The Molecular Dissection of Ataxias in Turkey and the Impact of Whole Exome Sequencing in Their Precise Differential Diagnosis

M.Sc. Thesis Defense
Cemile Koçoğlu
NDAL
18.11.2016

Overview

Ataxias

- Mechanisms and cerebellum

Next Generation Sequencing and applications

- Whole exome sequencing

Application of WES to Turkish ataxia cohort

The outcome of WES in Turkish ataxia cohort

How to improve?

Ataxias and the cerebellum

What is Ataxia?

A taxis: without order

Inability to control muscle movements

Imbalance

Cerebellar dysfunction

Cerebellum

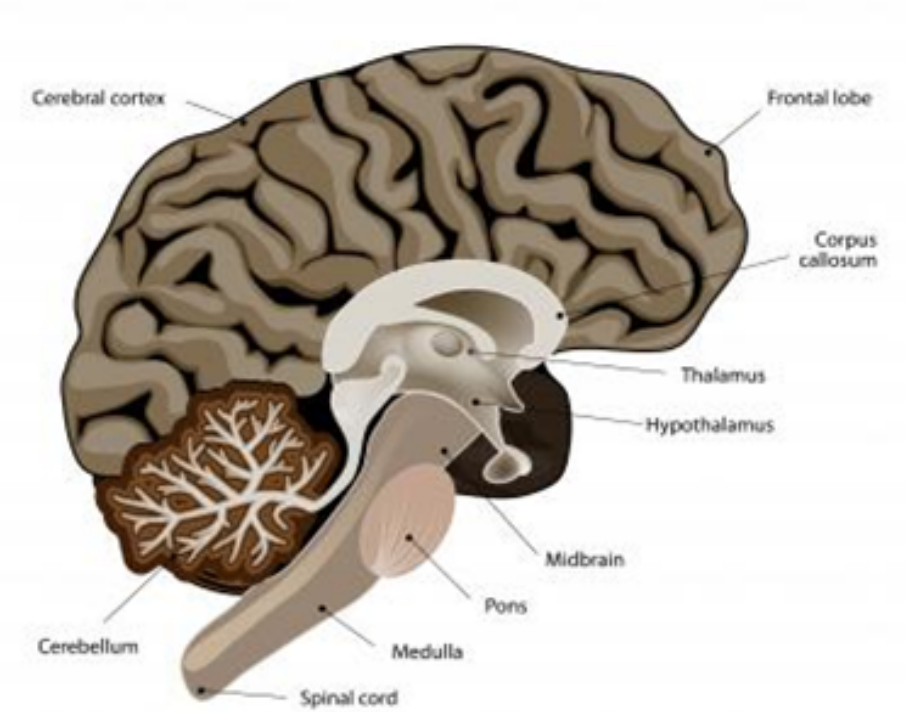
Motor input and output

Control over body movements

voluntary movements

balance and posture

Motor learning



smooth, balanced muscular activity

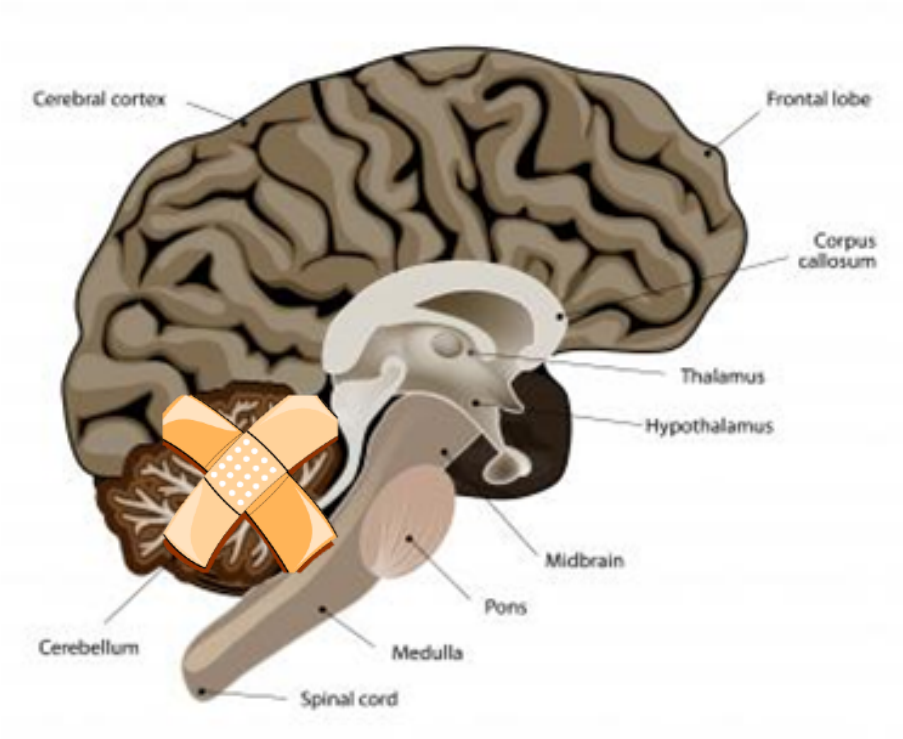
Cerebellum

Damage!

Imbalance

Slowed / uncoordinated limb movements

Tremor



“Ataxia”

Ataxias

Inherited ataxias

autosomal dominant

autosomal recessive

X-linked

mitochondrial

sporadic

Acquired ataxias

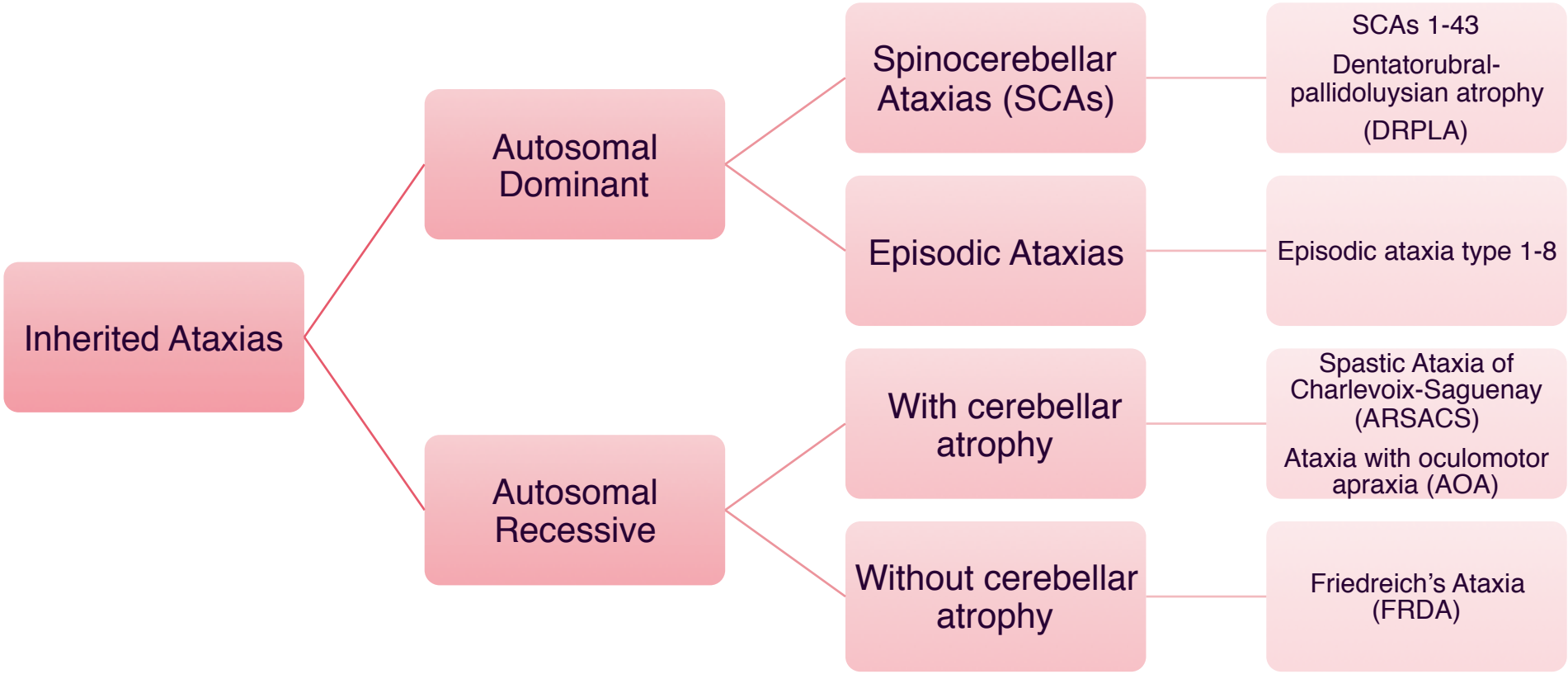
metabolic deficiencies

autoimmune conditions / infections

exposure to toxic substances or drugs

cancer

Inherited Ataxias



Mechanisms are Heterogeneous

Spinocerebellar ataxia:

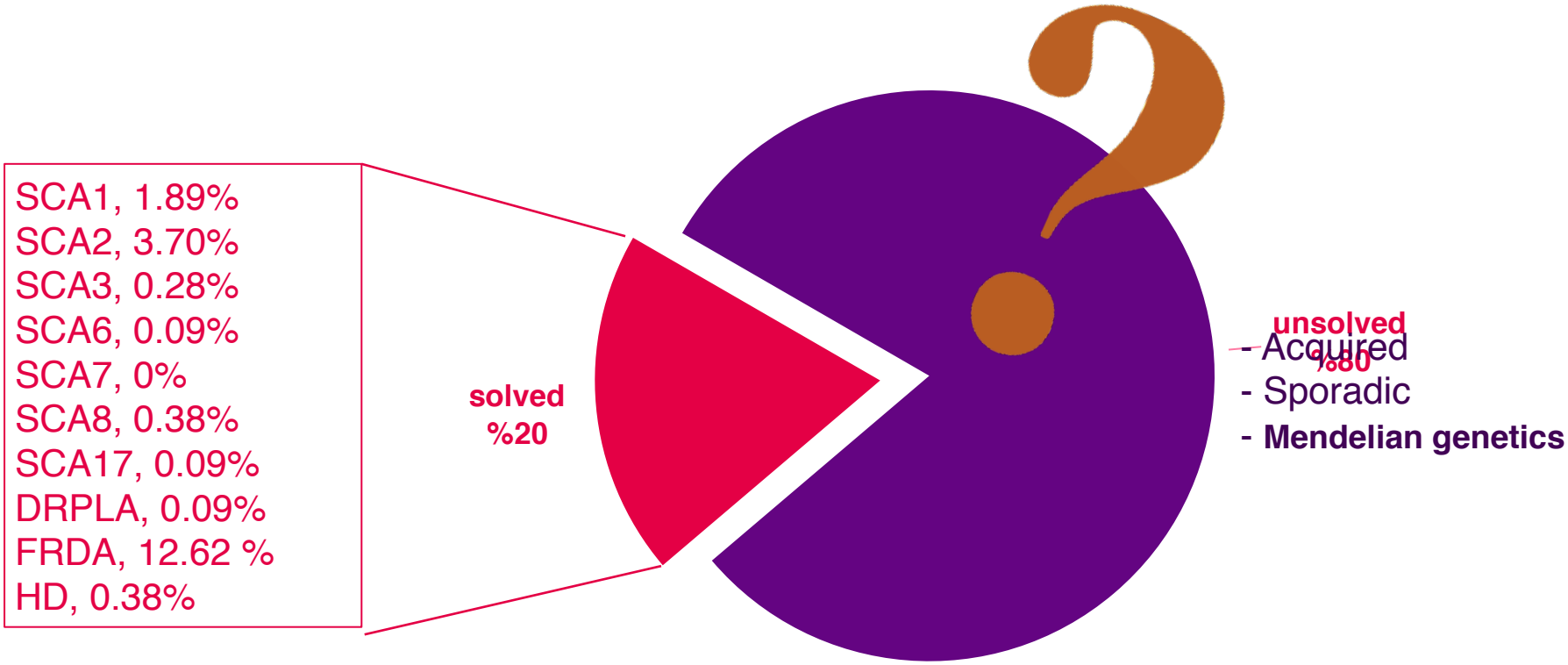
- PolyQ toxicity
- Ion-channel dysfunction
- RNA toxicity
- Signal transduction

Autosomal recessive ataxia

- DNA repair defect
- Mitochondrial dysfunction
- Protein & chaperone dysfunction
- Metabolic defects

Genetics in Turkey

A total of 1054 patients



How to solve the remaining patients?

