Riunione Regionale SIN Campania

Next generation sequencing in a series of patients with previously undiagnosed hereditary ataxias

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Napoli

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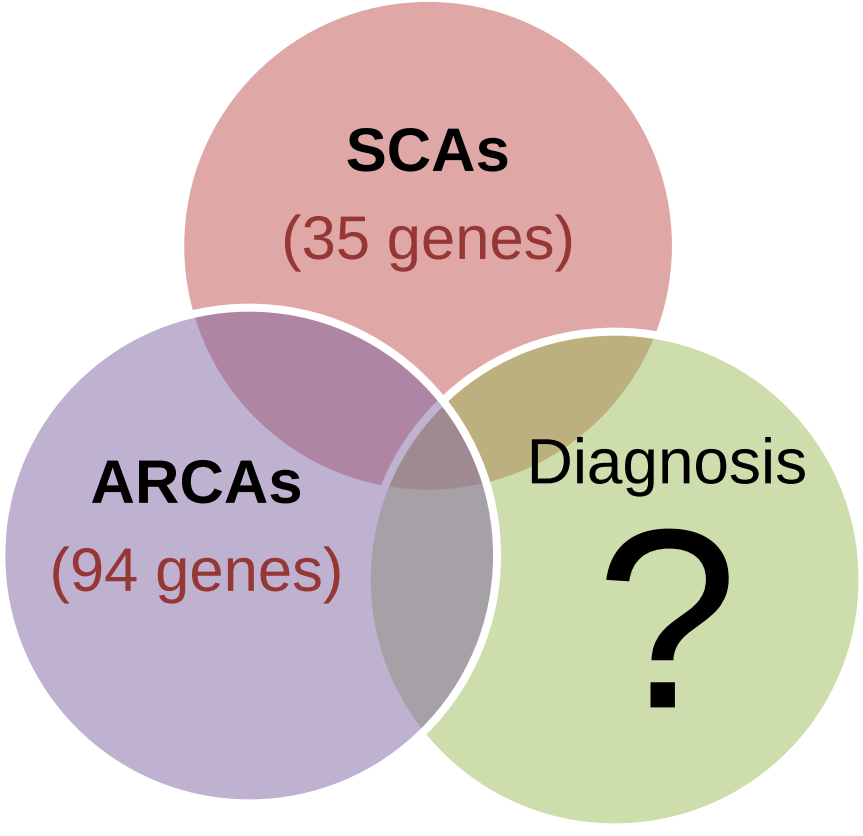
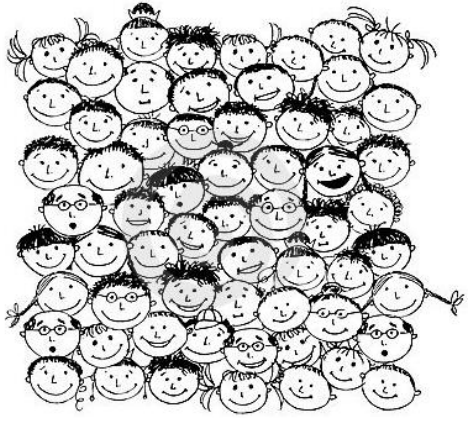
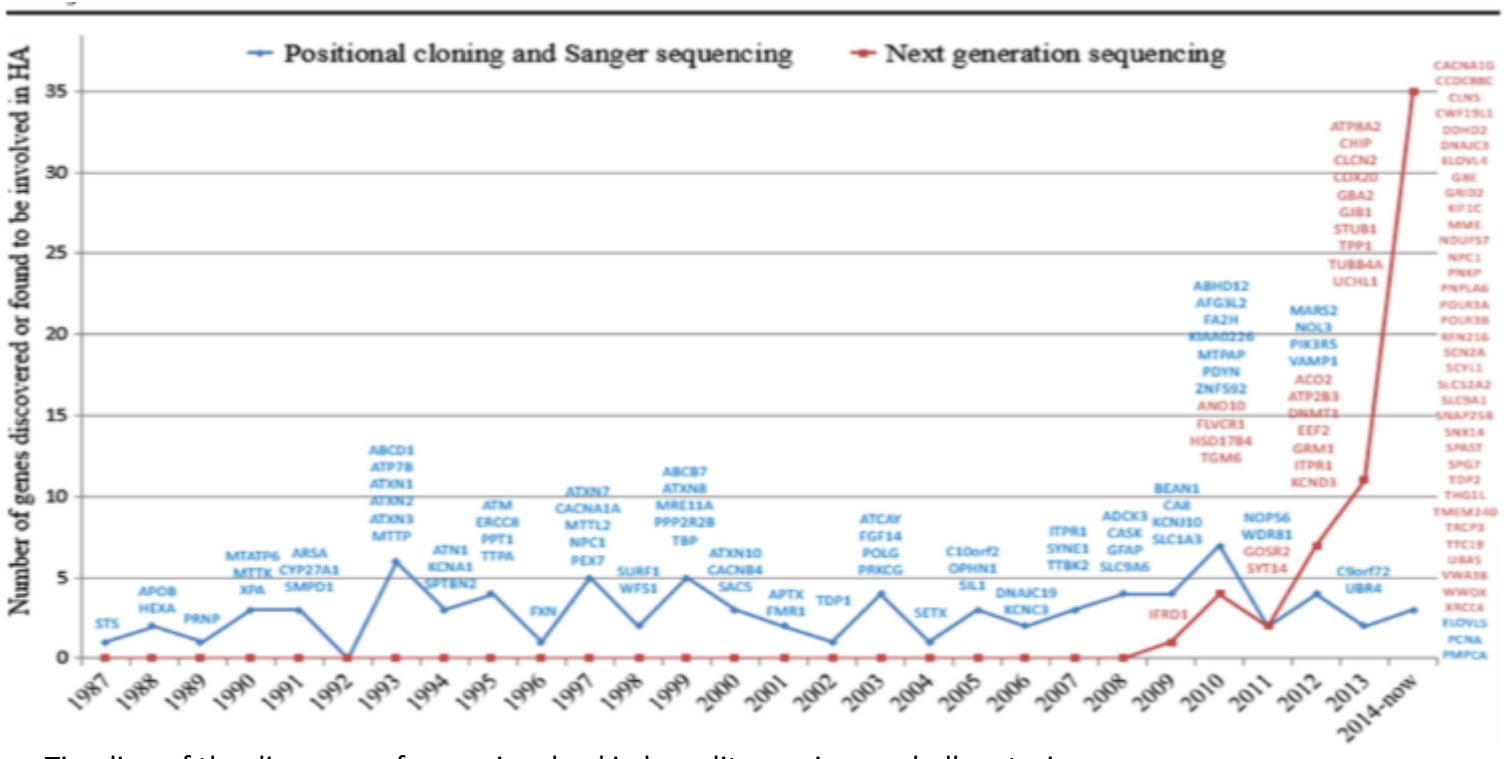
HEREDITARY ATAXIAS





