Investigation of Motor Neuron Diseases by WES: Genetic Dissection of a Turkish ALS Cohort

M.Sc. Thesis Defense
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NDAL
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Investigation of Motor Neuron Diseases by WES: Genetic Dissection of a Turkish ALS Cohort

Amyotrophic Lateral Sclerosis

- Genetic Basis of ALS

Gene discovery in ALS

- Linkage analysis
- Association studies
- NGS

Application of WES to Turkish MND cohort

- Bioinformatic Evaluation of the WES Data

Results

Discussion : Limitations and Possible Improvements

An overview of ALS

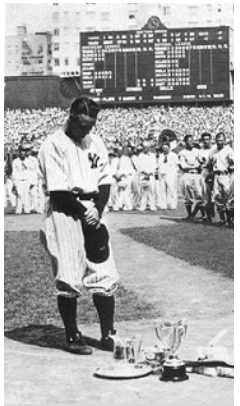
The Hallmarks of ALS

- Degeneration of upper and lower motor neurons

  in the brain and spinal cord

  Worldwide prevalance: less than 200,000

  1 in 500 adult deaths
  - The mean age of onset in ALS is 55
  - Fatal within the first 30 months from diagnosis



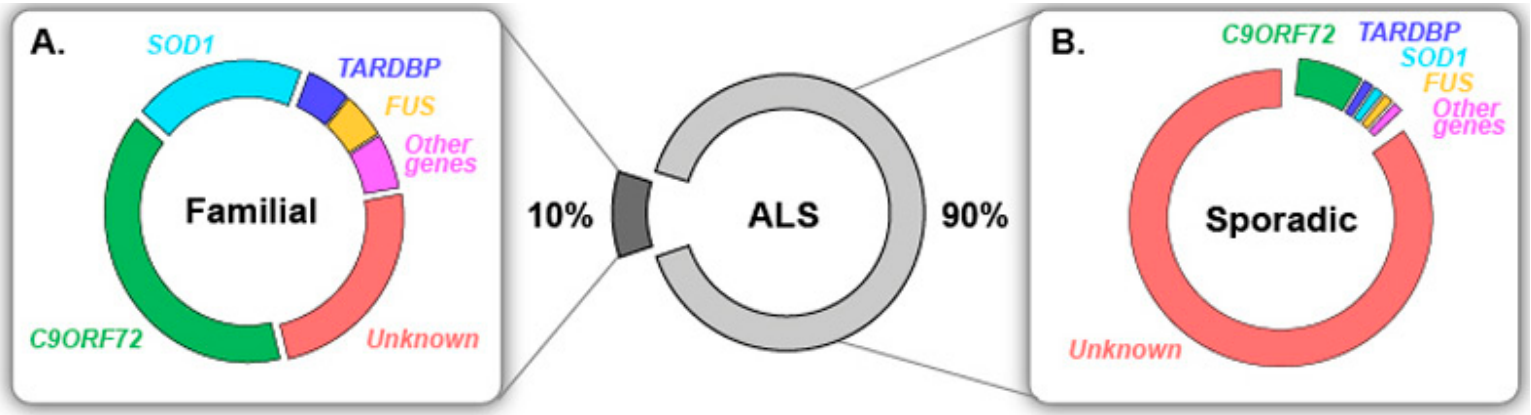
Lou Gehrig

- First described by the French neurobiologist JM Charcot
- Spasticity, atrophy and progressive weakness of muscles

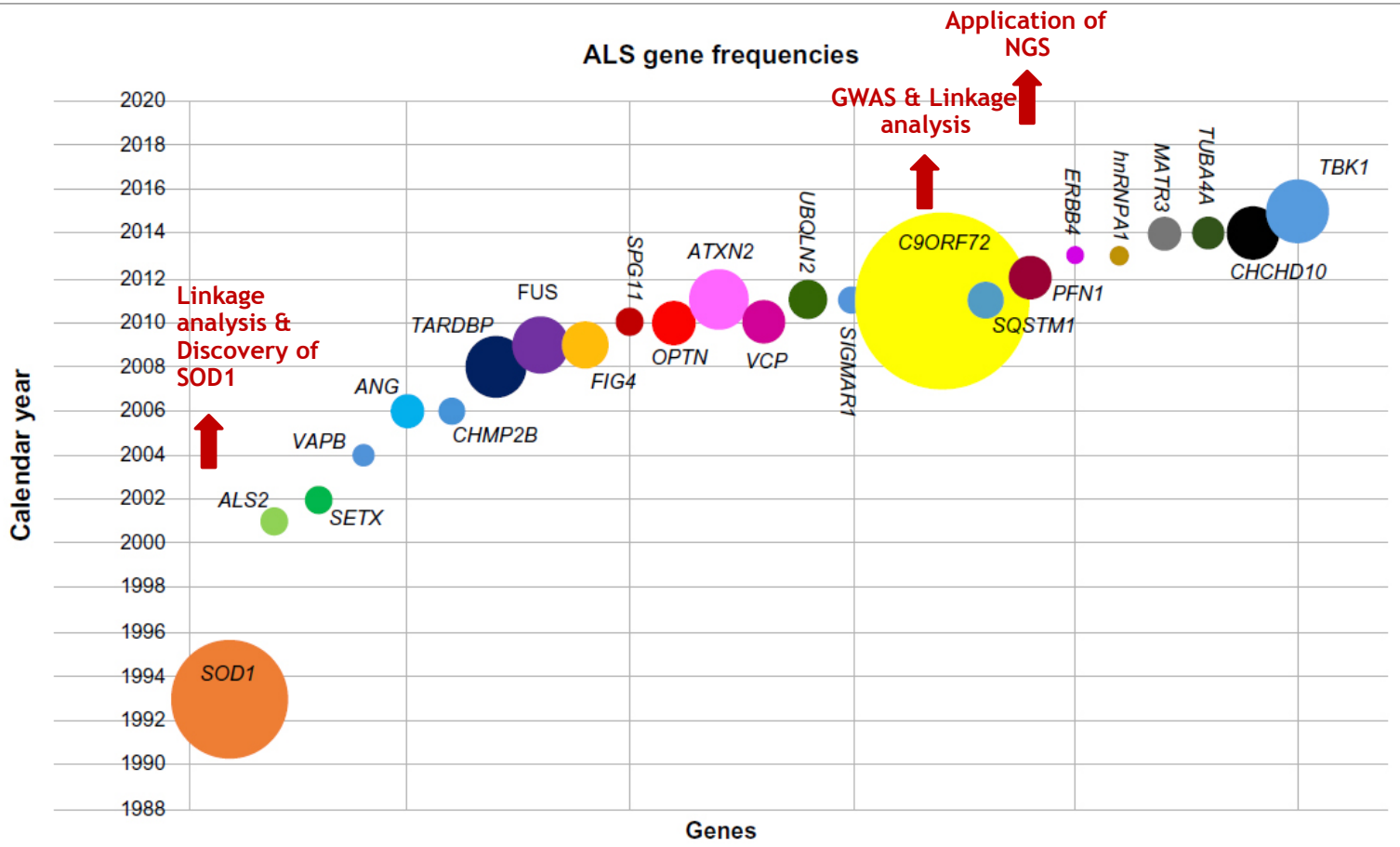
Stephan Hawking

The Genetic Basis of ALS

- ~ 90 % of ALS cases are sporadic (sALS)
- ~10 % are familial ALS (fALS)
- More than 40 genes associated with ALS