Next Generation Sequencing

1953 DNA structure (Watson & Crick)

1977 Maxam & Gilbert sequencing

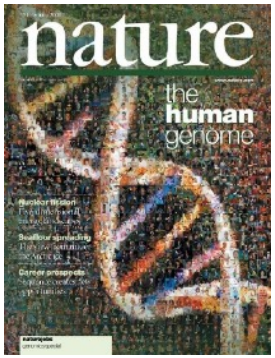
1977 Sanger sequencing

1990 Human Genome Project – start

1996 First NGS technology – Pyrosequencing

2003 HGP – ended

2007 NGS platforms routinely used



Whole Exome Sequencing (WES)

Journal of Human Genetics (2014) 59, 5–15

www.nature.com/jhg

REVIEW

The promise of whole-exome sequencing in medical genetics

Bahareh Rabbani[1], Mustafa Tekin[2] and Nejat Mahdieh[3]

3510

ARTICLES

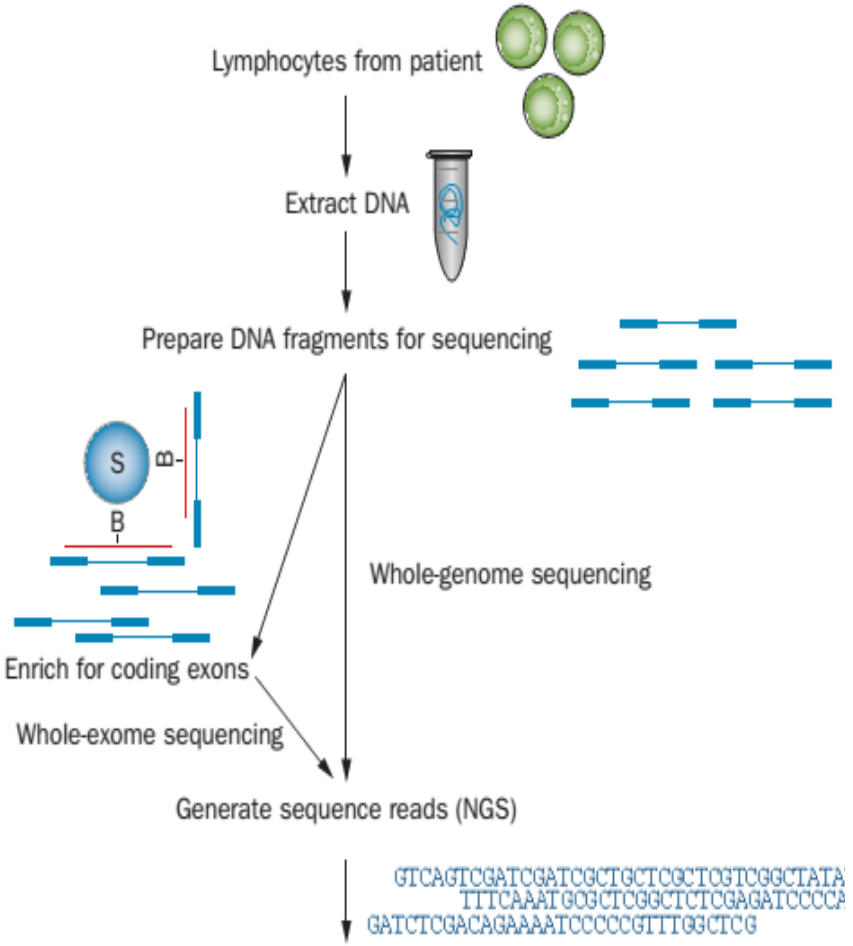
TGM6 identified as a novel causative gene of spinocerebellar ataxias using exome sequencing

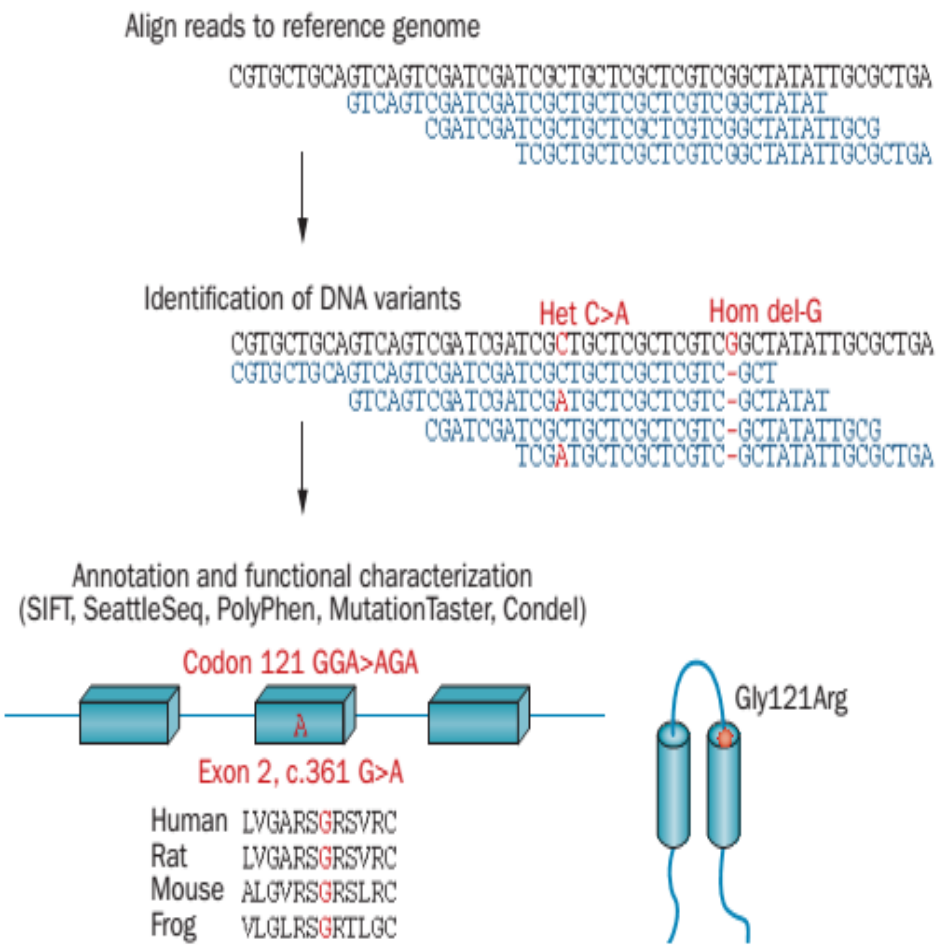
Jun Ling Wang,[1,2,*] Xu Yang,[3,*] Kun Xia,[2,*] Zheng Mao Hu,[2] Ling Weng,[1] Xin Jin,[3,4] Hong Jiang,[1,5] Peng Zhang,[3] Lu Shen,[1,5] Ji Feng Guo,[1,5] Nan li,[1] Ying Rui Li,[3] Li Fang Lei,[1] Jie Zhou,[1] Juan Du,[1] Ya Fang Zhou,[1] Qian Pan,[2] Jian Wang,[3] Jun Wang,[3,6] Rui Qiang Li[3,6] and Bei Sha Tang[1,2,5]

Exome sequencing identifies the cause of a mendelian disorder

Sarah B Ng[1,10], Kati J Buckingham[2,10], Choli Lee[1], Abigail W Bigham[2], Holly K Tabor[2,3], Karin M Dent[4], Chad D Huff[5], Paul T Shannon[6], Ethylin Wang Jabs[7,8], Deborah A Nickerson[1], Jay Shendure[1] & Michael J Bamshad[1,2,9]