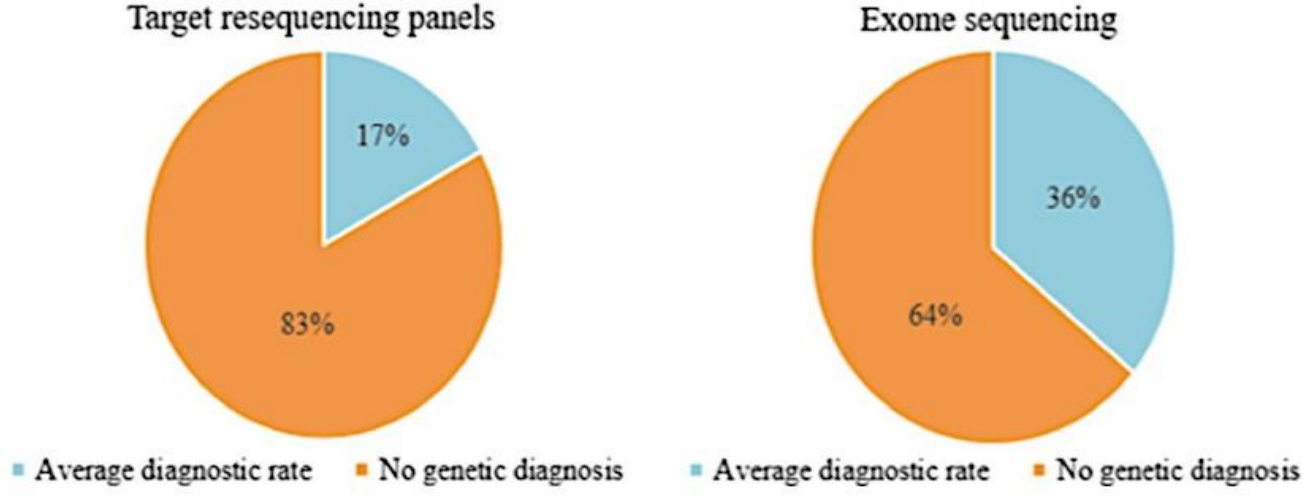
Ataxia: From Linkage analyses to NGS



Timeline of the discovery of genes involved in hereditary spinocerebellar ataxia
(Galatolo et al. 2017)

