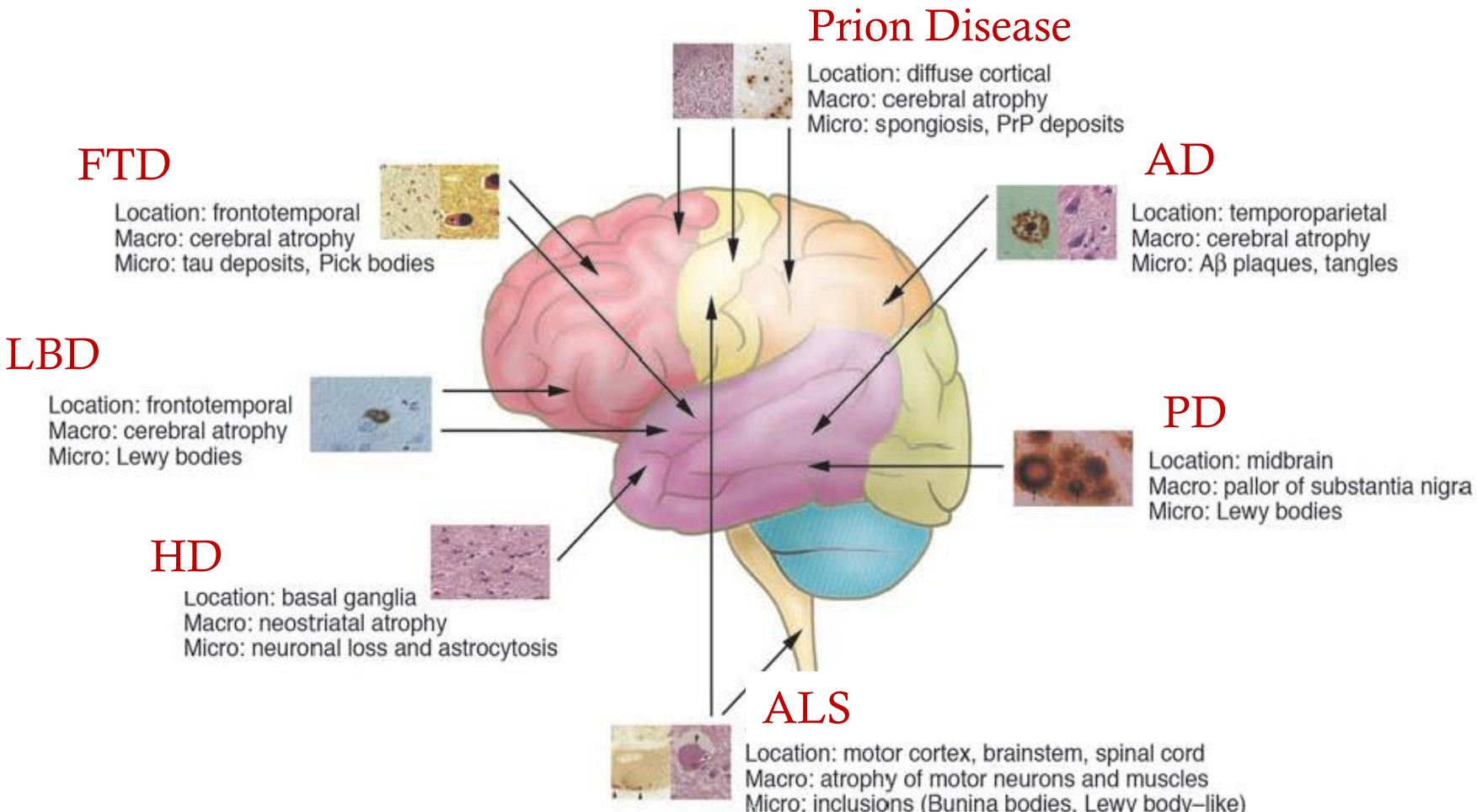
NDAL
Suna and İnan Kıraç Foundation
Neurodegeneration Research Laboratory

Identification of Pathogenic Mutations in Neurodegenerative Disorders:

Bioinformatic Analysis of Next Generation Sequencing Data

MSc Thesis Defence
Ece Kartal
March 17, 2015

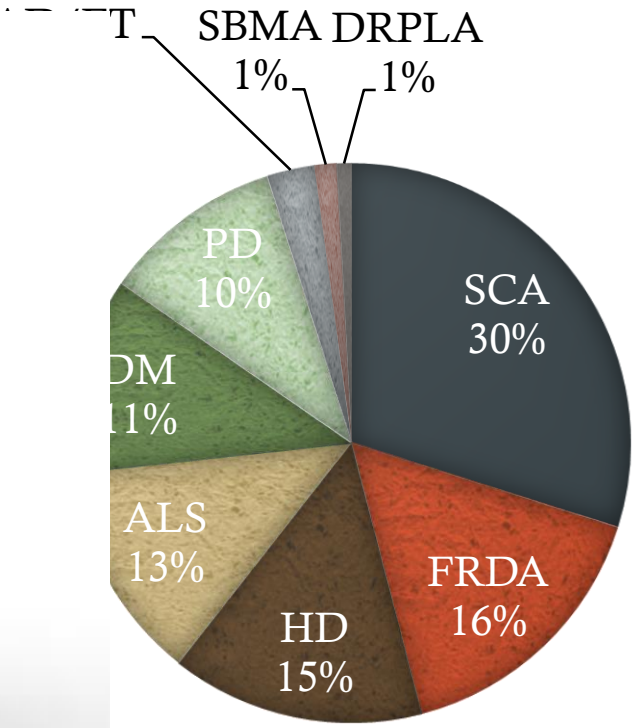
Selective neuron degeneration in different areas of the brain



Bertram and Tanzi, 2005

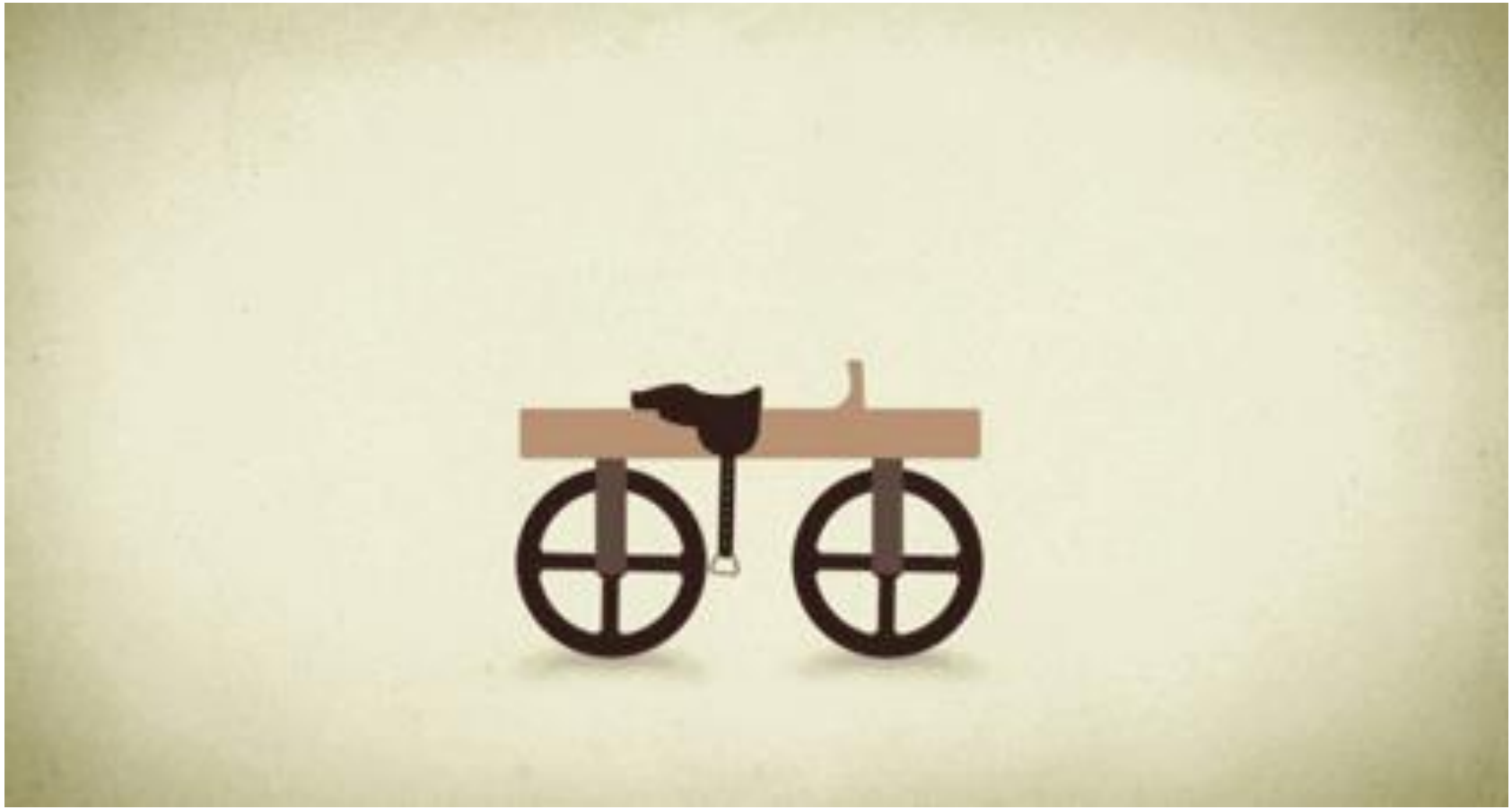
Patient cohort in Ndal

- Amyotrophic Lateral Sclerosis (ALS)
- Parkinson's Disea
- Alzheimer's Disea
- Frontotemporal D
- Ataxias
- Triplet repeat dis
  - Huntington
  - Spinocerebe
  - Friedreich A
  - Spinal-bulba
  - Dentatorubr
  - Myotonic Di



200 patient samples
>2000 control samples

Evolution of innovation



doi:10.1093/brain/awt236 Brain 2013: 136; 3106–3118 | 3106

BRAIN
A JOURNAL OF NEUROLOGY

Next generation sequencing for molecular diagnosis of neurological disorders using ataxias as a model

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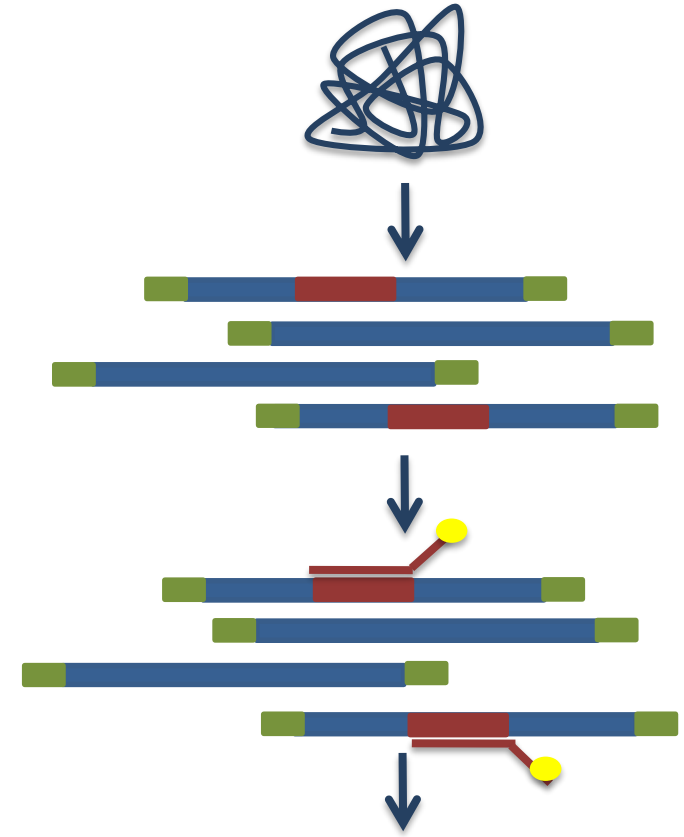
Exome sequencing

- 1% of genome, total length 30MB
- 85% of pathogenic variants
- Cost-effective
- Less data analysis