The Genetic Basis of ALS



Alsultan AA, Waller R et al., *Dove Medical Press*, 2016

The first breakthrough: The discovery of SOD1

- SOD1 is the first ALS gene,

- The locus was detected by linkage analysis in 1991,
  - The SOD1 gene was discovered in 1993

- Explains 20 % fALS and 3 % sALS with more than 170 mutations

The second breakthrough: The *C9ORF72* repeat expansion mutation

- G_4C_2 hexanucleotide repeat expansion
- The locus was detected by the combination of linkage analysis and association studies in 2006.
- The most common cause of ALS, FTD, ALS-FTD
  - Explains 10 % of sALS and 30 % of fALS cases

The third breakthrough: The advent of NGS

- Human Genome Project
  - the first map of the 3 billion bases in the human genome
  - identify genetic variants in an individual, which did not match the reference sequence
- An NGS platform can produce millions of short reads from 25 to 500 base pairs
  - An approach to detect SNVs and short INDELs
- WGS & WES

Aim

-To establish an efficient *in-silico* workflow to process the WES data.

-To characterize novel genotype-phenotype associations in MNDs by

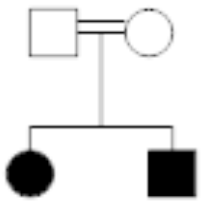
- identifying both known and novel mutations in known ALS-MND genes.
- describing mutations in novel genes associated with an MND phenotype.

Application of WES

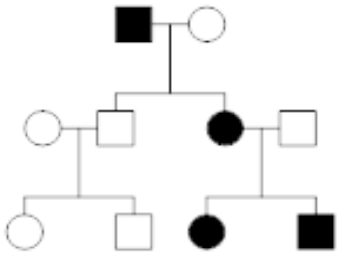
An overview of Turkish MND cohort

57 families
(81 patients + 66 unaffected family members)

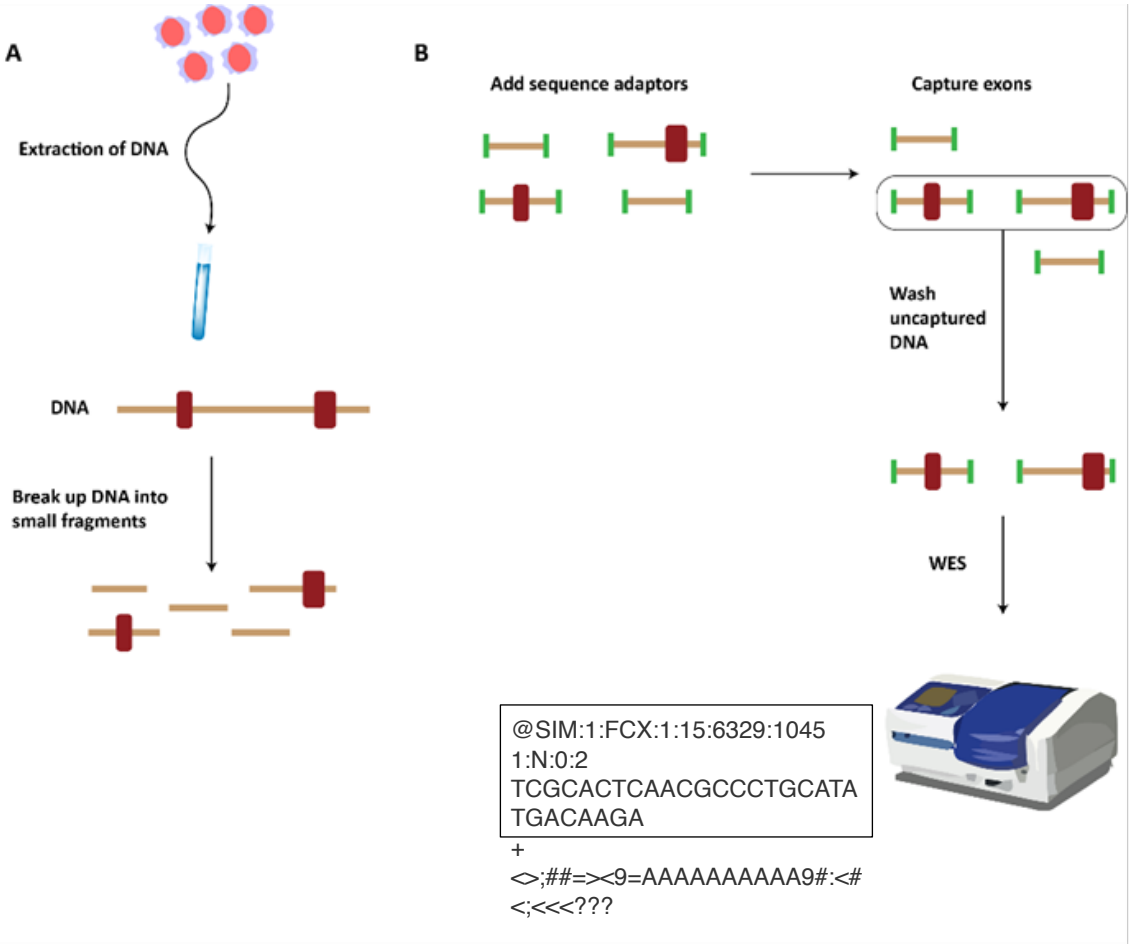
35 AR families with consanguineous marriage



