Complex Genetics and Disease Mechanisms in a Turkish Ataxia Cohort
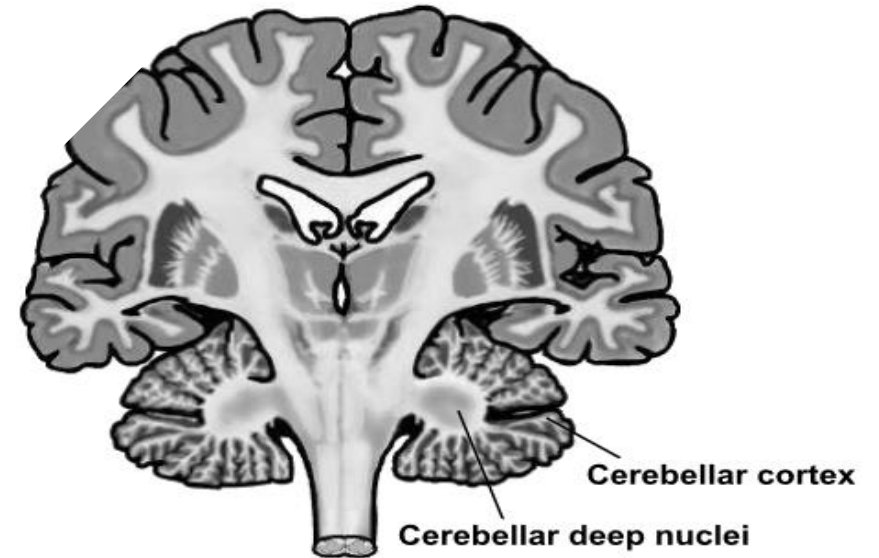
Gülşah Şimşir

MSc Thesis Defense

August 2nd, 2021

- Introduction

  – The Cerebellum

  – Ataxias

- Study Cohort

- Methods

- Results

- Discussion

- Conclusion

The Cerebellum

- Over 50% of the total number of neurons in the brain

- The cerebellum is involved in the following functions:
  - Maintenance of balance and posture
  - Coordination of voluntary movements
  - Motor learning
  - Cognitive functions

Knierim, J. (n.d.). Chapter 5: Cerebellum.

Figure 1. Major parts of the cerebellum

- Has 2 major parts:
  - Cerebellar deep nuclei: sole output structure of the cerebellum
  - Cerebellar cortex: has all the neurons in the cerebellum
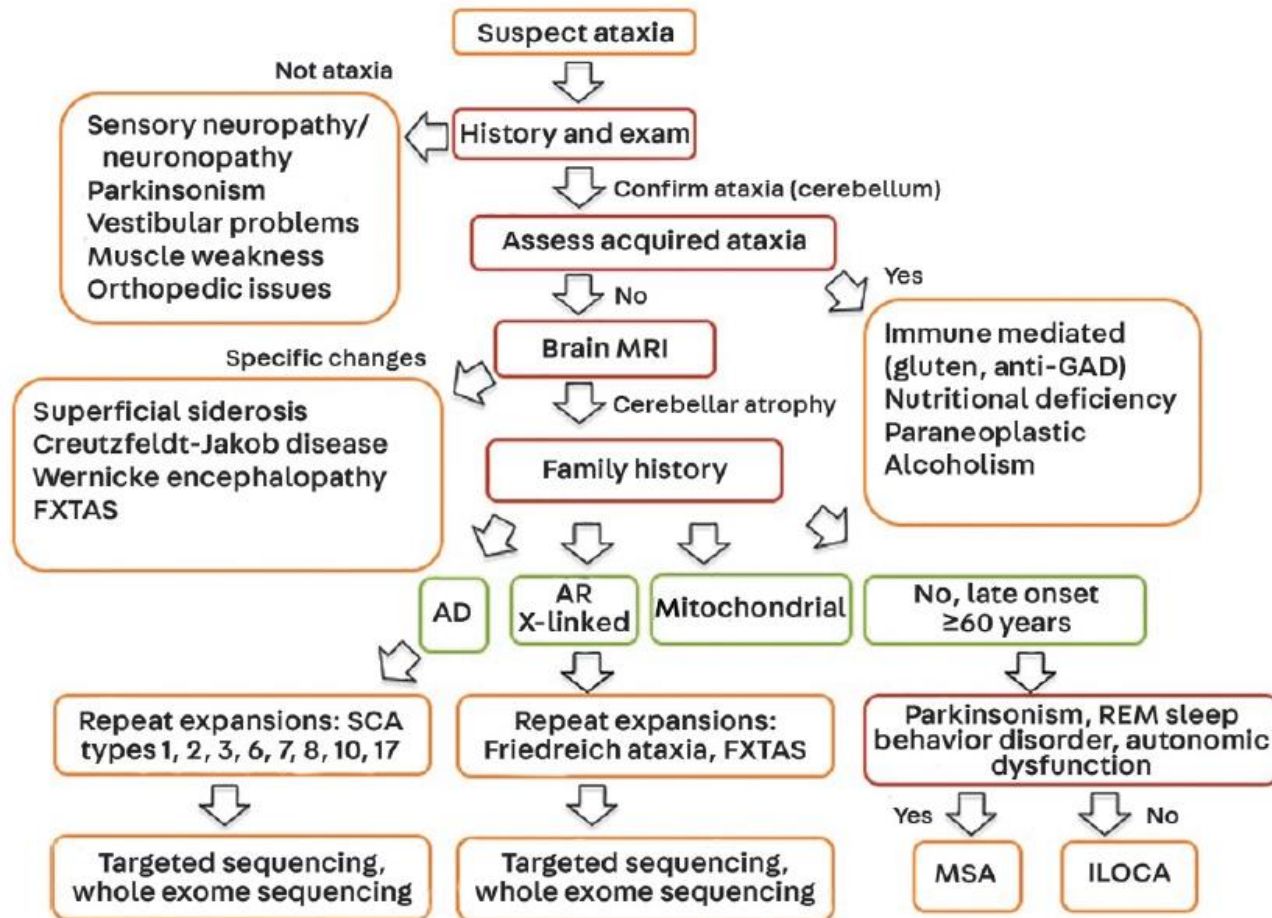
Ataxias: "a taxis"

- Heterogenous group of neurodegenerative diseases (NDD)

- Infectious and immune mediated to degenerative

- Genetic

- Overlapping with other NDD especially hereditary spastic paraplegias

Blood parameters	Cerebellar disorder
Reduced levels of ATM	Ataxia-telangiectasia
Reduced levels of vitamin E	AVED
Increased levels of cholestanol	CTX
Increased levels of oxysterols	Niemmann-pick type C
Increased levels of lactate	Mitochondrial ataxias

Table 1. Blood studies in cerebellar ataxias

Kuo, 2019. *CONTINUUM Movement Disorders.*

Figure 2. The diagnostic workflow for cerebellar ataxia

Dominant Ataxias (Spinocerebellar Ataxia-SCA)

- Prevalence: 2-4/100.000

- 48 types to date

- Repeat expansion vs non-repeat mutations

- Repeat expansion mutations cause anticipation

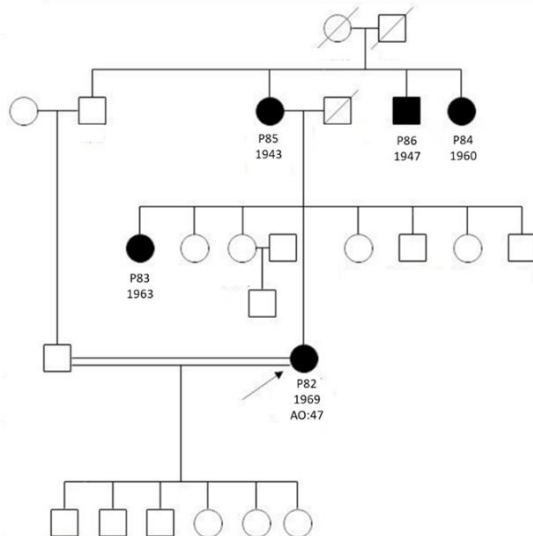- Polyglutamine accumulation, RNA toxic gain of function and protein function alteration

Figure 3. A sample dominant pedigree

Table 2. Autosomal Dominant Cerebellar Ataxias.

| Disease	Gene	Average AO (decade)	Clinical phenotype
Polyglutamine expansions			
SCA1	ATXN1	3-4	Spasticity, ophthalmoplegia, bulbar and sensory symptoms
SCA2	ATXN2	3 – 4	Slow saccades and sensory symptoms
SCA3	ATXN3	4	Spasticity, basal ganglia symptoms, sensory symptoms, amyotrophy including facial atrophy, and fasciculations
SCA6	CACNA1A	5 – 6	Pure cerebellar ataxia and downbeat nystagmus
SCA7	ATXN7	3 – 4	Visual loss, ophthalmoplegia, and spasticity
SCA17	TBP	4	Spasticity, basal ganglia symptoms, psychiatric disorders, and dementia
DRPLA	ATN1	4	chorea, seizures, dementia, myoclonus; often confused with Huntington disease
Non-coding expansions			
SCA8	ATXN8	4	Spasticity, sensory symptoms, cognitive and mood changes
SCA10	ATXN10	4	Epilepsy
SCA12	PPP2R2B	4	Tremor
SCA31	BEAN1	5 – 6	Pure cerebellar ataxia
SCA36	NOP56	5	Amyotrophy and hearing loss
Conventional SCAs			
SCA5	SPTBN2	3-4	Pure cerebellar ataxia
SCA11	TTBK2	3	Mild, remain ambulatory
SCA13	KCNC3	Childhood or adulthood	Intellectual disability
SCA14	PRKCG	3-4	Myoclonus
SCA15/16	ITPR1	4	Pure cerebellar ataxia
SCA19/22	KCND3	4	Slowly progressive, rare cognitive impairment, myoclonus, hyperreflexia
SCA27	FGF14	2	Early-onset tremor; dyskinesia, cognitive deficits
SCA28	AFG3L2	2	Spasticity, ophthalmoplegia, and ptosis
SCA35	TGM6	4	Hyperreflexia, Babinski responses, spasmodic torticollis