Sequencing steps of whole exome



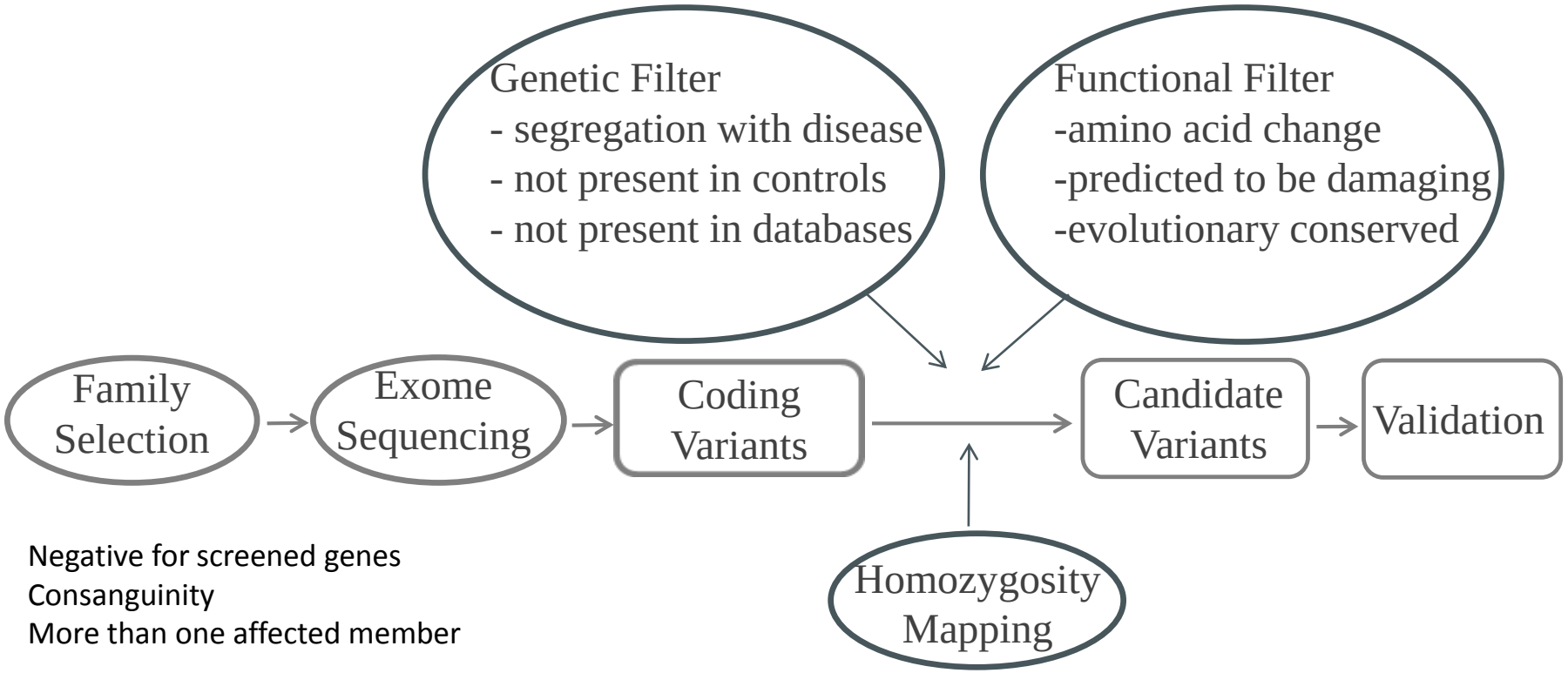
Genomic DNA

Adapter adding

Capturing exons

Sequencing

Exome sequencing workflow



Negative for screened genes
Consanguinity
More than one affected member

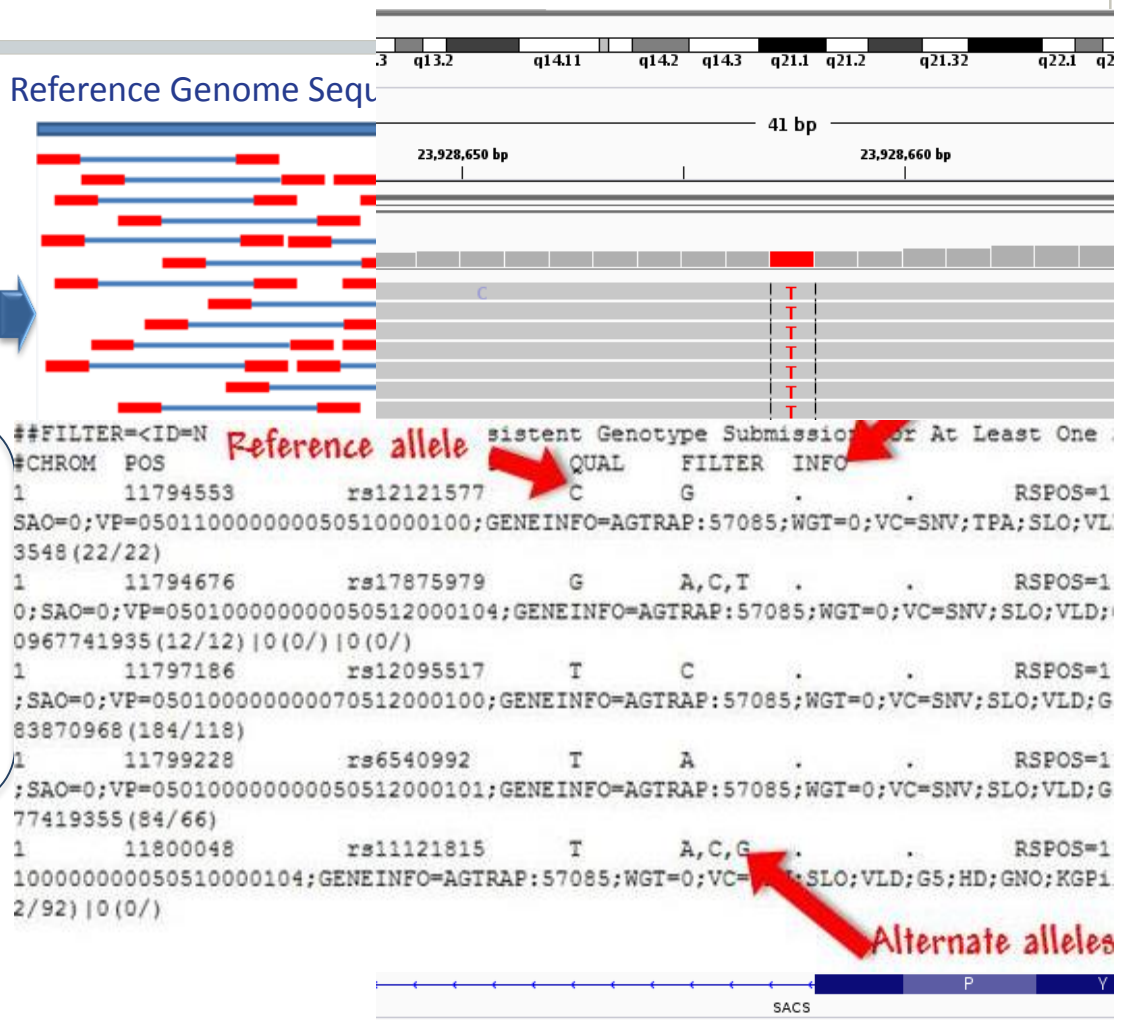
Bioinformatic analysis steps

Base Calling

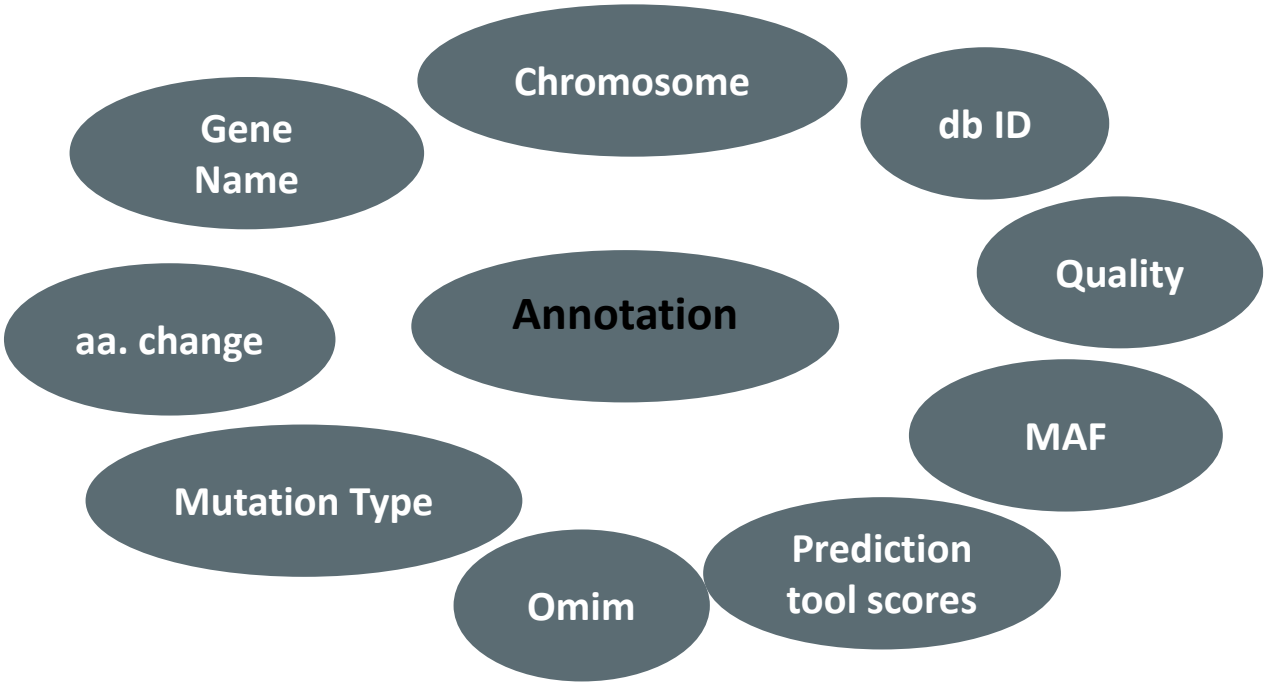
Sequencing signal to nucleotides
-Result:.short reads in *fastq files

Alignment

Alignments of reads to human reference genome
- Result: SAM/BAM



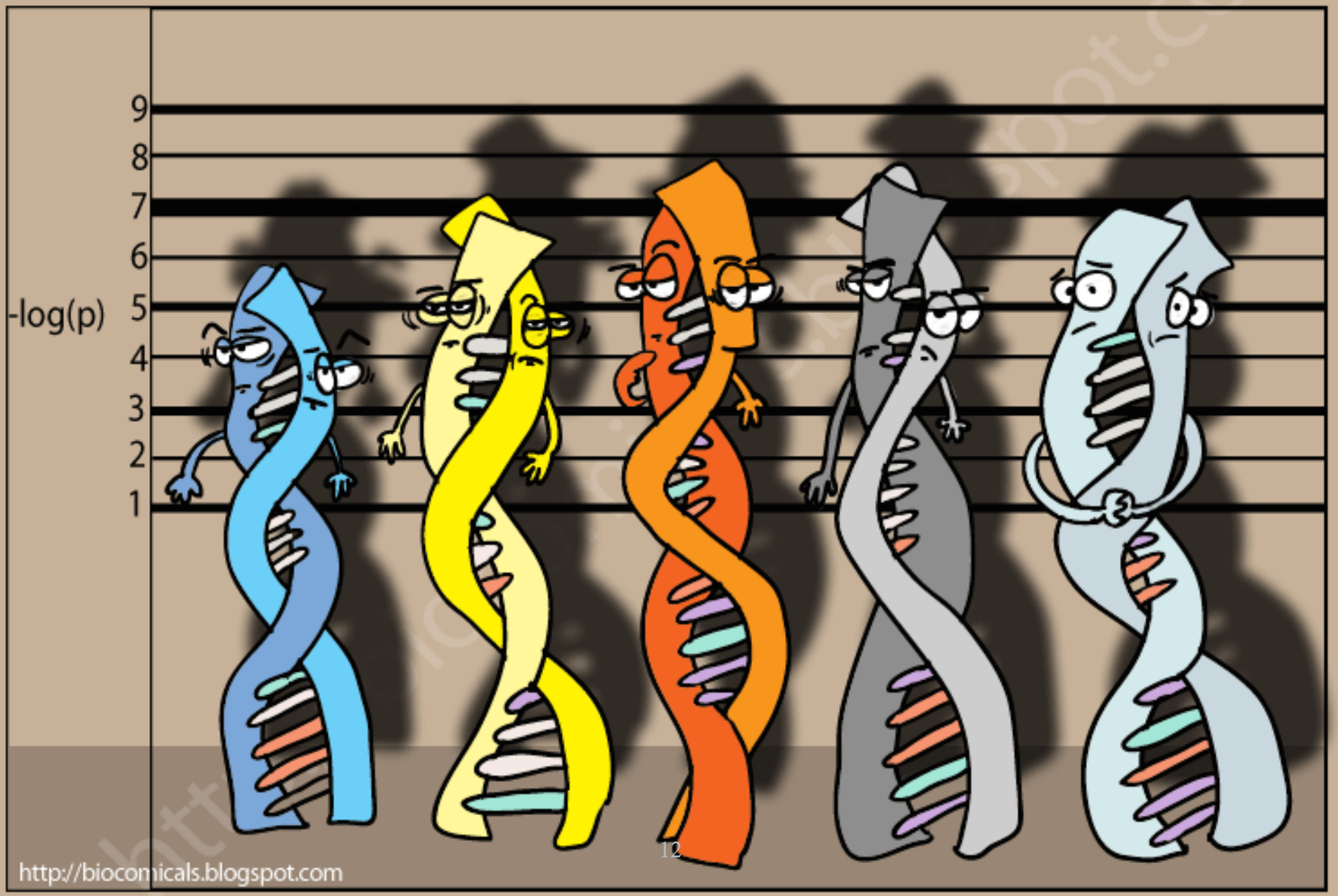
Annotation Step



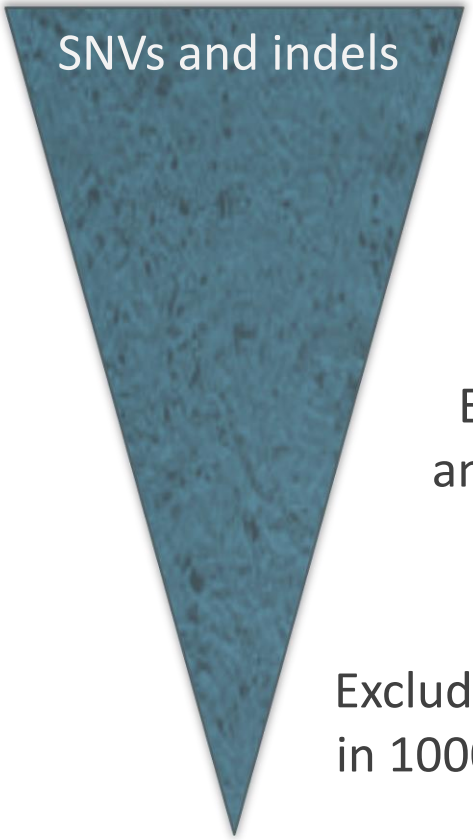
Annotated .vcf file

Chr	chr1	**AA_Change**	exon1:c.G166C:p.A56P
LeftFlank	54355553	**Conserved_LOD**	483
RightFlank	54355555	**Segmental_Duplication**	0
Gene_name	YIPF1	**ESP6500_MAF**	0
muttype	SNP	**1000_Genome_MAF**	0
dbID	rs36013100	**PhyloP_score**	0.062946
ref_allele	A	**SIFT_score**	0.69
var_allele	C	**PolyPhen2_score**	0.026
QUAL	5593.41	**Gerp++**	-6.89
FILTER	PASS	**Mutation Taster**	9.00E-06
Type	exonic_nonsynonymous	**OMIM**	Hypomagnesemia 3

USUAL SUSPECTS: Rare variants



Elimination of variants



Filter 1
Mode of inheritance

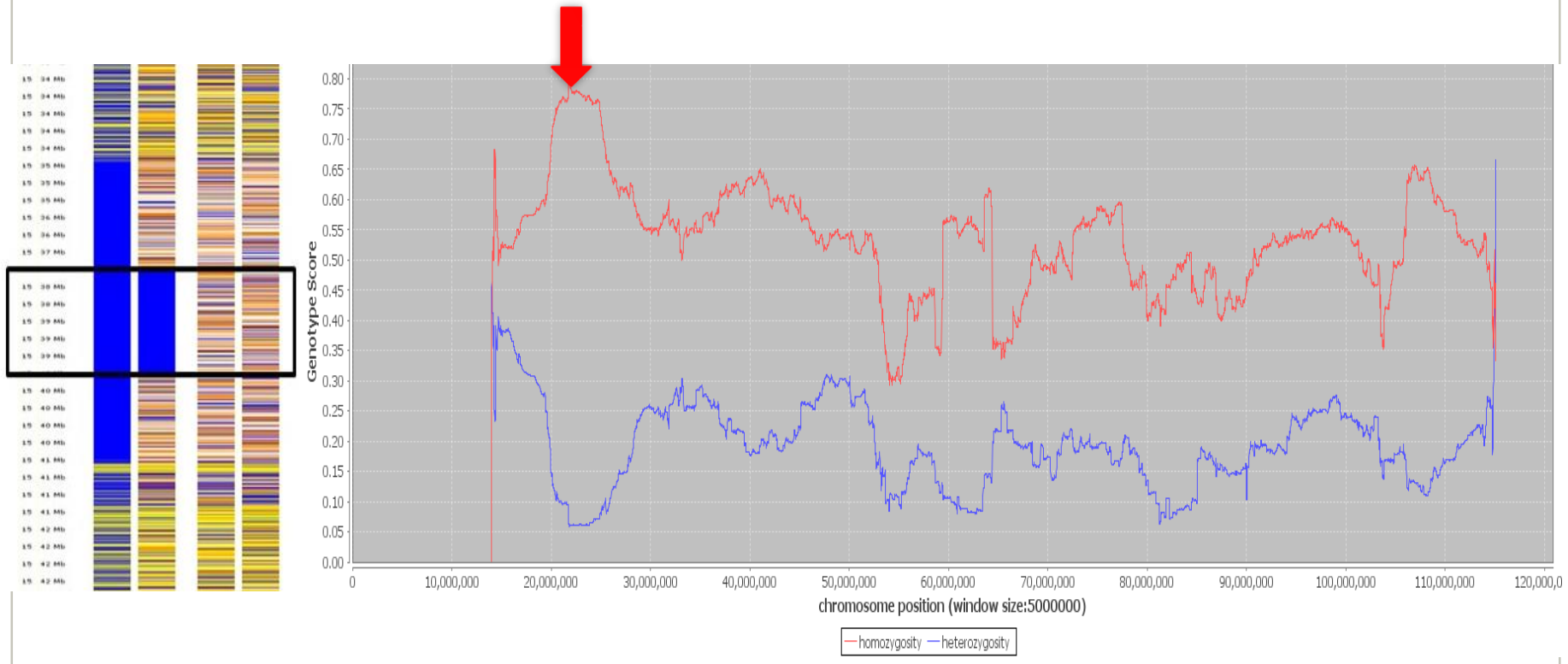
Filter 2
Exclude all non-coding
and synonymous variants

Filter 3
Exclude all nonsynonymous variants
in 1000genomes and EVS databases

List of putative pathogenic variants

Homozygosity mapping

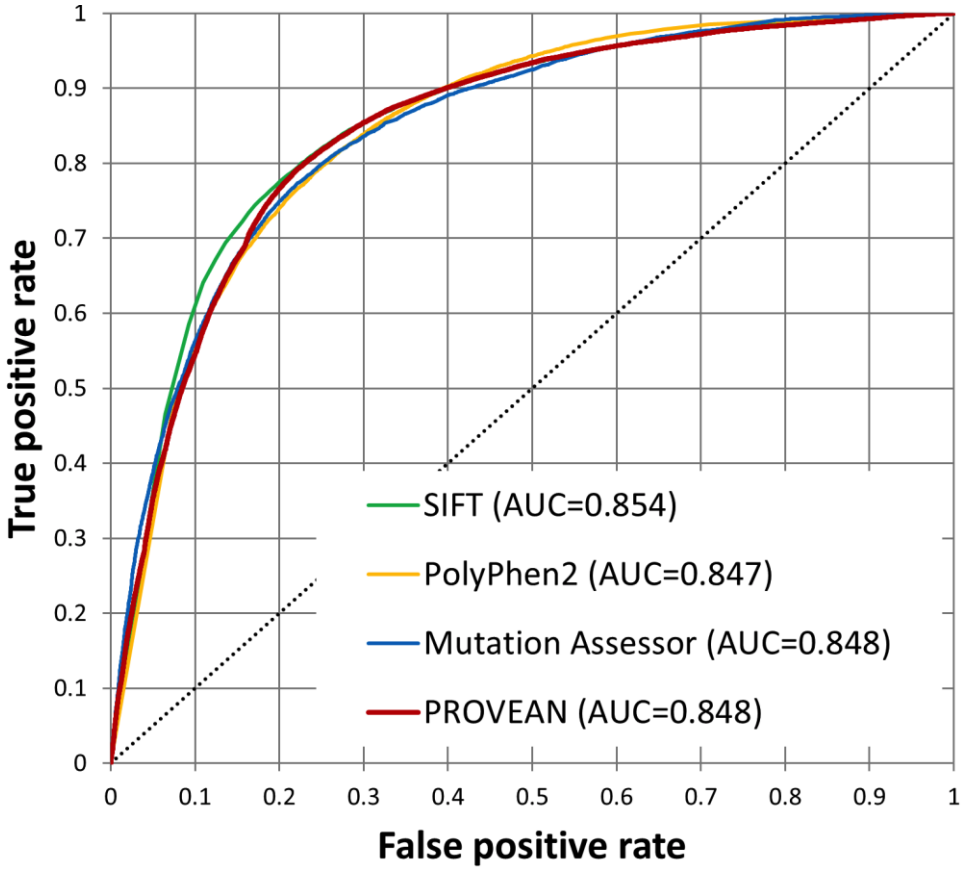
- Homozygous Stretch Identifier (HomSI) (Tübitak)



http://www.igbam.bilgem.tubitak.gov.tr/en/softwares/HomSI/index.html

Prediction Tools

- SIFT
- Polyphen2
- Mutation Taster
- Provean
- SNP & Go
- Pylop



Purpose

- Identification the genes/variants responsible for different neurodegenerative diseases, which could not be solved by conventional PCR-based techniques.
- This study includes 13 families:
  - Amyotrophic Lateral Sclerosis and related syndromes
  - Recessive ataxias

Study cohort

Family	Clinical Diagnosis
1	ALS
2	ALS
3	ALS
4	ALS
5	ALD-PD
6	SCA & FRDA
7	SCA & FRDA
8	SCA & FRDA
9	SCA & FRDA
10	FRDA
11	SCA
12	SCA & FRDA
13	SCA

STUDY COHORT

ALS

- Most common adult-onset motor neuron disease
- Selective death of
  - ✓ upper motor neurons in the motor cortex
  - ✓ lower motor neurons in the brainstem and spinal cord
- Symptoms:
  - spasticity
  - muscle weakness
  - muscle atrophy
  - twitching
  - breathing and talking difficulties

Ataxias

- Coordination of muscle movements
- dysfunction of the cerebellum
- Types
  - Friedrich's ataxia
  - Spinocerebellar ataxias
  - Ataxia oculomotor apraxia
  - Ataxia-telangiectasia
  - Episodic ataxia

Family 1

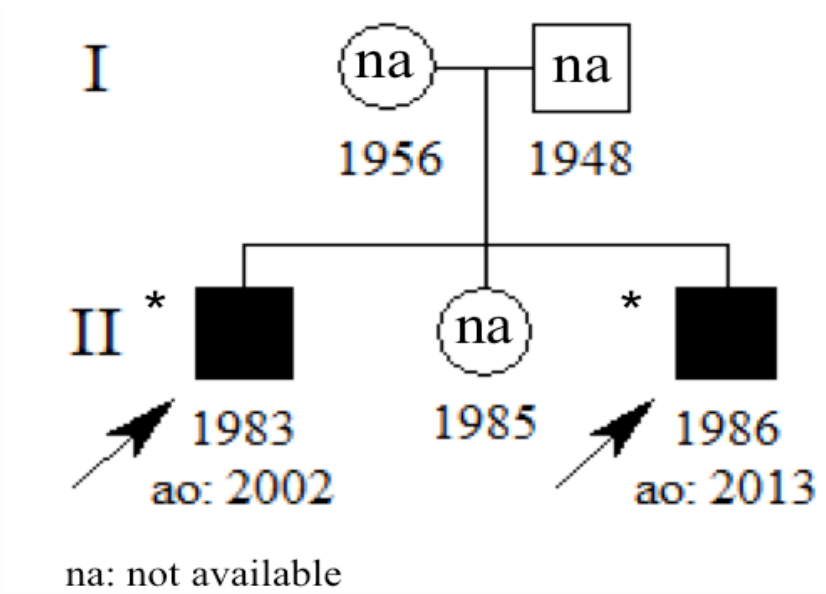
Symptoms: limb onset disease

- weakness in right hand fingers
  - atrophy of hand muscles
  - atrophy in the right arm
- fasciculations

Clinical diagnosis: ALS

Genetics: dominant

- C9Orf72, SOD1, UBQLN2, TDP-43 and FUS (-)

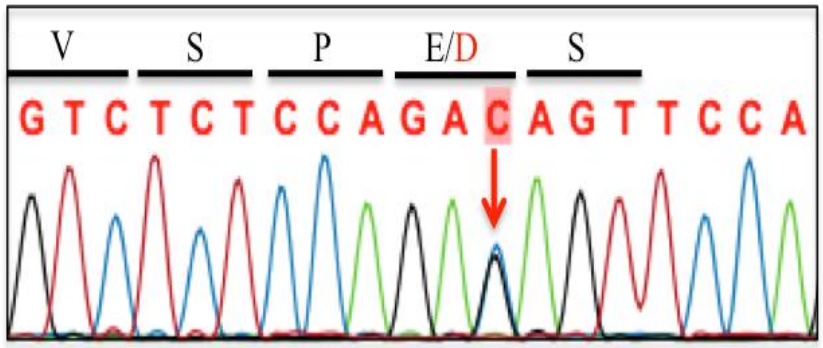


Clinician: Dr. Yeşim Parman, Çapa

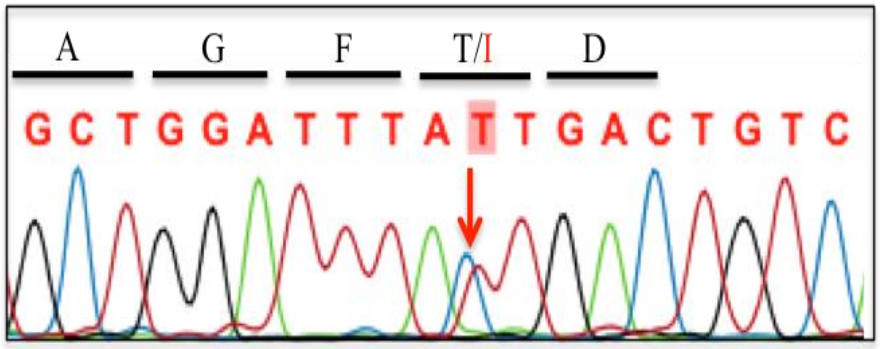
Exome results/validation: Family 1

Candidate variations in ALS-Family 1.