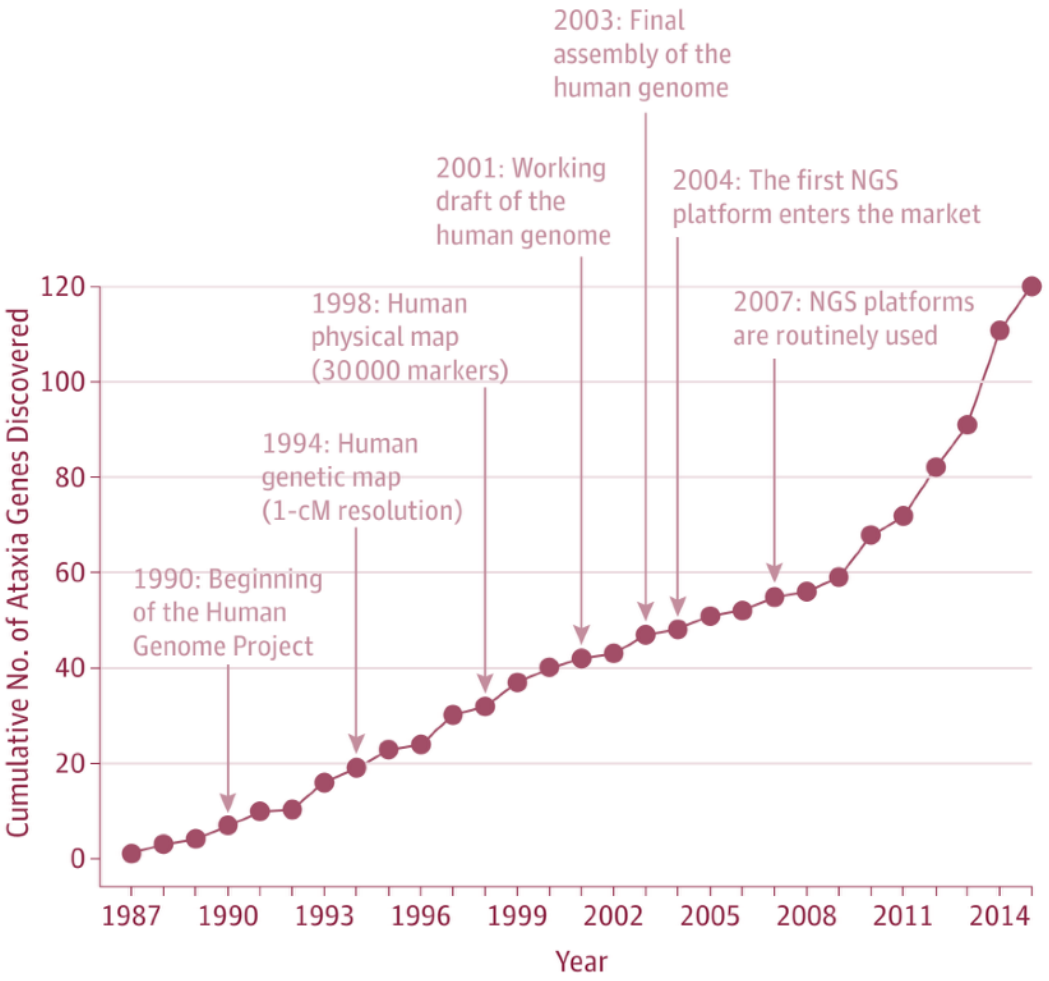
Whole Exome Sequencing





Foo et al., Nature Reviews Neurology, 2012

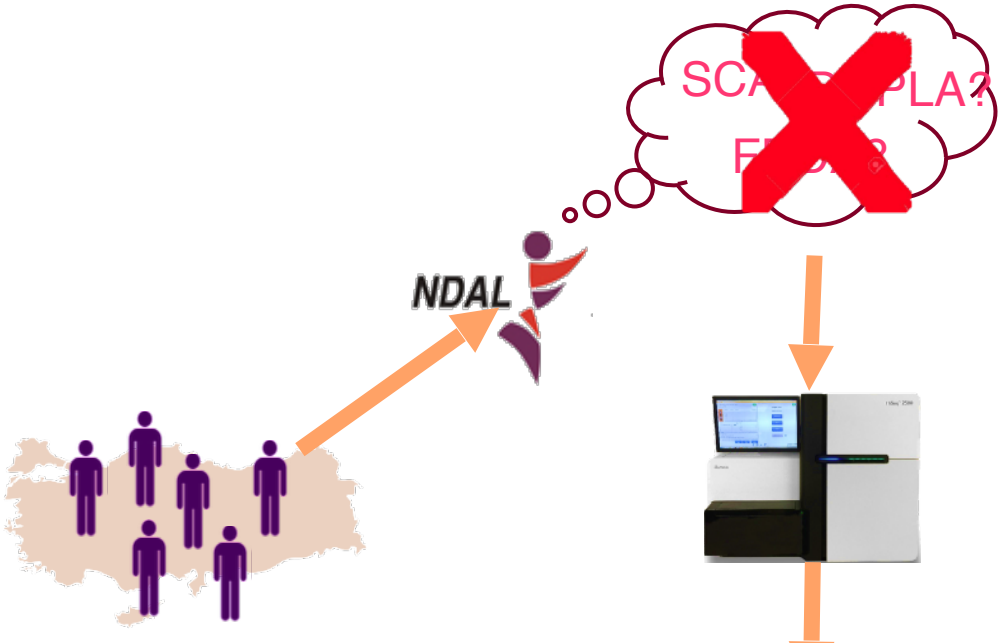
NGS and Ataxia Genetics



DiDonna & Opal, 2016

Dissect the disease cause by whole exome sequencing

Study Design



vcf
files

Convert to Plink

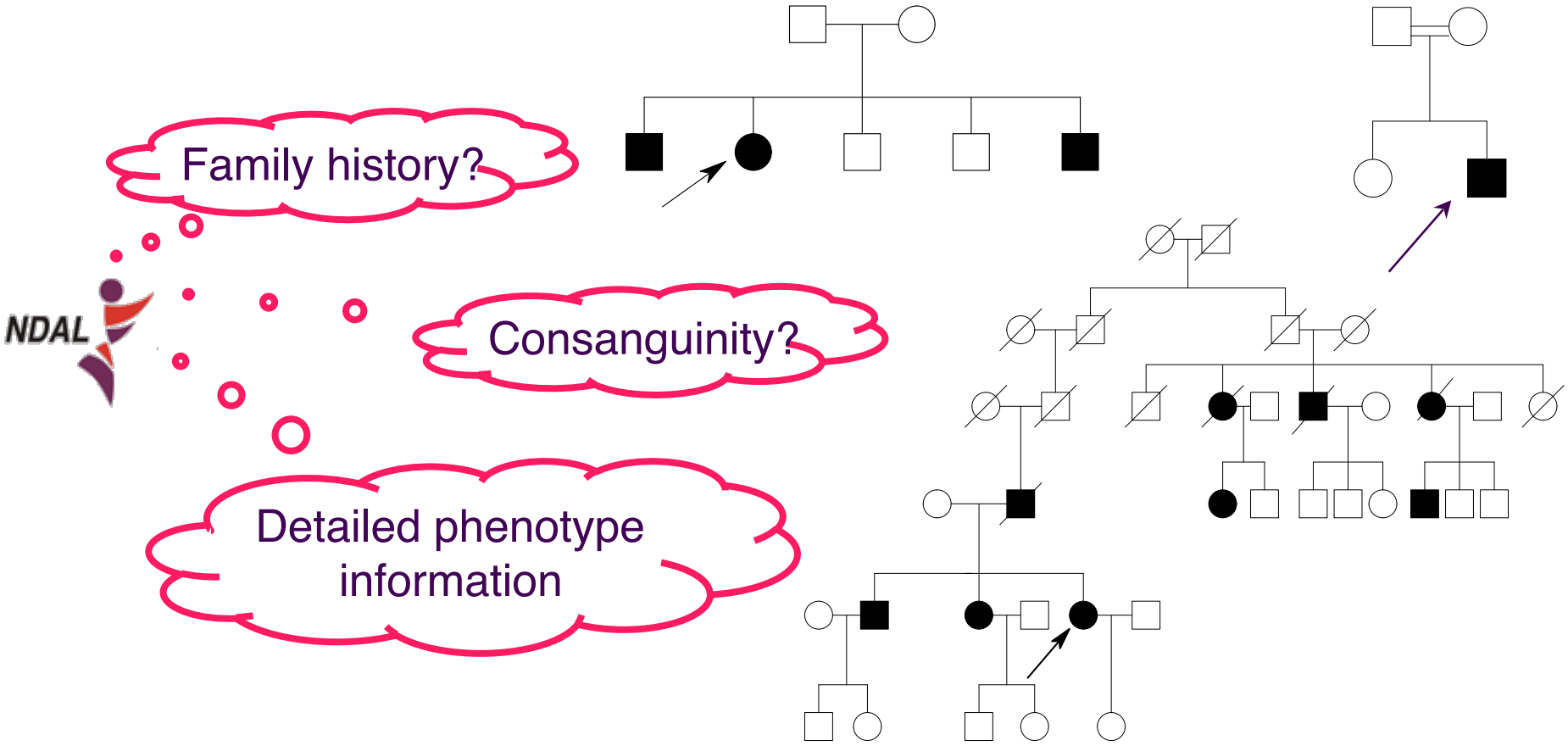
Confim relationships

Family vcf

AR: Homozygosity mapping

Variant prioritization

Sample Selection



Study Cohort

47 families - 150 individuals

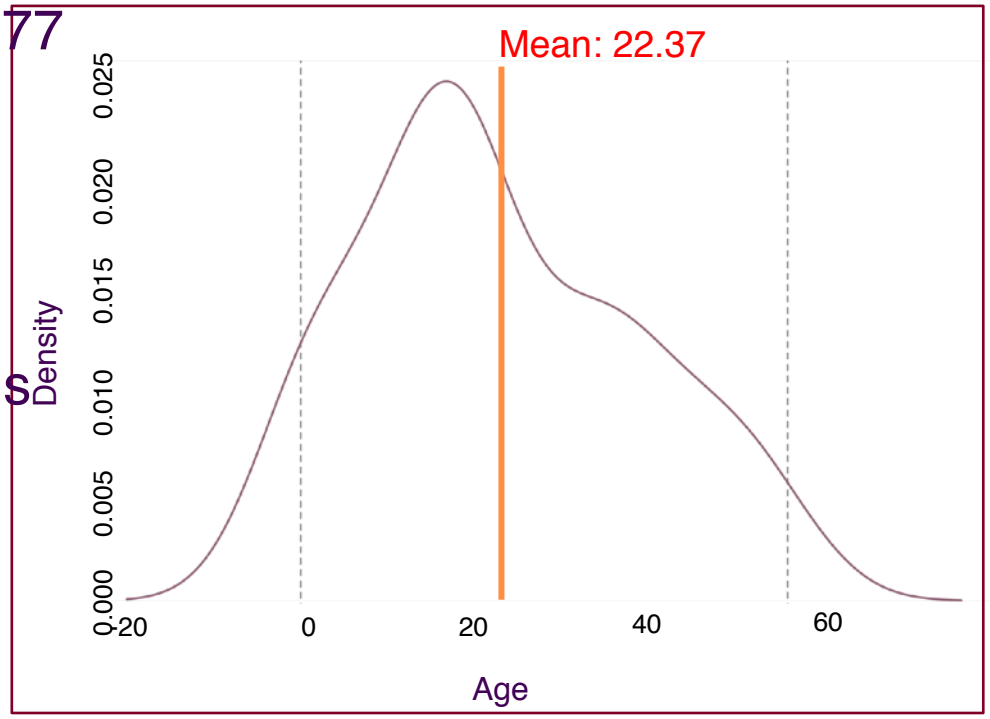
91 patients & 59 family members

Mean age at onset: 22.37±15.77

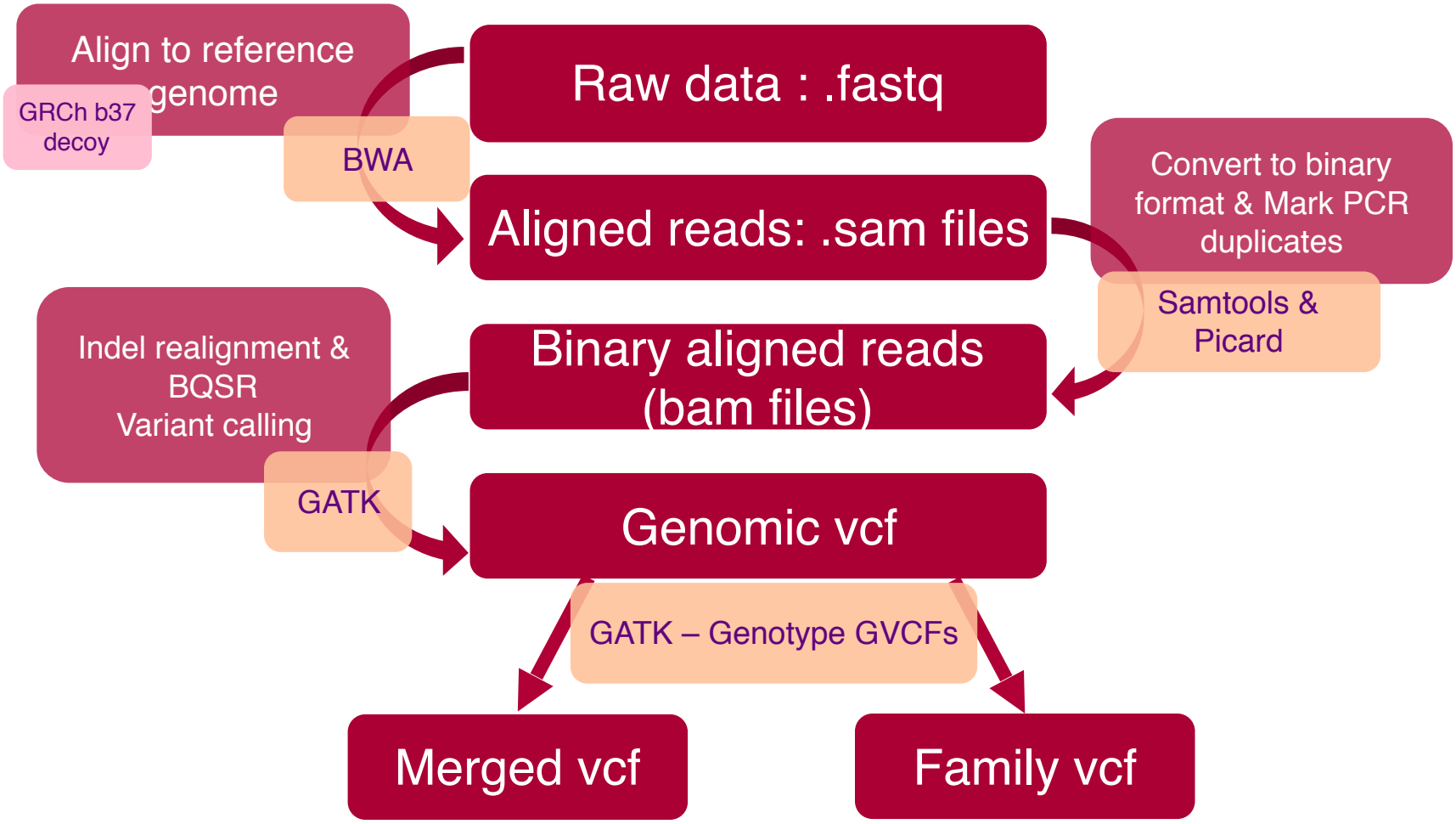
53 males & 38 females

12 AD / 34 AR / 1 both modes

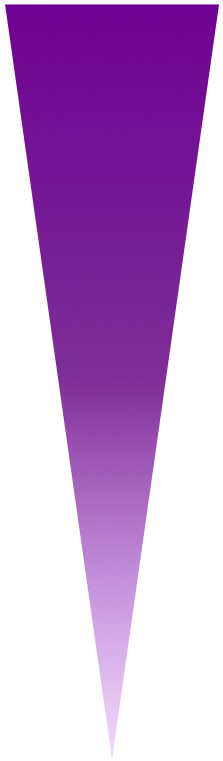
Negative for repeat expansions



Exome data analysis pipeline – data processing



WES data analysis – variant prioritization



Functional annotation of variants - ANNOVAR

Reducing target by filtering according to inheritance pattern

Homozygosity mapping

Variant filtration

- Allele frequencies from 1000 genomes, ExAC, Exome Variant Server
- Protein prediction tools (SIFT, LRT, Polyphen-2)