Synofzik, 2019. *Neuron*
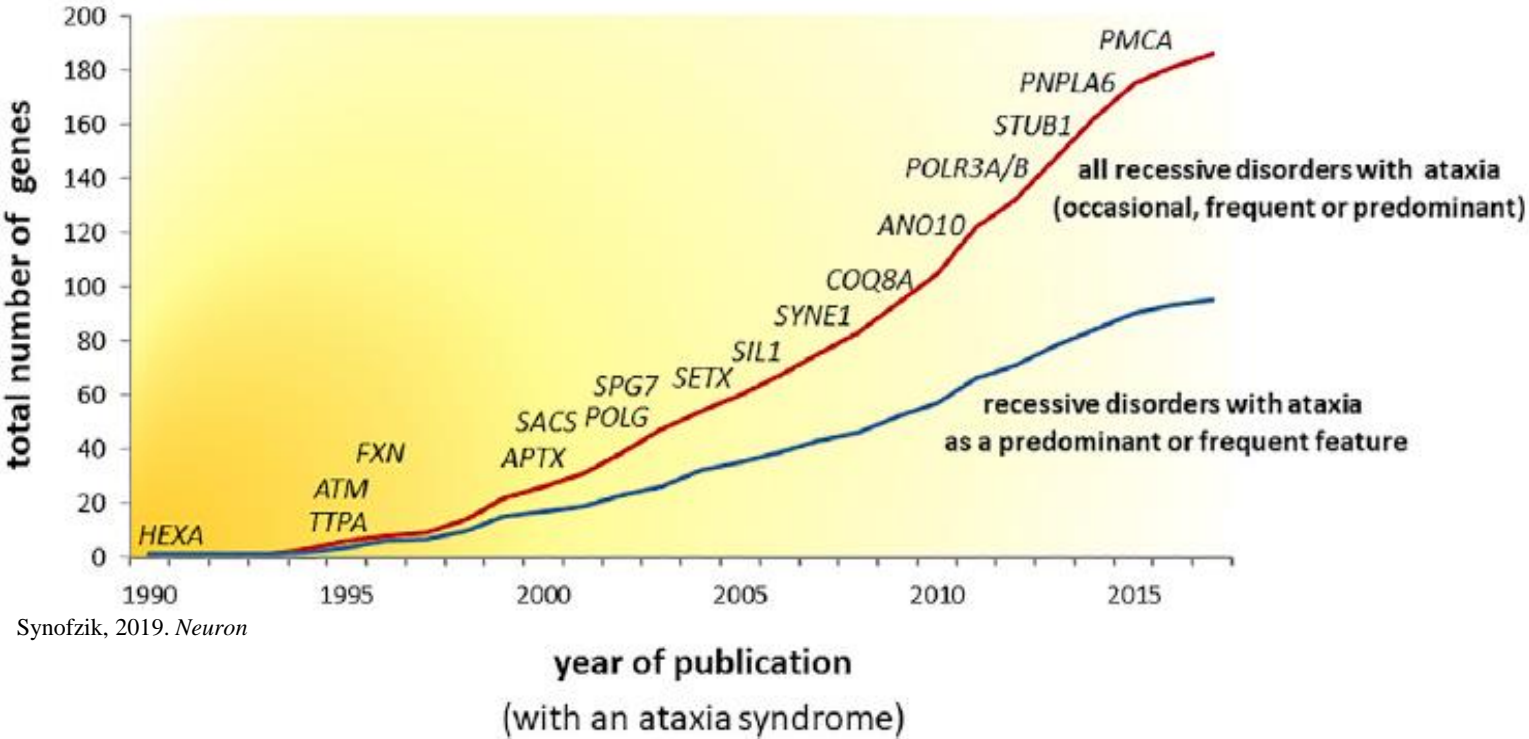
Figure 4. Rapidly increasing numbers of ARCA genes
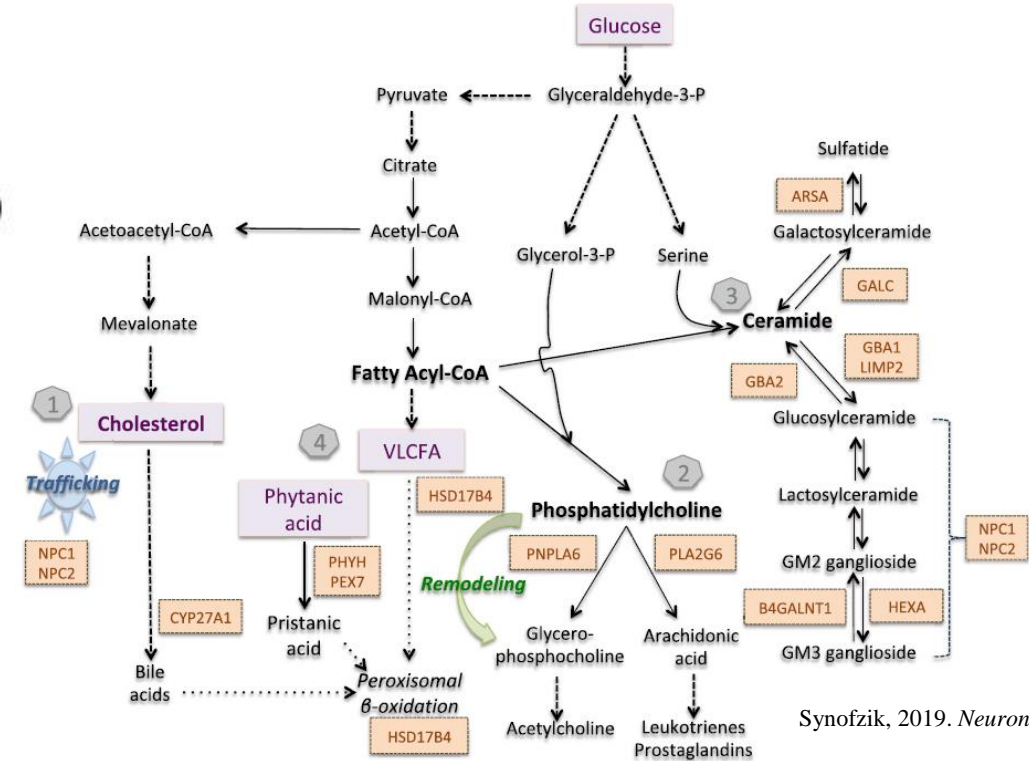

Synofzik, 2019. *Neuron*

Figure 5. ARCA genes involved in complex lipid metabolism

Some SCAR genes take role in certain pathways or cell clusters. For example:

1. DNA Damage Repair: ATM, SETX, APTX, PNKP
2. Mitochondrial Homeostasis (including mtDNA replication and repair): Frataxin, POLG, TWNK/C10ORF2, SPG7
3. Phospholipid Metabolism: PNPLA6, PLA2G6, ABHD12, DDHD1, DDHD2, CYP2U1
4. Sphingolipid Metabolism: FA2H, GBA2, GALC, HEXA, ASA, PSAP, GLB1
5. Autophagy-Lysosomal Activity: Niemann–Pick disease types C1 and C2, ATP13A2, SPG15, SPG11
6. Cilial Disorders: Joubert syndrome and related conditions