Chr	Position	Gene name	dbSNP ID	Ref/Alt	Mutation
chr5	179260099	SQSTM1	rs55793208	G/C	c.G822C:p.E274D
chr15	50878630	TRPM7	rs8042919	G/A	c.C4445T:p.T1482I

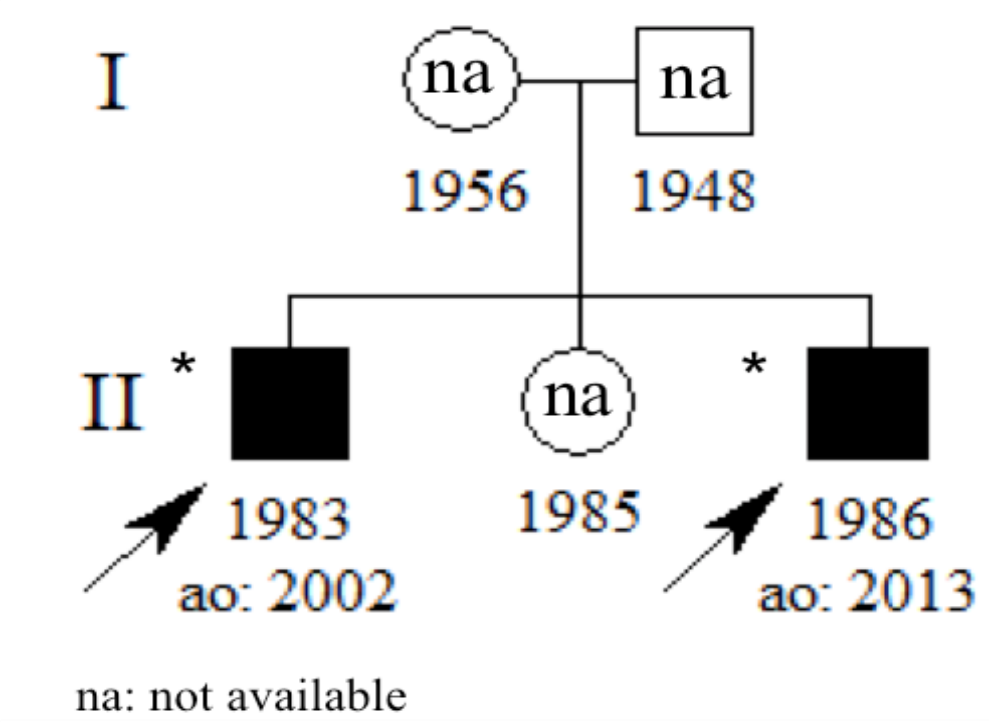


SQSTM1 gene



TRPM7 gene

Overall results-Family 1: ALS



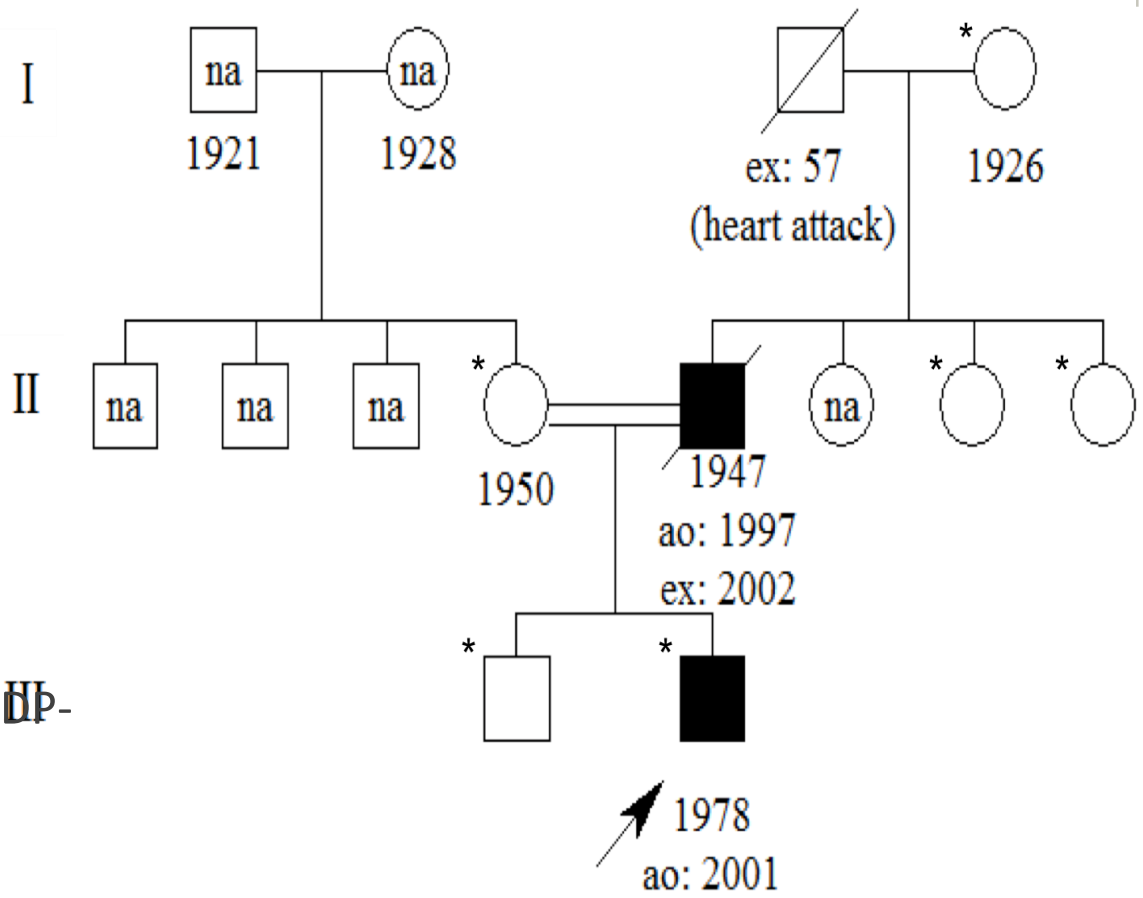
Family 2

- Symptoms:
  - cognitive impairment
  - bilateral leg weakness
  - upper extremity weakness
  - AO: 23

Clinical diagnosis: ALS

Genetics: recessive

- C9Orf72, SOD1,UBQLN2, TDP-43, FUS and FRDA (-)



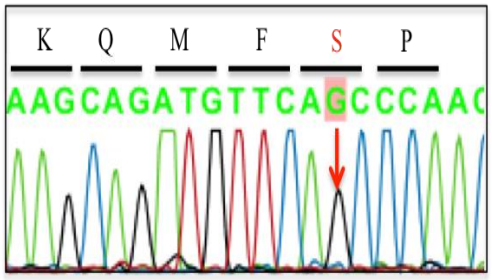
Clinician: Dr. Mefkure Eraksoy, Çapa

Exome results/validation: Family 2

Candidate variations in ALS-Family 2.

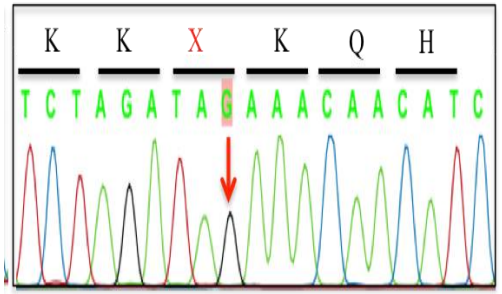
Chr	Position	Gene name	dbSNP ID	Ref/Alt	Mutation
chr15	44855496	SPG11	-	A/C	c.T6816G:p.Y2272X
chr15	44865000	SPG11	rs140824939	T/C	c.A5885G:p.N1962S
chr5	73205717	ARHGEF28	rs17634865	C/T	c.C3703T:p.P1235S

SPG11 p.N1982S mutation



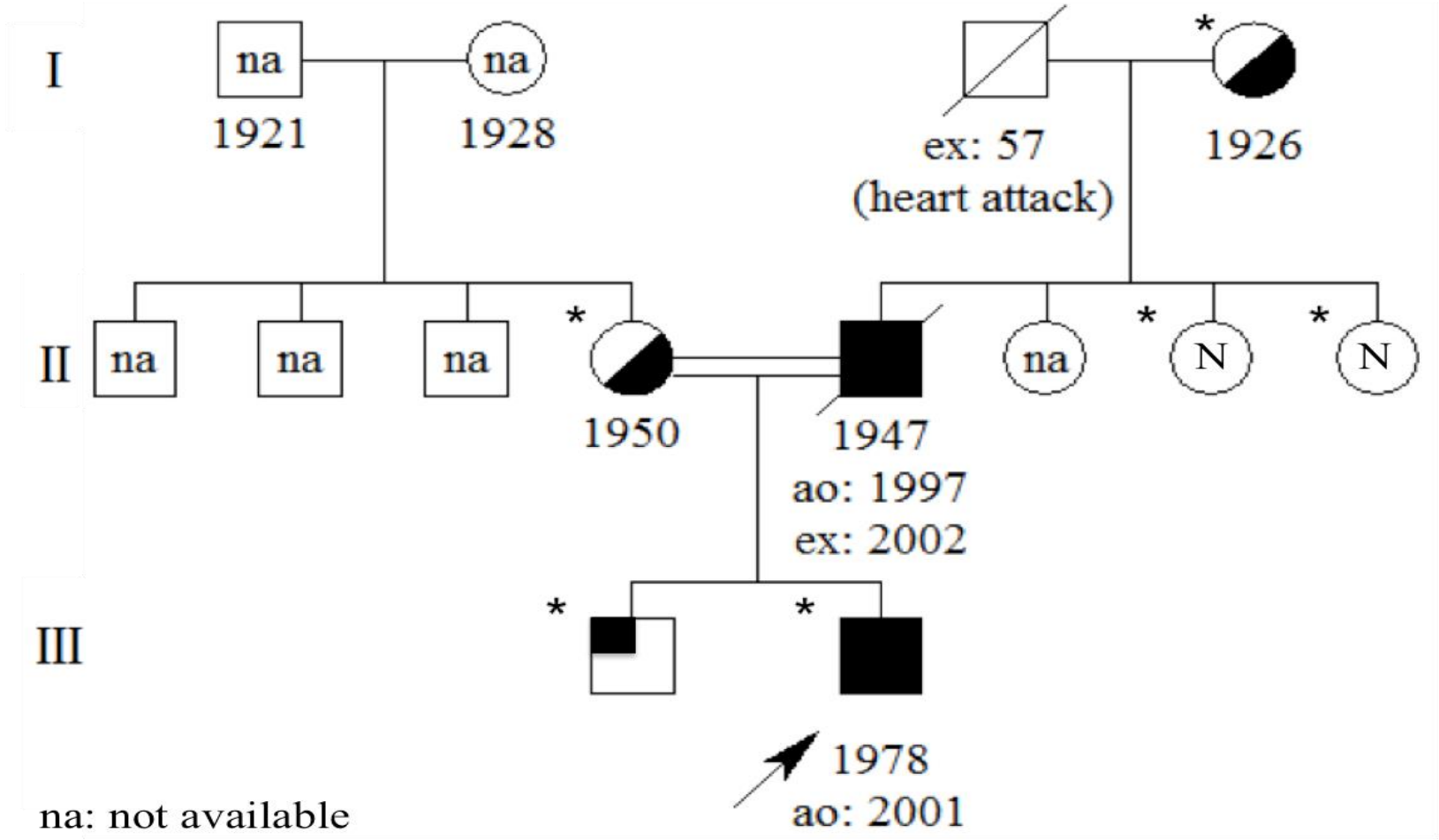
index case

SPG11 p.Y2272X mutation



index case

Overall results-Family 2: ALS



Family 3

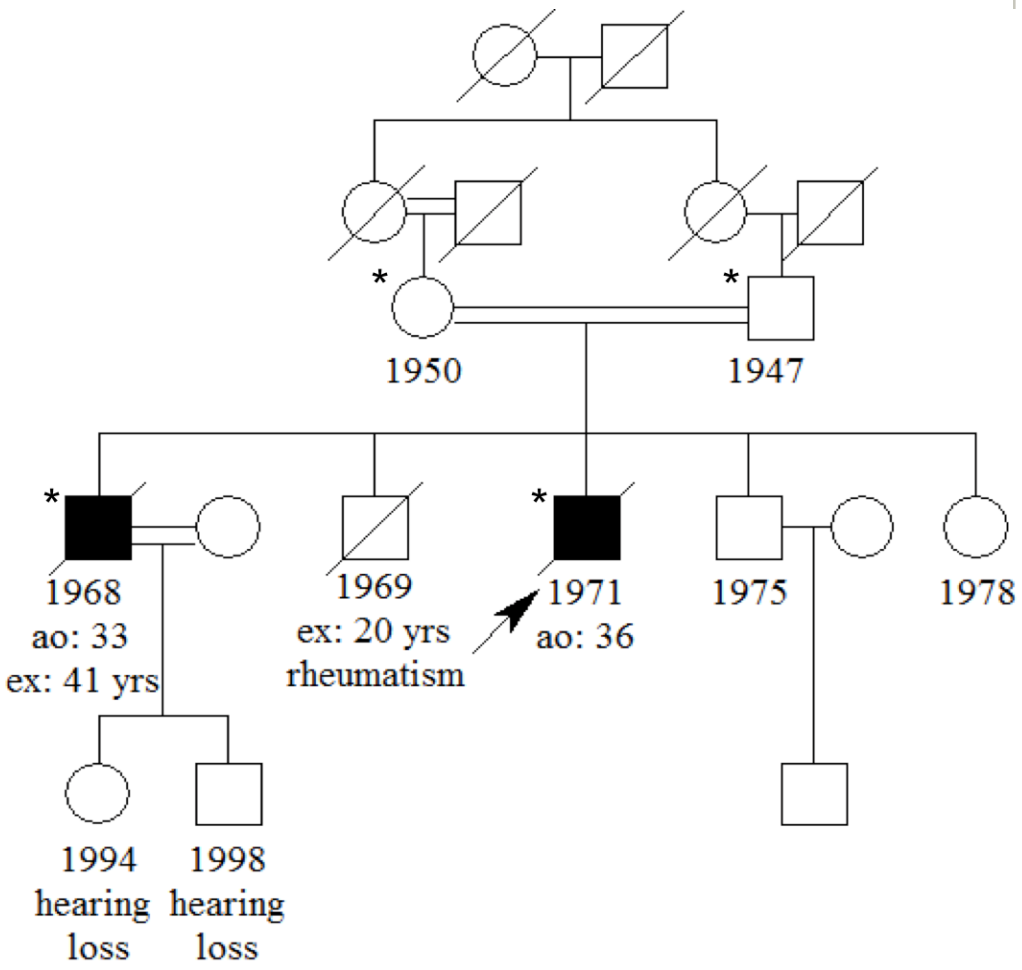
Symptoms:

- involvement of anterior horn

Clinical diagnosis: ALS

Genetics: recessive

- C9Orf72, SOD1,UBQLN2, TDP-43, FUS, FRDA (-)

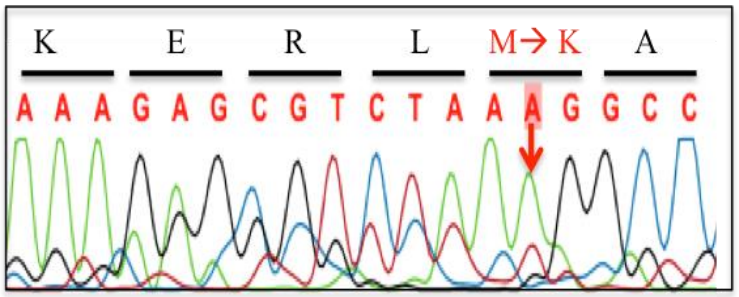


Exome results/validation: Family 3

Candidate variations in ALS-Family 3.