- Association with the disease

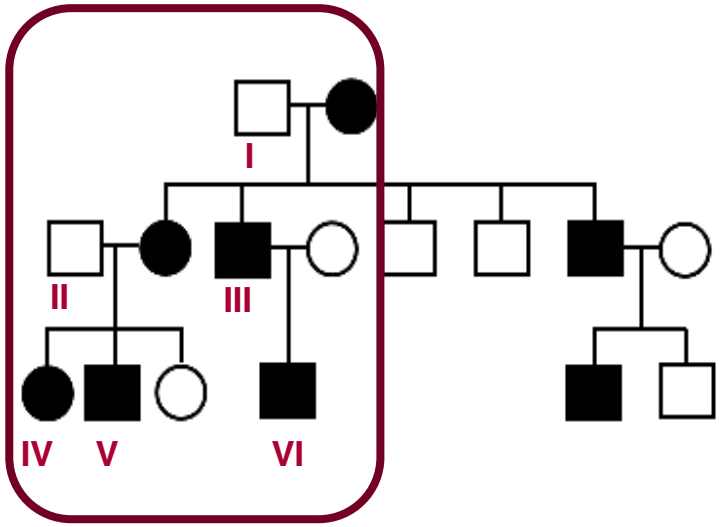
Confirmation of relationships

Identity-by-descent (IBD)

- Identity by state (IBS): Identical nucleotide sequences in two or more individuals in an DNA segment
- IBD: Alleles descended from a common ancestor

Estimation of relatedness of the individuals by pi-hat values among individuals

Identity-by-Descent



Expected Pi-hat values:

IV – V : 0.500

IV – II : 0.500

IV – I : 0.250

IV – III : 0.250

IV – VI : 0.125

Plink - setup

Study cohort
391 individuals

Genotyping rate: 0.015
sites: 4785494

Filter out sites
missing > 0.1

Prune in LD

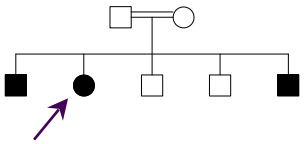
Genotyping rate: 0.936
sites: 35219

Working data set:
391 individuals
16742 variant sites

--genome

IBD estimations

PLINK – Runs of Homozygosity



Fa

Create p

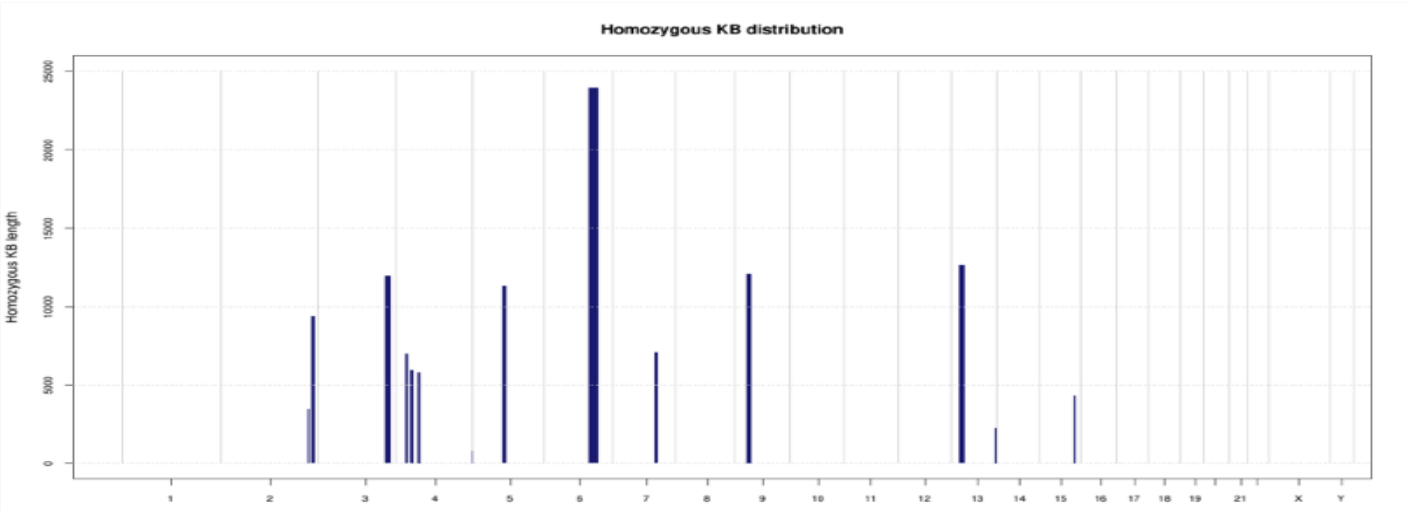
Binary plink files

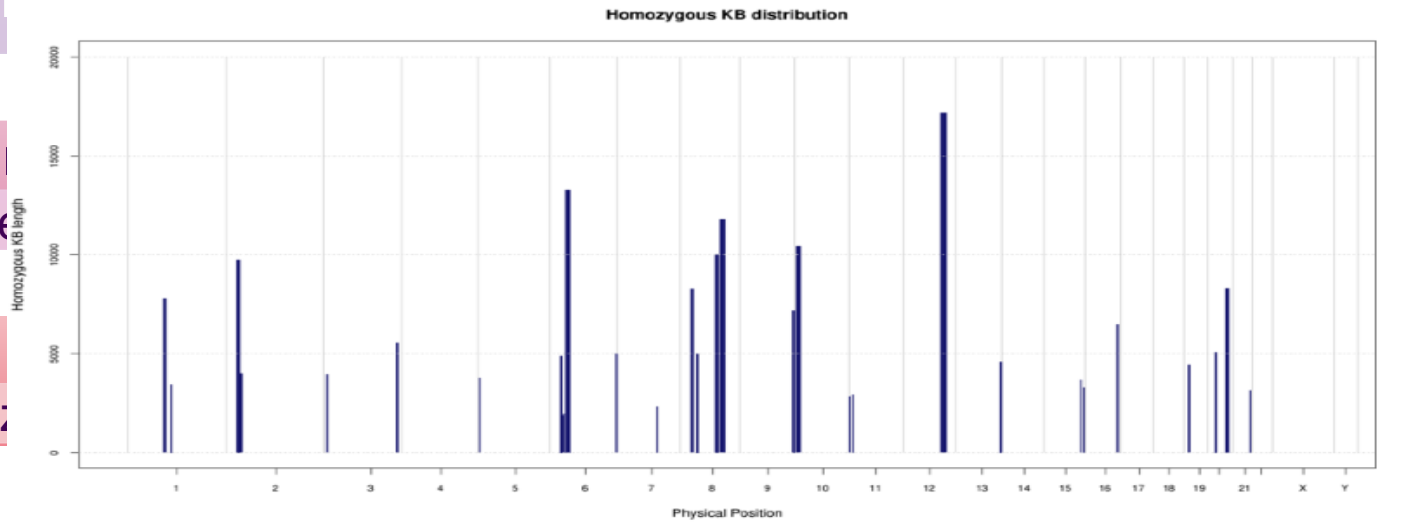
Pru

Remove

Runs of

Find homoz





The impact of WES in disease gene identification

Results from a Turkish cohort

WES Results

WES applied to 47 ataxia families

Mutations identified in:

2/12 dominant pedigrees

18/34 recessive pedigrees

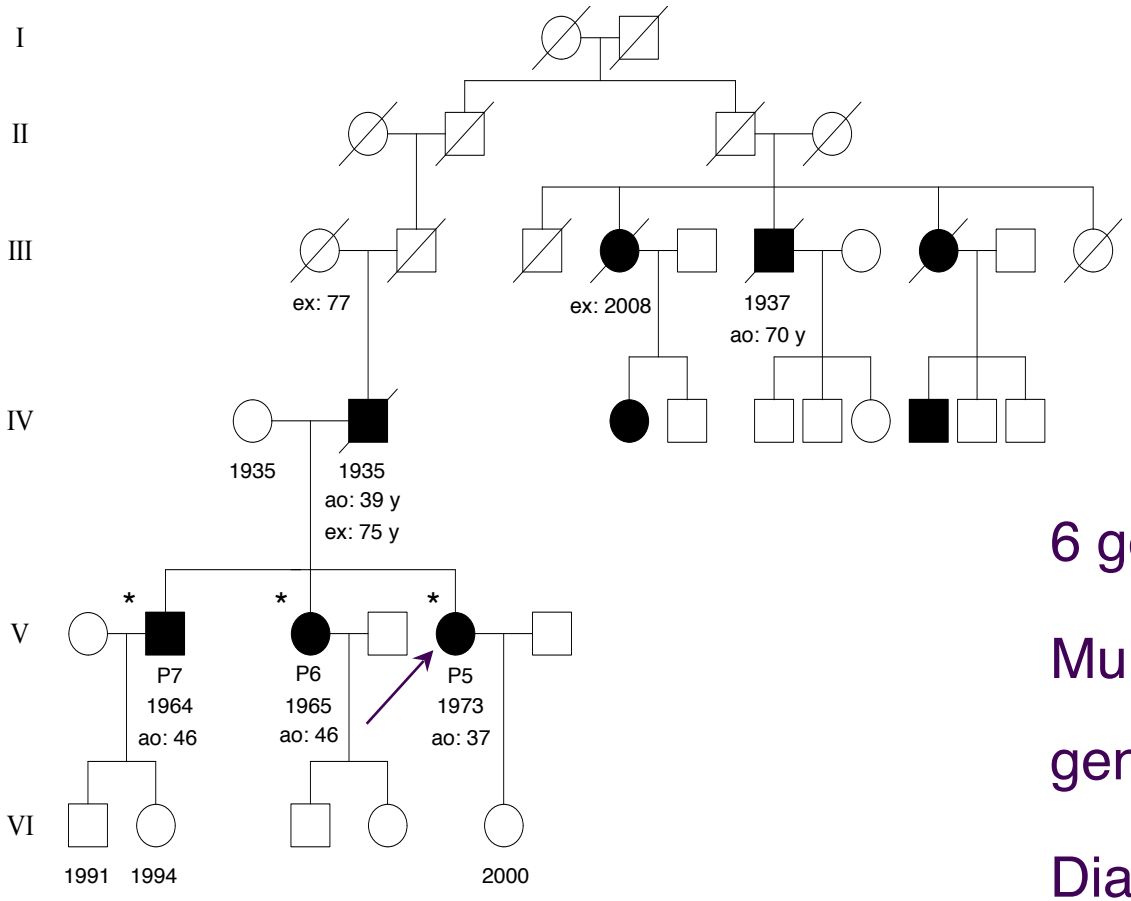
WES Results

Family	Initial diagnosis	Mutated gene	Mutation		Associated Disease (OMIM)
			Coding sequence	Protein sequence	
1	SCA	AFG3L2	c.C2062A	Pro688Thr	SCA28
2	SCA	KIF1B	c.G4712A	Arg1571Gln	CMT2A1
3	SCA	SYNE1	c.C20263T	Arg6755Ter	ARCA-1
4	SCA	SACS	c.19923_12927delAAGAA	Lys4308fs*21	ARSACS
5	SCA	SACS	c.C11374T	Arg3792Ter	ARSACS
6	FA	APTX	c.T569G	Val190Gly	AOA1
7	SCA / FA	SETX	c.5549-2A>G	NA	AOA2
8	FA	SETX	c.5635delG	Val1879Ter	AOA2
9	FA	SETX	c.5249dupT	Leu1750fs*7	AOA2
10	SBMA / FA	SETX	c.T6686C	Met2229Thr	AOA2
11	FA	SETX	c.5249dupT	Leu1750fs*7	AOA2
12	SCA / FA	CAPN1	c.G994A	Gly332Arg	ARHSP76
13	SCA	CAPN1	c.G1176A	Trp392Ter	ARHSP76
14	SCA	SPG11	c.1966_1967delAA	Lys656fs*11	ARHSP-TCC
15	SCA	SPG7	c.G1972A	Ala658Thr	ARHSP-7
16	SCA	SPG7	c.C1763T	Thr588Met	ARHSP-7
17	FA	KIF1C	c.G38A	Arg13Gln	SPAX-2
18	FA / SCA	ADCK3	c.G1009A	Ala337Thr	ARCA-2
19	SCA	ADCK3	c. C1013T	Ala338Val	ARCA-2
20	FA	TTPA	c.487delT	Trp163fs*13	AVED

WES Results

Family	Initial diagnosis	Mutated gene	Mutation		Associated Disease (OMIM)
			Coding sequence	Protein sequence	
1	SCA	AFG3L2	c.C2062A	Pro688Thr	SCA28
2	SCA	KIF1B	c.G4712A	Arg1571Gln	CMT2A1
3	SCA	SYNE1	c.C20263T	Arg6755Ter	ARCA-1
4	SCA	SACS	c.19923_12927delAAGAA	Lys4308fs*21	ARSACS
5	SCA	SACS	c.C11374T	Arg3792Ter	ARSACS
6	FA	APTX	c.T569G	Val190Gly	AOA1
7	SCA / FA	SETX	c.5549-2A>G	NA	AOA2
8	FA	SETX	c.5635delG	Val1879Ter	AOA2
9	FA	SETX	c.5249dupT	Leu1750fs*7	AOA2
10	SBMA / FA	SETX	c.T6686C	Met2229Thr	AOA2
11	FA	SETX	c.5249dupT	Leu1750fs*7	AOA2
12	SCA / FA	CAPN1	c.G994A	Gly332Arg	ARHSP76
13	SCA	CAPN1	c.G1176A	Trp392Ter	ARHSP76
14	SCA	SPG11	c.1966_1967delAA	Lys656fs*11	ARHSP-TCC
15	SCA	SPG7	c.G1972A	Ala658Thr	ARHSP-7
16	SCA	SPG7	c.C1763T	Thr588Met	ARHSP-7
17	FA	KIF1C	c.G38A	Arg13Gln	SPAX-2
18	FA / SCA	ADCK3	c.G1009A	Ala337Thr	ARCA-2
19	SCA	ADCK3	c. C1013T	Ala338Val	ARCA-2
20	FA	TTPA	c.487delT	Trp163fs*13	AVED

Fam 2: KIF1B



6 generation family

Multiple affected in all generations

Diagnosis: SCA

Fam 2: KIF1B

WES: *KIF1B :* Kinesin family member 1B

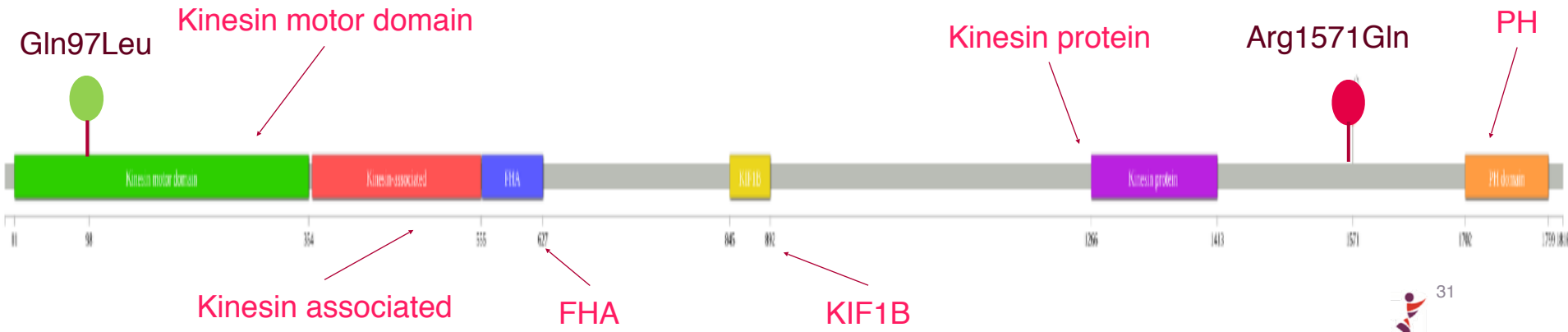
p.Arg1571Gln (ExAC: 8.237e-06)

Cell, Vol. 105, 587–597, June 1, 2001, Copyright ©2001 by Cell Press

Charcot-Marie-Tooth Disease Type 2A Caused by Mutation in a Microtubule Motor KIF1Bβ

Chunjie Zhao,[1] Junko Takita,[2] Yosuke Tanaka,[1] Mitsutoshi Setou,[1] Terunaga Nakagawa,[1] Sen Takeda,[1] Hong Wei Yang,[1] Sumio Terada,[1] Takao Nakata,[1] Yosuke Takei,[1] Masaaki Saito,[3] Shoji Tsuji,[3] Yasuhide Hayashi,[2] and Nobutaka Hirokawa[1,4]

GERP: 5.53
PolyPhen2: 1.0
SIFT: 0.17



Fam 2: KIF1B

Zhao et al., 2001 (Reported by Saito et al., 1997)

Muscle weakness

Pes cavus

Tendon reflexes reduced in lower extremities

Mild sensory disturbance

Patients of this study (P5-P7)

Cerebellar findings

EMG normal

Pes cavus

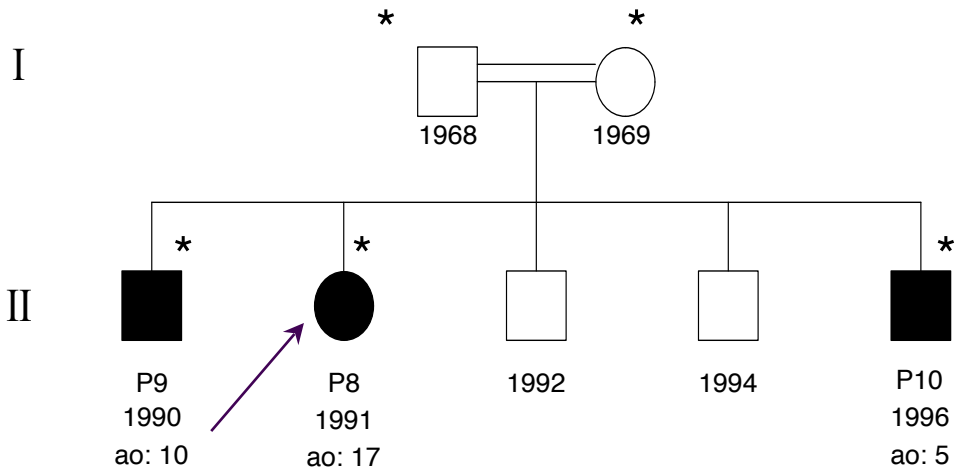
Hammer toe

Loss of vibration sense

Fam 3: SYNE1

Diagnosis: SCA

SMA type 3 suspicion in the youngest brother



Fam 3: SYNE1

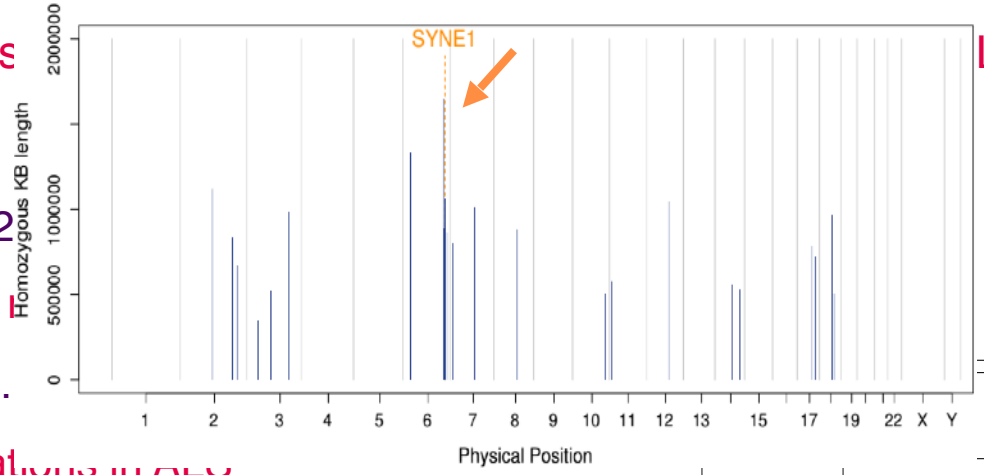
SYNE1: Nesprin 1

Homozygosity mapping

p.Arg6755Stop (ExAC: 8.243e-06 - hom)

Autos

Louis et al., 2007)



Izumi et al., 2

ARCA-1plus

Ozoguz et al.

