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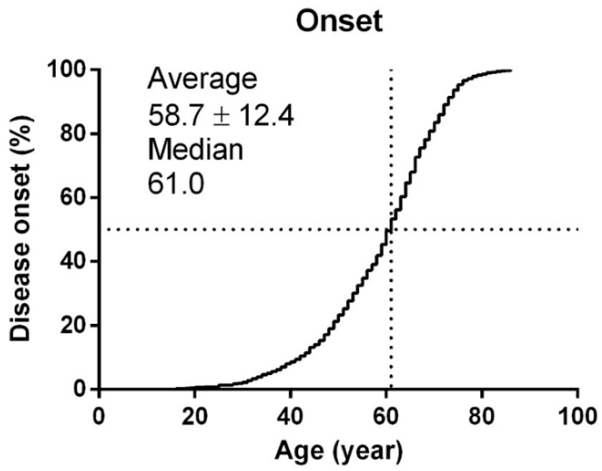
Whole genome sequencing a tool to unravel rare variants associated with ALS survival

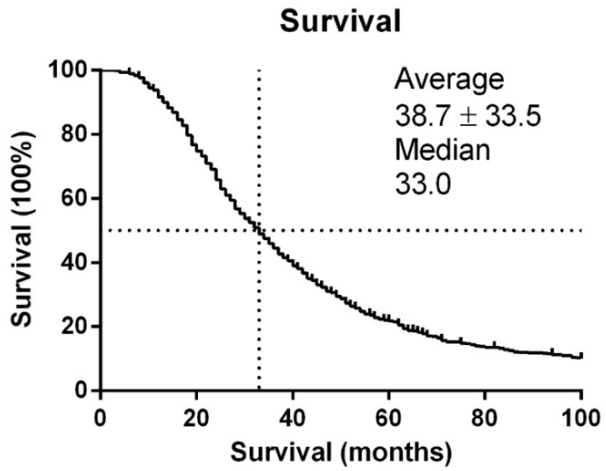
Philip Van Damme & Matthieu Moisse

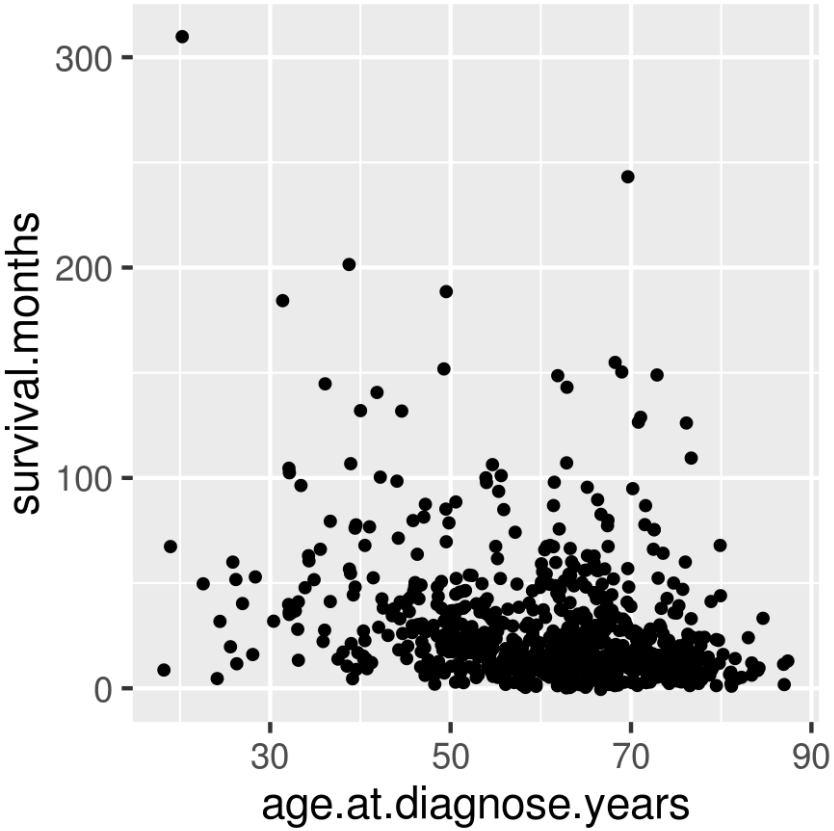
VIB KU LEUVEN

Clinical presentation of ALS

- Third most frequent neurodegenerative syndrome
- Epidemiology
  - Incidence 2/100,000 a year
  - Prevalence 4-8/100,000
  - Life time risk 1/400
  - Variability in clinical presentation