Next generation sequencing and Ataxia: the state of art

- Target Resequencing Panels (TRP)
- Exome Sequencing (ES)
- Whole Genome Sequencing (WGS)



Our Project

To use NGS strategies (Target Resequencing Panel, Exome Sequencing, Whole Genome Sequencing) to define the molecular characterization of a group of 106 patients with undiagnosed ataxia

Patients and Methods

Clinical evaluation

Diagnostic evaluation

- Causes of secondary ataxias were ruled out

Initial genetic screening

- Patients were tested for pathological CAG repeats in **SCA genes** and for the intronic GAA expansion in **FXN**

106 patients

Patients and Methods

Diagnosis

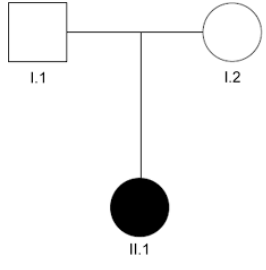
Target Resequencing Panel

- A gene panel containing 273 genes known or supposed to be related to hereditary ataxias was created using *SureDesign*

Exome Sequencing

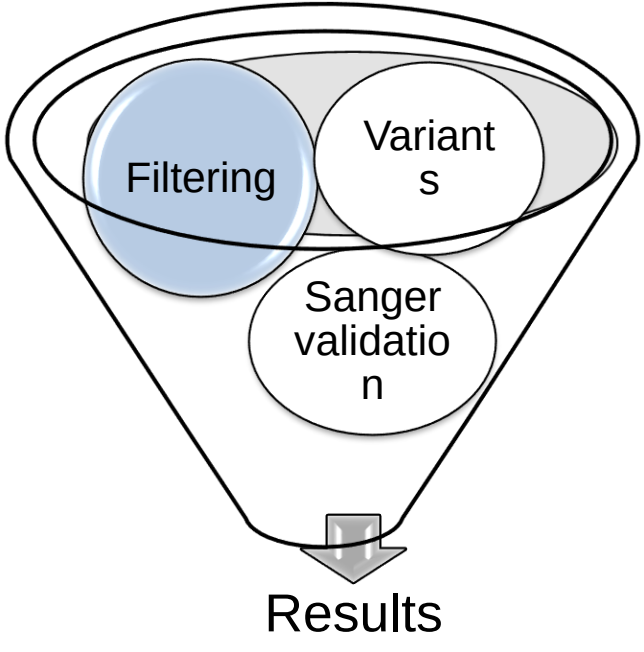
Whole Genome Sequencing

Trio GS



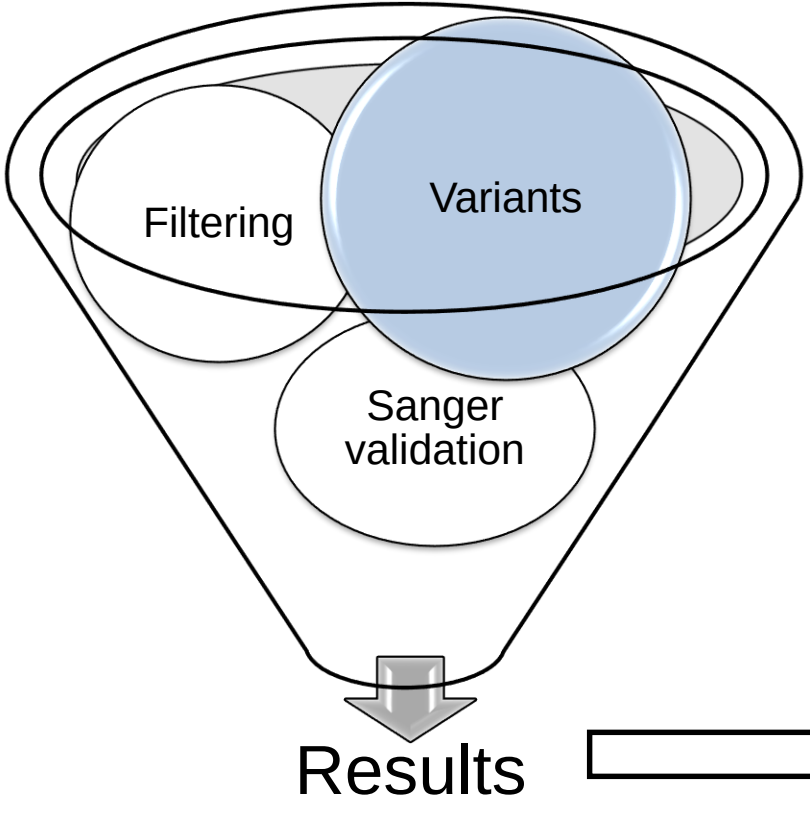
Data Analysis: Filtering

Using software *SureCall* which allows to align sequence raw data with reference human genome sequence



- ✓ Variants found are analyzed using *Ingenuity variant analysis* and filtered
  - Call quality <30
  - Read depth <20
  - Frequency > 1%
  - False positives (variants shared by more than 3 samples or more)
- ✓ Algorithms *SIFT* e *PolyPhen2* for missens mutations, *NeteGene2* e *BDGP* for splicing variants

Data Analysis: Variants



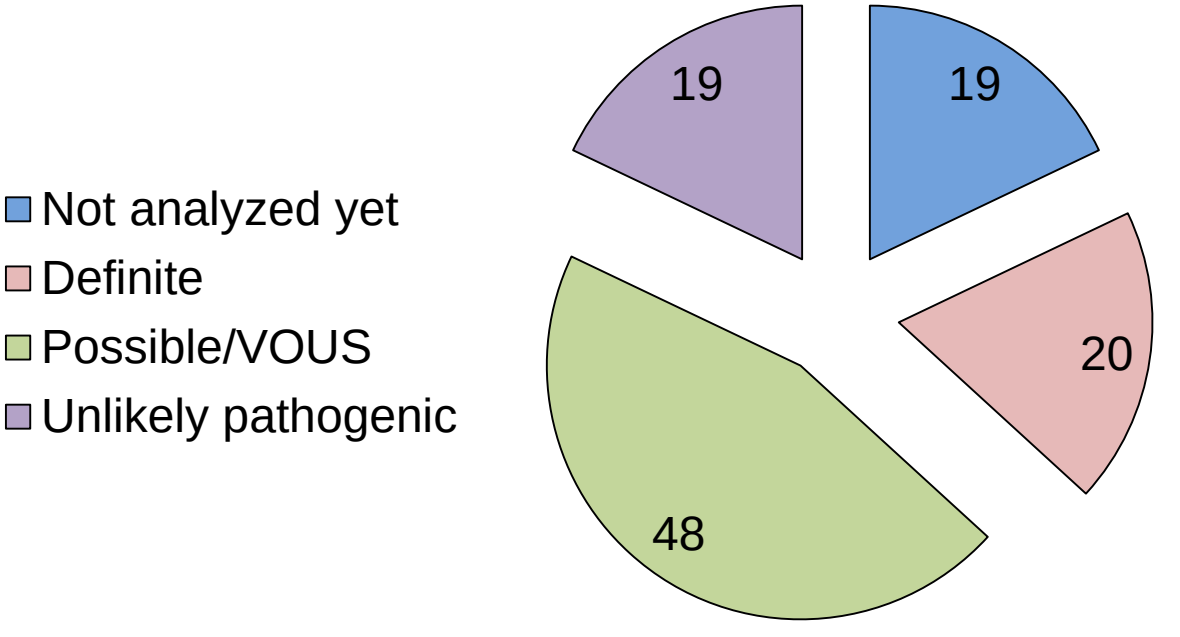
Confirmed pathogenic variants

Variants of uncertain significance (VOUS)