SYNE1 mutations in ALS

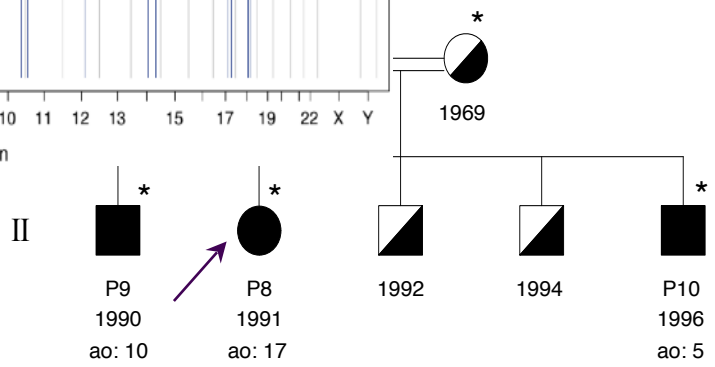
Synofzik et al., 2016

Ataxia with motor neuron involvement

Chromosome 6

SYNE1

p.Arg6755Stop

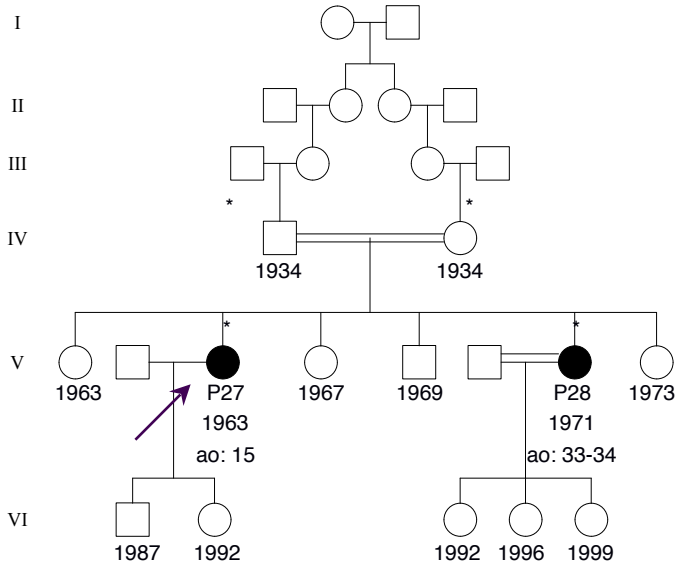


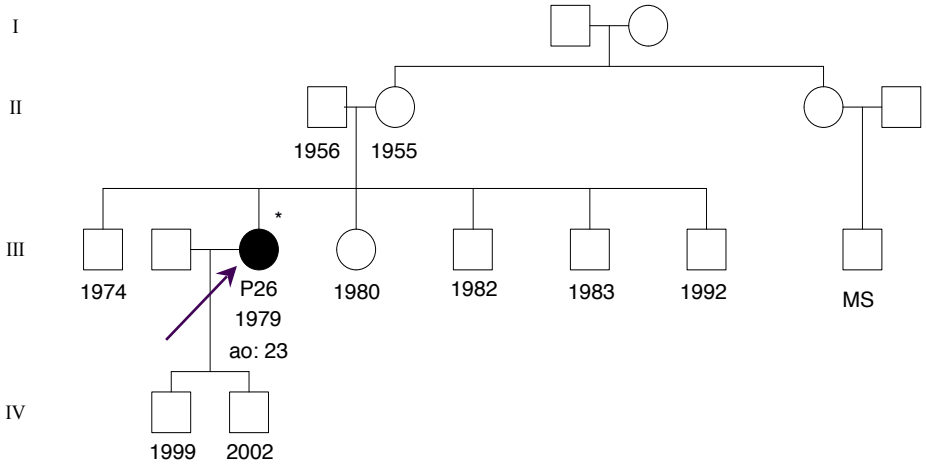
Fam 12 & 13: CAPN1

Common feature: spasticity

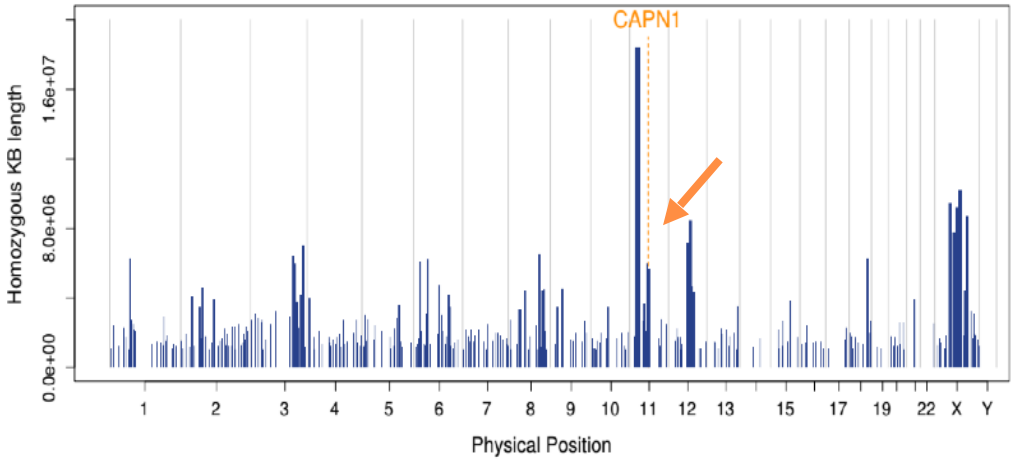
ao < 40

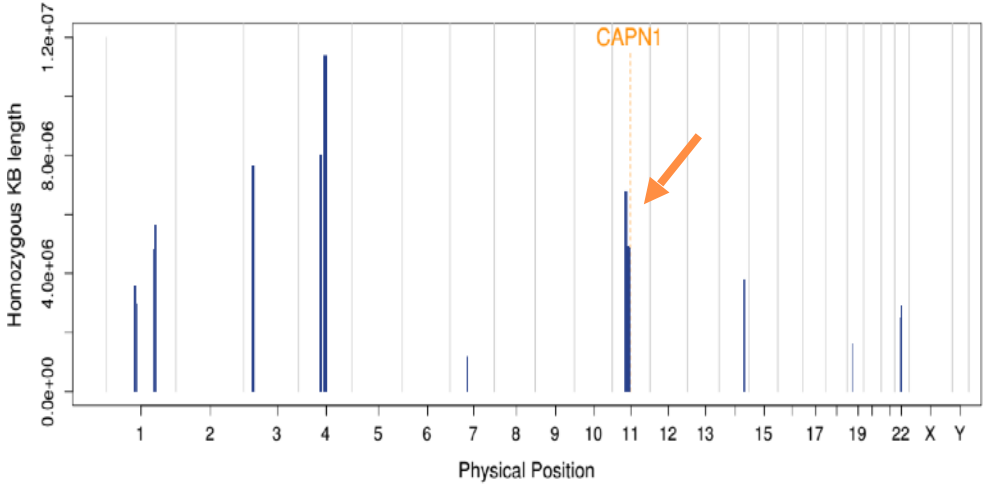
Diagnosis: SCA





Fam 12 & 13: CAPN1





Homozygosity mapping:
Chromosome 11

CAPN1
Fam12 p.Gly332Arg
Fam13 p.Trp392Stop

Fam 12 & 13: CAPN1

CAPN1: Calpain-1

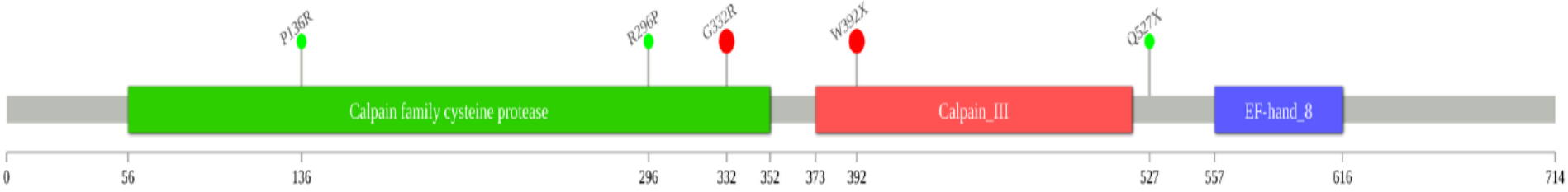
Fam12 p.Gly332Arg (ExAC: not present - hom)

Fam13 p.Trp392Stop (ExAC: 8.513e-06 - hom)