project MinE
Make it yours

Worldwide Project MinE

More and more countries join this groundbreaking genetic ALS research!

goal
22,500.00 DNA profiles

collected so far
8,335.98 DNA profiles

The Netherlands	Turkey	United Kingdom	USA	Australia	Belgium	Ireland
101% of goal raised	38% of goal raised	68% of goal raised	58% of goal raised	51% of goal raised	100% of goal raised	64% of goal raised
3,008.3	189.8	1,206.9	577.1	503.2	750.4	667.2
€5,866,129 RAISED	€370,056 RAISED	£1,945,471 RAISED	$1,500,460 RAISED	$AU1,400,023 RAISED	€1,463,360 RAISED	€1,301,050 RAISED
€5,850,000 GOAL	€975,000 GOAL	£2,901,600 GOAL	$2,600,000 GOAL	$AU2,782,000 GOAL	€1,462,500 GOAL	€2,047,500 GOAL

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https://www.projectmine.com/

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Survival analysis

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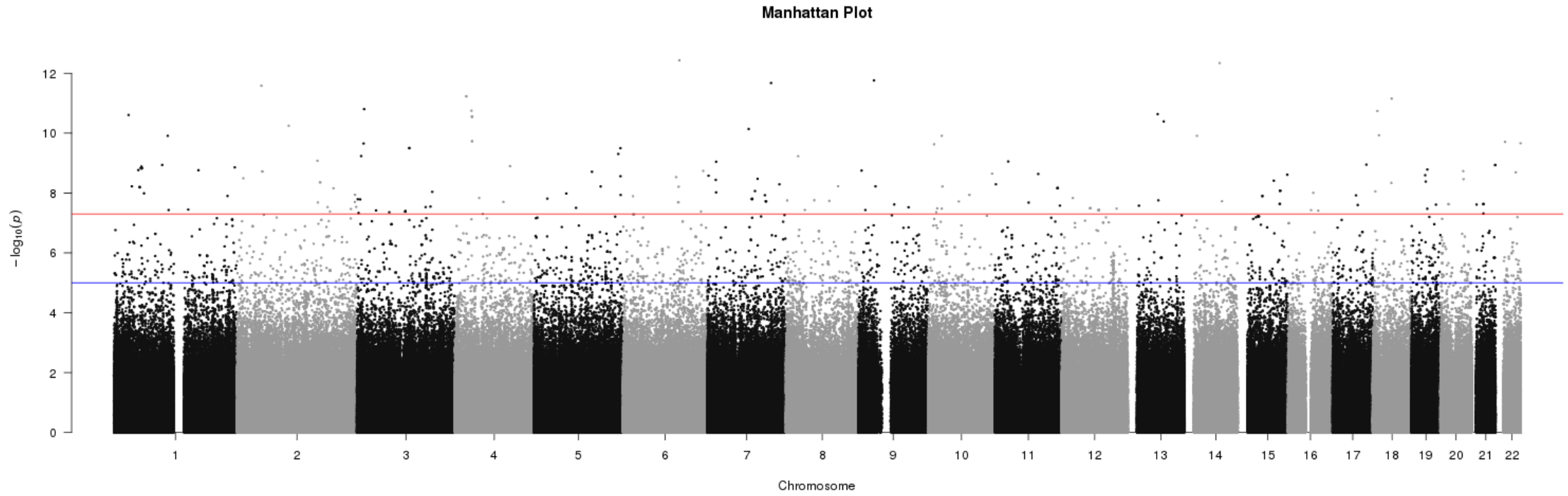
ALS and survival

