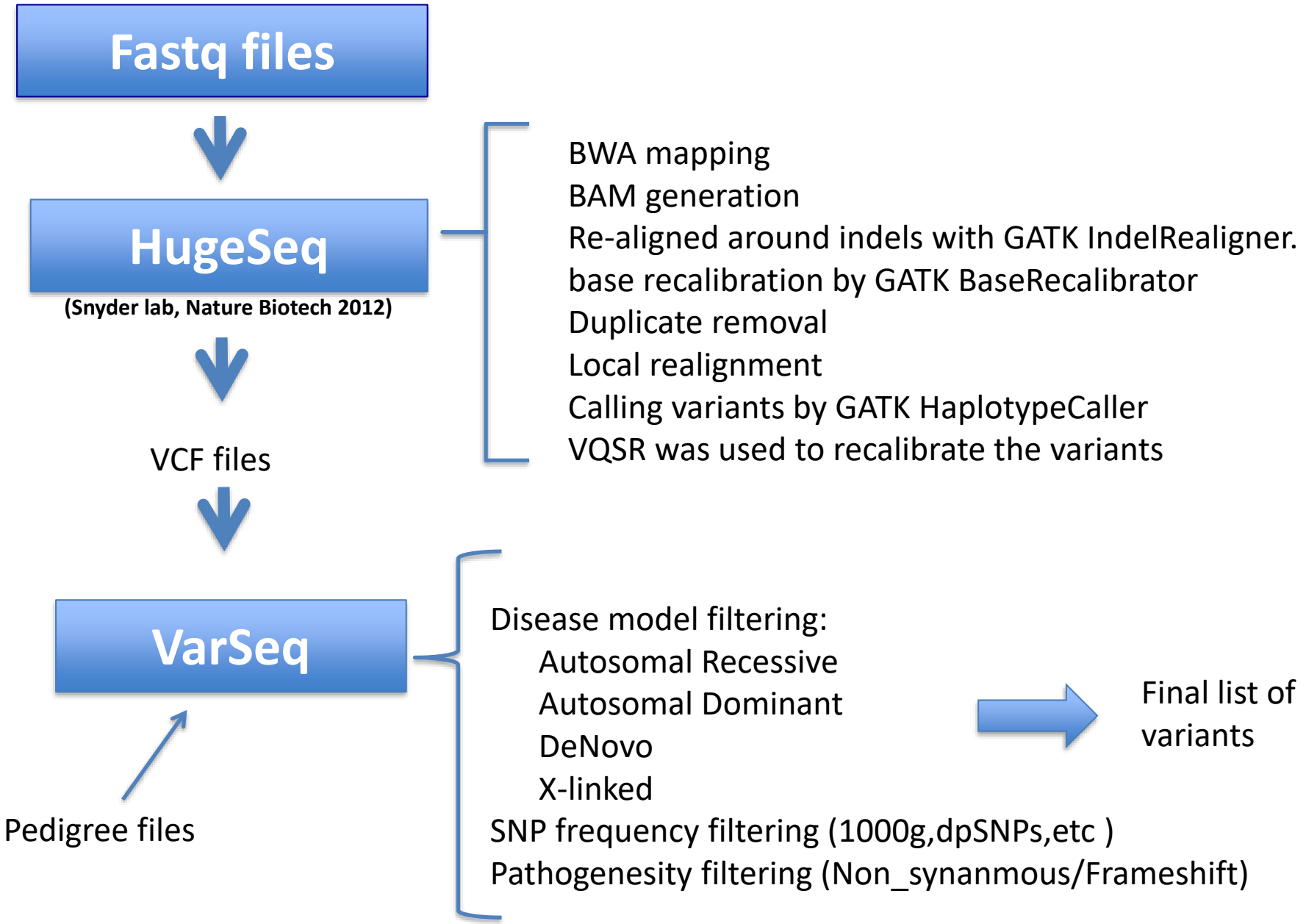
WISP3 mutation associated with Pseudorheumatoid Dysplasia

Here we report genetic characterization of a family segregating an uncharacterized from of skeletal dysplasia.



The patients were asymptomatic at birth, with normal growth, development and intelligence as well as no facial, joint, and skeletal system deformity. However, the disease started to manifest at four to six years of age in affected individuals and progressively worsened.