Autosomal Recessive
Hereditary Spastic Paraplegia
76

REPORT May 2016

Mutations in *CAPN1* Cause Autosomal-Recessive Hereditary Spastic Paraplegia



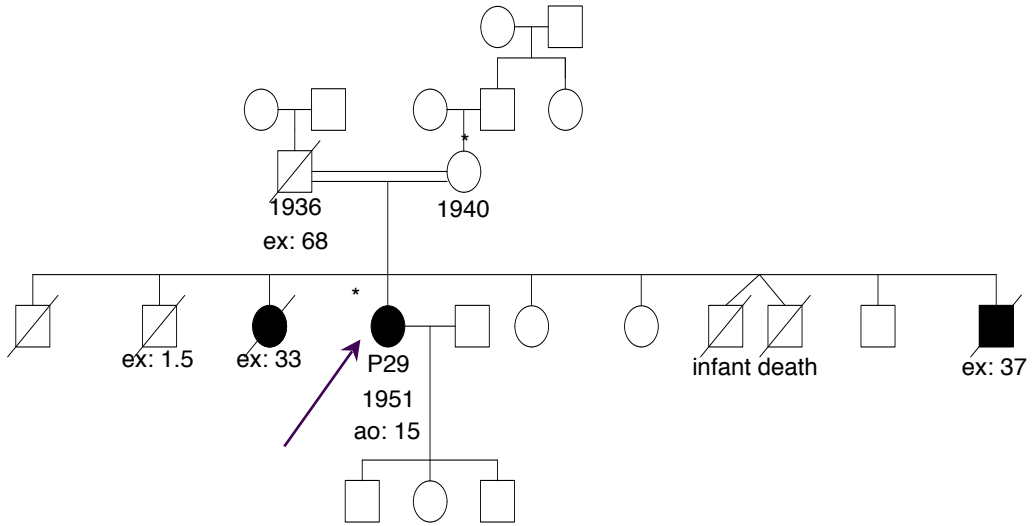
Fam 14: SPG11

Juvenile onset patient

Cerebellar atrophy

Cognitive dysfunction

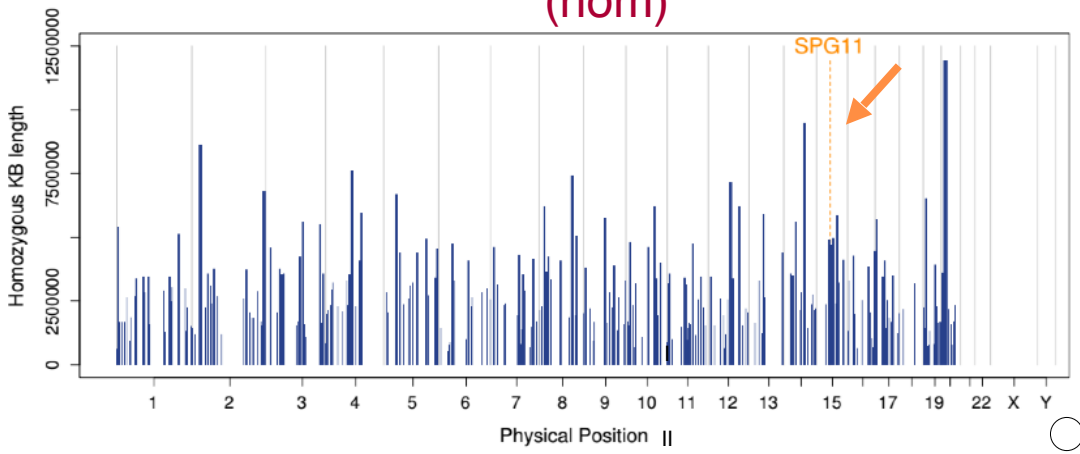
Diagnosis: SCA

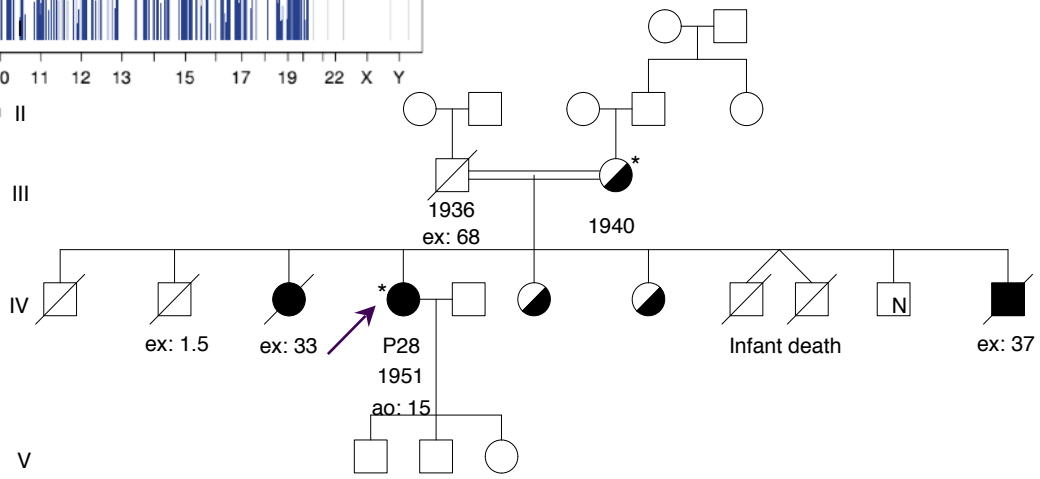


Fam 14: SPG11

Homozygosity mapping:
Chromosome 15

SPG11: p.Lys626fs*11 (hom)





Fam 14: SPG11

Turkish families with juvenile motor neuron disease broaden the phenotypic spectrum of *SPG11*

OPEN

Ceren Iskender, MSc*
Ece Kartal, MSc*
Fulya Akcimen, BSc
Cemile Kocoglu, BSc
Aslihan Ozoguz, PhD
Dilcan Kotan, MD
Mefkure Eraksoy, MD
Yesim G. Parman, MD
Ayse Nazli Basak, PhD

ABSTRACT

Objective: Identification of causative mutations in 3 consanguineous families (with 4 affected members) referred to our center with young-onset motor neuron disease and overlapping phenotypes resembling autosomal recessive juvenile amyotrophic lateral sclerosis (ARJALS) and autosomal recessive hereditary spastic paraplegia (ARHSP).

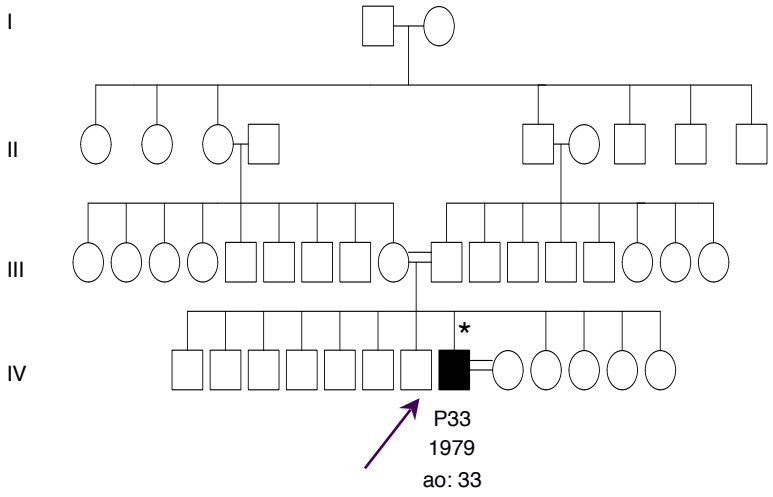
Methods: Patients have a slowly progressive motor neuron disease with upper and lower motor neuron dysfunction. There is distal muscle weakness and atrophy associated with pyramidal signs. Whole-exome sequencing was performed on the patients and the unaffected parent samples to identify disease-causing mutations. Variants were prioritized according to their predicted pathogenicity and their relevance to the clinical phenotypes.

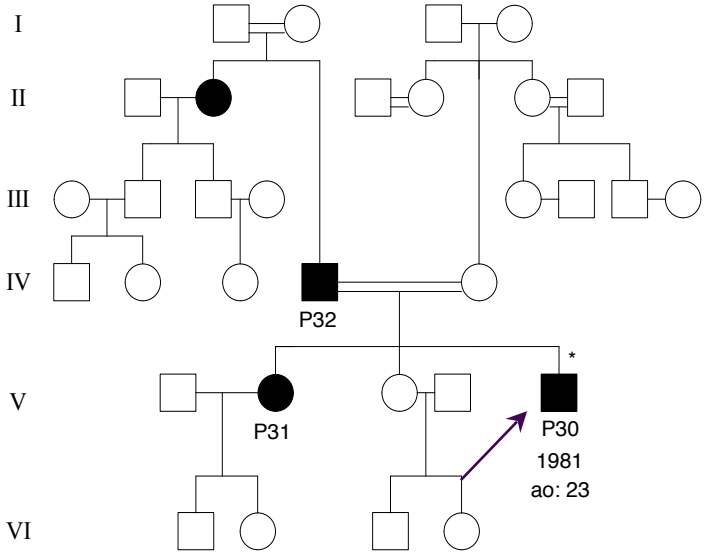
Results: Five distinct homozygous mutations within the SPG11 gene were identified, 3 of which

Fam 15 & 16: SPG7

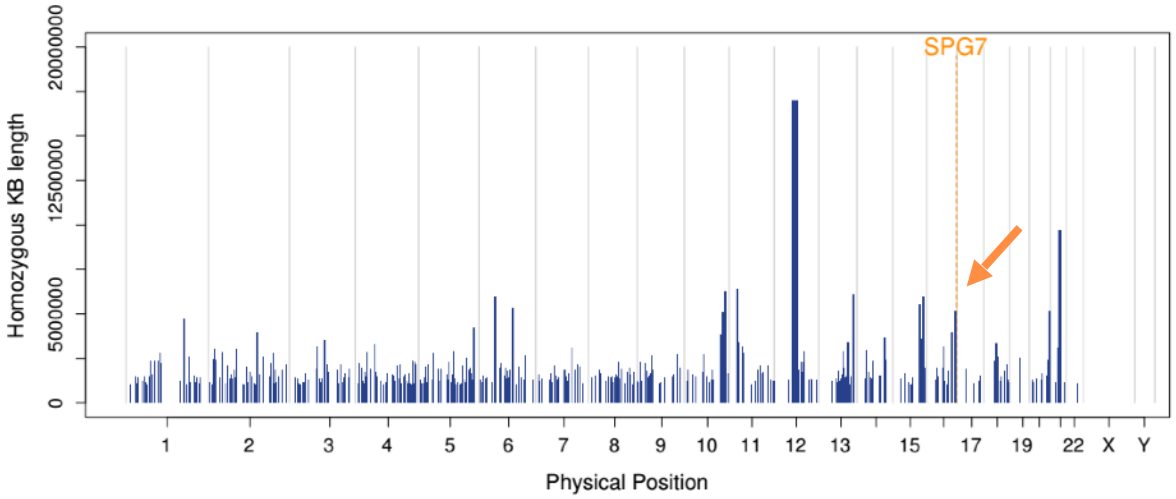
Adult onset ataxia

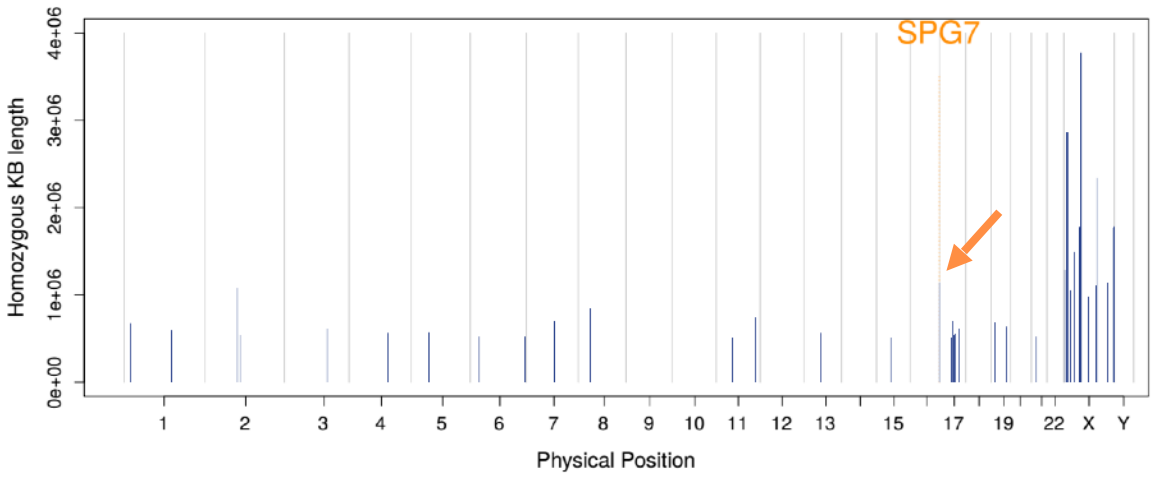
P33 developed spasticity





Fam 15 & 16: SPG7





Homozygosity mapping: Chromosome 16

SPG7

Fam 15 p. Ala658Thr

Fam 16 p.Thr588Met

Fam 15 & 16: SPG7

SPG7: Paraplegin

Fam 15 p. Ala658Thr (hom)