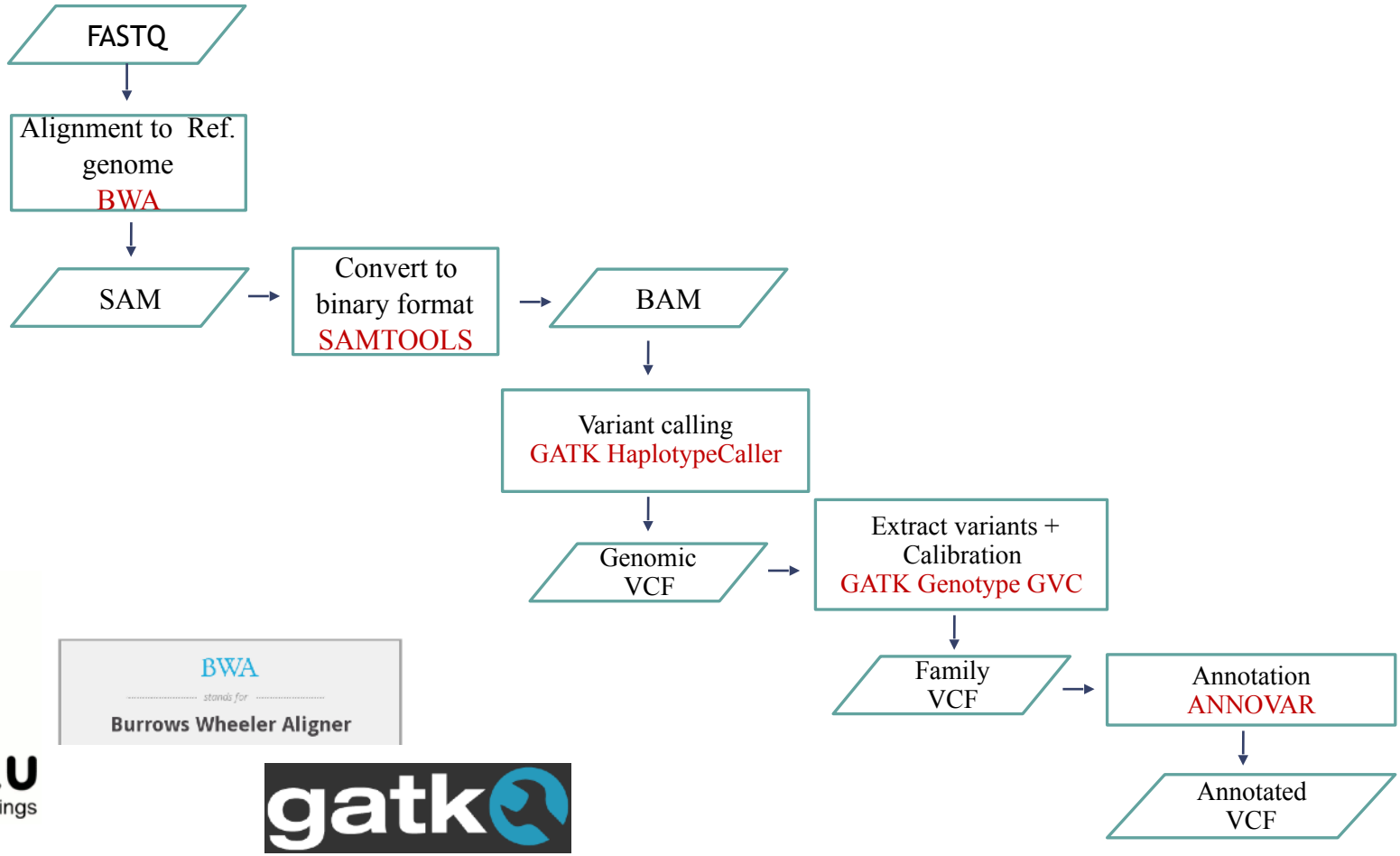
22 AD + 1 XLD families



General Workflow of Exome Sequencing



Bioinformatic Pipeline I



ubuntu
linux for human beings

BWA
stands for
Burrows Wheeler Aligner

gatk

Bioinformatic Pipeline II

- Variant Prioritization
  - Mode of inheritance
- Filtration-Allele Frequency
  - 1000G, dbSNP
  - ExAC
  - In house control db
  - Prediction tools (Polyphen, SIFT, Gerp ++, OMIM)
- Homozygosity Mapping

50 YEARS OMIM
Human Genetics Knowledge for the World

plink...

Whole genome association analysis toolset

Quality Check- QC metrics

- **Cohort based Sample QC (147 individuals)**
  - Mean depth of coverage
  - Missingness Rate
  - Ts/Tv ratio
- **Population Stratification**
  - PCA - to generate population clusters

Homozygosity Mapping

Create plink hard calls

- Binary plink files (ped, bim, fam)