Variant Calling Pipeline



VarSeq filtering Steps:

As an **autosomal recessive mode of inheritance** is indicated by the pattern of segregation of disease in this family, we began by searching for **homozygous variants**.

We selected variants with minor allele frequency (MAF) less than 0.01 in public databases:

- dbSNP Common 144 (Database of Single Nucleotide Polymorphism, NCBI),
- 1000 Genome project phase 3 (http://www.1000genomes.org),
- Exome Aggregation Consortium version (ExAC)
- Exome Variant Server-NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (http://evs.gs.washington.edu/EVS/),

VarSeq filtering Steps:

Individual ID	I-2	II-1	II-2	II-3	II-4
Shared variants	163,116				
Homozygote variants in affected but heterozygote variants in parents	1,064				
1KG MAF < 0.01	373				
EXaC MAF < 0.01	325				
dbSNP 144 MAF < 0.01	322				
NHLBI MAF < 0.01	320				
UK 10K twins	286				
UK 10K ALSPAC	286				
Exonic Variants	26				
Pathogenic (Missense or Stop gain/loss)	1				
Candidate	Chr6:112,382,301; WISP3;c.210C>A; p.Cys70*				

MAF minor allele frequency. 1 kg, 1000 Genome project phase 3,. EXaC, Exome Aggregation Consortium version 0.3. dbSNP 144, Database of Single Nucleotide Polymorphism, NCBI. NHLBI, Exome Variant Server; NHLBI GO Exome Sequencing Project (ESP)

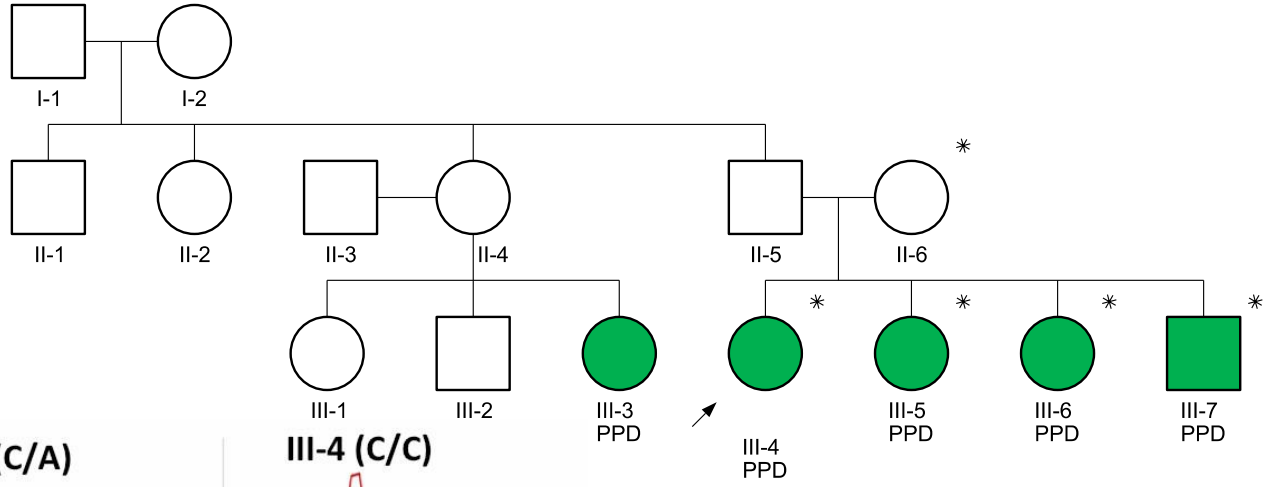
Our study is the first to report a homozygote loss of function mutation for rs121908901; WISP3;c.210C>A; p.Cys70*.