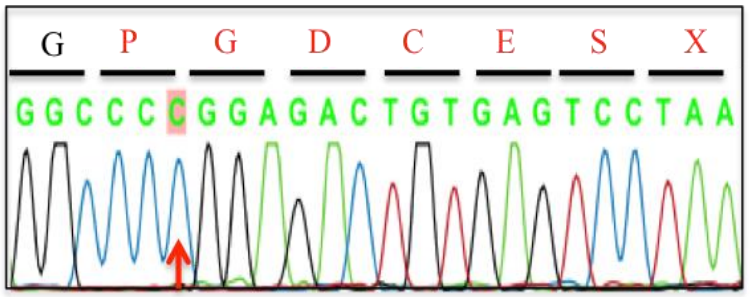
Chr	Position	Gene name	dbSNP ID	Ref/Alt	Mutation
chr10	13152400	OPTN	rs11258194	T/A	c.T293A:p.M98K
chr10	13164477	OPTN	-	G/GC	c.873dupC:p.G291fsX6

OPTN p.M98K mutation



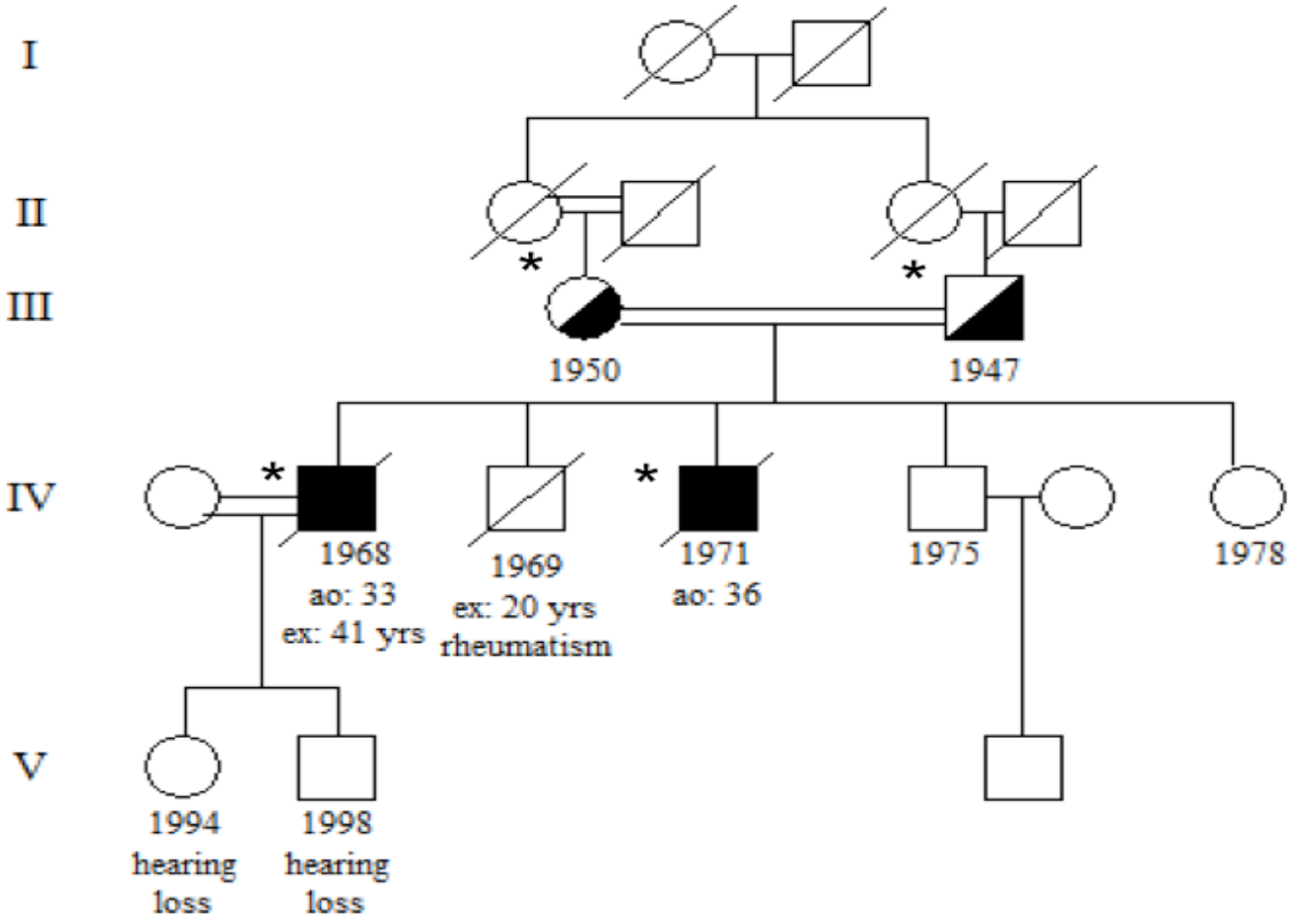
index case

OPTN p.G291fsX6 mutation

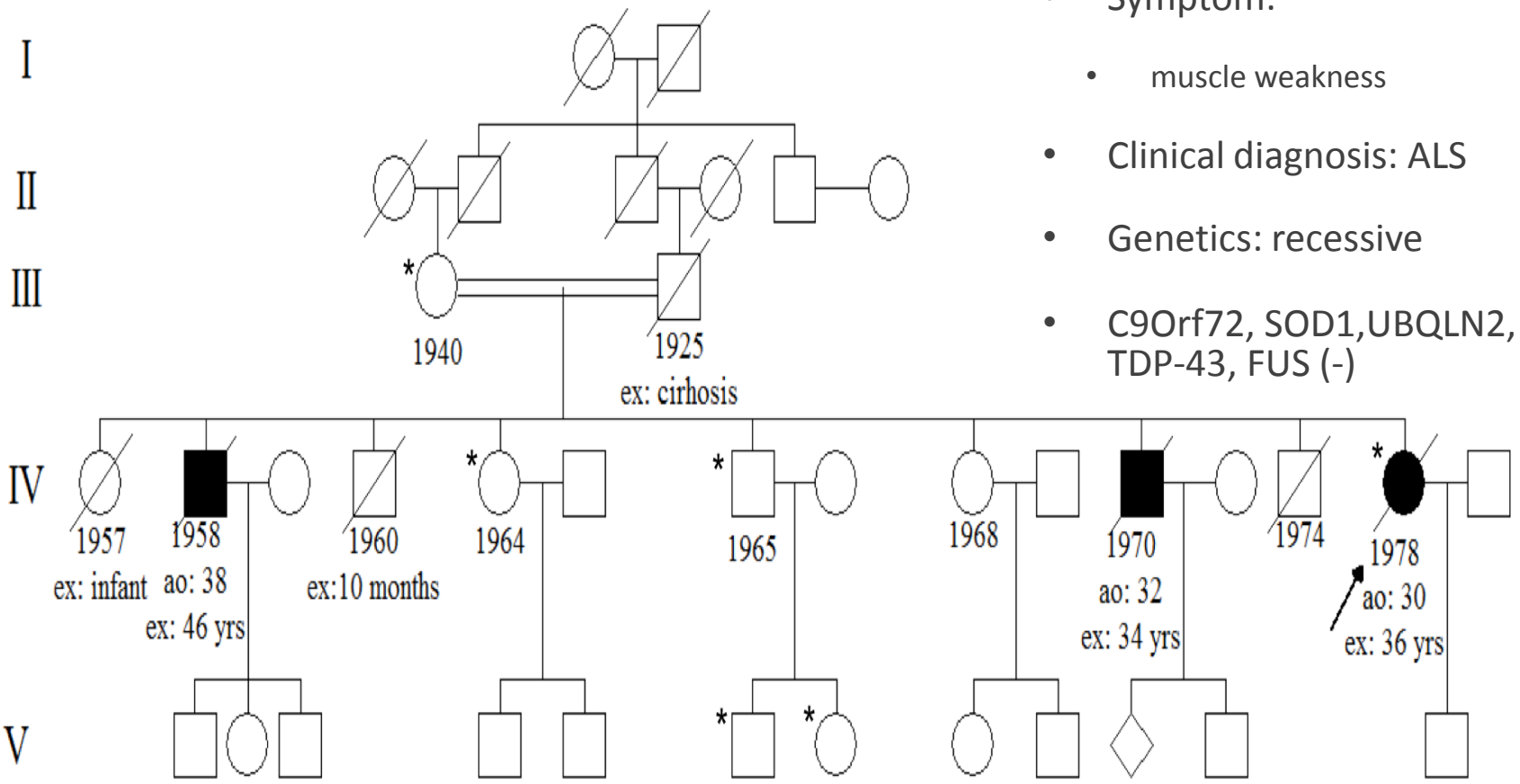


index case

Overall results-Family 3: ALS



Family 4



- Symptom:
  - muscle weakness
- Clinical diagnosis: ALS
- Genetics: recessive
- C9Orf72, SOD1,UBQLN2, TDP-43, FUS (-)

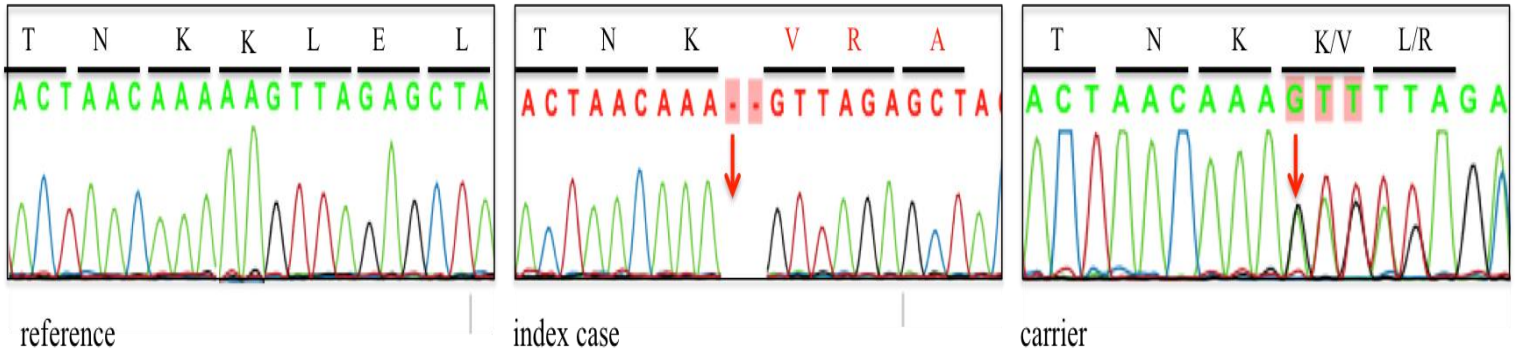
Clinician: Dr. Halil Idrisoğlu, Çapa

Exome results/validation: Family 4

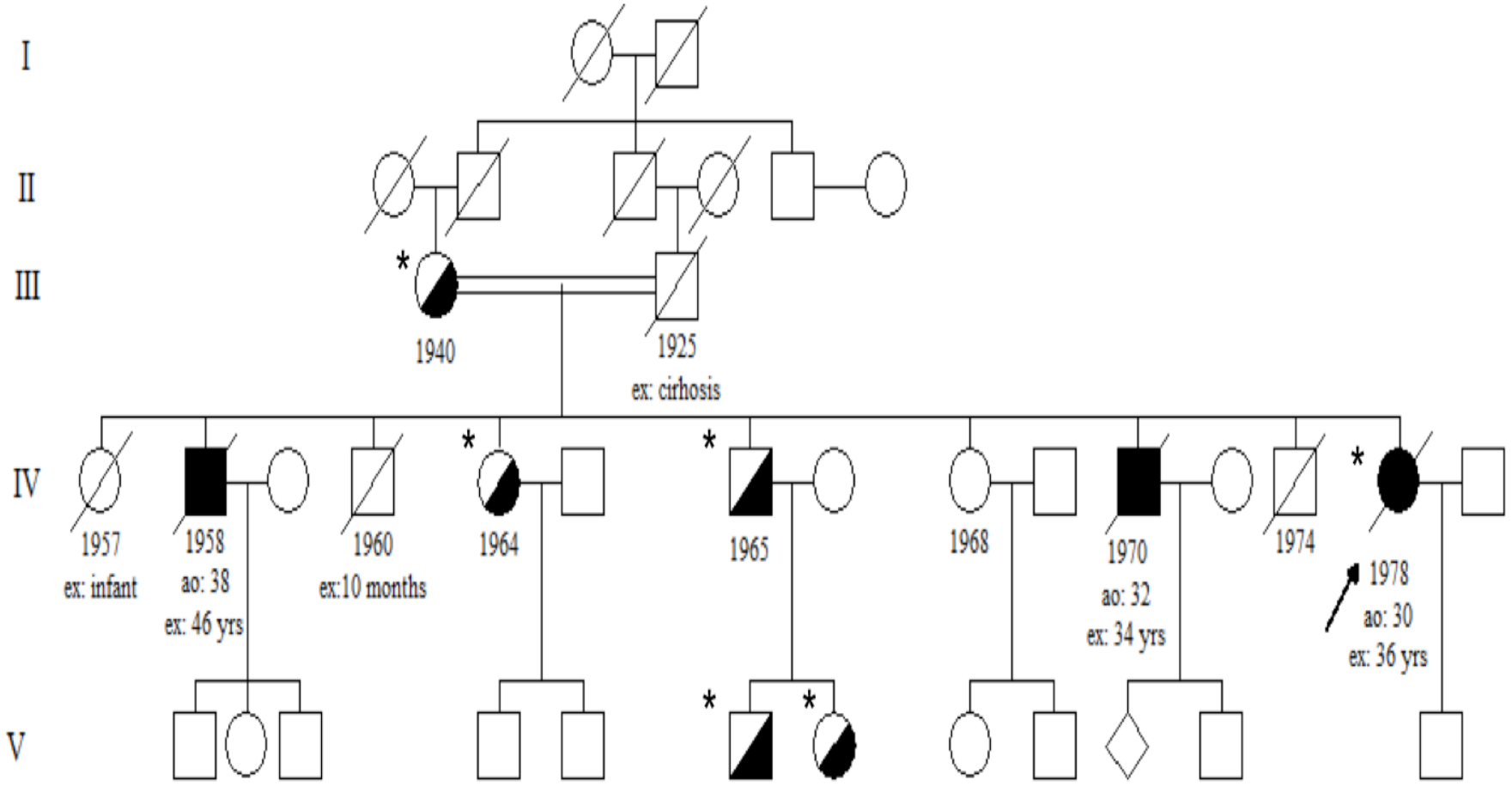
Candidate variation in ALS-Family 4.

Chr	Position	Gene name	dbSNP ID	Ref/Alt	Mutation
chr10	13167492	OPTN	-	CAA/A	p.359_359del

OPTN p.359_359del mutation

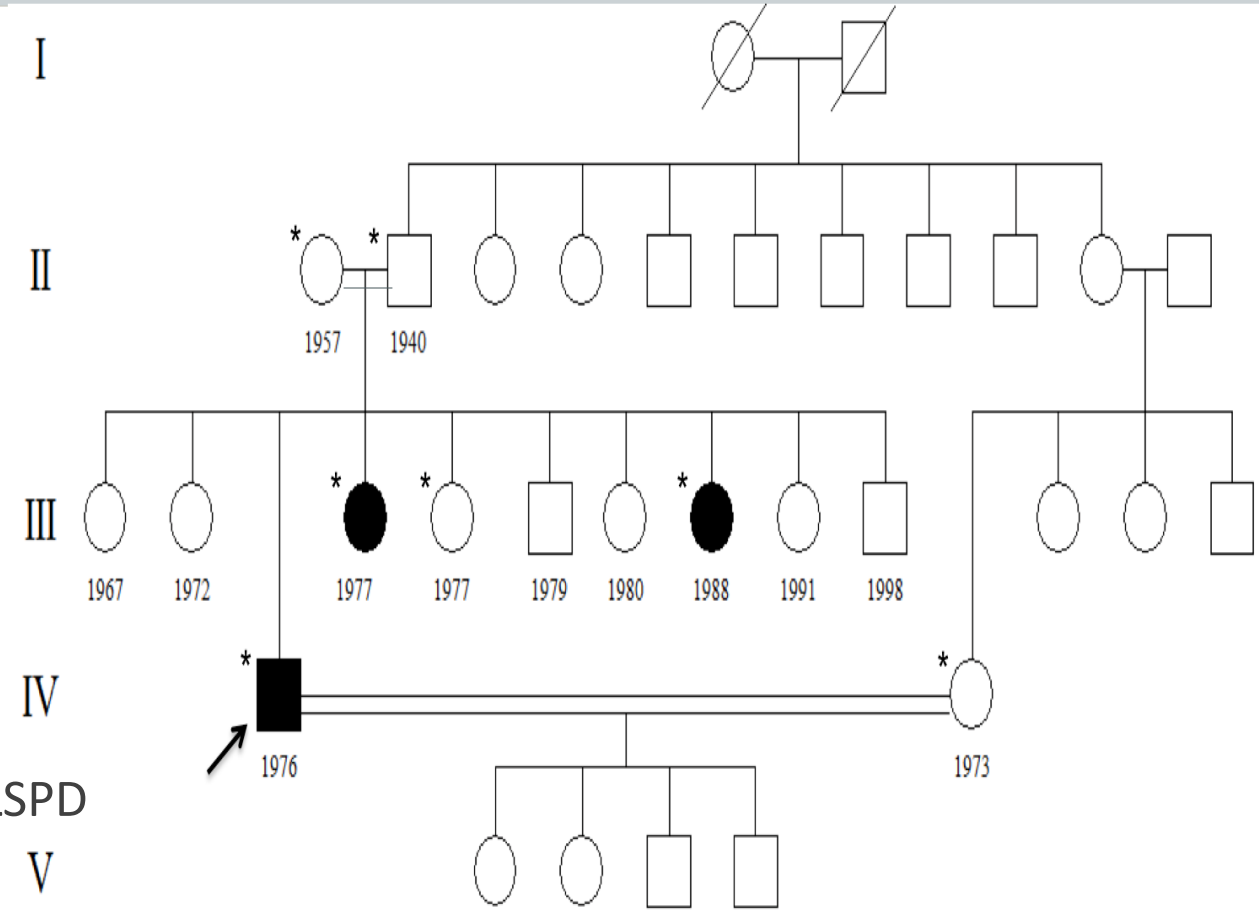


Overall results-Family 4: ALS



Family 5: ALS with parkinsonism

- Symptoms:
  - bradykinesia
  - rigidity
  - tremor
  - speech problems
  - blepharospasm
  - psychosis
  - hallucination
  - AO: 24 years
- Clinical diagnosis: ALSPD
- Genetics: recessive

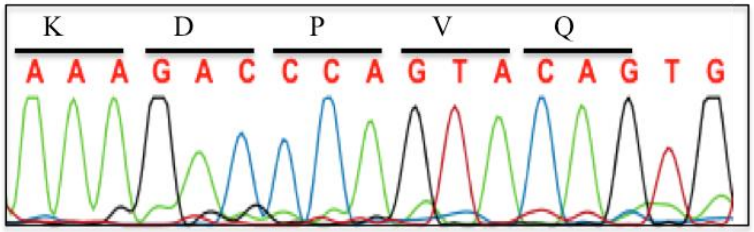


Clinician: Dr. Haşmet Hanağası, Çapa

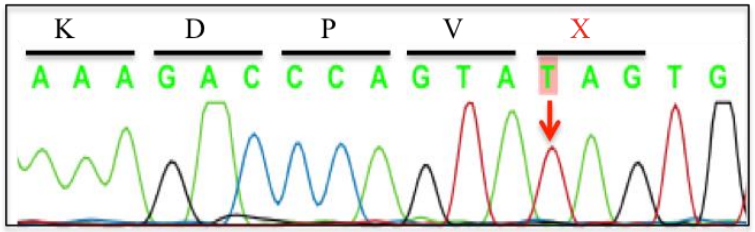
Exome results/validation: Family 5

Candidate variation in DJ1 Family.