No candidate variants or variants with unlikely pathogenicity

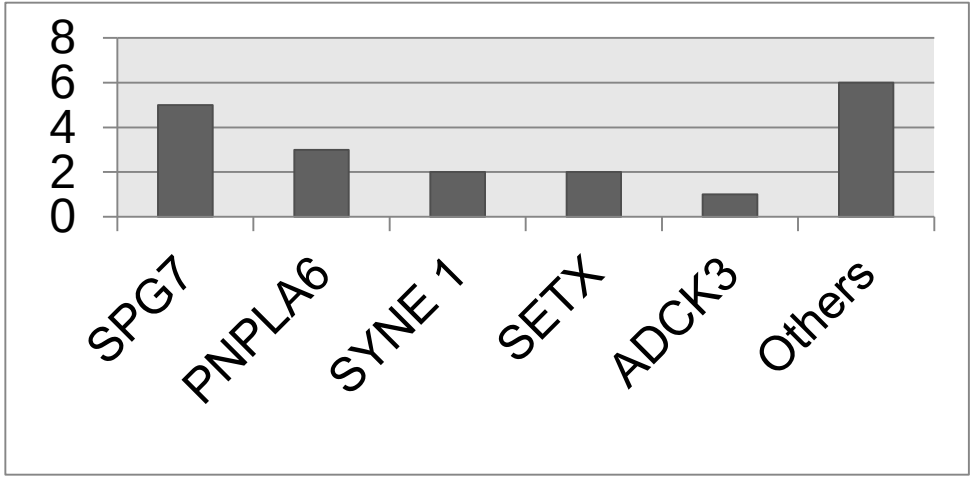
(Incidental findings)

Data Analysis: The State of Art



Total: 106 cases

Preliminary Results from Target Resequencing Panel: Confirmed Pathogenic Variants



Others: RNF216, ZFYVE26, ANO10, PMM2, TGM6, ATP13A2, SCL2A1

TOTAL 20

SPG7

paraplegin

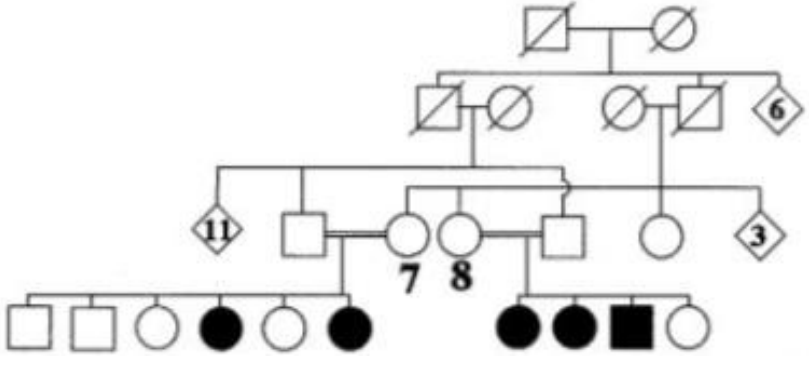
Am. J. Hum. Genet. 63:135–139, 1998

European Journal of Human Genetics (2016) 24, 1016–1021

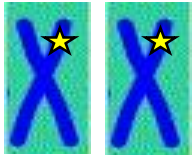
www.nature.com/ejhg

ARTICLE

SPG7 mutations explain a significant proportion of French Canadian spastic ataxia cases