Prune SNPs

- Remove SNPs in LD

IBD calculation

- Confirm relationships

Runs of homozygosity & R plots

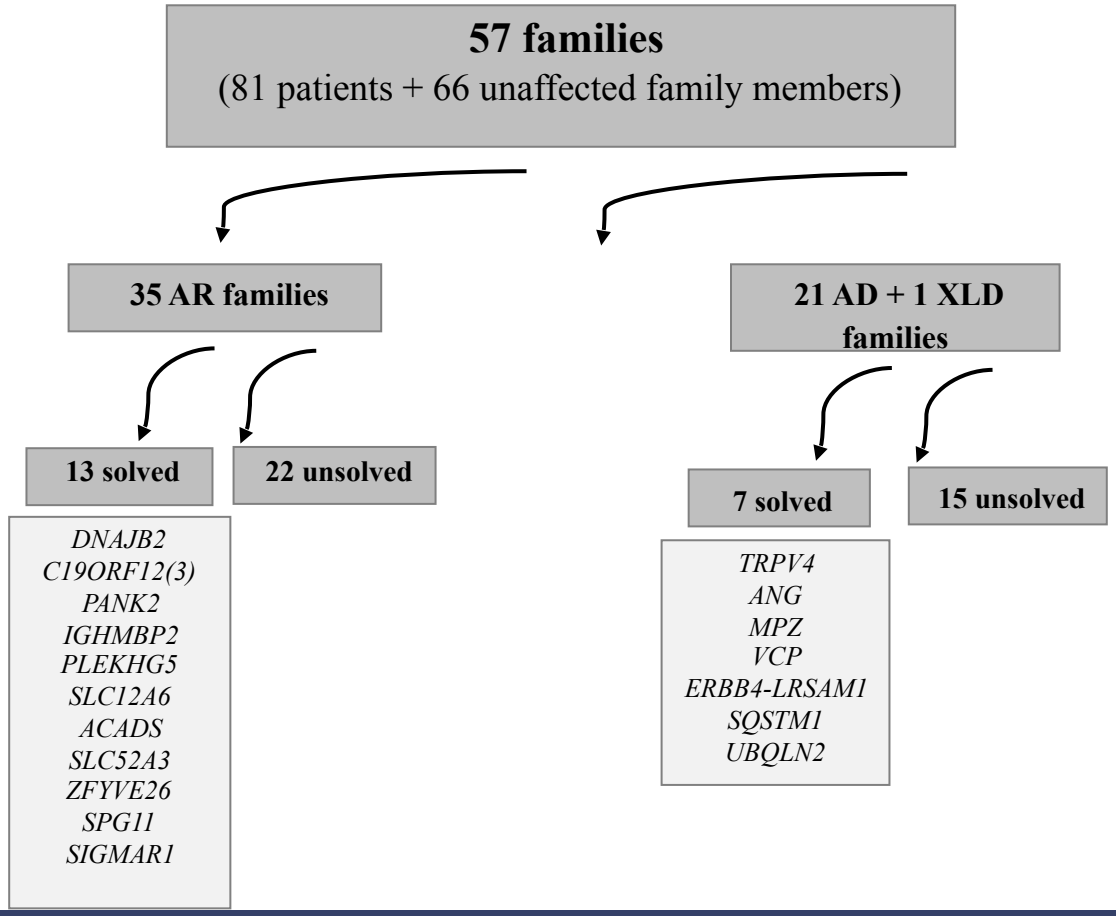
- Optimized parameters

Homozygosity Mapping-Runs of Homozygosity parameters

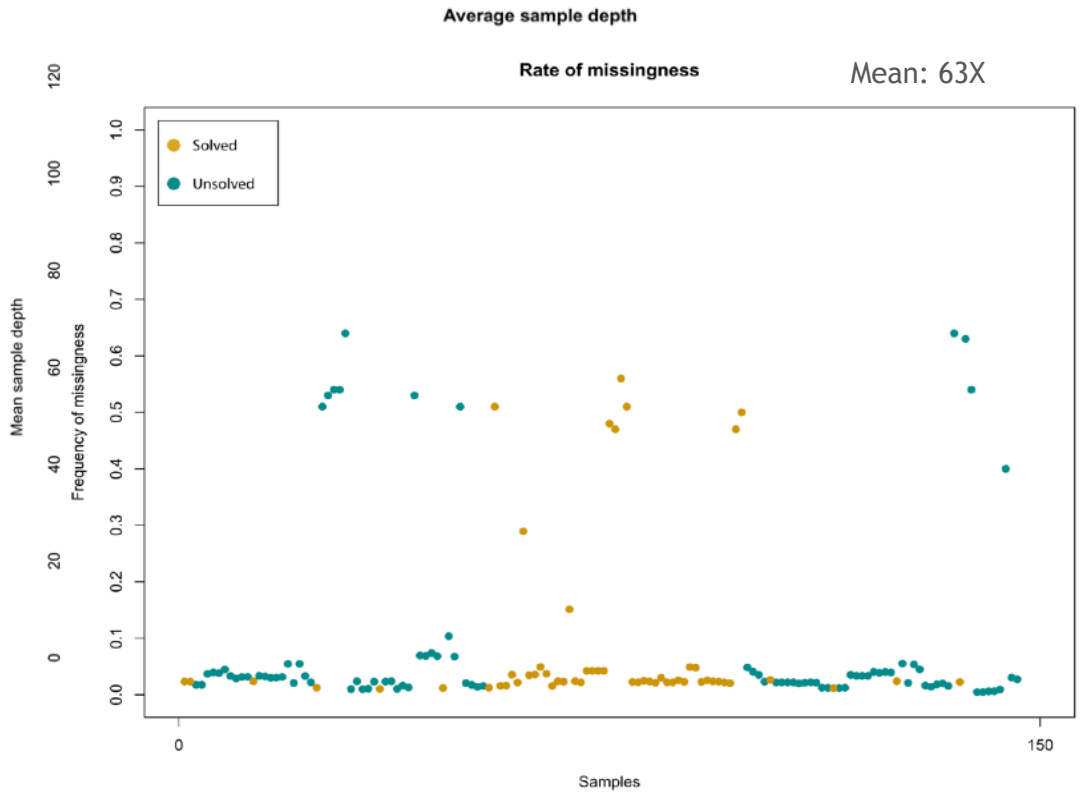
Parameter	HOMWES by Kancheva et al	UMCU Neurogenetics lab.	NDAL Optimizied
Size threshold (kb) to call on ROH	1000	750	500
SNP number threshold to call an ROH	10	30	10
Sliding window size in SNPs	20	5	20
Allowed missing SNPs	5	10	10
Proportion of homozygous window threshold	0.05	0.05	0.05
Minimum SNP density to call an ROH	50	50	200
Maximum allowed gap	500	4000	2000
Allowed heterozygous SNPs	1	1	1, 2

Results

An overview of Turkish MND cohort



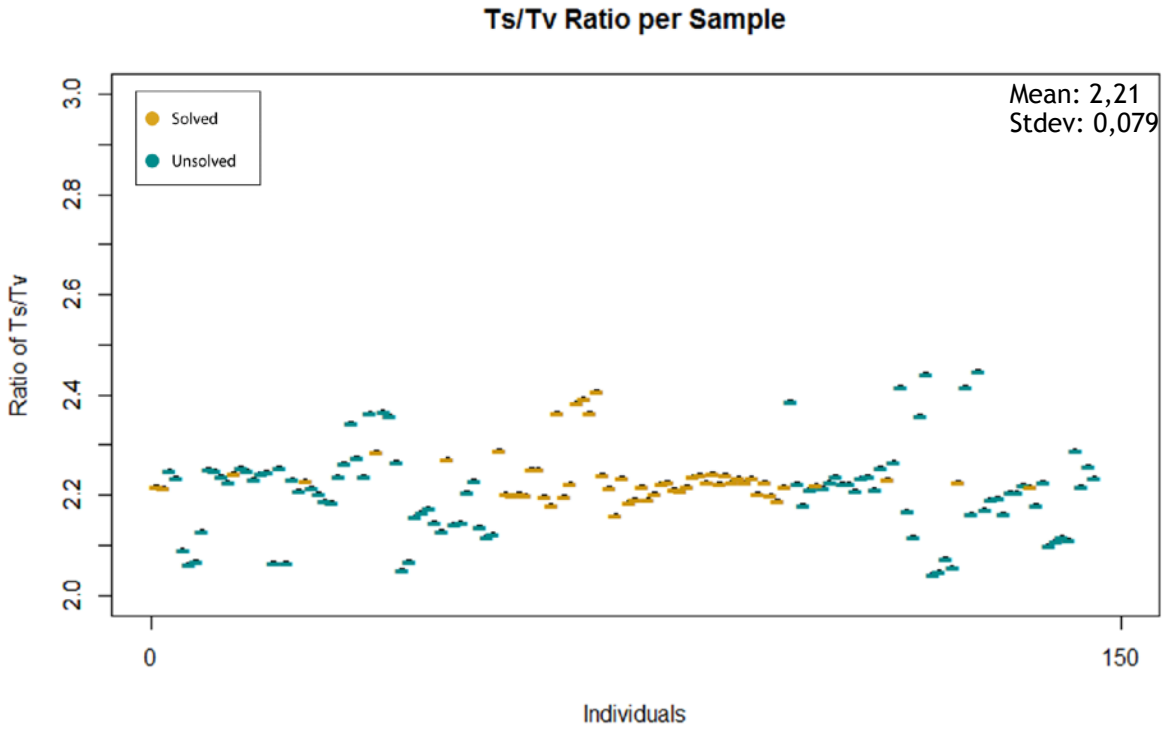
An overview of Turkish MND cohort- Sample Quality Metrics



Mean: 63X

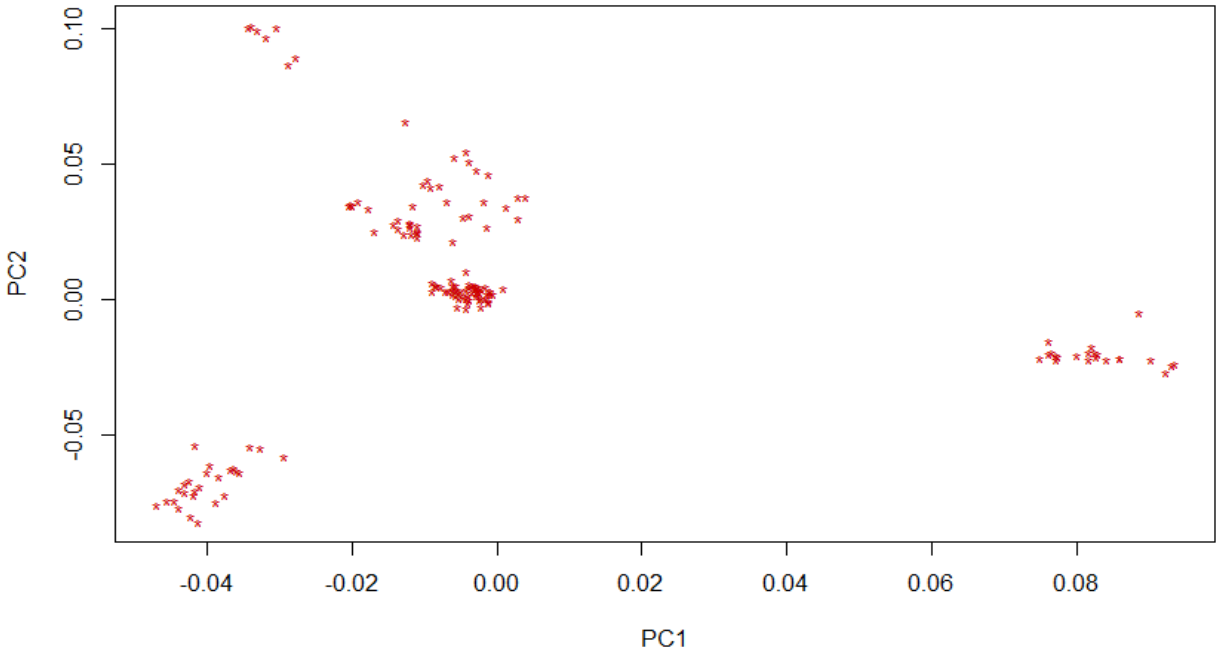
The average missingness
Mean: 0,0925
Stdev: 0,16