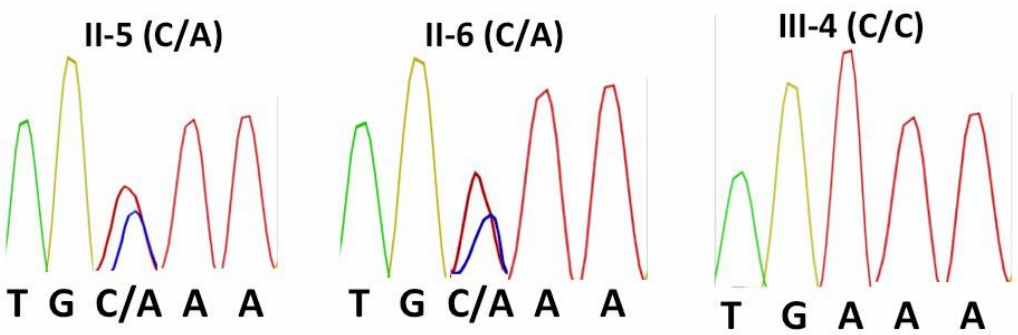
rs121908901

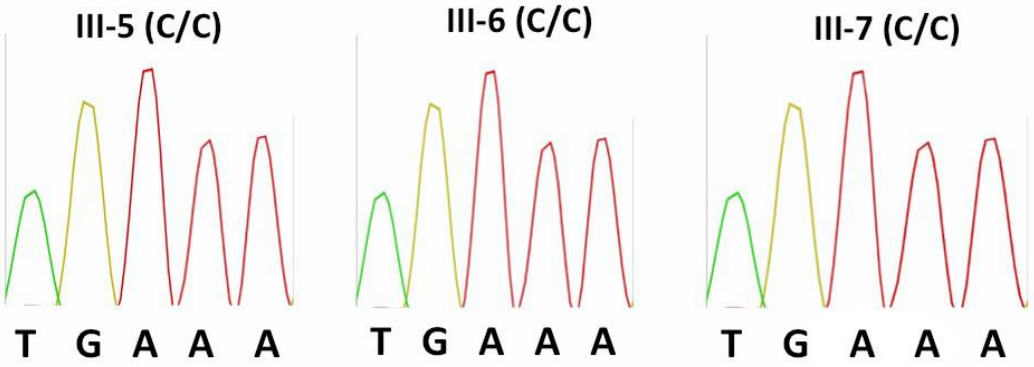
Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
South Asian	1	16512	0	6.056e-05
European (Non-Finnish)	3	66736	0	4.495e-05
African	0	10406	0	0
East Asian	0	8654	0	0
European (Finnish)	0	6614	0	0
Latino	0	11560	0	0
Other	0	908	0	0
Total	**4**	**121390**	**0**	**3.295e-05**

We confirmed the homozygosity of this variant by Sanger sequencing and showed that the variant segregates in the family







Loss-of-function mutations in the *WISP3* gene has been associated with Progressive pseudorheumatoid dysplasia (PPD)

In 1998, linkage studies in consanguineous families segregating PPD (El-Shanti et al, 1998; Fischer, J. et al 1998) mapped the candidate region on a 3-cM interval on chromosome 6q22.
One year later, Hurvitz et al 1999 showed that mutations in the WISP3 gene that fall within the linkage region are the strongest candidate to cause this autosomal recessive condition (Hurvitz et al 1999).

letter

Mutations in the CCN gene family member *WISP3* cause progressive pseudorheumatoid dysplasia

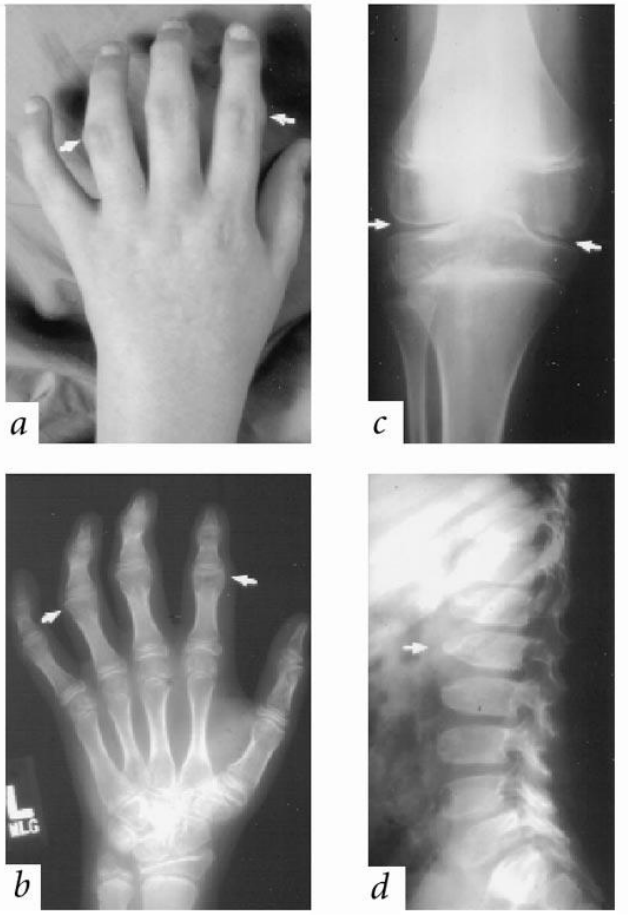
Jennifer R. Hurvitz[1], Wafaa M. Suwairi[1,2,3], Wim Van Hul[4], Hatem El-Shanti[5], Andrea Superti-Furga[6], Jean Roudier[7], Daniel Holderbaum[8], Richard M. Pauli[9], J. Kenneth Herd[10], Els Van Hul[4], Hossien Rezai-Delui[11], Eric Legius[12], Martine Le Merrer[13], Jamil Al-Alami[14], Sultan A. Bahabri[3] & Matthew L. Warman[1]