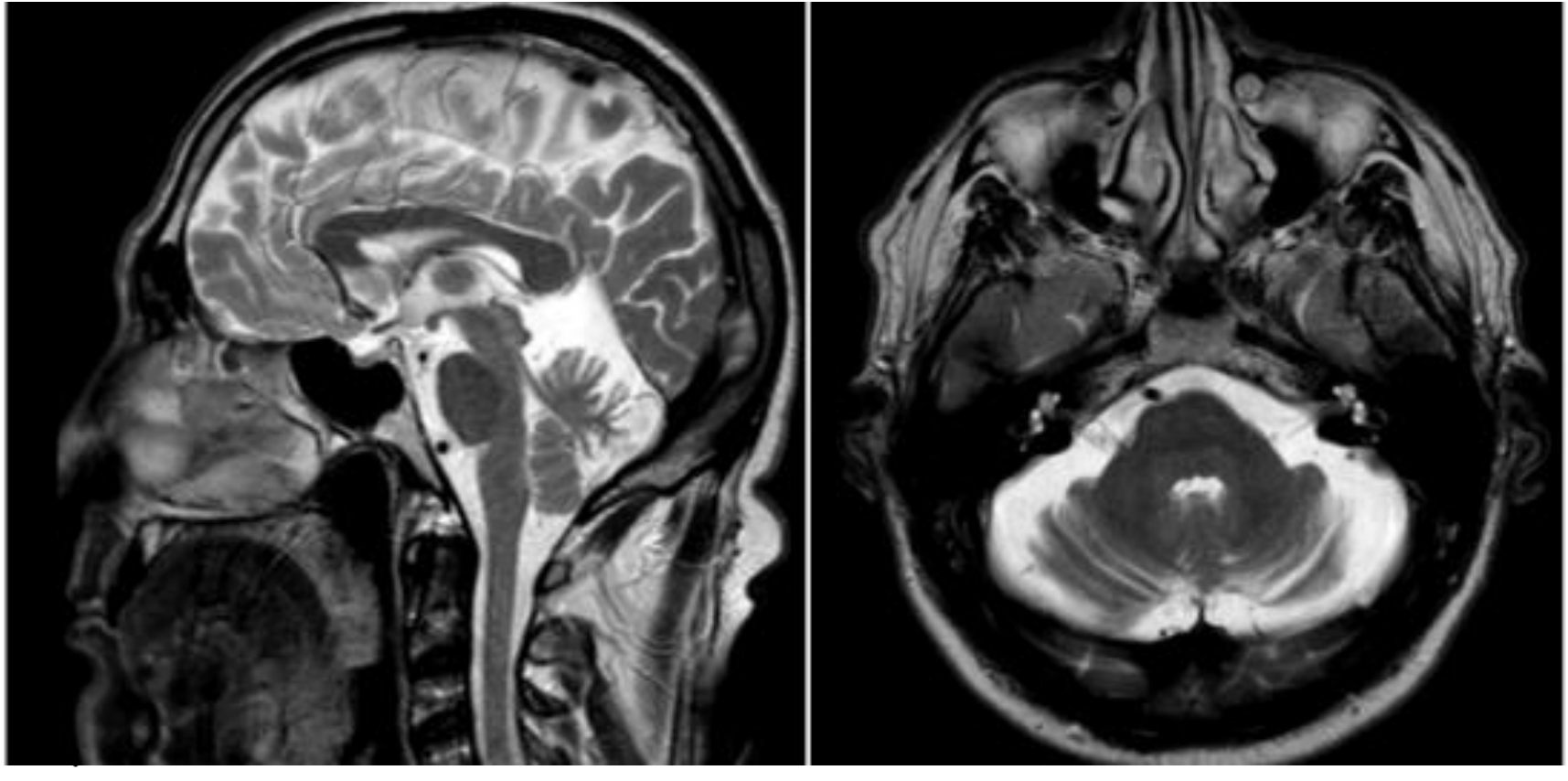


SPG7
paraplegin



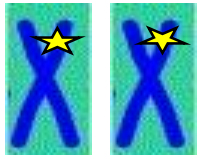
Onset 38 y (10-63)