An overview of Turkish MND cohort- Sample Quality Metrics



An overview of Turkish MND cohort- Sample Quality Metrics

Principal Component Analysis



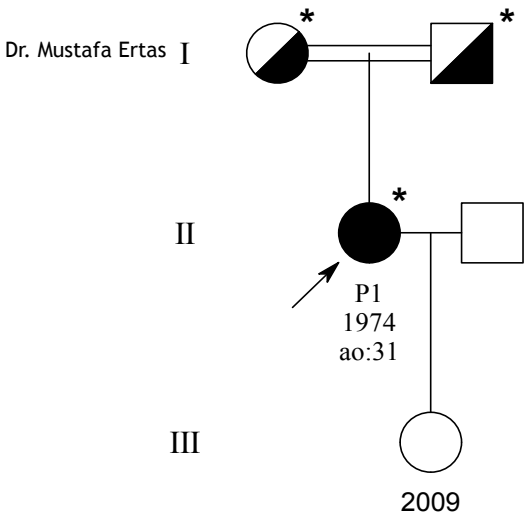
Identified Mutations in AR- inherited families

	Initial diagnosis	Variation			OMIM Association
		Gene	Coding sequence	Protein sequence	
Family 1	distal motor neuropathy	*DNAJB2*	c.757G>A	p.Glu253Lys	distal spinal muscular atrophy
Family 2	Atypical ALS	*C19ORF12*	c.194G>T	p.Gly65Val	NBIA4
Family 3	Atypical ALS	*C19ORF12*	c.194G>T	p.Gly65Val	NBIA4
Family 4	ALS	*C19ORF12*	c.32C>T	p.Thr11Met	NBIA4
Family 5	HSP	*PANK2*	c.427G>A	p.Ala143Thr	NBIA1
Family 6	MND	*IGHMBP2*	c.638A>G	p.His213Arg	SMARD1
Family 7	ALS	*PLEKHG5*	c.1648C>T	p.Gln550Ter	distal spinal muscular atrophy
Family 8	HSP	*SLC12A6*	c.1073+G>A	-	Andermann syndrome
Family 9	MND	*ACADS*	c.1108A>G	p.Met370Val	(SCAD) deficiency
Family 10	BVVL	*SLC52A3*	c.802C>T	p.Arg268Trp	BVVL1
Family 11	MND	*ZFVYE26*	c.2074delC	p.L692fs	SPG15
Family 12	MND	*SPG11*	c.1423C>T	p.Gln478Ter	SPG11, ARJALS
Family 13	MND	*SIGMAR1*	c.308G>A	p.Gly103Glu	ALS-16

Identified Mutations in AD-XLD inherited families

	Inheritance	Initial diagnosis	Variation			OMIM Association
			Gene	Coding sequence	Protein sequence	
Family 14	AD	Scapuloperoneal SMA/CMT	*TRPV4*	c.943C>T	p.Arg315Trp	scapuloperoneal SMA / hereditary motor and sensory neuropathy type 2
Family 15	AD	ALS	*ANG*	c.208A>G	p.Ile70Val	ALS-9
Family 16	AD	CMT	*MPZ*	c.293G>A	p.Arg98His	CMT1B
Family 17	AD	ALS/FTD	*VCP*	c.572G>C	p.Arg191Pro	ALS-14 w/wo FTD
Family 18	AD	ALS	*ERBB4*	c.3334C>T	p.Arg1112Cys	ALS-19
			LRSAM 1	c.578G>A	p.Cys193Tyr	CMT2P
Family 19	AD	ALS	*SQSTM 1*	c.374A>G	p.Asn125Ser	ALS/FTD/Paget disease of bone
Family 20	XLD	ALS/MMND	*UBQLN 2*	c.374A>G	p.Met391Ile	ALS-15 w/wo FTD

DNAJB2 mutation (p. Glu253Lys) in a patient with distal SMA



*: exome data available
P: patient
ao: age of onset

Homozygous KB length

doi:10.1093/brain/aww028 BRAIN 2016: 139; 1417–1432 | 14
BRAIN
A JOURNAL OF NEUROLOGY

The heat shock response plays an important role in TDP-43 clearance: evidence for dysfunction in amyotrophic lateral sclerosis

doi:10.1093/brain/aww154 BRAIN 2016: Page 1 of 2
BRAIN
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LETTER TO THE EDITOR

The role of *DNAJB2* in amyotrophic lateral sclerosis

doi:10.1093/brain/aww155 BRAIN 2016: 139; 1–2 | e58
BRAIN
A JOURNAL OF NEUROLOGY

LETTER TO THE EDITOR

Reply: The role of *DNAJB2* in amyotrophic lateral sclerosis

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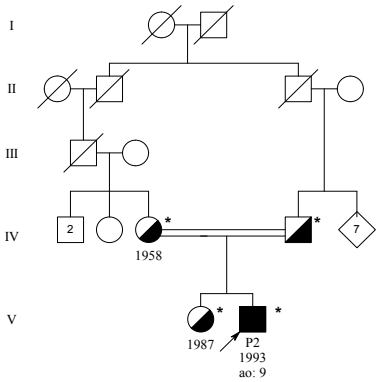
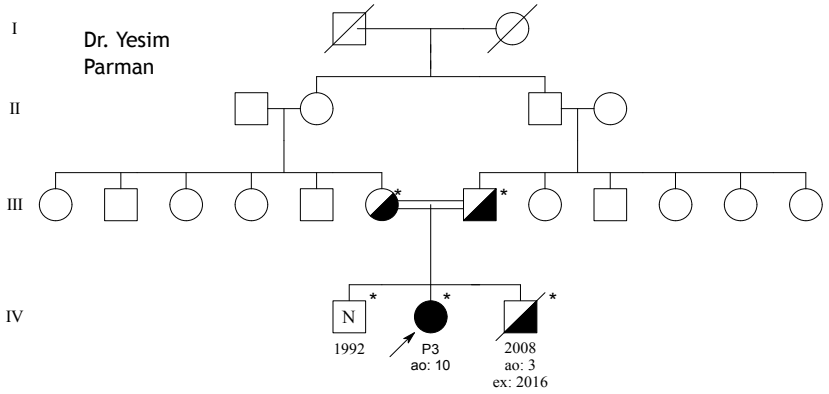
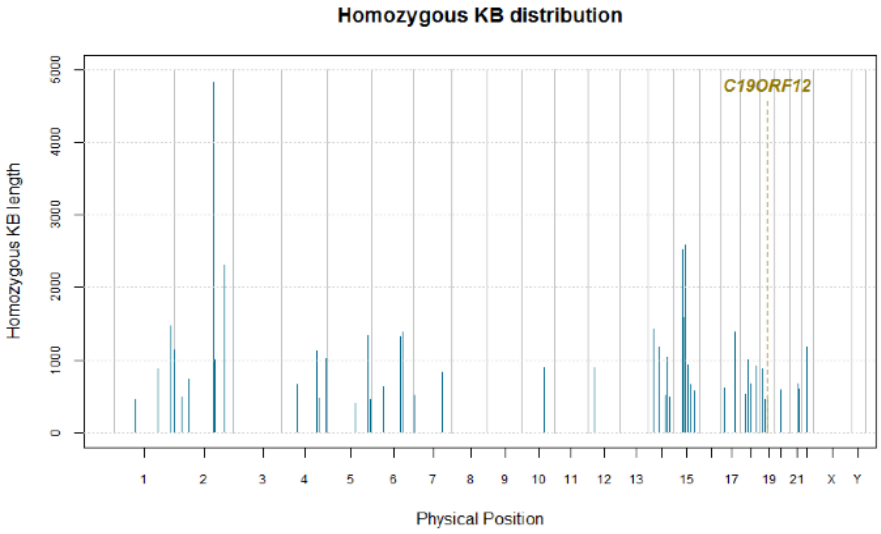
C19ORF12 mutations (p. Gly65Val) in patients with atypical ALS