Investigating the complex genetic structure of an ataxia cohort by using

- whole exome sequencing

- bioinformatic analysis and tools

Figure 2. The diagnostic workflow for cerebellar ataxia

Kuo, 2019. *CONTINUUM Movement Disorders.*
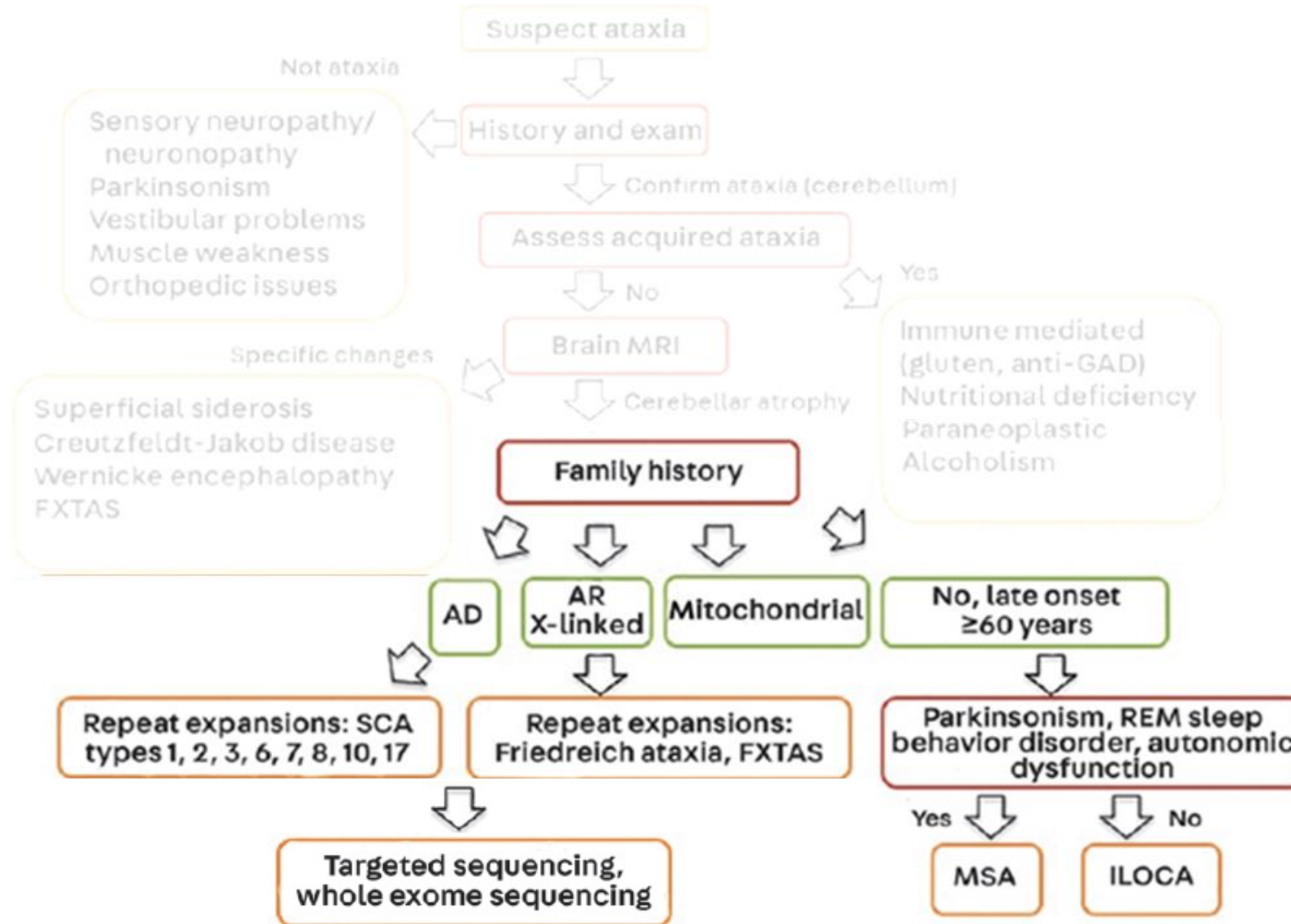
- 83 index patients with complex ataxia phenotype
- 59 out of 83 patients have consanguinity in family
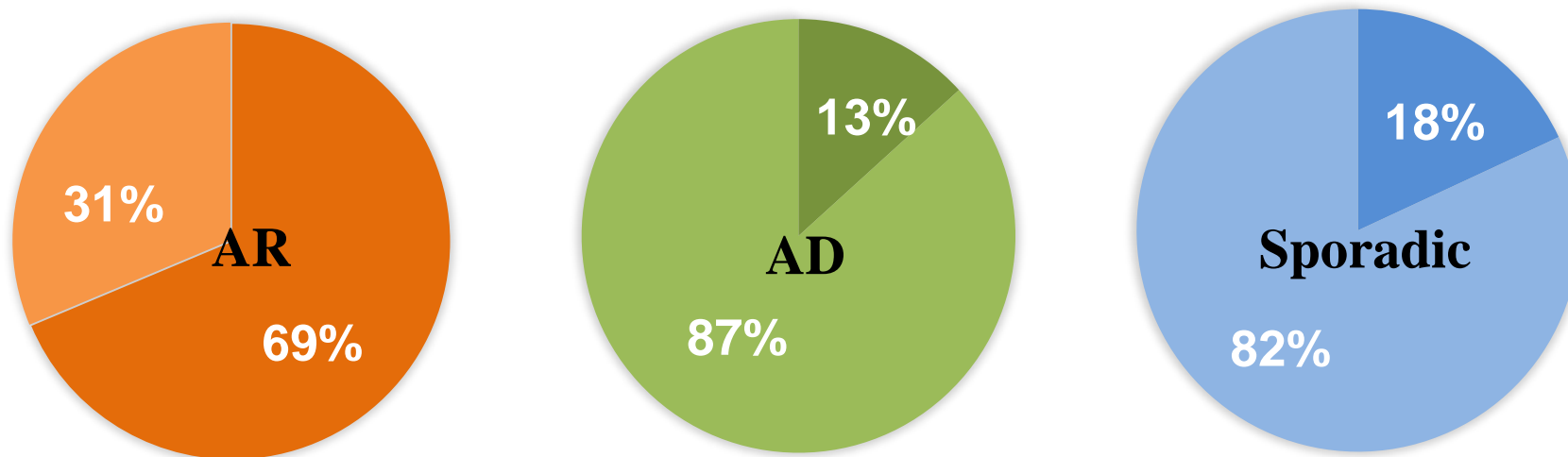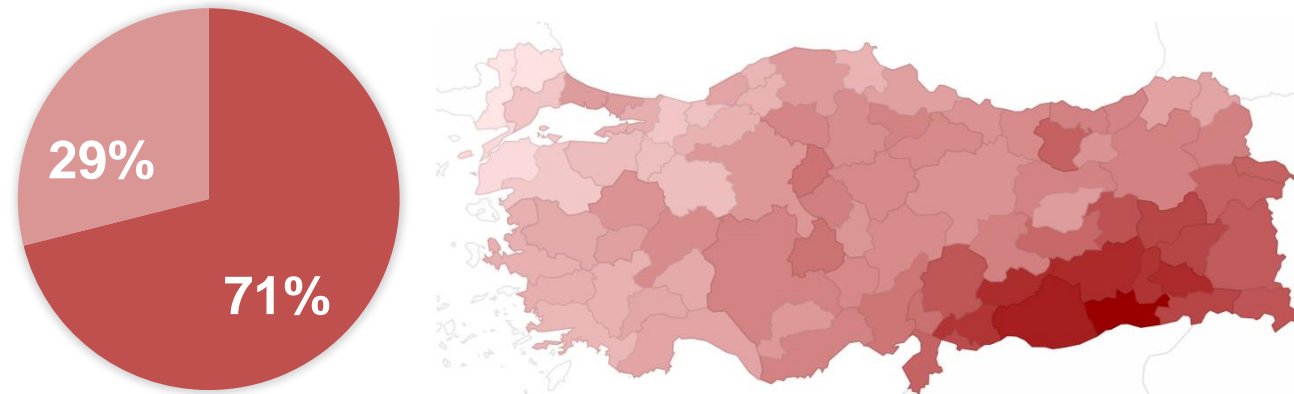

Figure 6. Distribution of inheritance of families

Figure 7. Distribution of consanguinity rate in the cohort and in Turkey

Whole Exome Sequencing (WES)

- Sequencing the protein-coding regions of the genome.

- 180.000 exons

- Covers 95% of the exons

- More than 150 genes were discovered by WES

- Cannot identify large structural variations and repeats

- Validated by Sanger seq and restriction enzyme digestion
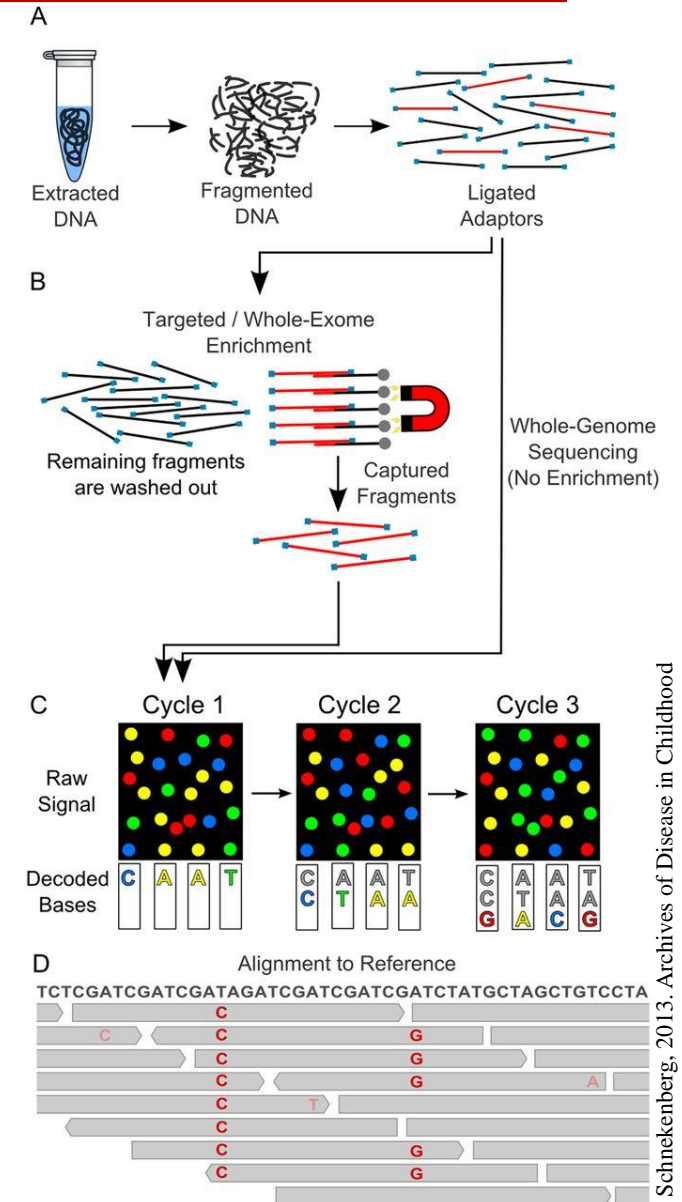
Schnekenberg, 2013. Archives of Disease in Childhood