Chr	Position	Gene name	dbSNP ID	Ref/Alt	Mutation
chr1	8025425	DJ1	-	C/T	c.C133T:p.Q45X

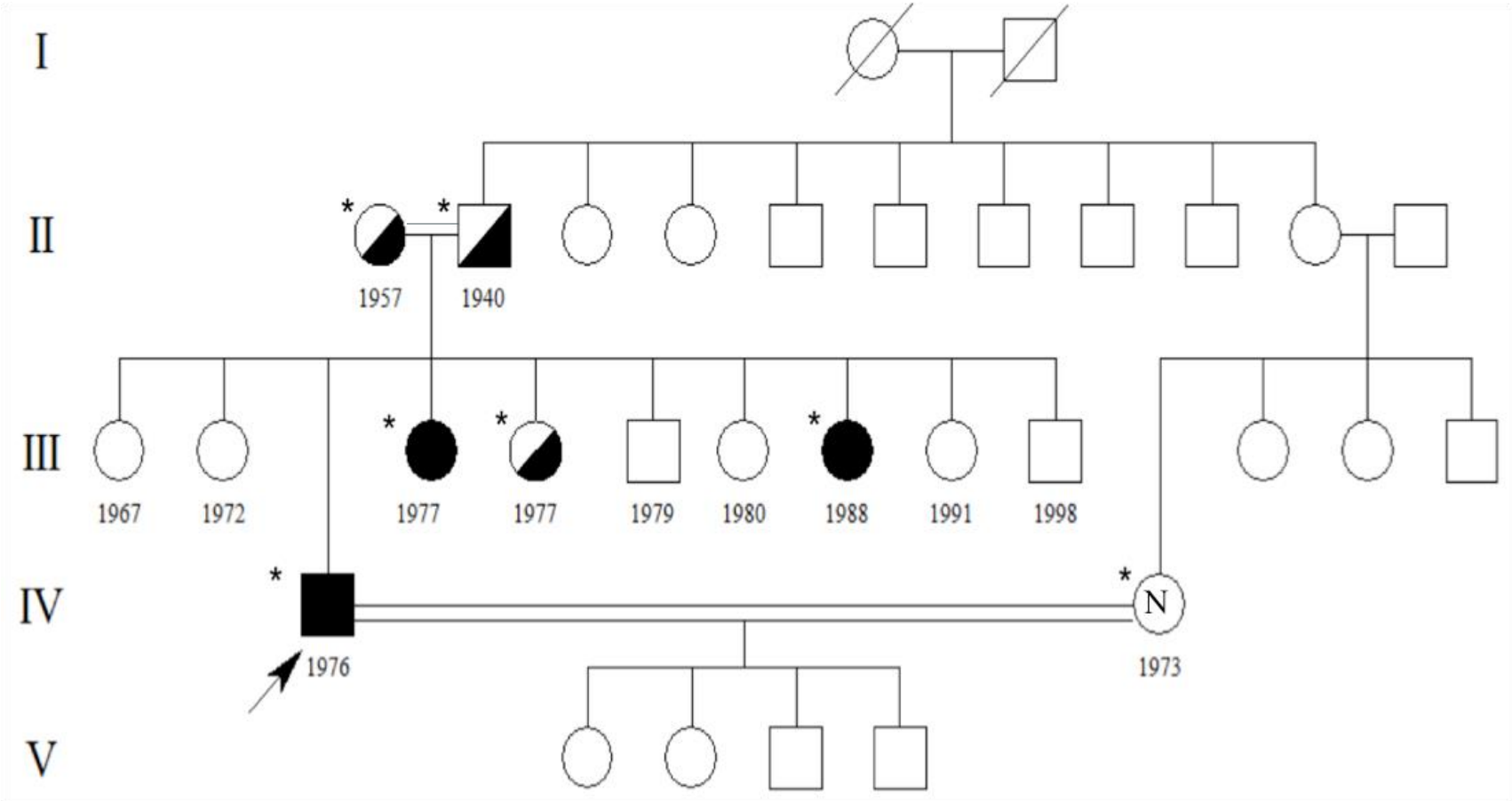


reference



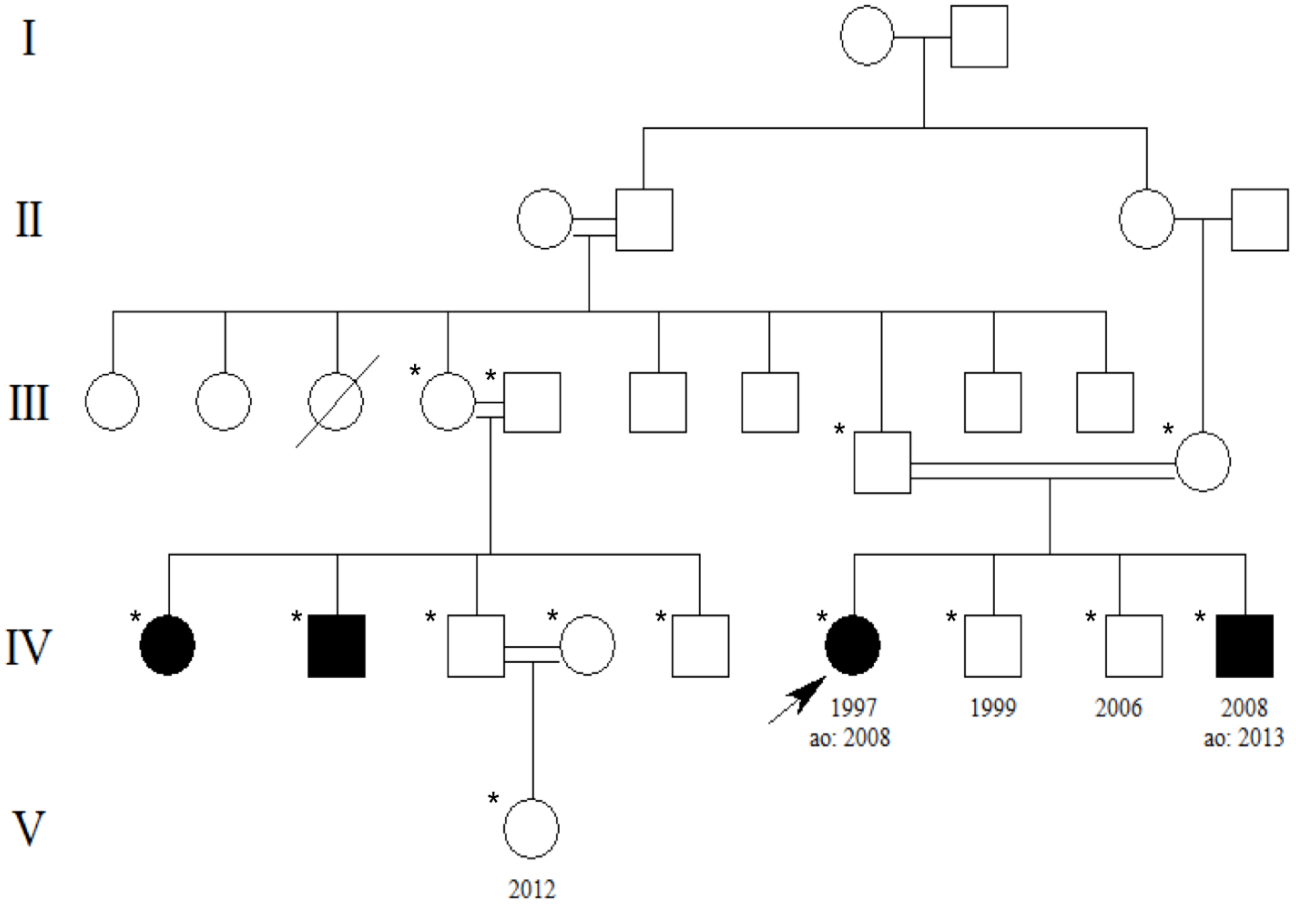
index

Overall results-Family 5: ALD-PD



Family 6

- Symptoms:
  - balance disorder
  - walking difficulties
  - cerebellar atrophy
  - polyneuropathy
- Clinical diagnosis: SCA and FRDA
- Genetics SCA1,3 and FRDA(-)

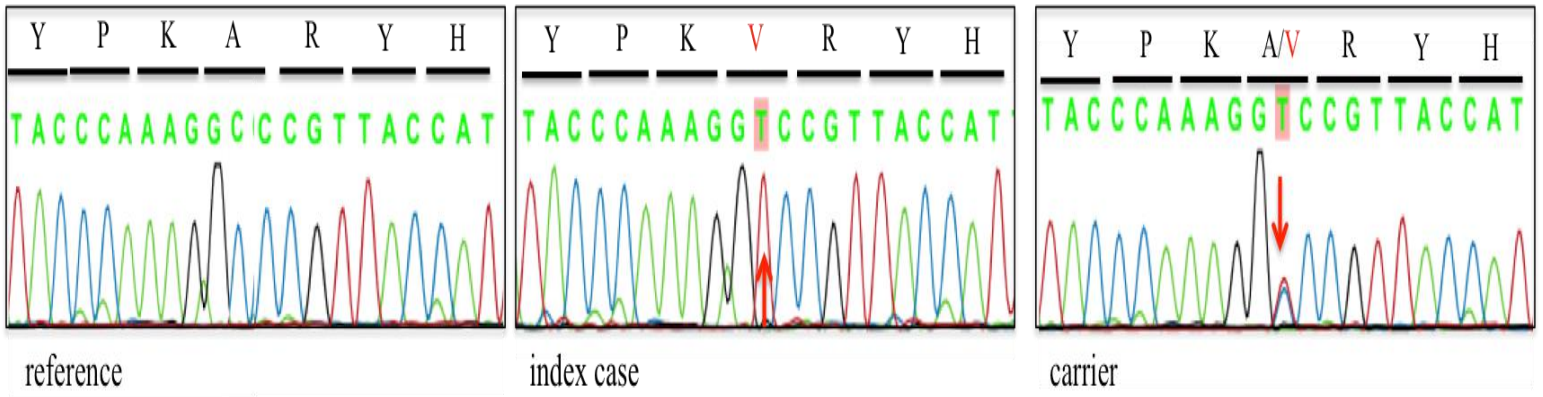


Clinician: Dr. L. Harika Çalışkan, Kanuni Sultan Süleyman

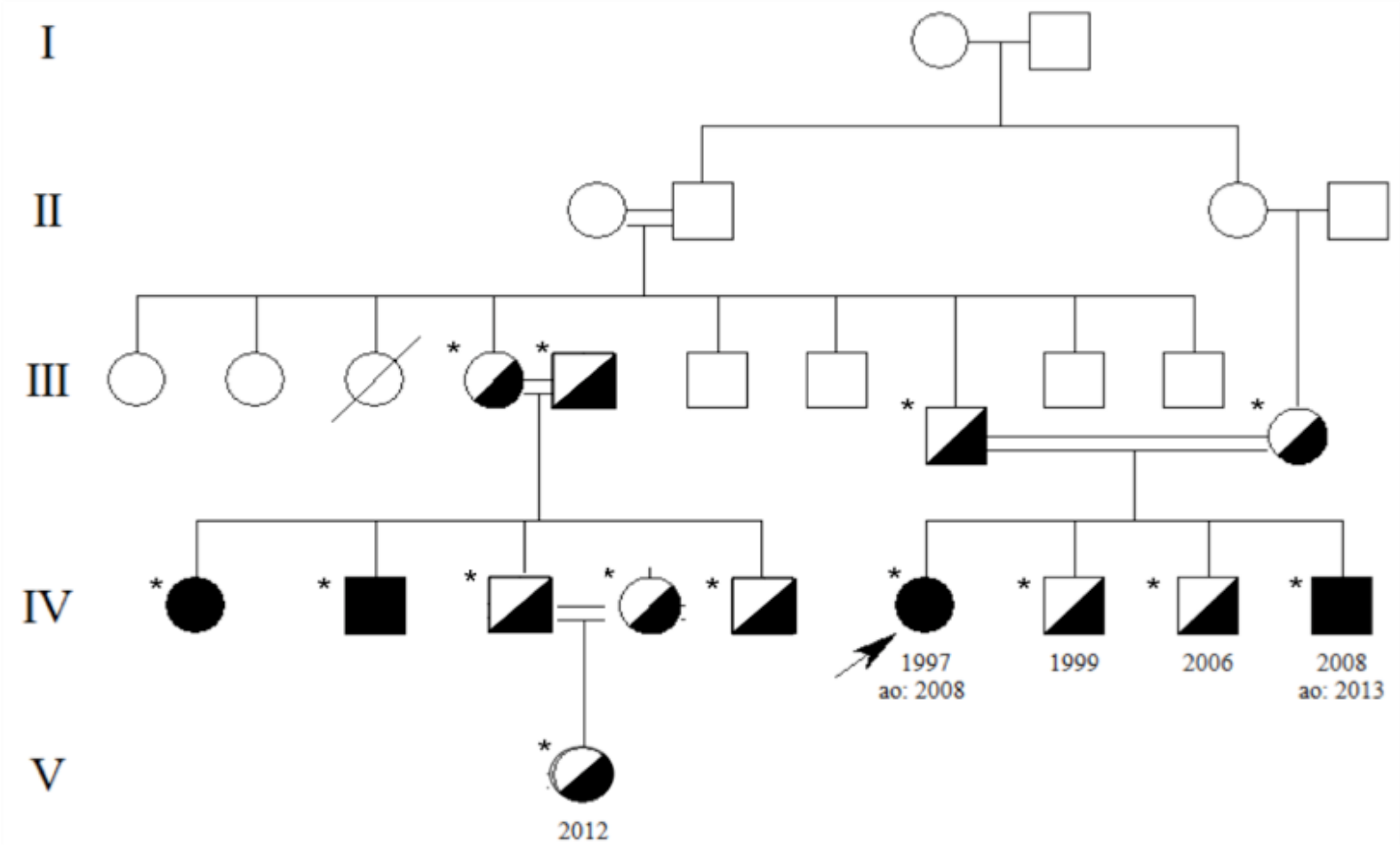
Exome results/validation: Family 6

Candidate variation in AOA1-Family 1.

Chr	Position	Gene name	dbSNP ID	Ref/Alt	Mutation
chr9	32984806	APTX	-	G/A	c.C473T:p.A158V



Overall results-Family 6:
Ataxia with Oculomotor Apraxia type 1



Family 7

- V.1: AO, 14 years

mental retardation

walking difficulties

- V.2: AO, 13 years

walking difficulties

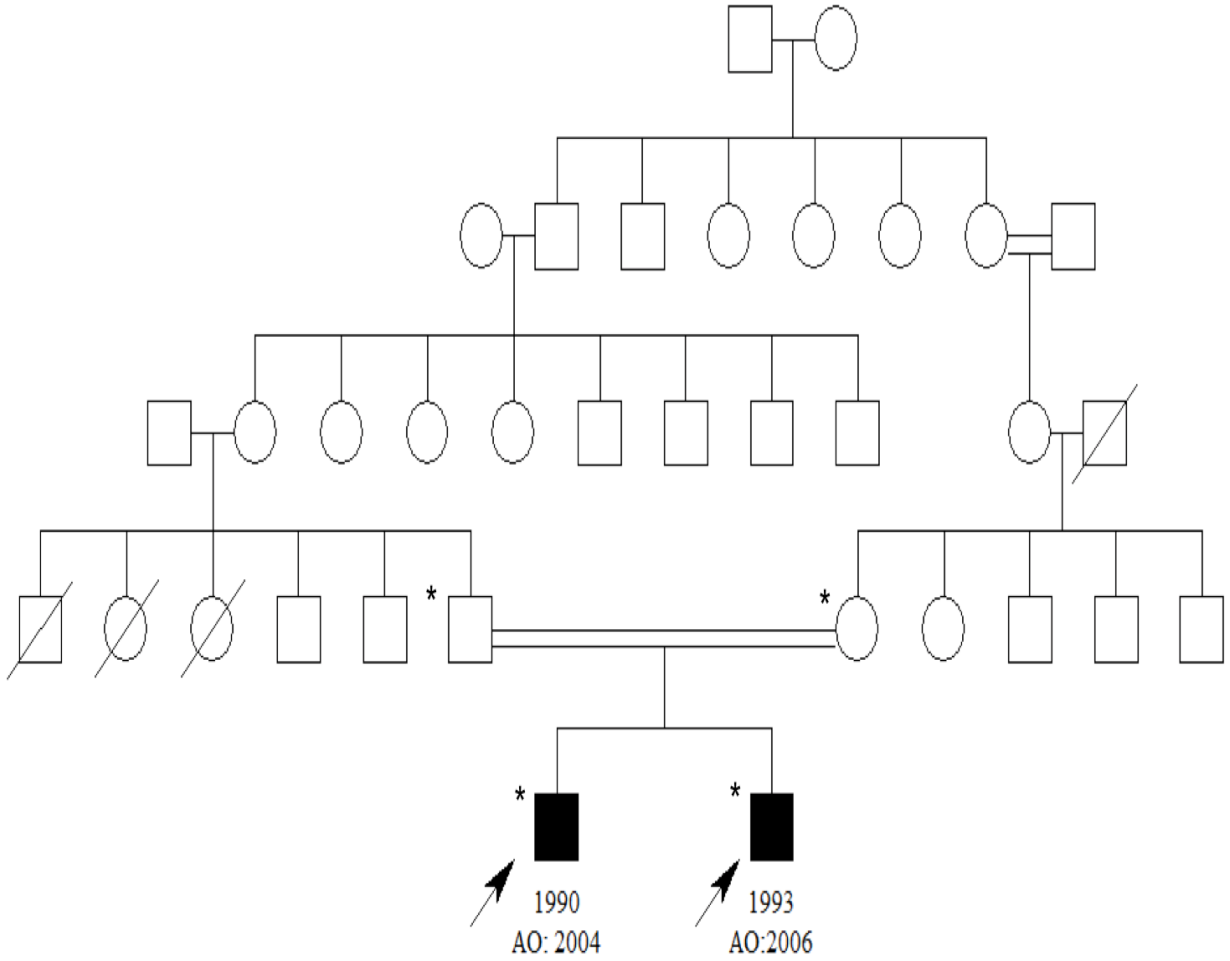
no mental retardation

Clinical diagnosis:

SCA and FRDA

Genetics: recessive

SCA 1,2,3,6,7,17 and FRDA (-)