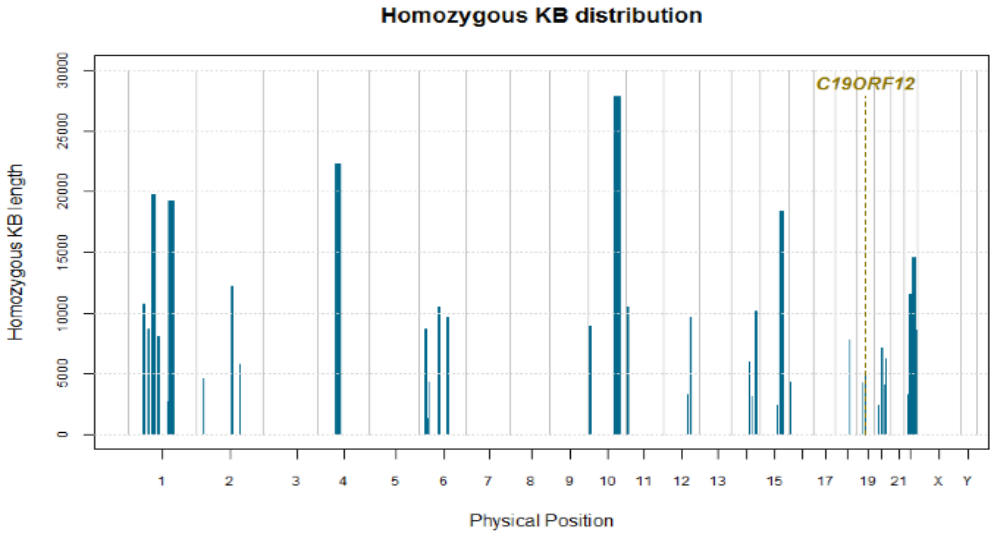
Dr. Yesim Parman



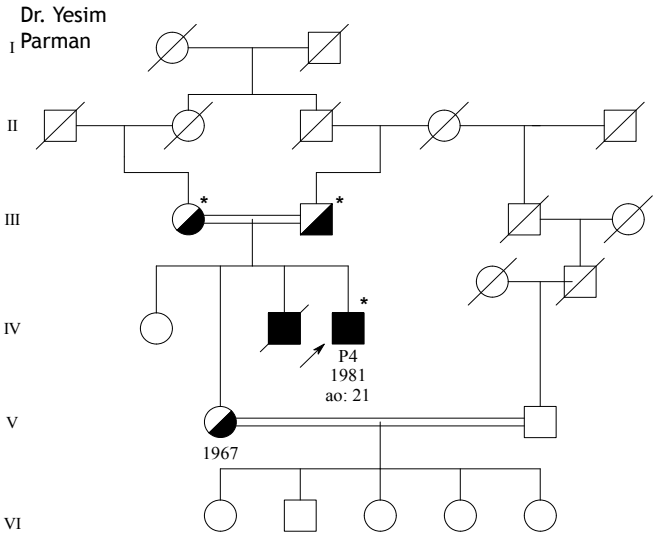
Dr. Yesim Parman

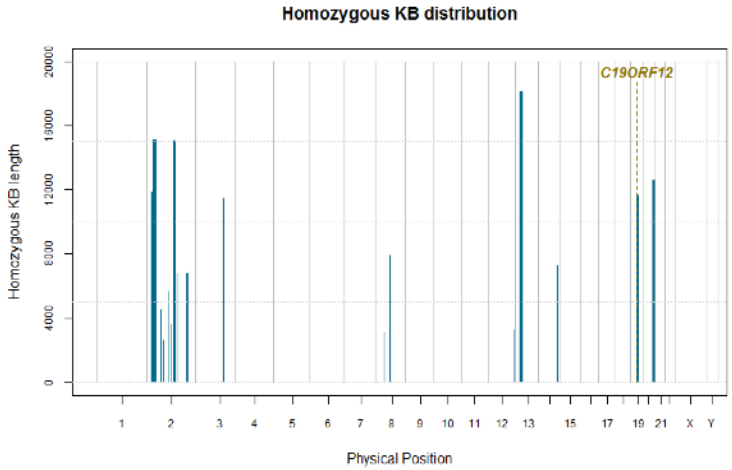






C19ORF12 mutations (p. Thr11Met) in patients with early onset ALS





J Neurol (2012) 259:2434–2439
DOI 10.1007/s00415-012-6521-7

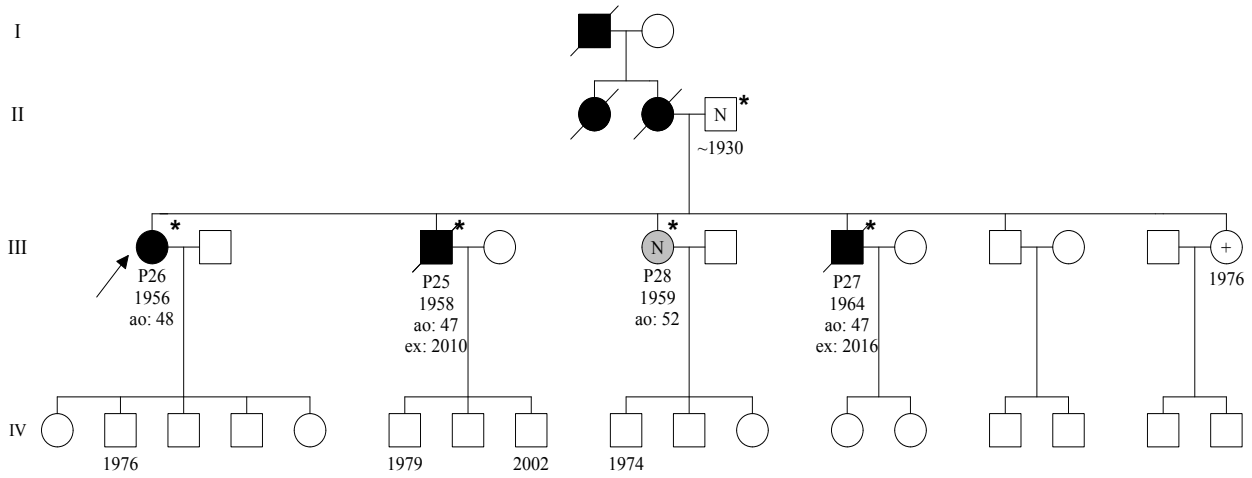
ORIGINAL COMMUNICATION

C19orf12 mutations in neurodegeneration with brain iron accumulation mimicking juvenile amyotrophic lateral sclerosis