Figure 8. Workflow of WES

Bioinformatic Pipeline I
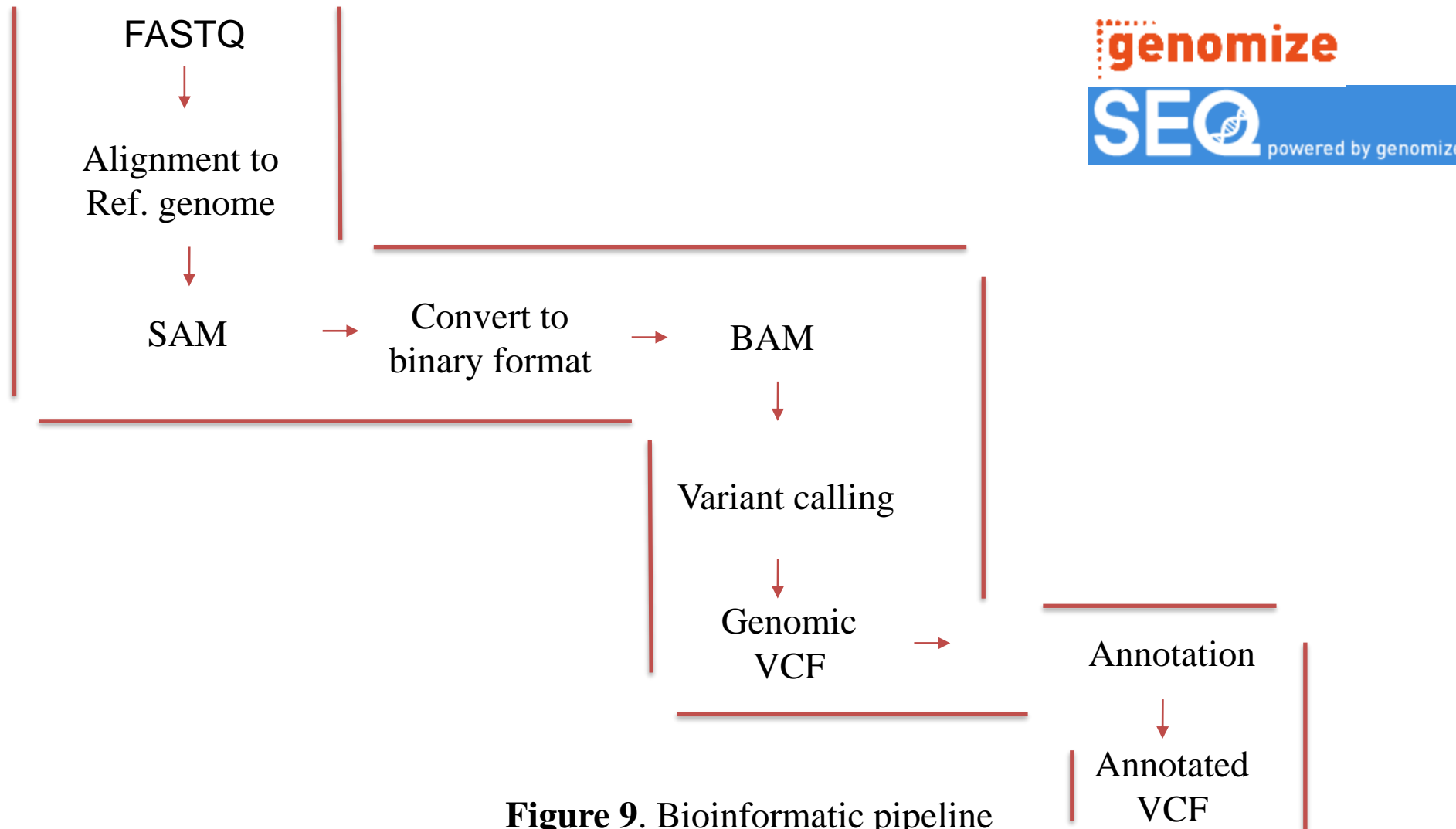
Figure 9. Bioinformatic pipeline

Bioinformatic Pipeline II

- Variant Prioritization

  - Mode of Inheritance

  - Variant Filtration: MAF< 1%

- MAF: 1000 Genomes, ExAc, gnomAD

  - In-house control

- Variant Classification: Pathogenicity tools

  - DANN, GERP, SIFT, MutationTaster, REVEL, MetalR, CADD

- Clinical Information: OMIM, ClinVar

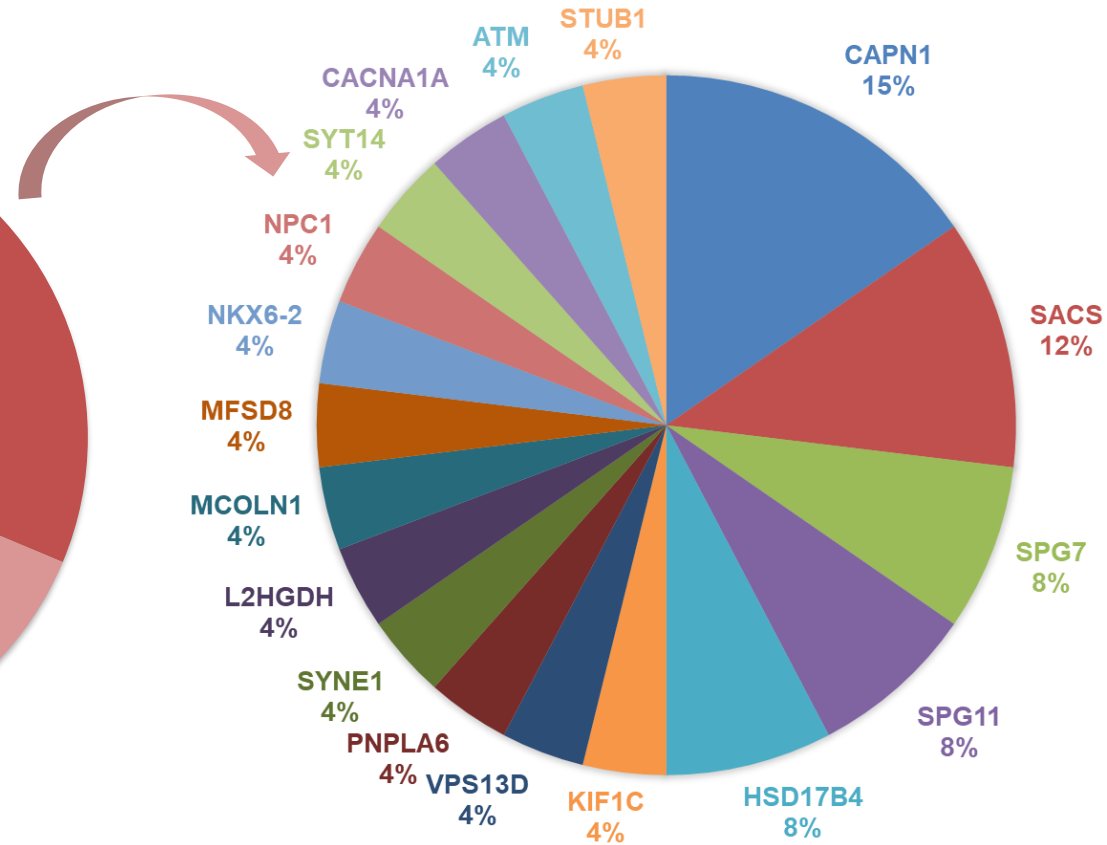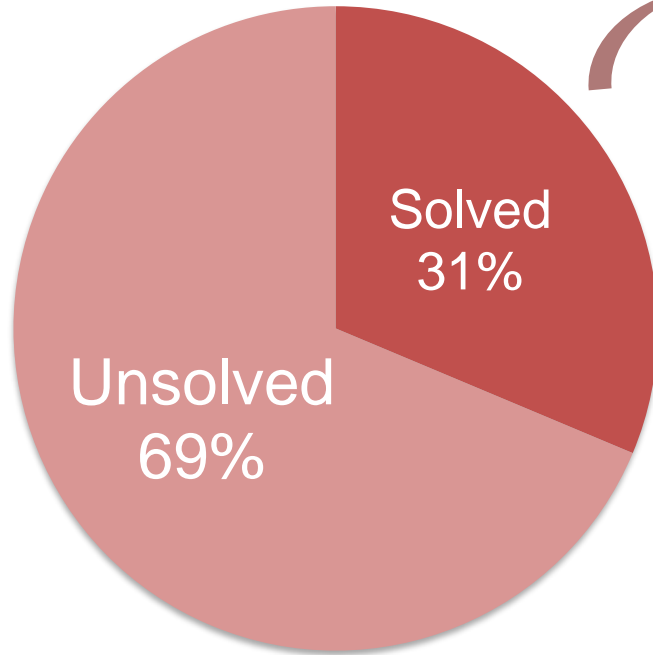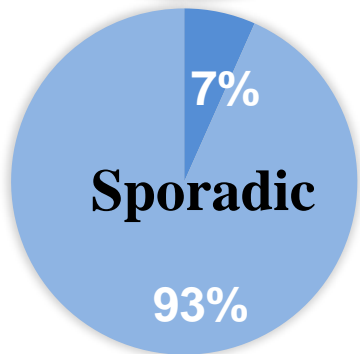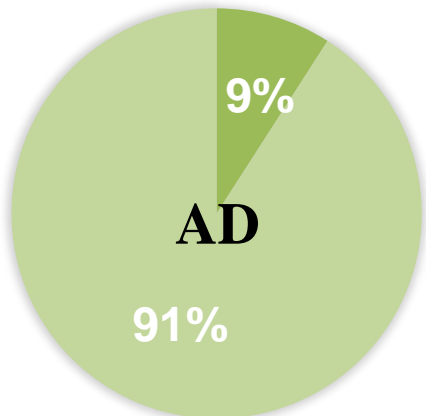- Pathogenic > Likely Pathogenic > Variant of Uncertain Significance (VUS) > Likely Benign > Benign
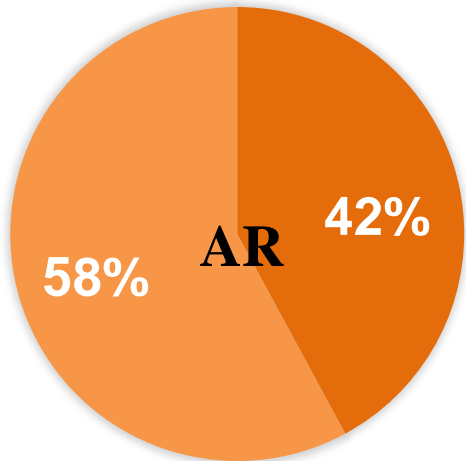
Figure 12. Diagnostic yield of WES (26/83)