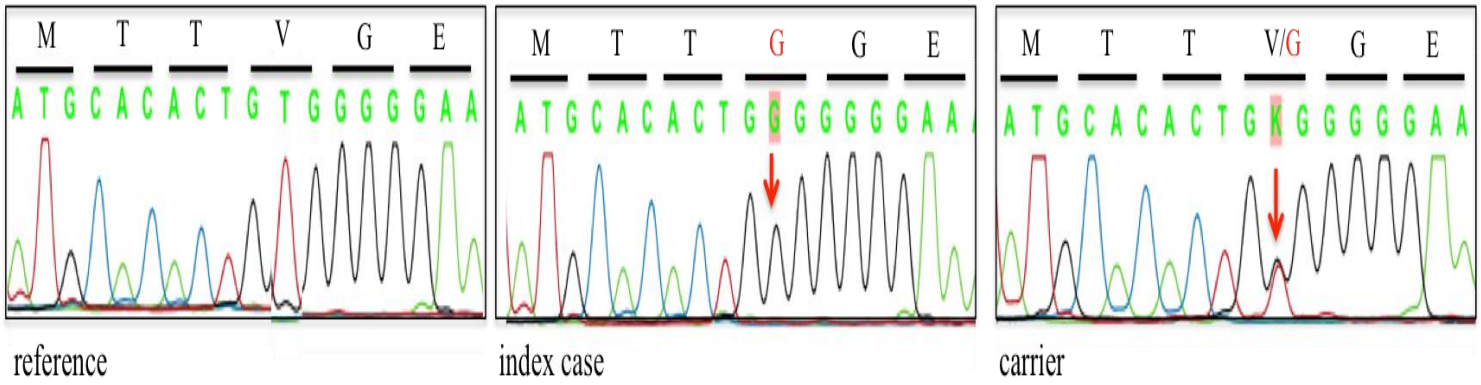


Clinician: Dr. Elif Acar Arslan, Hacettepe

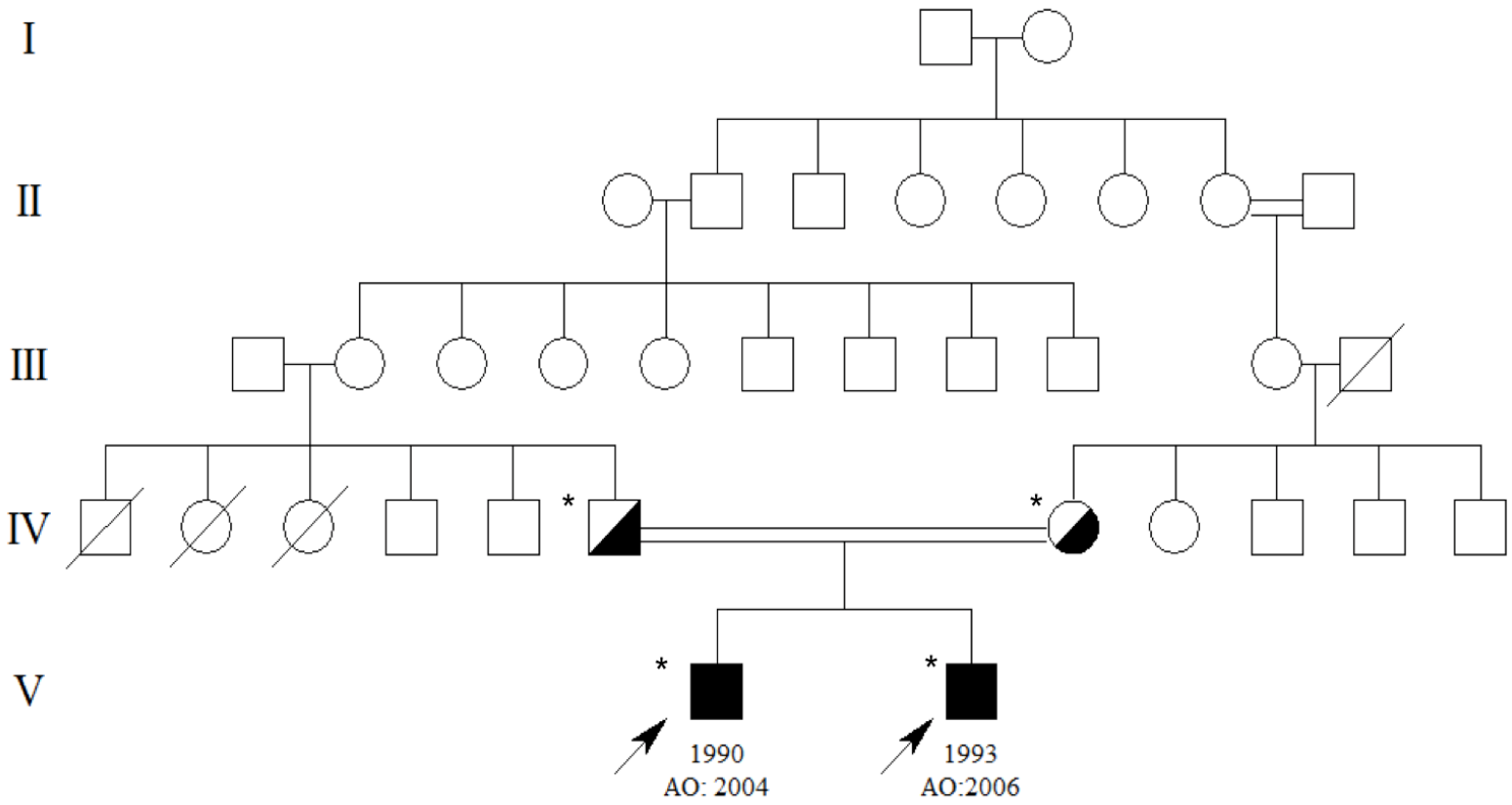
Exome results/validation: Family 7

Candidate variation in AOA1-Family 2.

Chr	Position	Gene name	dbSNP ID	Ref/Alt	Mutation
chr9	32984710	APTX	-	A/C	c.T569G:p.V190G

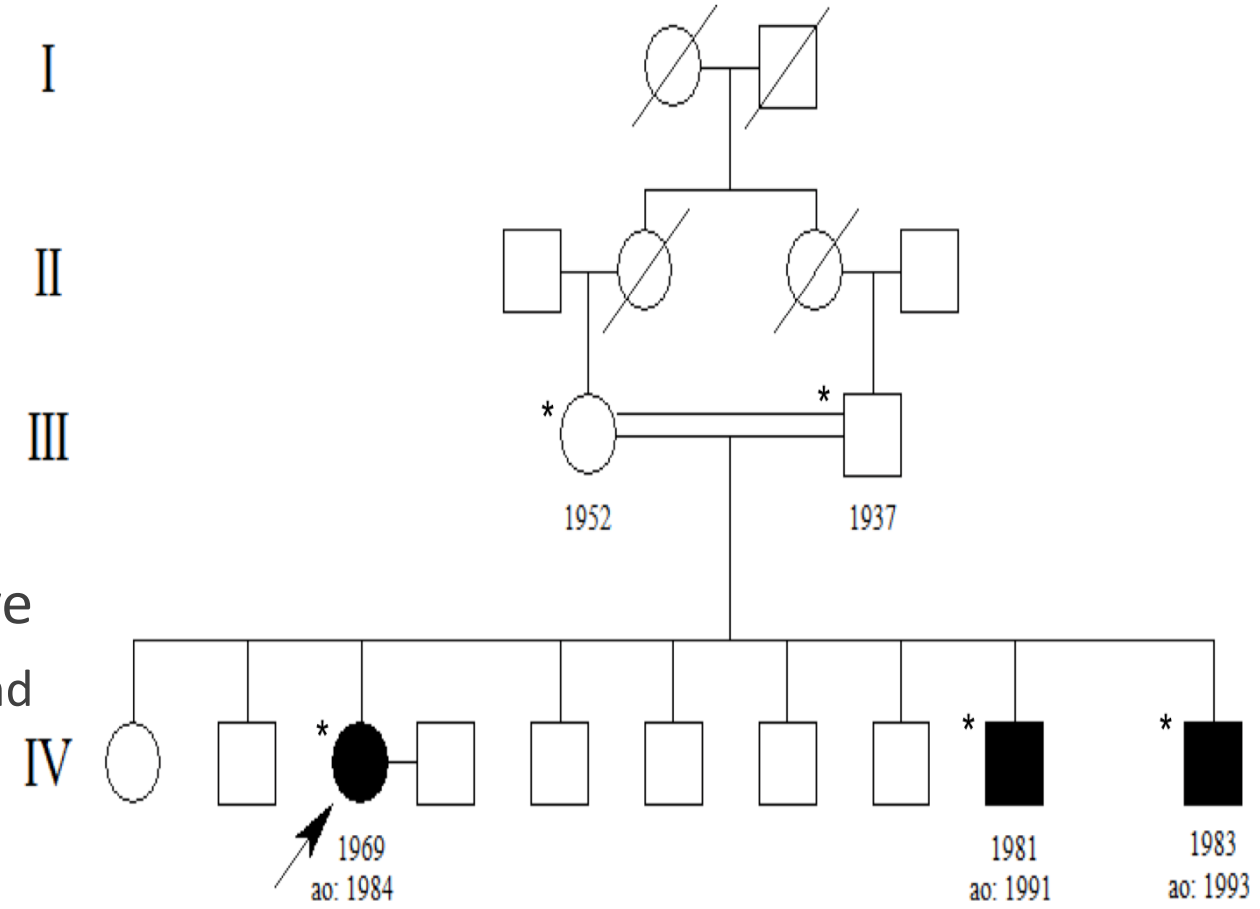


Overall results-Family 7:
Ataxia with Oculomotor Apraxia type 1



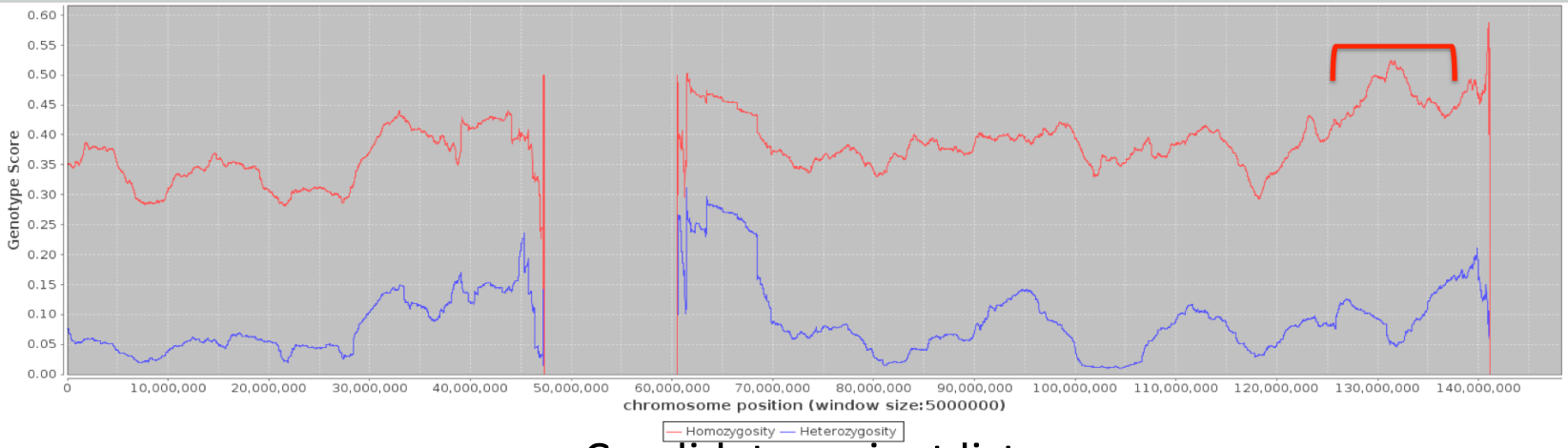
Family 8

- Symptoms:
  - speech problems
  - walking difficulties
  - cerebellar ataxia
- Clinical diagnosis:
  - SCA and FRDA
- Genetics: recessive
  - SCA 1,2,3,6,7,17 and FRDA (-)



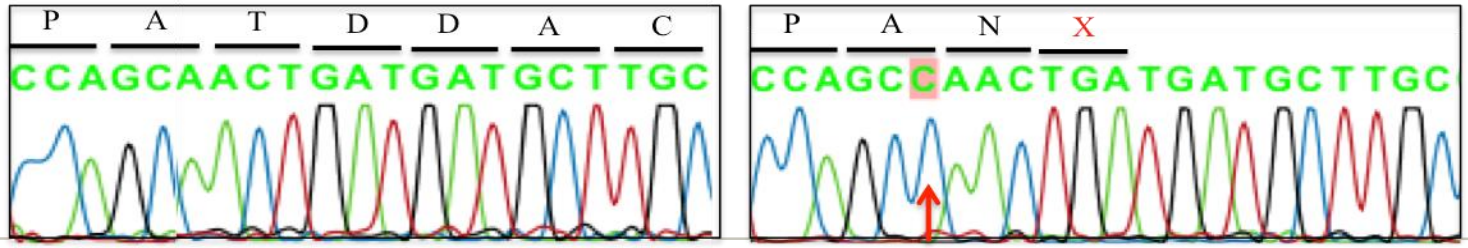
Clinician: Dr. Piraye Oflazer, Çapa

Exome results/validation: Family 8



Candidate variant list

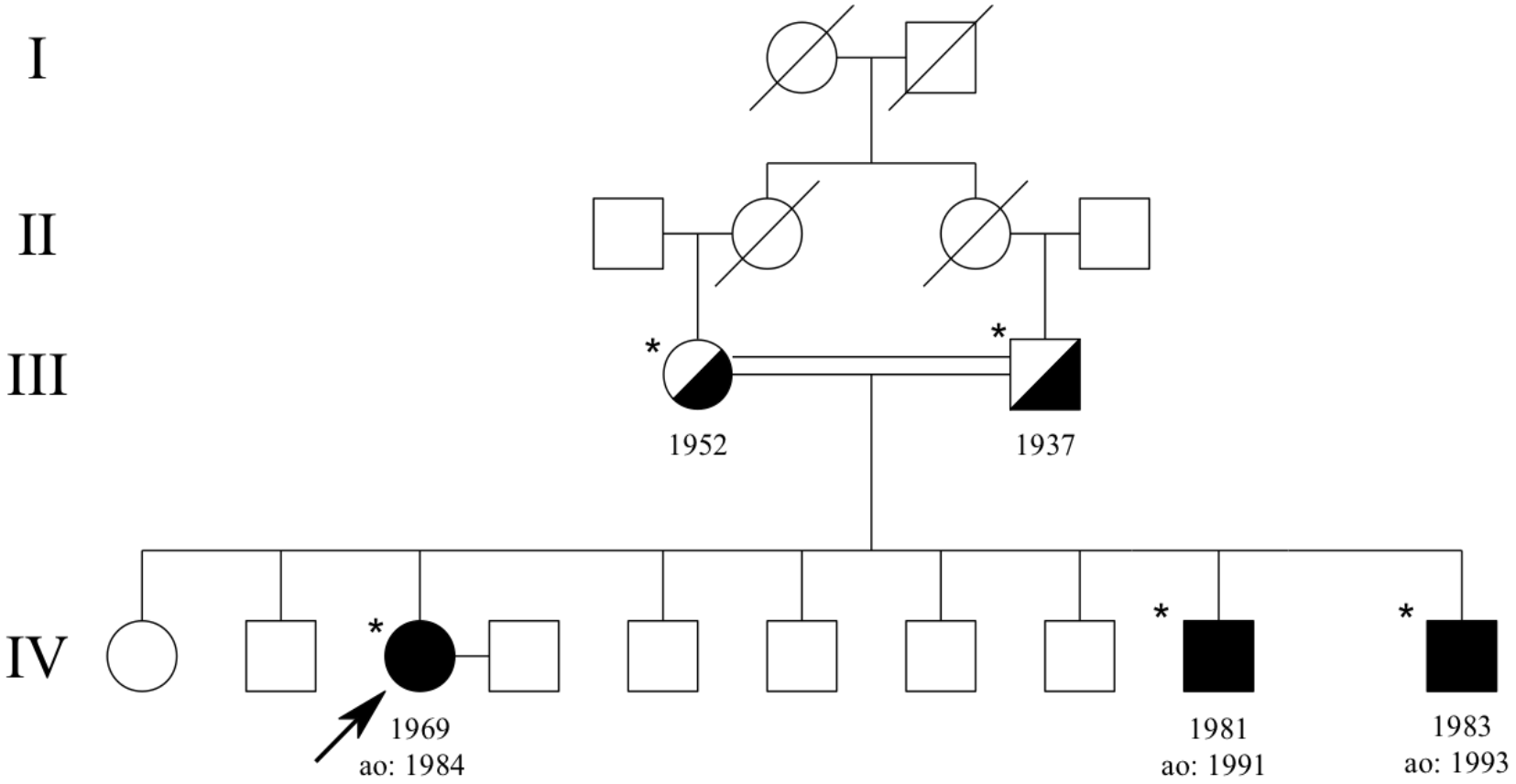
Chr	Position	Gene name	dbSNP ID	Ref/Alt	Mutation
chr9	135202689	SETX	-	T/TG	c.4296_4297insC:p.A1432fs



reference

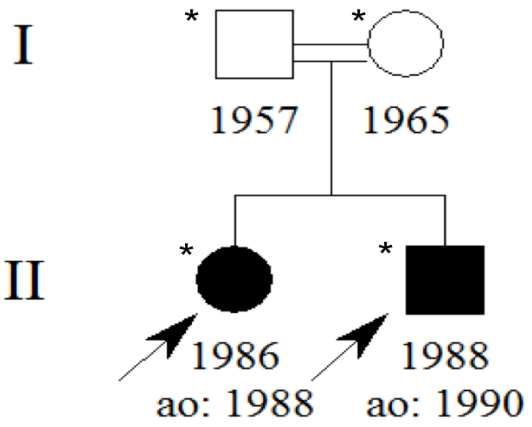
index case

Overall results-Family 8: Ataxia with Oculomotor Apraxia type 2



Family 9

- Symptoms:
  - walking difficulties at the age of two
  - dysarthria
  - spinocerebellar atrophy
- Clinical diagnosis: SCA and FRDA
- Genetics: recessive
  - SCA 1,2,3,6,7,17 and FRDA (-)

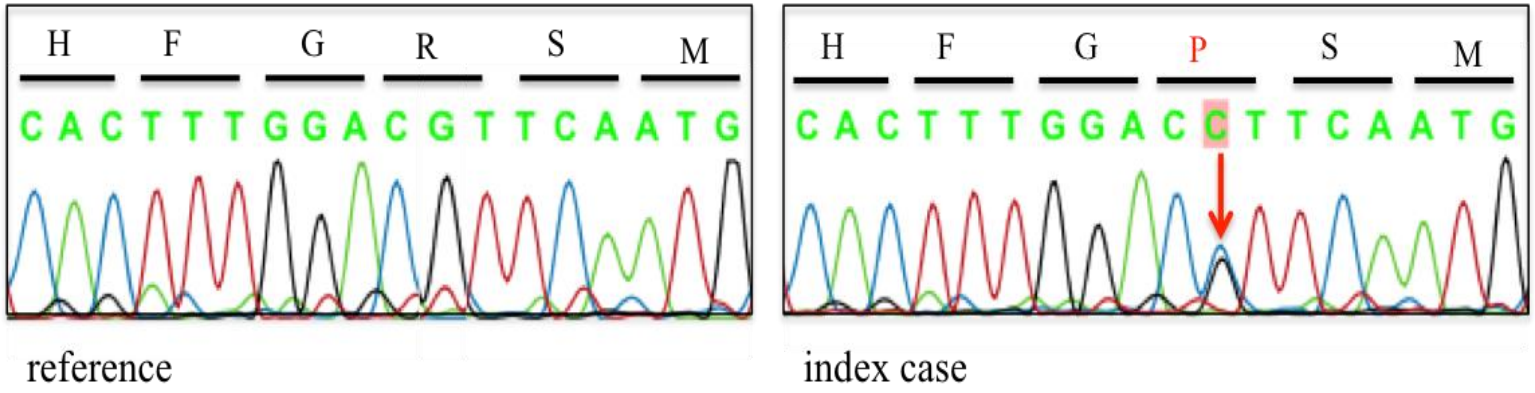


Clinician: Dr. Banu Anlar, Hacettepe

Exome results/ validation: Family 9

Candidate variation in the ALTD1-Family.