Autosomal recessive

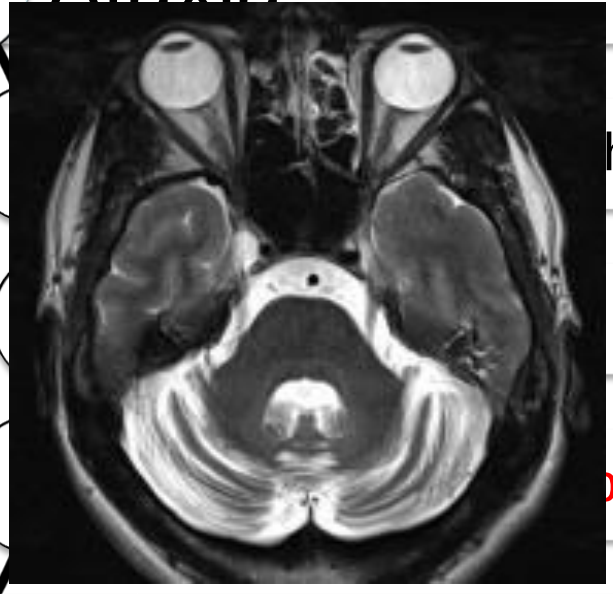


Boucher-Neuhäuser/SPG39
PNPLA6

Onset < 8 y



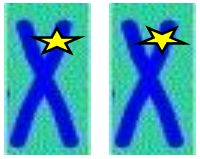
Autosomal recessive

- Ataxia
- 
- hy
- 
-
- hy
- Hypogonadism
- 

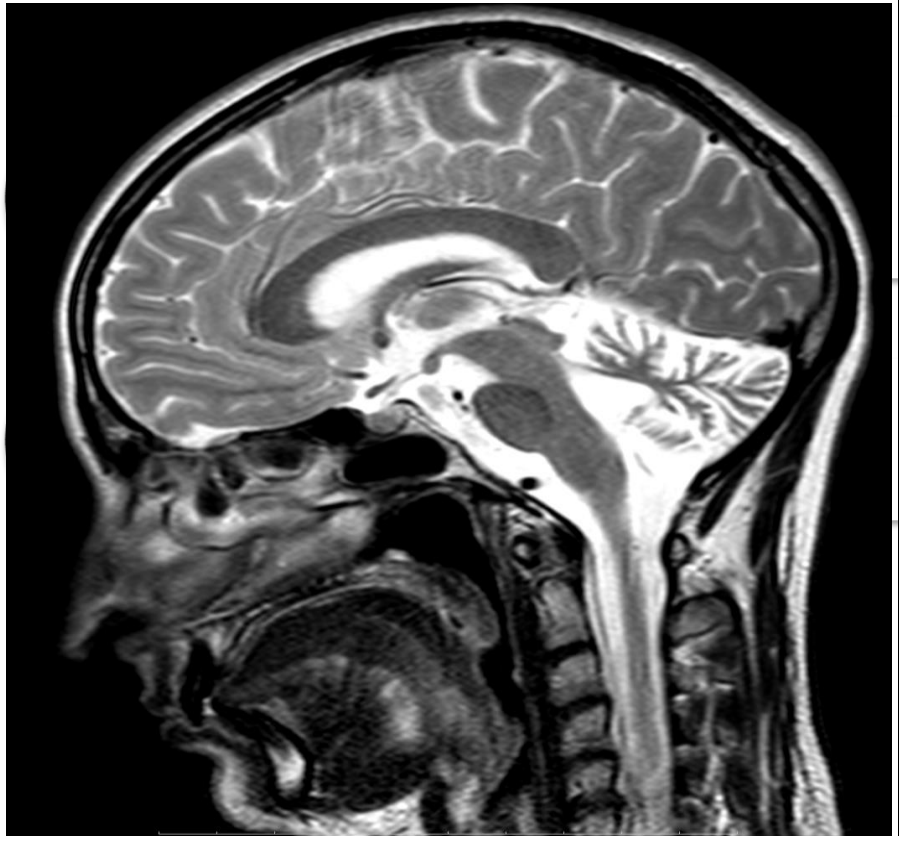
ARCA1/BEAUCE ATAXIA/SCAR8

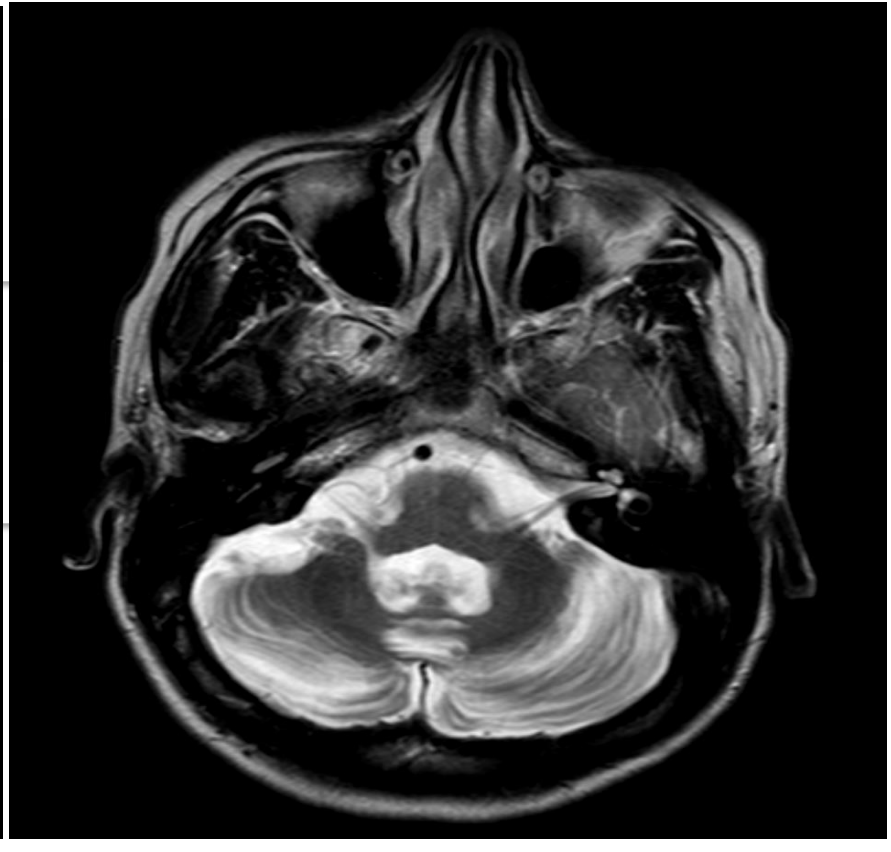
SYNE1/Nesprin1

Onset 22y (6-40)



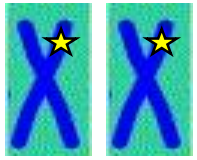
Autosomal recessive





ARCA2
COQ8A/ADCK3/CABC1

Onset 0-20 y

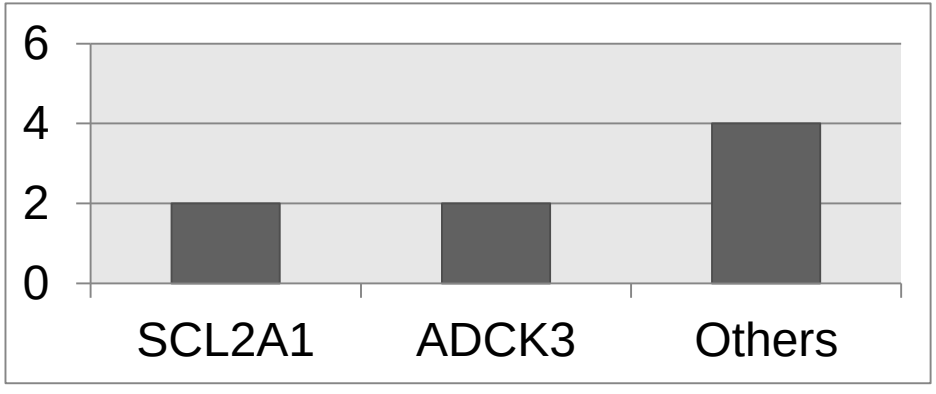


Autosomal recessive

- Ataxia
  - Spasticity
  - Dystonia/tremor/myoclonus
  - Migraine
  - Cognitive.Epilepsy