Table 3. Online prediction tool scores and frequencies of the variants.

Chr. Location	Gene	Variant	ACMG (Varsome)	CADD	DANN	GERP ++	Mutation Taster	REVEL	MetalR	SIFT	gnomAD	Fam. Seg.
6:152 771784	SYNE1	p.Asp1124Gly	VUS: PM2, PP3	25.1	0.99	5.78	Disease causing	B	D	D	-	+
19:76 05926	PNPLA6	p.Arg314Trp	LP: PM1, PM2, PP5, PP2, PP3	29.1	0.99	5.38	Disease causing	B	T	D	-	+
5:118 813112	HSD17B4	p.Asp142Gly	LP: PP3, PM1, PM2	32	0.99	6.02	Disease causing	Pat	D	D	-	+
4:128 863274	MFSD8	p.Thr160Asn	LP: PM2, PM5, PP2, PP3	24.6	0.99	4.86	Disease causing	B	T	D	-	+
18:21 136368	NPC1	p.Arg389Cys	LP: PM2, PP5, PP2, PP3	30	0.99	5.97	Disease causing	Pat	D	D	0.000 003981	n.a

Pat: Pathogenic, LP: Likely pathogenic, VUS: Variant of uncertain significance, D: Damaging, T: Tolerated, B: Benign, n.a: not available

**Table 4.** Disease mechanisms of certain genes

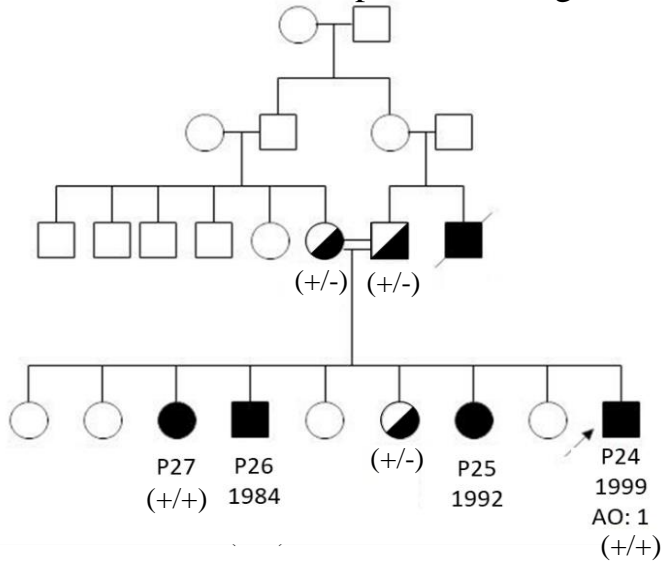| Mechanism	Gene
Mitochondrial Metabolism	SACS
	SPG7
	L2HGDH
DNA Repair Pathway	ATM
Autophagy and Lysosomal Activity	SPG11
Lipid Metabolism	HSD17B4
	PNPLA6
	NPC1
Proteolysis of substrates involved in cytoskeletal remodeling and signal transduction	CAPN1
Autophagy and Mitochondrial Clearance	VPS13D
Membrane trafficking in synaptic transmission	SYT14

Genes in Mitochondrial Metabolism:

- SACS: intermediate filament regulator

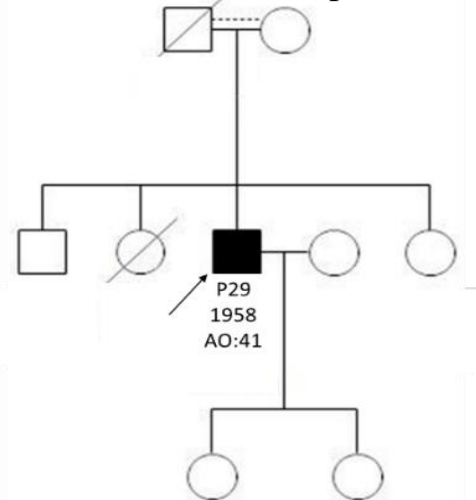- SPG7: mitochondrial protein control

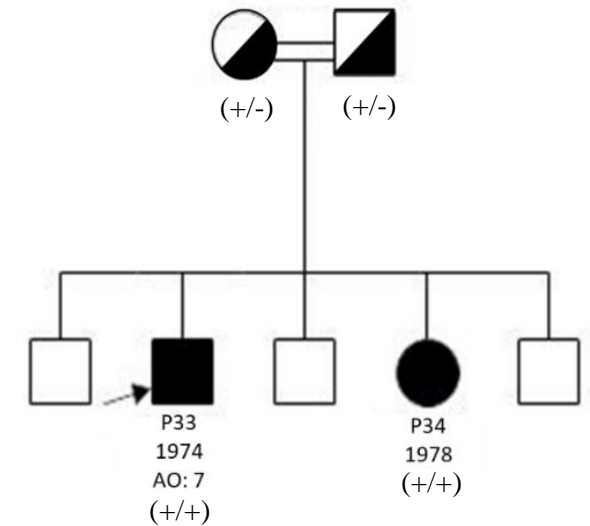- L2HGDH: energy production
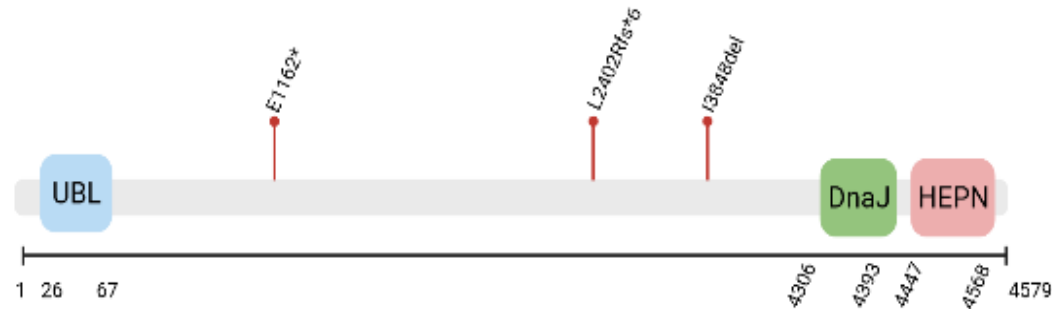
Fam. 16: SACS p.Leu2402ArgfsTer6

Fam. 14: SPG7 p.Gln621Ter **N**
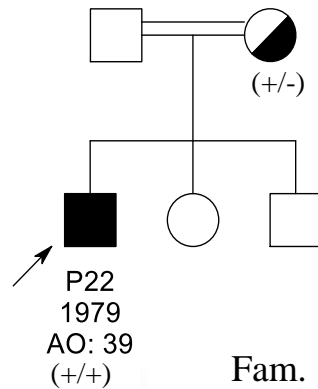
Fam. 18: SPG7 p.Ala572Val