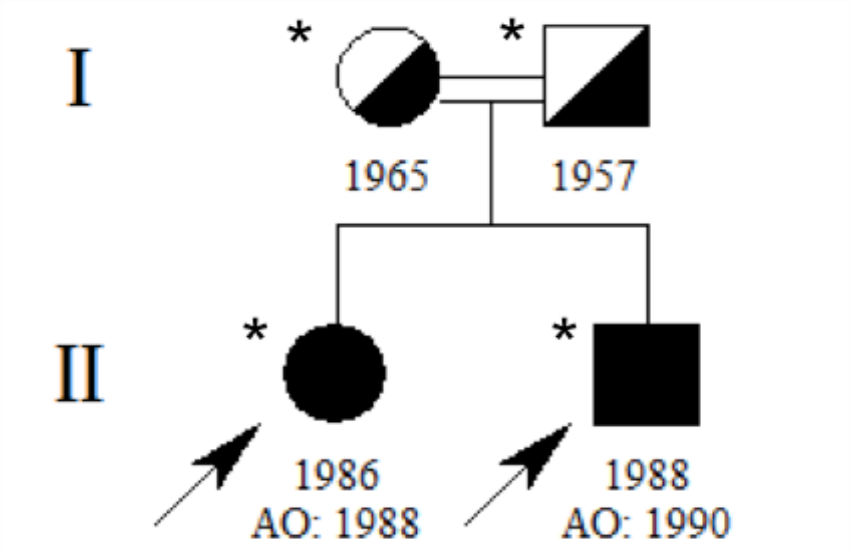
Chr	Position	Gene name	dbSNP ID	Ref/Alt	Mutation
chr11	94211981	MRE11A	-	C/G	c.G464C:p.R155P



reference

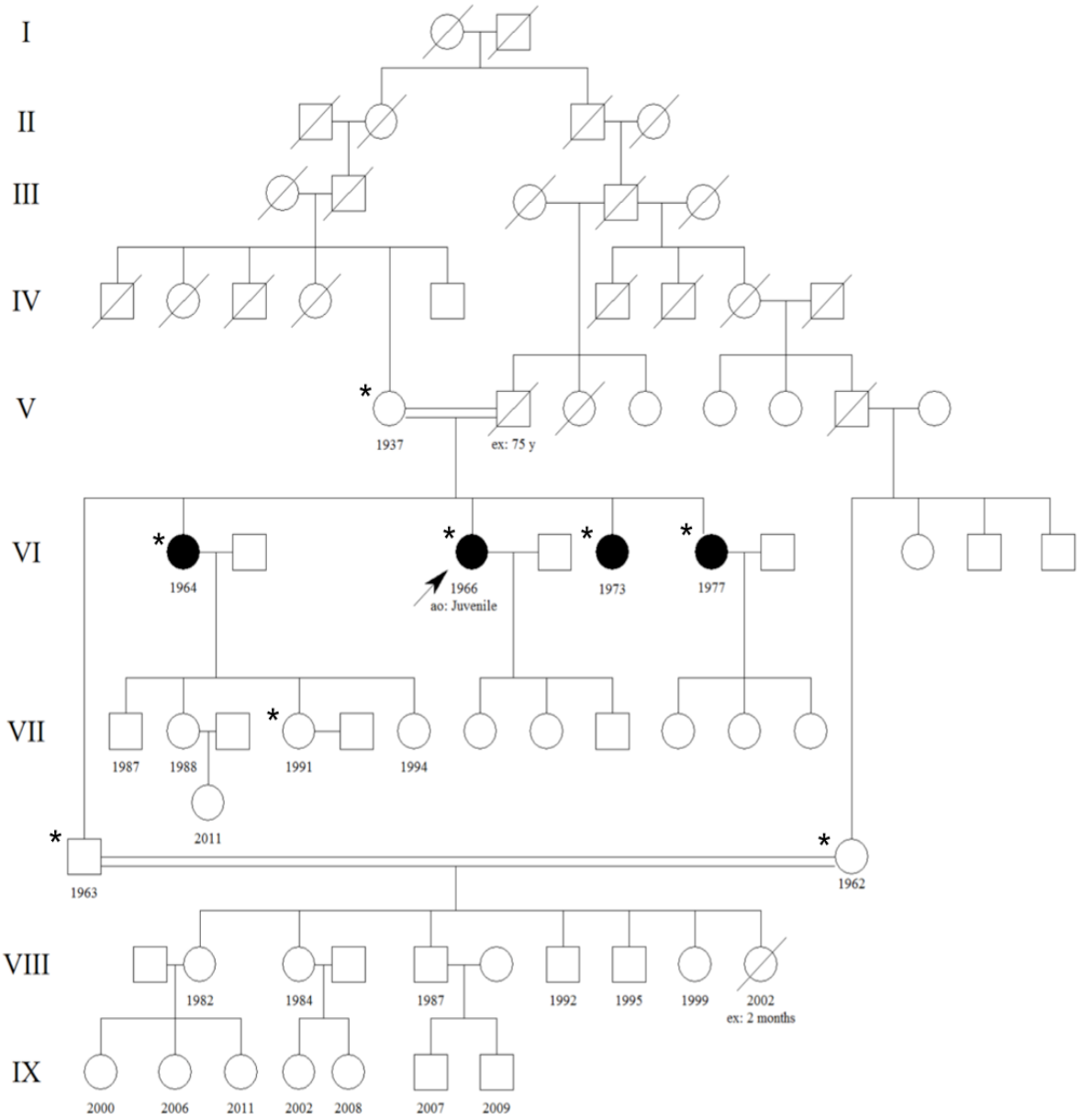
index case

Overall results: Family 9
Ataxia-telangiectasia-like Disorder-1



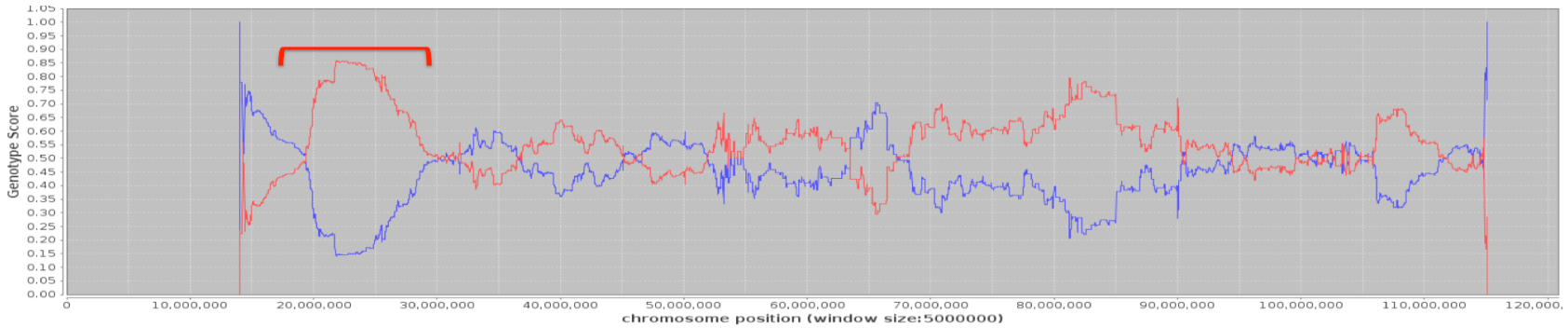
Family 10

- Symptoms:
  - tenar
  - hypotenar
  - Interosseal
  - tibialis anterior
  - intrinsic foot
  - muscle atrophy
  - symmetrical weakness
  - dysarthria
  - spondylosis
- Clinical diagnosis: FRDA
- Genetics: recessive
  FRDA (-)



Clinician: Dr. Semiha Kurt, Gaziosmanpaşa Uni.

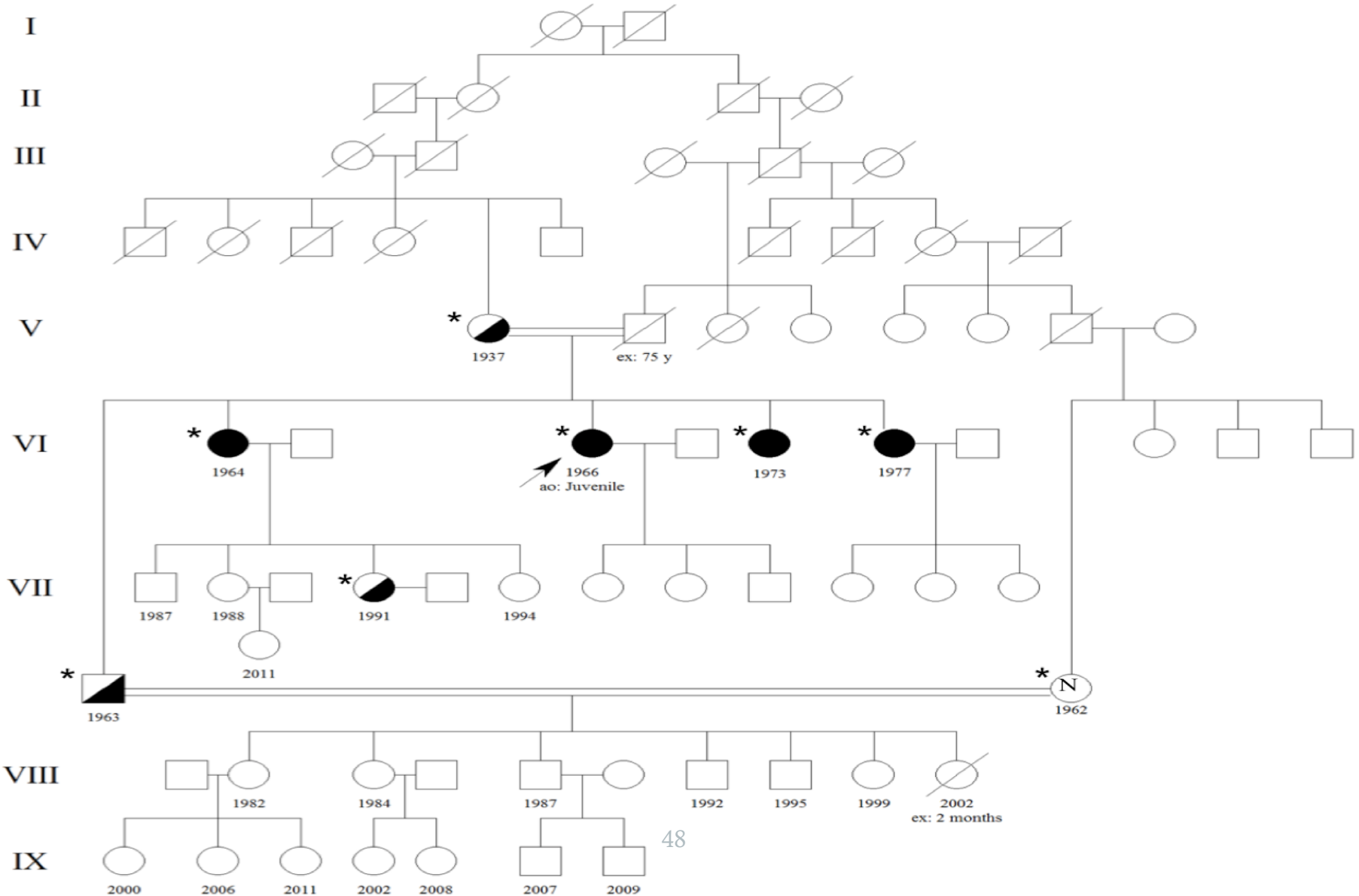
Exome results/validation: Family 10



Candidate variant list in ARSACS-Family 1.

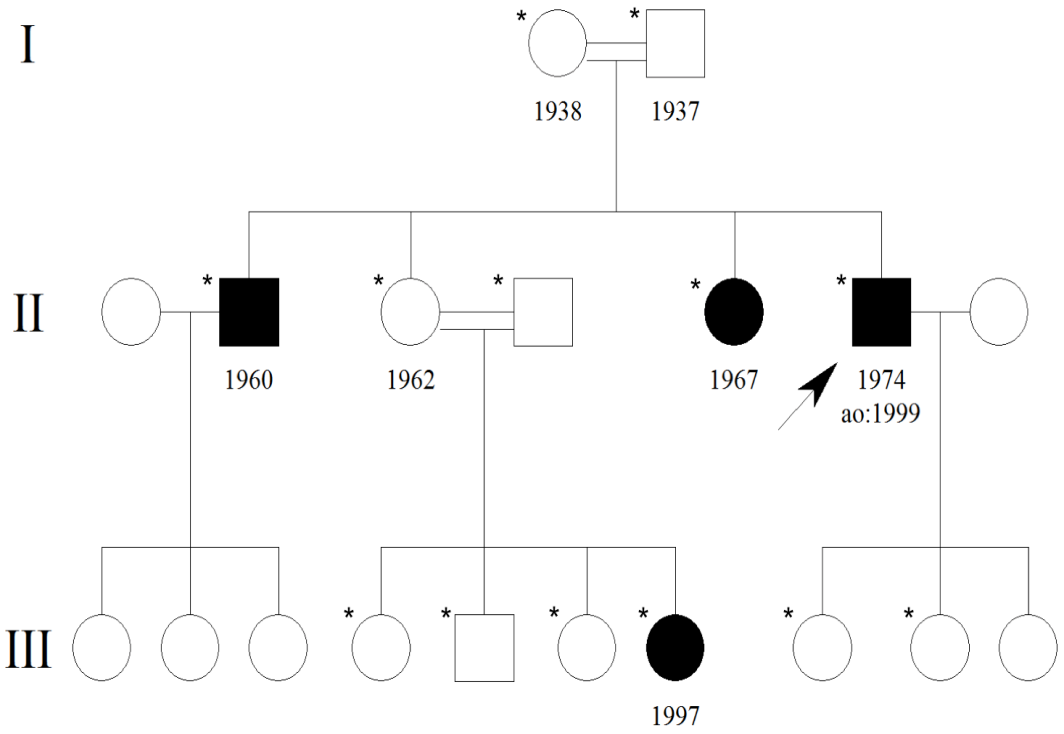
Chr	Position	Gene name	dbSNP ID	Ref/Alt	Mutation
chr13	23909699	SACS	-	C/G	c.G8315C:p.G2772A
chr13	23928656	SACS	-	C/T	c.2093+1G>A
chr15	89399451	ACAN	-	C/T	c.C3635T:p.T1212I
chr16	89399454	ACAN	-	C/T	c.C3638T:p.A1213V

Overall results-Family 10
Autosomal recessive spastic ataxia of Charlevoix-Saguenay



Family 11

- Symptoms: -
- Clinical diagnosis: SCA
- Genetics: recessive
  - SCA 1,2,3,6,7,17 (-)

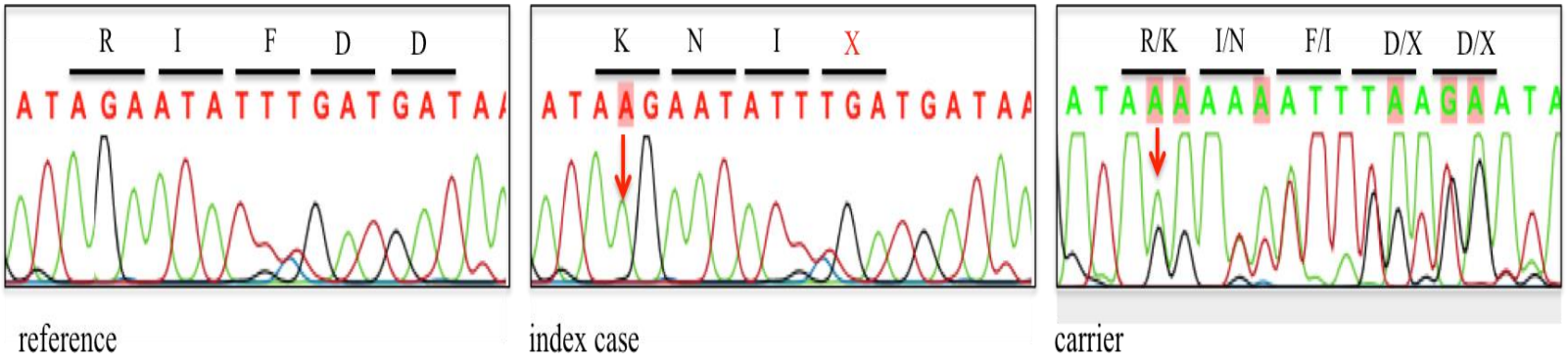


Clinician: Dr. Başar Bilgiç, Çapa

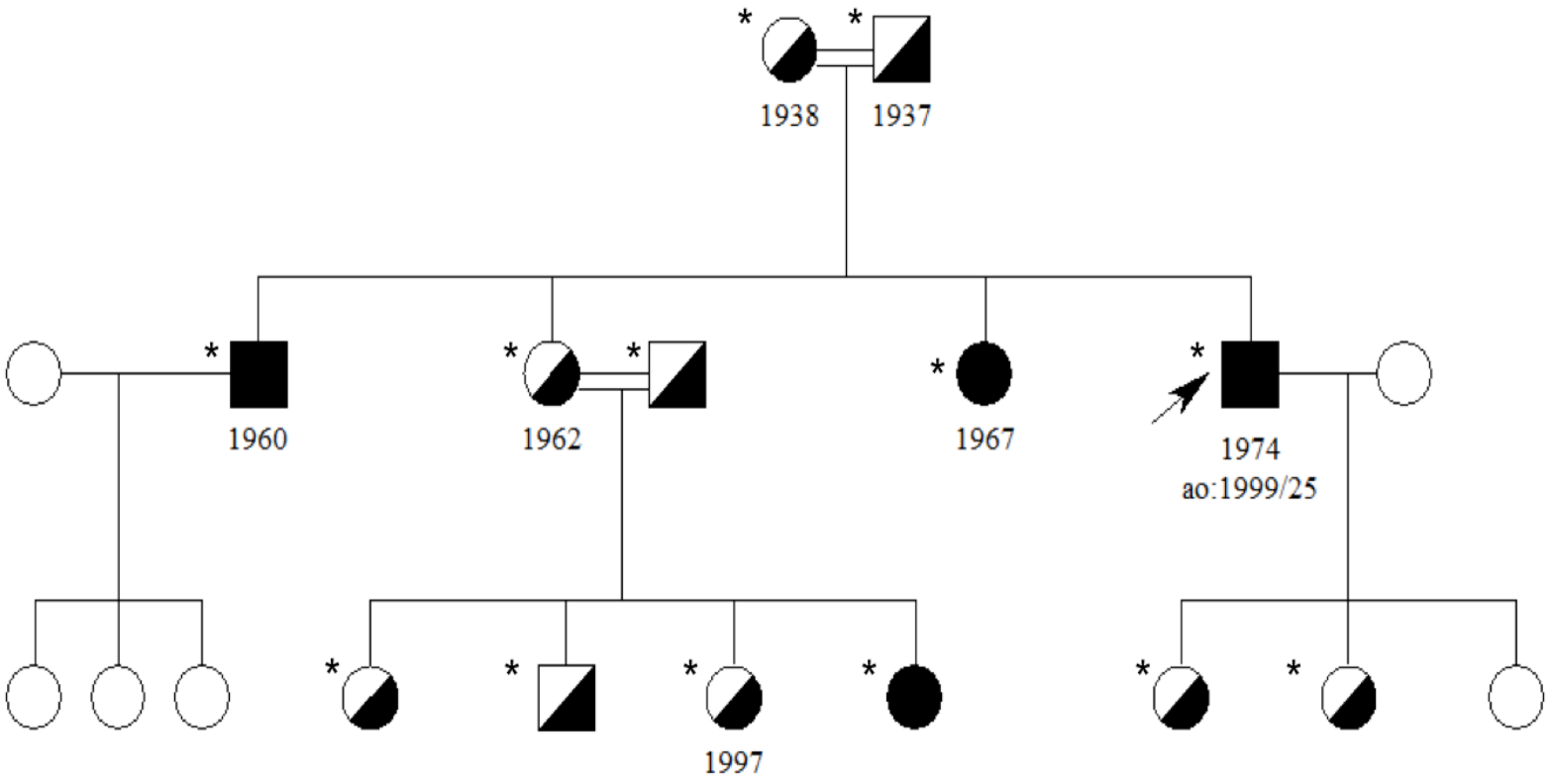
Exome results/validation: Family 11

Variation list in ARSACS-Family 2.