Progressive pseudorheumatoid dysplasia (PPD):

A rare skeletal dysplasia characterized by predominant involvement of articular cartilage with progressive joint stiffness.

is an autosomal recessive genetic disease.

Clinical and radiographic findings in a 13-year-old male PPD patient



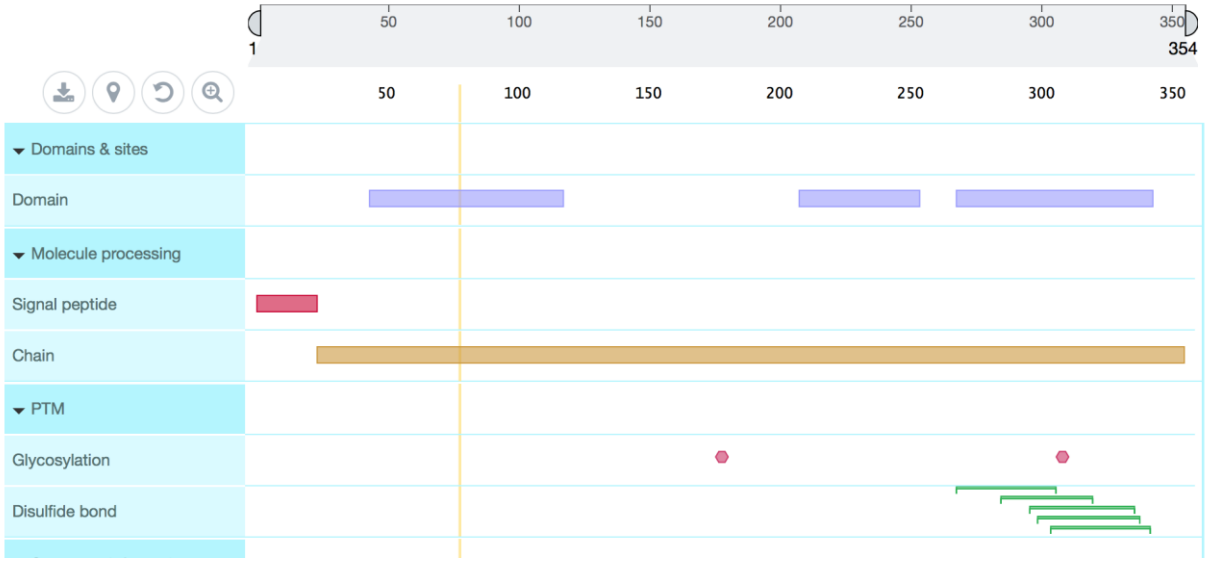
PPD remains a diagnostic challenge because of the rarity of the disease and the heterogeneity in the phenotypes, as well as having similarities with other disorders.

PPD is caused by Wnt1-inducible signaling protein 3 (WISP3) mutations.

Nature Genetics 23, 94 - 98 (1999)

WISP3 encodes Wnt1-inducible signaling protein 3, a cysteine-rich, multidomain, secreted protein

WISP3:c.210C>A p.Cys70* occurs in IGFBP N-terminal domain



The variant identified in this study at p.Cys70* would, if not subjected to nonsense mediated mRNA decay, produce a significantly truncated protein that misses major part of the protein making it unlikely that normal protein function would be retained.

Conclusion:

Combining our whole exome sequencing data with clinical documentation (familial histories, genetic data, clinical and radiological findings), we have diagnosed the families with Progressive Pseudorheumatoid Dysplasia (PPD).