Fam. 22: L2HGDH p.Pro302Leu
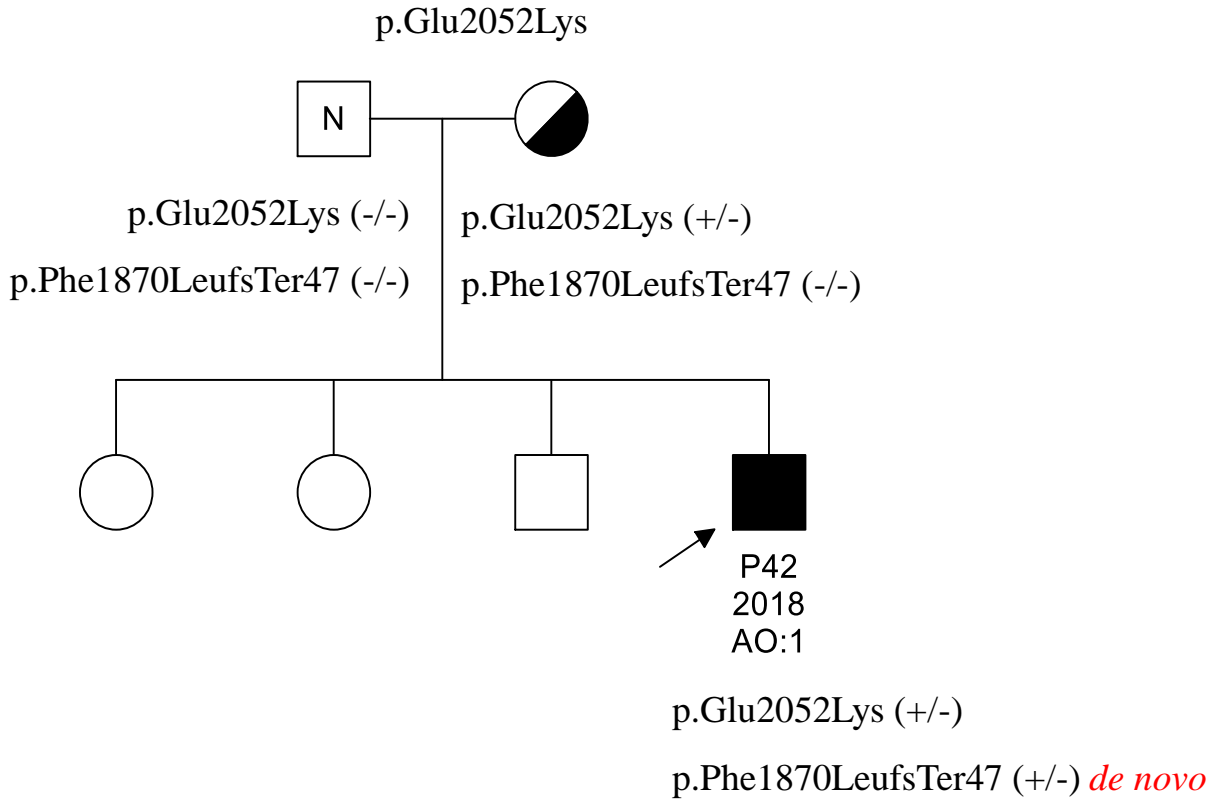
Genes in DNA Repair Pathway:

- ATM: double-strand break repair mechanism

Fam. 28: ATM p.Phe1870LeufsTer47

p.Glu2052Lys

p.Glu2052Lys (-/-)

p.Phe1870LeufsTer47 (-/-)   p.Glu2052Lys (+/-)

p.Phe1870LeufsTer47 (-/-)

P42
2018
AO:1

p.Glu2052Lys (+/-)

p.Phe1870LeufsTer47 (+/-) *de novo*

FUS variant from father confirmed the parenthood

Genes in Autophagy and Lysosomal Activity

- SPG11: autophagic lysosome reformation

Fam. 8: SPG11 p.His2388ThrfsTer6 **N**

P12
1987
AO: congenital
(+/+)
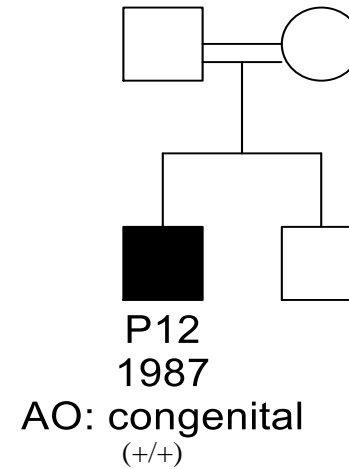
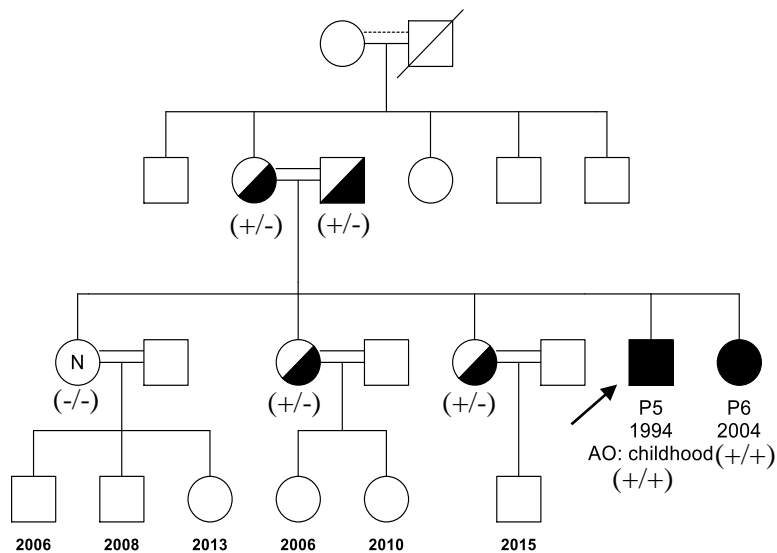Fam. 12: SPG11 p.Val1270Asp

(+/-)   (+/-)

P19
1992
AO: 7
(+/+)
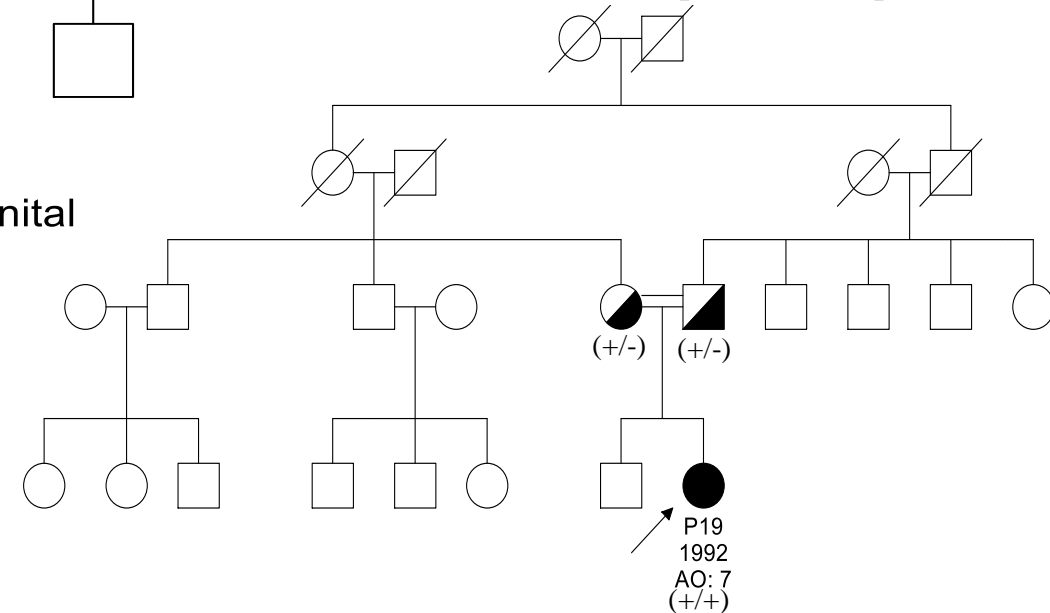
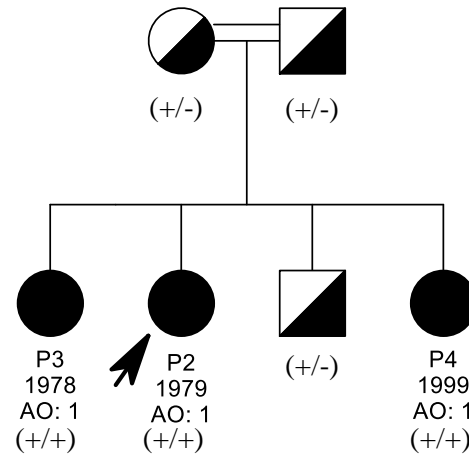Genes in Lipid Metabolism:

- HSD17B4: β-oxidation of VLCFA

- PNPLA6: de-esterification of phosphatidylcholine

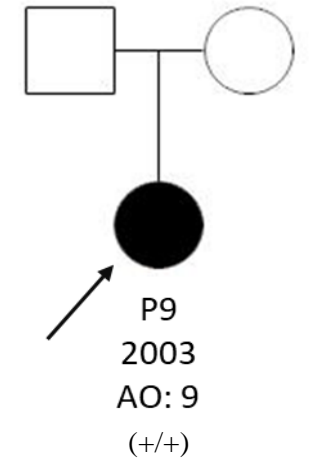- NPC1: trafficking of intracellular cholesterol