M. Deschauer · C. Gaul · C. Behrmann · H. Prokisch · S. Zierz · T. B. Haack

An *ERBB4* mutation with an intra-familial clinical variation

Dr. Nalan Gunes
Dr. Atay Vural



ERBB4	c.3334C>T	p.Arg1112Cys	ALS-19
LRSAM1	c.578G>A	p.Cys193Tyr	CMT2P

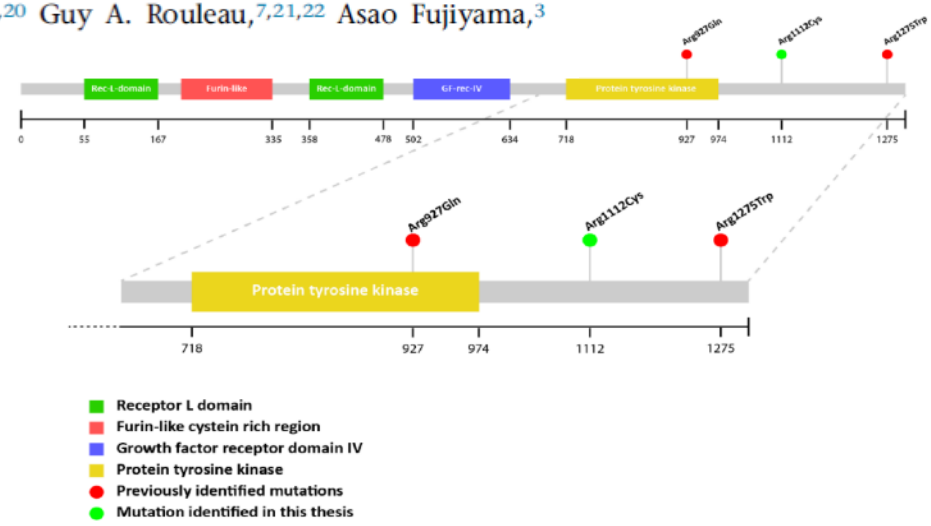
ERBB4 mutations in ALS

REPORT

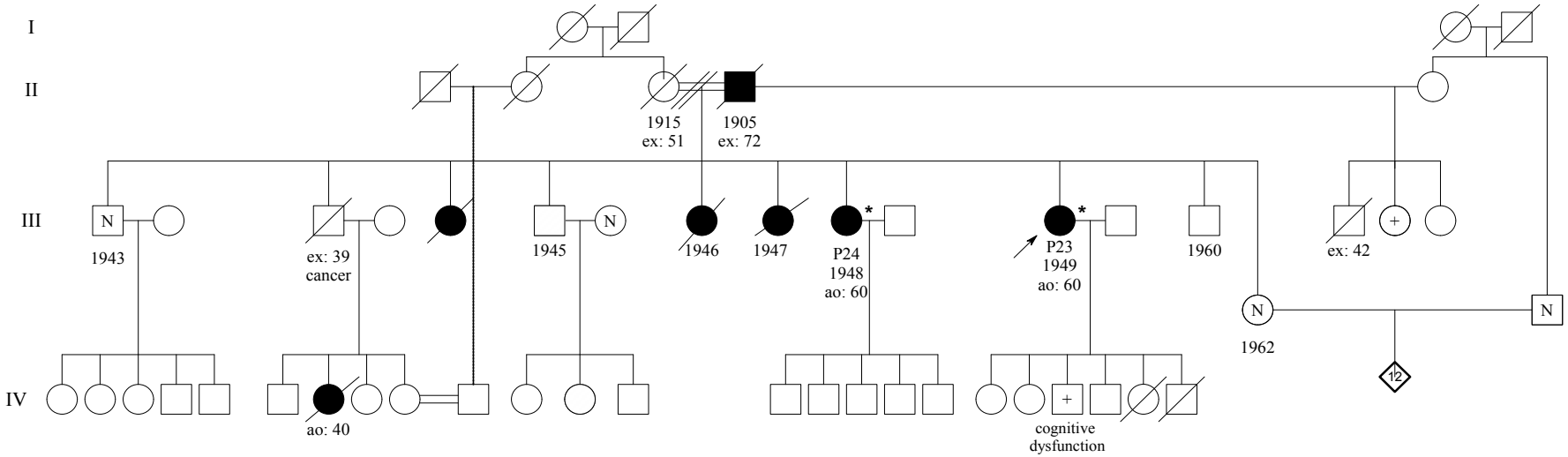
November, 2013

ERBB4 Mutations that Disrupt the Neuregulin-ErbB4 Pathway Cause Amyotrophic Lateral Sclerosis Type 19

Yuji Takahashi,[1] Yoko Fukuda,[1] Jun Yoshimura,[2] Atsushi Toyoda,[3] Kari Kurppa,[4,5] Hiroyoko Moritoyo,[6] Veronique V. Belzil,[7] Patrick A. Dion,[7,8] Koichiro Higasa,[2] Koichiro Doi,[2] Hiroyuki Ishiura,[1] Jun Mitsui,[1] Hidetoshi Date,[1] Budrul Ahsan,[1] Takashi Matsukawa,[1] Yaeko Ichikawa,[1] Takashi Moritoyo,[6] Mayumi Ikoma,[9] Tsukasa Hashimoto,[9] Fumiharu Kimura,[10] Shigeo Murayama,[11] Osamu Onodera,[12] Masatoyo Nishizawa,[12] Mari Yoshida,[13] Naoki Atsuta,[14] Gen Sobue,[14] JaCALS,[15] Jennifer A. Fifita,[16,17,18] Kelly L. Williams,[16,17,18] Ian P. Blair,[16,17,18] Garth A. Nicholson,[16,17] Paloma Gonzalez-Perez,[19] Robert H. Brown, Jr.,[19] Masahiro Nomoto,[6] Klaus Elenius,[4,20] Guy A. Rouleau,[7,21,22] Asao Fujiyama,[3] Shinichi Morishita,[2] Jun Goto,[1] and Shoji Tsuji[1,23,*]