Fam 16 p.Thr588Met (hom)

Autosomal Recessive Hereditary Spastic Paraplegia 7

OPEN

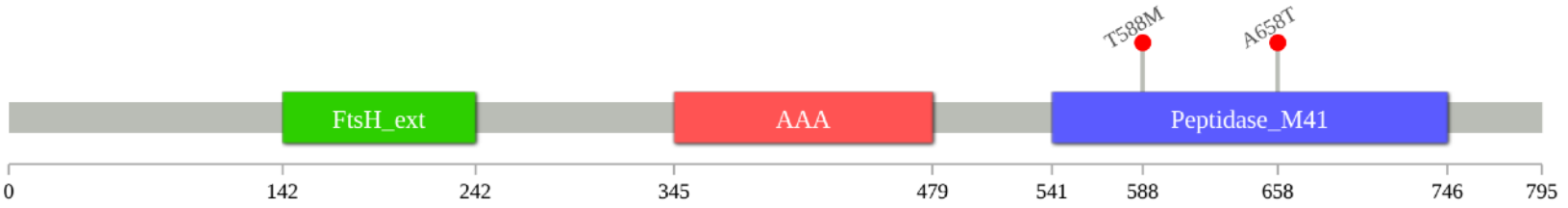
Citation: Human Genome Variation (2015) 2, 15012; doi:10.1038/hgv.2015.12

www.nature.com/hgv

DATA REPORT

Predominant cerebellar phenotype in spastic paraplegia 7 (SPG7)

Hiroyuki Yahikozawa[1], Kunihiro Yoshida[2], Shunichi Sato[1], Norinao Hanyu[1], Hiroshi Doi[3,4], Satoko Miyatake[4] and Naomichi Matsumoto[4]

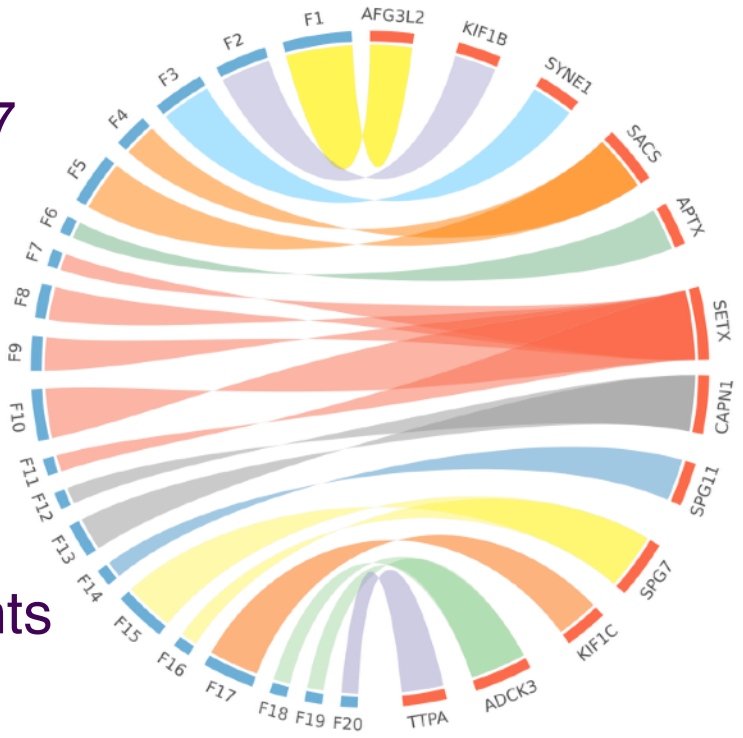


Overall Results

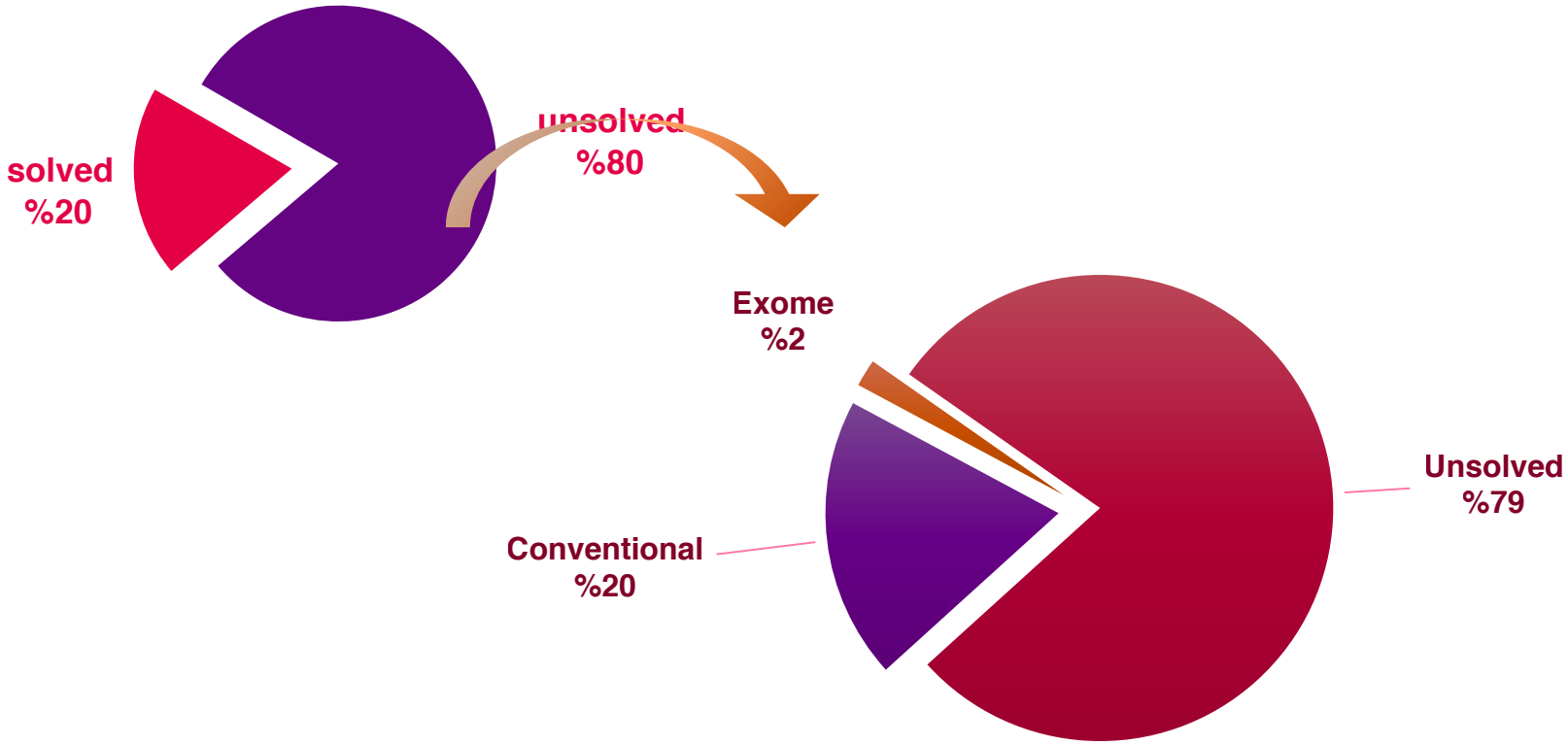
Disease cause identified in 20 / 47

1 case later diagnosed with HD

Ongoing analysis in 26 / 47 patients



Ataxia Genetics in Turkey - updated



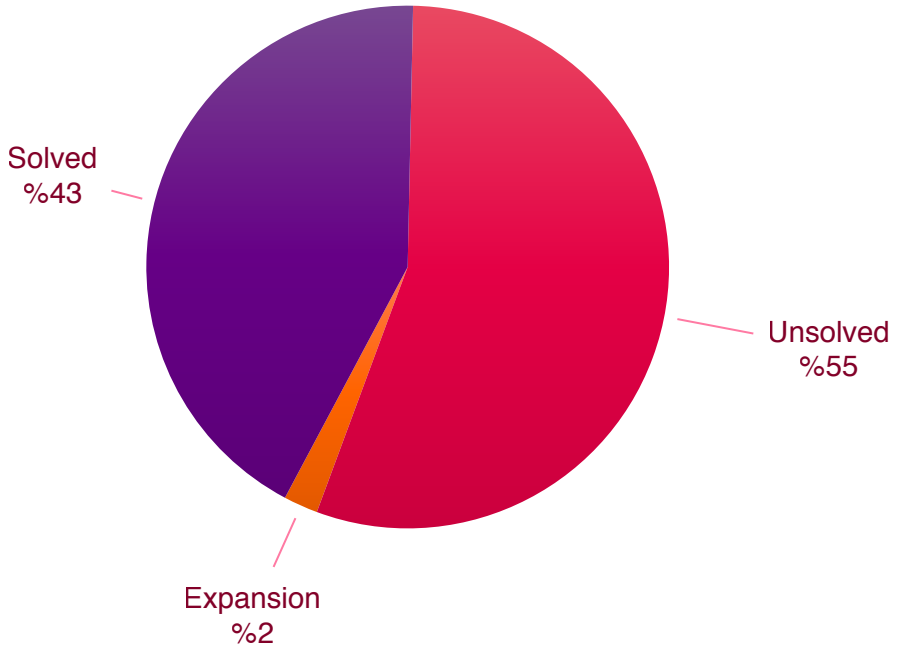
The Outcome of WES in Investigated Ataxia Families

High yield

Novel genotype-phenotype associations

Precise diagnosis

Possible treatments



Why 55% unsolved: WES Technical Shortcomings

Noncoding regions of the genome

(~99%)

Inability to detect

- repeats
- large indels
- copy number variants

Choice of software

How to proceed in 55%?

Variants of unknown significance (VUS)

- need of more detailed clinical information
- further confirmation of suspected variants

Oligogenic inheritance

Epigenetics

Environmental factors

Novel disease genes

Path to novel disease gene discovery

Detailed phenotyping of all affected individuals

Increase sample size with

similar clinical features

Alternative strategies

- Network analysis
- Combine with epigenome

Dissection of ataxia: Conclusion

Detailed phenotyping of patients CRUCIAL

- One gene multiple phenotypes

Keeping pace with developing tools in bioinformatics

IMPORTANT

Interdisciplinary collaborations on several levels - a MUST

Translational research / Precision medicine – the FUTURE

Acknowledgements

Supervisor: Prof. A. Nazlı Başak

Prof. Hande Çağlayan

Prof. Esra Battaloğlu

Prof. Sibel Ertan

Clinicians

Family members

Suna and İnan Kıraç Foundation

Atay Vural

Jan H. Veldink

Kristel Kool van Eijk

Sara L. Pulit

Thank you!