Chr	Position	Gene name	dbSNP ID	Ref/Alt	Mutation
chr13	23910294	SACS	-	C/CT	c.7279dupA:p.R2574fs

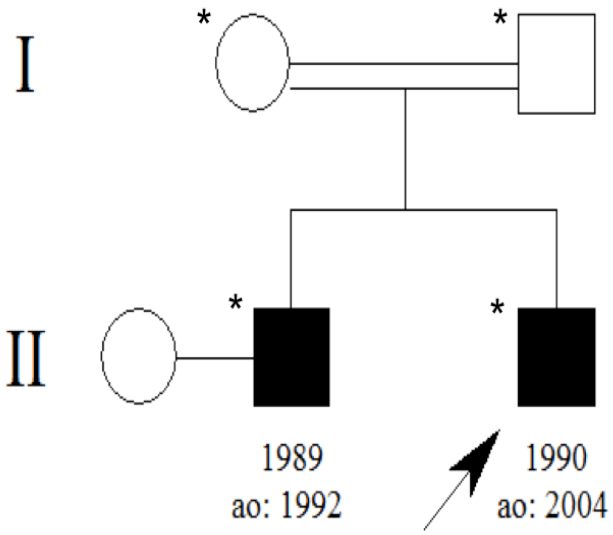


Overall results-Family 11:
Autosomal recessive spastic ataxia of Charlevoix-Saguenay



Family 12

- Symptoms:
  - cerebellar dysfunction
  - nystagmus
  - speech problems
- Clinical diagnosis: SCA and FRDA
- Genetics: recessive
  - SCA 1,2,3,6,7,17 (-)
  - II.2 GAA expansion in FXN

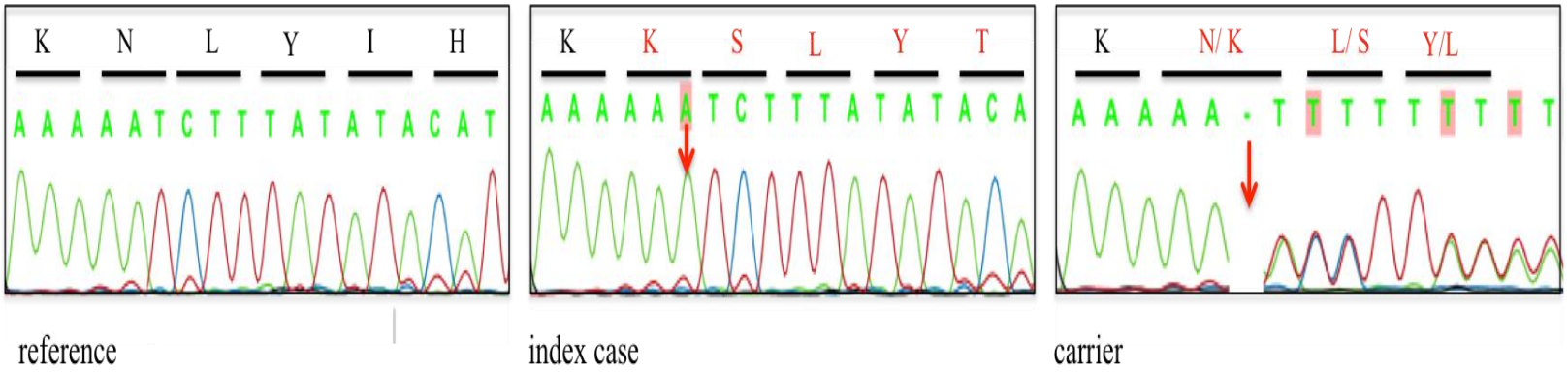


Clinician: Dr. İhsan Şengün, Dokuz Eylül

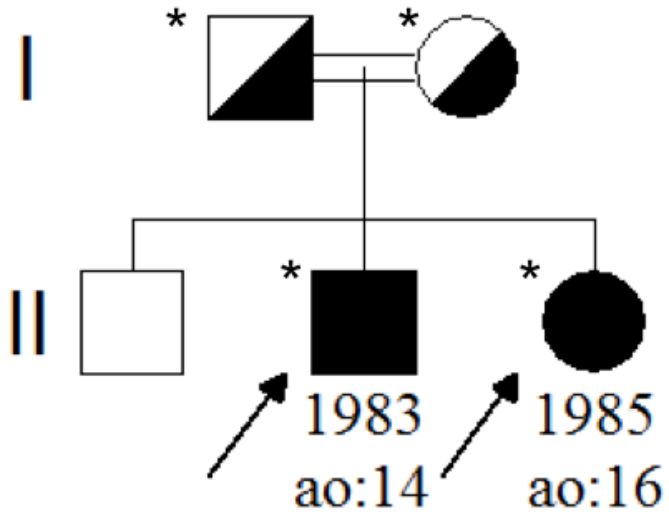
Exome results/validation: Family12

Candidate variation in ARSACS-Family 3.

Chr	Position	Gene name	dbSNP ID	Ref/Alt	Mutation
chr13	23915660	SACS	-	A/AT	c.2355_2356insA:p.N785fsX12

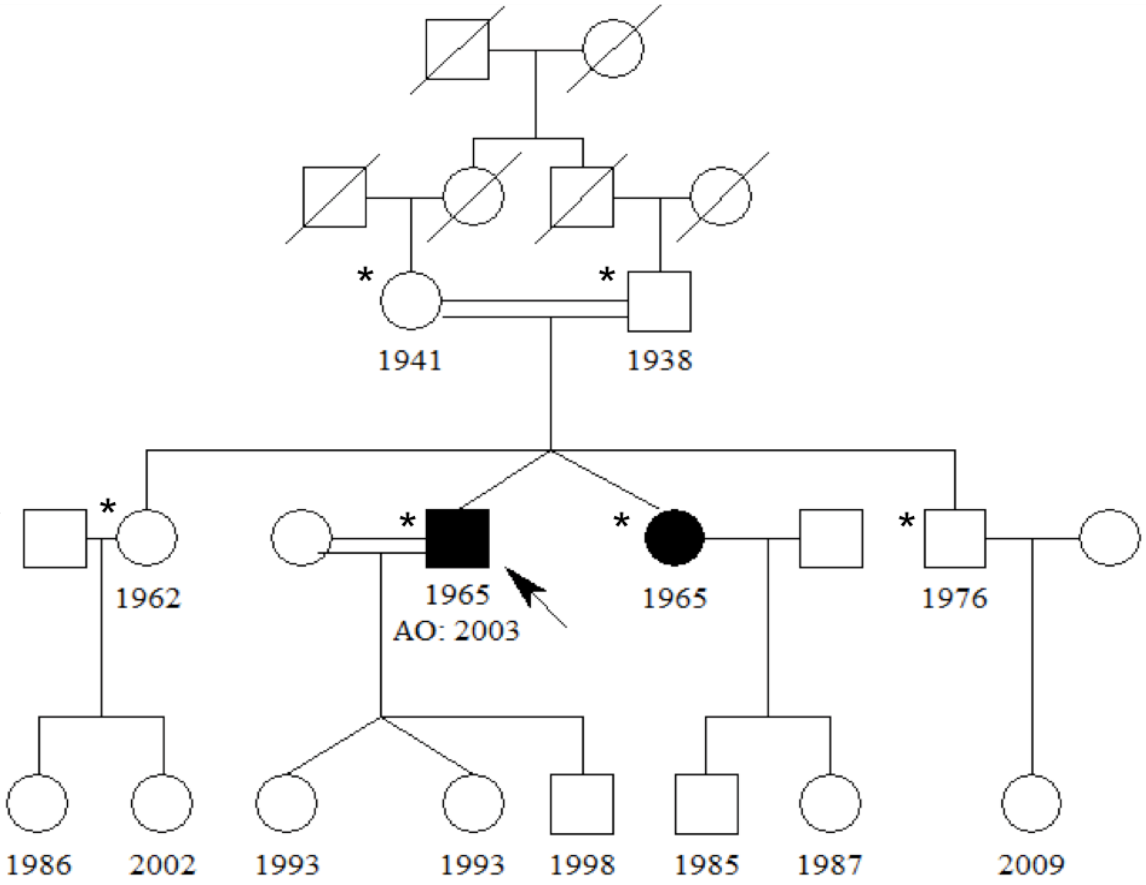


Overall results-Family 12:
Autosomal recessive spastic ataxia of Charlevoix-Saguenay



Family 13

- Symptoms:
  - slowly progressive cerebellar ataxia
  - dementia
  - speech, balance and walking problems
- Clinical Diagnosis: SCA
- Genetics: recessive

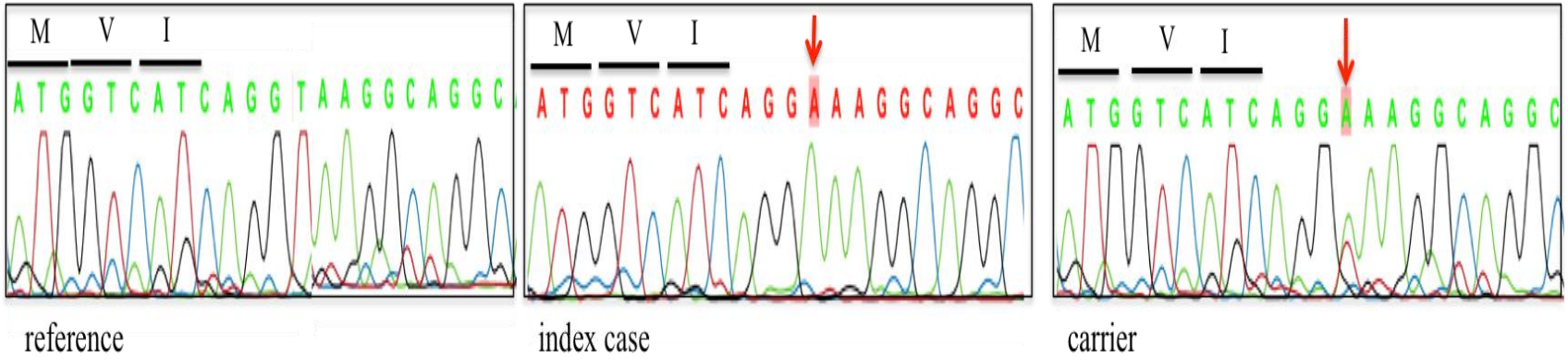


Clinician: Dr. Mefkure Eraksoy, Çapa

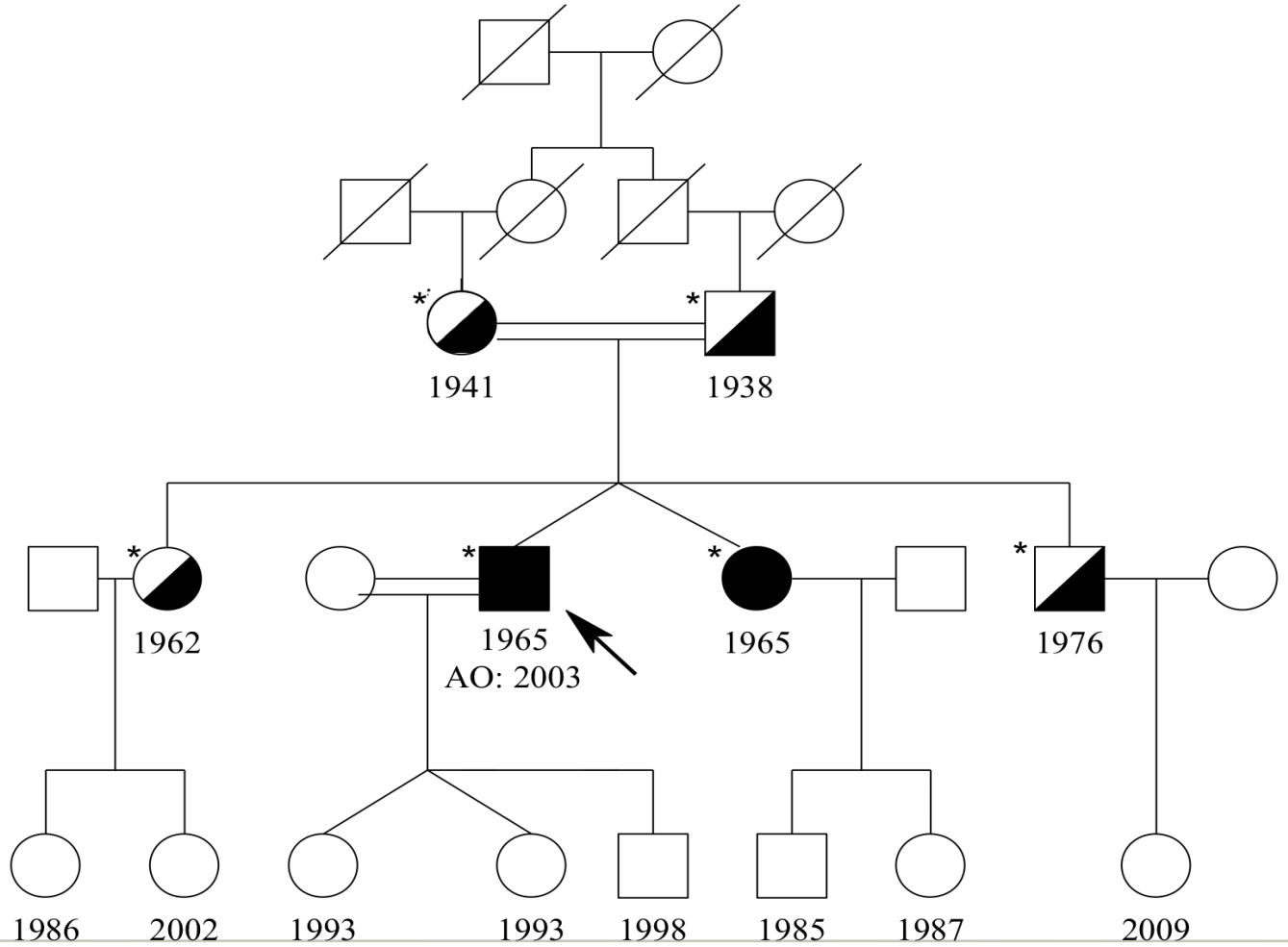
Exome results/validation: Family 13

Variant list in the family.

Chr	Position	Gene name	dbSNP ID	Ref/Alt	Mutation
chr 1	17314815	ATP13A2	-	A/T	c.2747+2T>A



Overall results-Family 13: Kufor-Rakeb Syndrome



Overall results

Family	Diagnosis	Results	Final diagnosis
Family 1	ALS	SQSTM1 p.E274D & TRPM7 p.T1482I	ALS
Family 2	ALS	SPG11 p.Y2272X & p.N1962S	ALS
Family 3	ALS	OPTN p.M98K & p.G291fsX	ALS
Family 4	ALS	OPTN p.359del AA	ALS
Family 5	ALD-PD	DJ1 p.Q45X	ALS-PD
Family 6	SCA & FRDA	APTX p.A158V	AOA1
Family 7	SCA & FRDA	APTX p.V190G	AOA1
Family 8	SCA & FRDA	SETX p.A1482fsX	AOA2
Family 9	SCA & FRDA	MRE11 p.R155P	A-TLD
Family 10	FRDA	SACS p.G2772A & ACAN p.T1212I-p.A1213V	ARSACS
Family 11	SCA	SACS p.R2574fsX	ARSACS
Family 12	SCA & FRDA	SACS p.N785fsX12	ARSACS
Family 13	SCA	ATP13A2 c.2747+2T>A	KRS

Discussion & Conclusion

Computational Challenges of Exome Sequencing

- Missing regions: repeat expansions, copy number variations (CNVs), repetitive and satellite regions
- Exon capture problem (GC-rich region)
- False-positive and false-negative rates
- Algorithms and coverage
- Educated people

What do we need???

Better tools for:

- annotating variants
- manipulating variant data
- prioritizing variants
  (both coding & non-coding)
- prioritizing candidate genes
- pathway analysis for genetic heterogeneity
- methods for functional analysis

Biological Challenges of Exome Sequencing

- Differential diagnosis in NDD
- Consanguinity, inter- and intra-familial heterogeneity, oligogenic inheritance pattern, incomplete penetrance, de novo mutations
- High number of variations in sporadic and dominant cases
- MAF in open-source databases
- Variant frequency of Turkish population
- Complex genetic and epigenetic factors

A new era in molecular diagnosis

- Mechanisms leading to neurodegeneration
- Genetic counseling
- Personalized medicine
- More targeted therapies

Acknowledgements

Thesis supervisor: Prof. A. Nazlı Başak

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Prof. Uğur Özbek

Prof. Duran Üstek

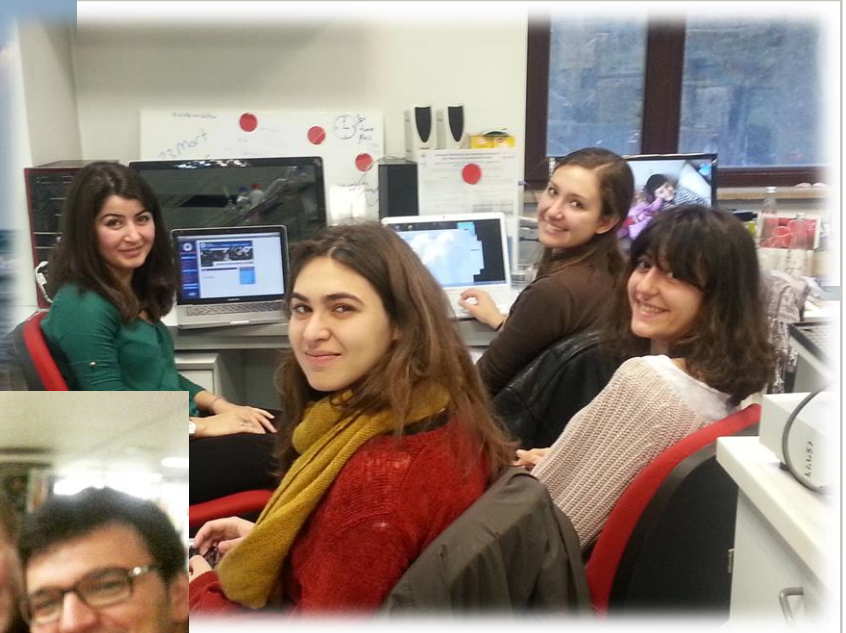
Assoc. Prof. Sibel Aylin Uğur İşeri

MBG Members

Clinicians

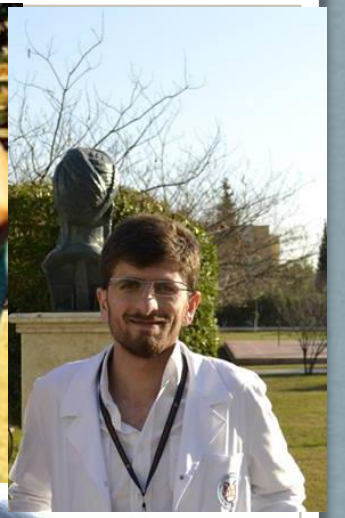
Pınar Kavak

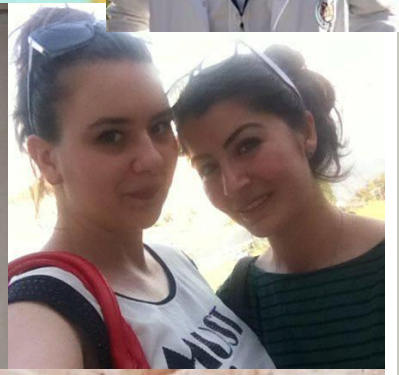
Patients and families




jolly



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Thank you for listening

Any Questions?