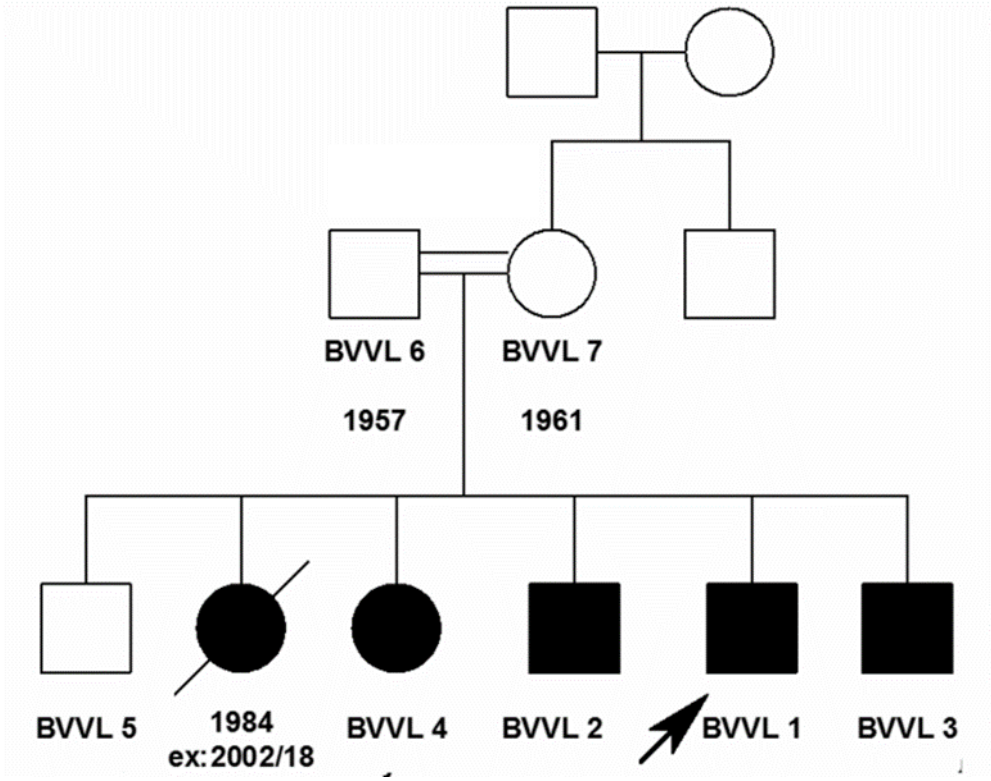


A Novel *VCP* mutation causing ALS and cognitive dysfunction



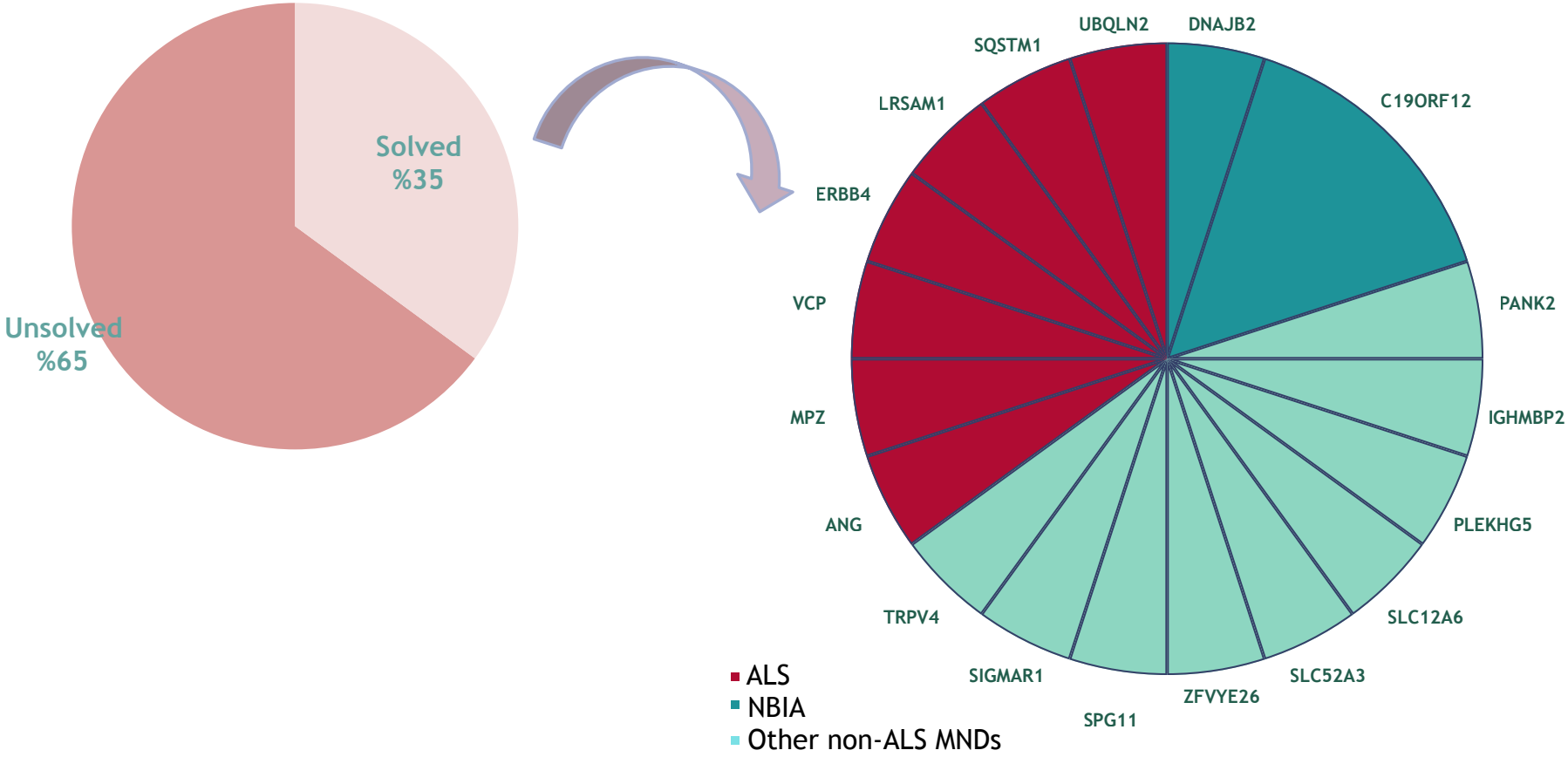
VCP	c. 572G>C	p.Arg191P ro	ALS-14 w/wo FTD

A Novel gene causing BVVL – KIF13A2

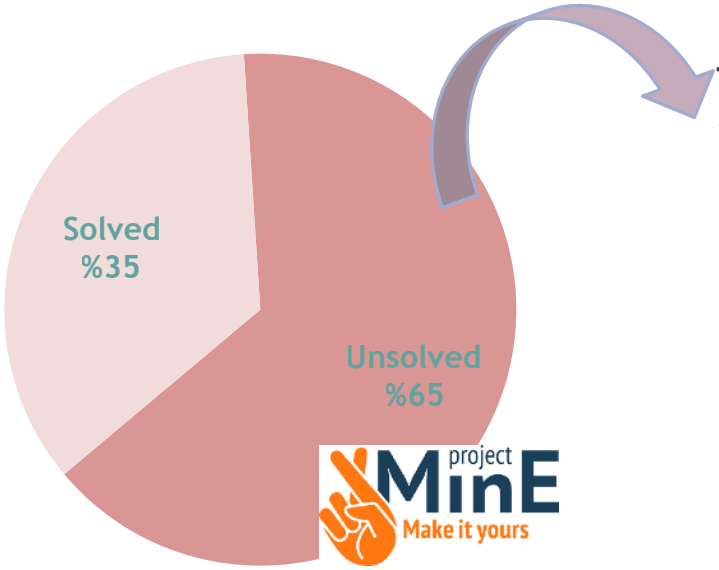


Summary and Discussion

Overall Results



Remaining Cases to be Solved



Technical Limitations of WES I
-Short-read sequencing approach in NGS
Inability to detect structural variations

Technical Limitations of WES II
-Low coverage problem
Especially for heterozygous variants

Data processing
-False positives

Sample Size
-Discovery of novel disease causative genes
Association studies

Detailed and Correct Pedigree information
-Misleading or missing clinical information
-Importance of deep phenotyping

Project MinE

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Prof. Sibel Ertan

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Family members
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Suna, Ayse
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