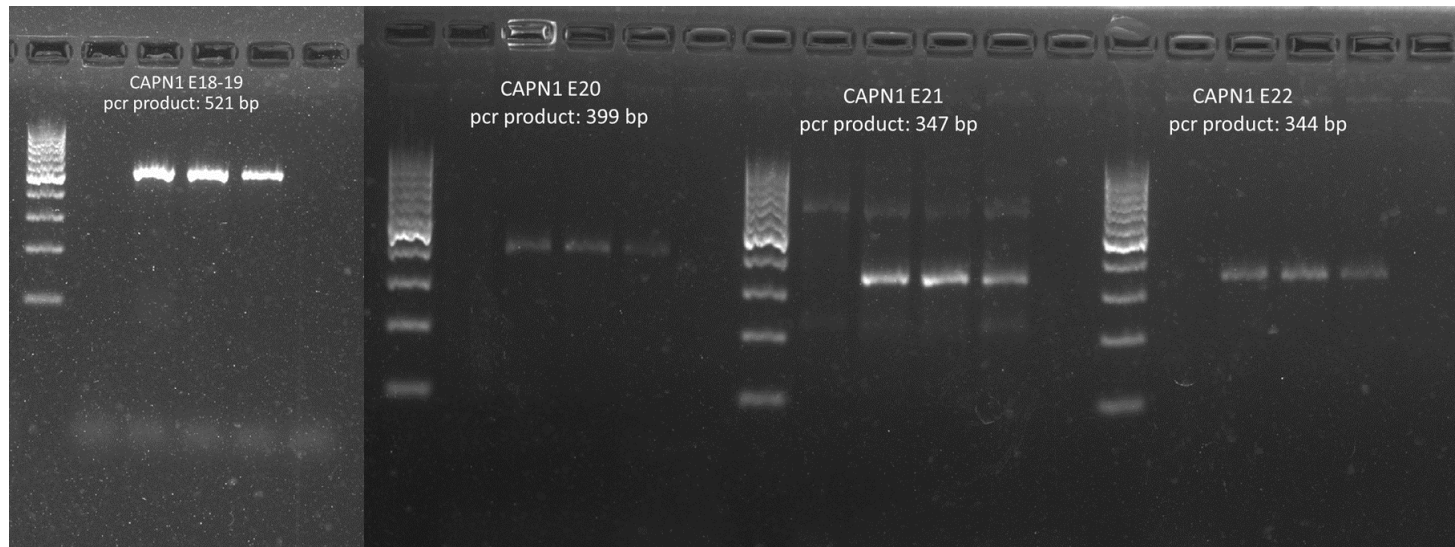Fam. 3: HSD17B4 p.Asp142Gly **N**
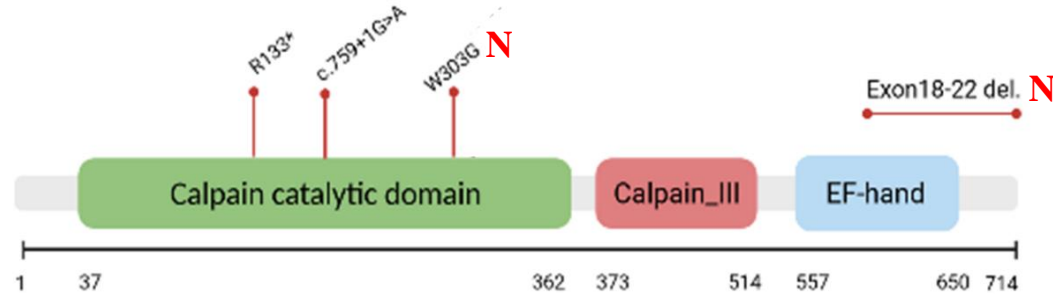

Fam. 2: PNPLA6 p.Arg314Trp
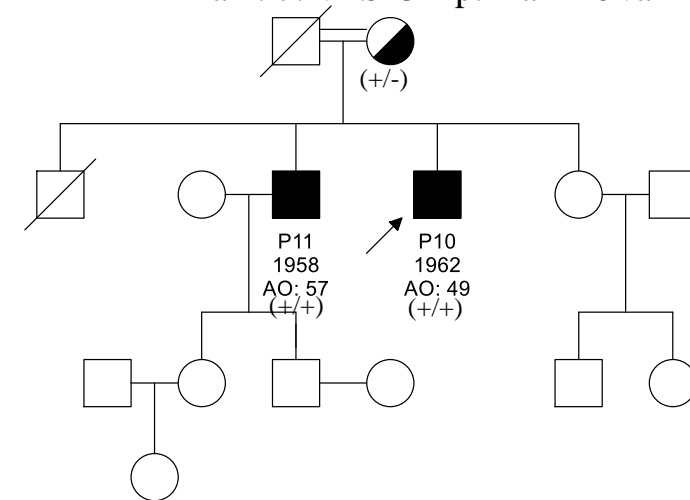

Fam. 6: NPC1 p.Arg389Cys
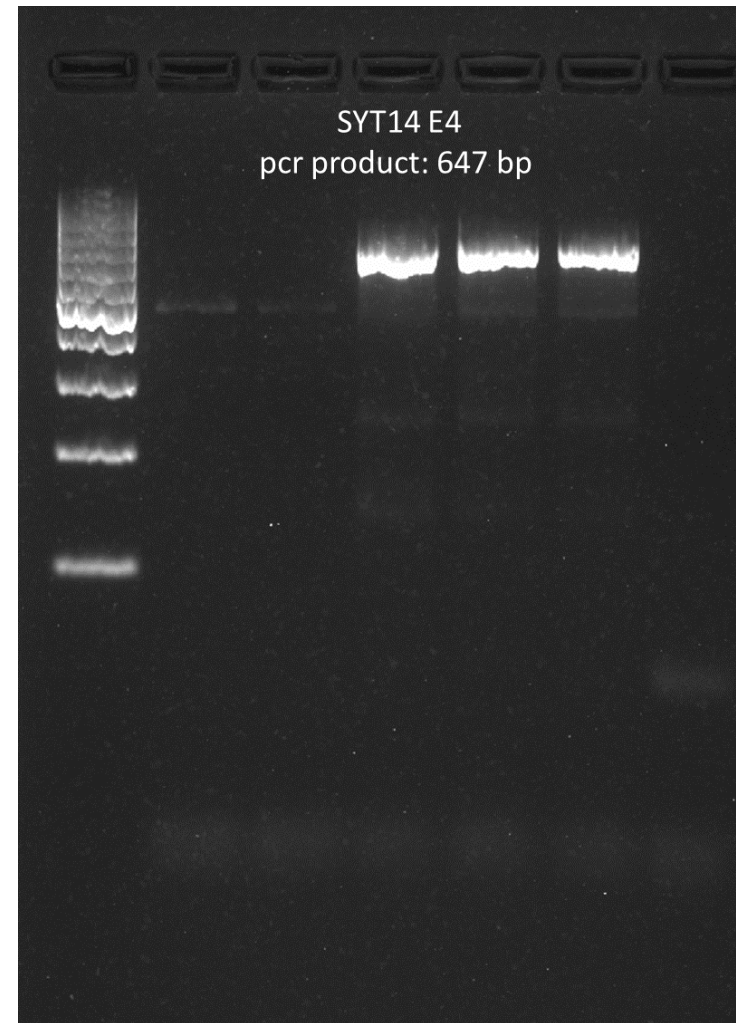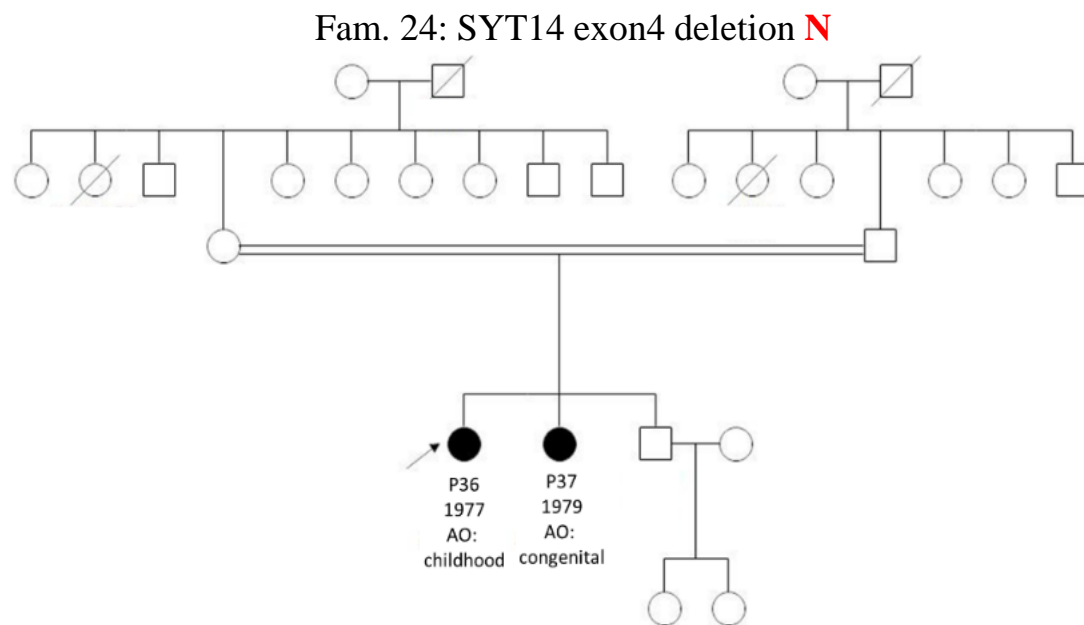
Genes with Different Mechanisms:

- CAPN1: proteolysis of substrates involved in cytoskeletal remodeling and signal transduction