  - Peripheral neuropathy absent

Q10

Preliminary Results from Target Resequencing Panel:
Variants of Uncertain Significance (VOUS)



Others: KIF1C, PRKCG, KCND3, HARS

TOTAL: 48

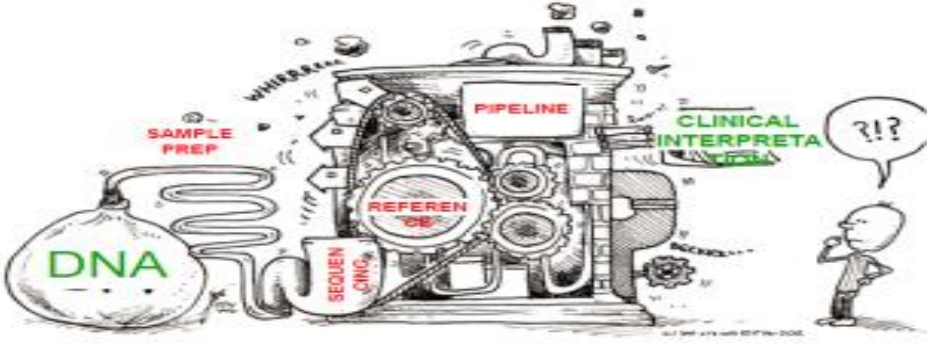
SCL2A1/GLUT1

De novo mutation
/Autosomal dominant

Autosomal recessive

- Gait abnormalities (ataxia, spastic ataxia)
- Dysarthria
- Infantile epilepsy
- Dystonia, tremor, myoclonus
- Developmental delay

Next Future



- To analyze remaining data from TRP
- To complete the investigation through ES and WGS and TRIOS study

<u>Hoping to increase the diagnostic yield!</u>


THANK YOU