- VPS13D: autophagy and mitochondrial clearance

Fam. 7: VPS13D p.Ala4210Val

- SYT14: mediating membrane trafficking in synaptic transmission

Fam. 24: SYT14 exon4 deletion N

P36
1977
AO:
childhood

P37
1979
AO:
congenital

SYT14 E4
pcr product: 647 bp

WES: Advantages
- Identifies variants across a wide range of applications
- Comprehensive coverage of coding regions
- Cost-effective alternative to whole-genome sequencing
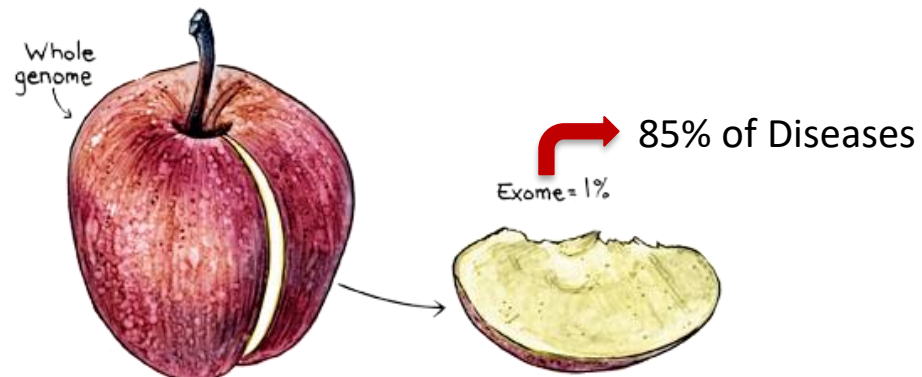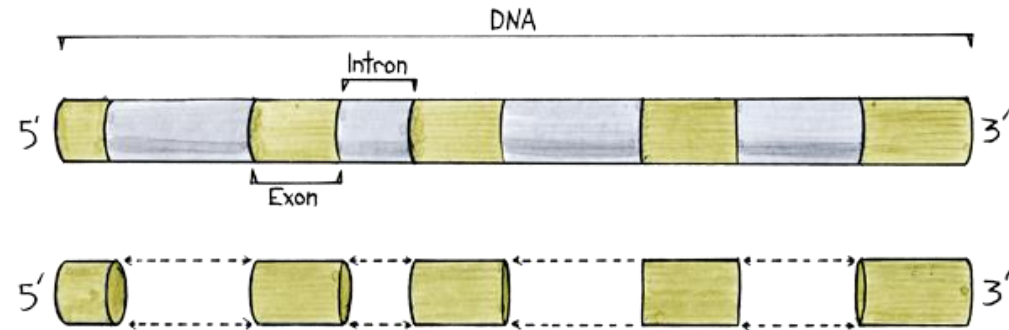- Less data, faster analysis

Figure 13. WES vs WGS

- WES: Shortcomings
    why 69% unsolved?
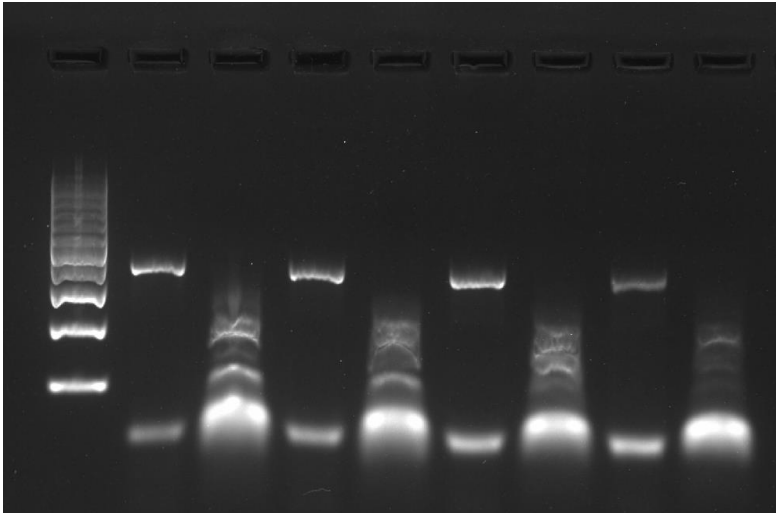
Figure 15. Restriction enzyme digestion gel image

- How to solve remaining patients?
    - Variants of Uncertain Significance (VUS)
    - More detailed clinical info
    - Oligogenic inheritance
    - Epigenetics
    - Environmental factors
    - Novel genes

- Molecular basis of ataxias in Turkey
- Precise Diagnosis
- Novel genotype-phenotype associations
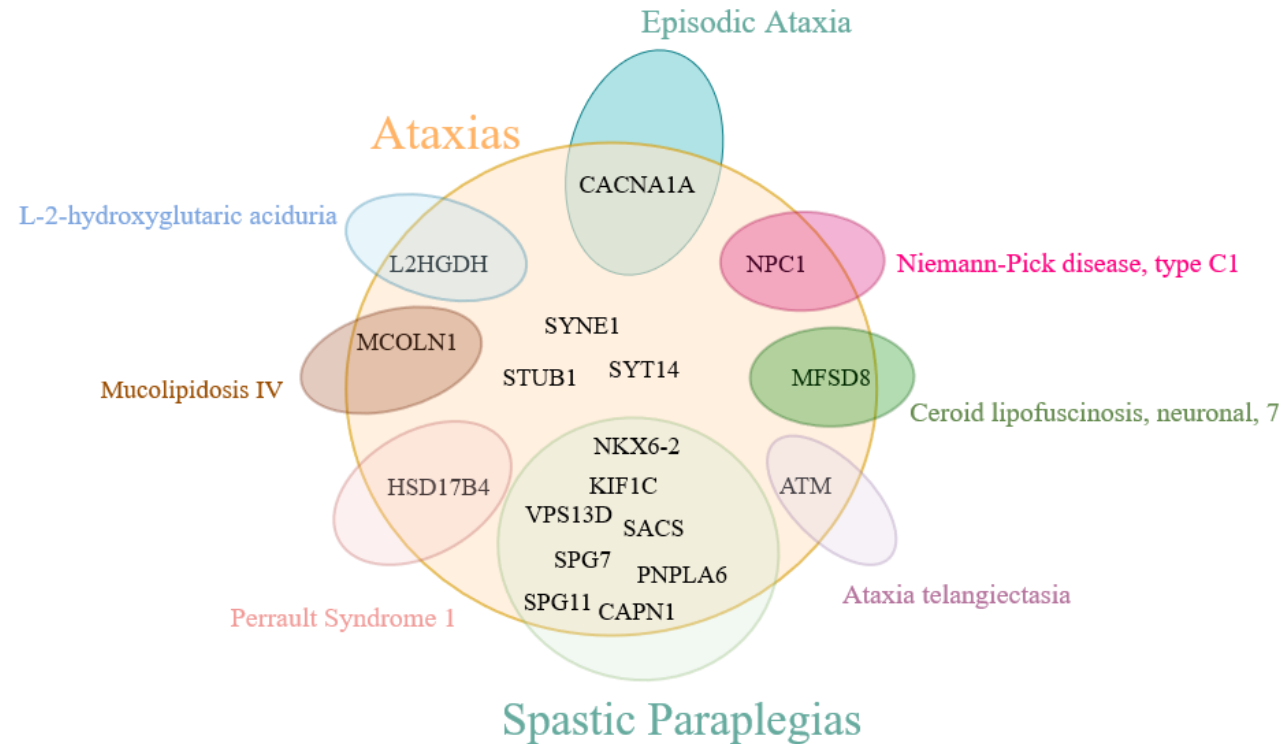- Different mechanisms
- Possible treatments

Figure 14. The genes identified in the cohort

- Causative variants were found in 26/83 probands, corresponding to a diagnostic yield of 31%

- High heterogeneity and complexity

- Genetic overlap among different NDD

Molecular Complexity of Spastic Ataxias and Hereditary Spastic Paraplegias in Turkey

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ORIGINAL ARTICLE

Cerebellar cognitive-affective syndrome preceding ataxia associated with complex extrapyramidal features in a Turkish SCA48 family

R. Palvadeau[1] · Z. E. Kaya-Güleç[2] · G. Şimşir[1] · A. Vural[3] · Ö. Öztop-Çakmak[3] · G. Genç[4] · M. S. Aygün[5] · O. Falay[6] · A. Nazlı Başak[1] · S. Ertan[3]

RESEARCH ARTICLE

The Complex Genetic Landscape of Hereditary Ataxias in Turkey and Implications in Clinical Practice

Atay Vural, MD, PhD,[1] Gülşah Şimşir, BSc,[2] Şeyma Tekgül, BSc,[2] Cemile Koçoğlu, MSc,[3] Fulya Akçimen, MSc,[3]
Ece Kartal, PhD[3] Neşli E. Şen, MSc[3] Suna Lahut, PhD[3] Özgür Ömür, MSc[3] Nazan Şener, PhD[3] Tuğçe Gül, MSc[2]

BRIEF COMMUNICATION

A novel PNPLA6 mutation in a Turkish family with intractable Holmes tremor and spastic ataxia

Ahmed S. Emekli[1] · Bedia Samanci[1] · Gülşah Şimşir[2] · Hasmet A. Hanagasi[1] · Hakan Gürvit[1] · Başar Bilgiç[1] · A. Nazlı Başak[2]

Thank You!