Single variants associated with survival

- 1552 cases from BE and NL origin had the required clinical info and used in the analysis
- Cox-regression with the following cofactors:
  - Age
  - Gender
  - Sequencing technology
  - Population through pca1-10
  - C9orf72
  - Site of onset

Findings

- 138 loci have a p-value < 5e-8
- Smallest p-value 3.7e-13
- HR between 1.7 and 44
- MAF from 0.07% up to 4.5%



Findings

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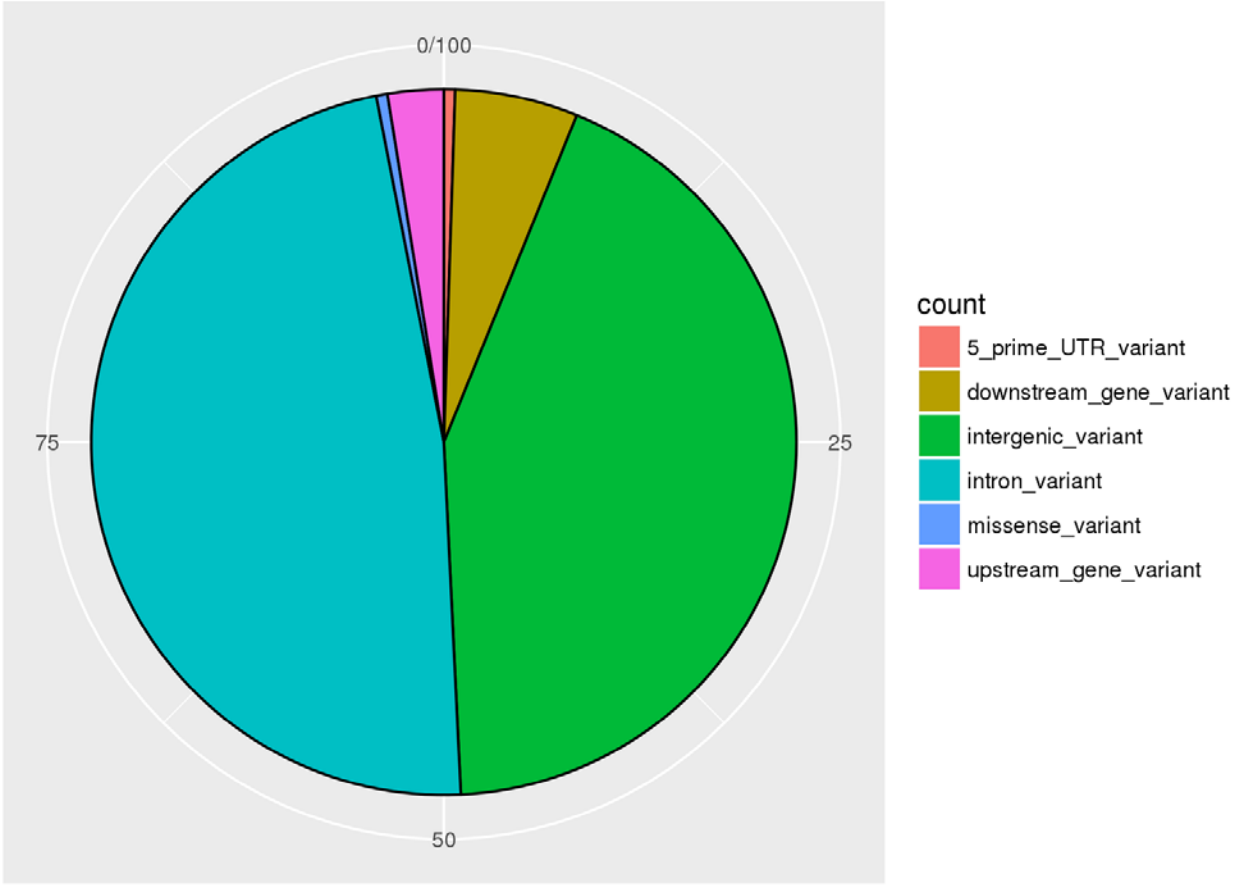

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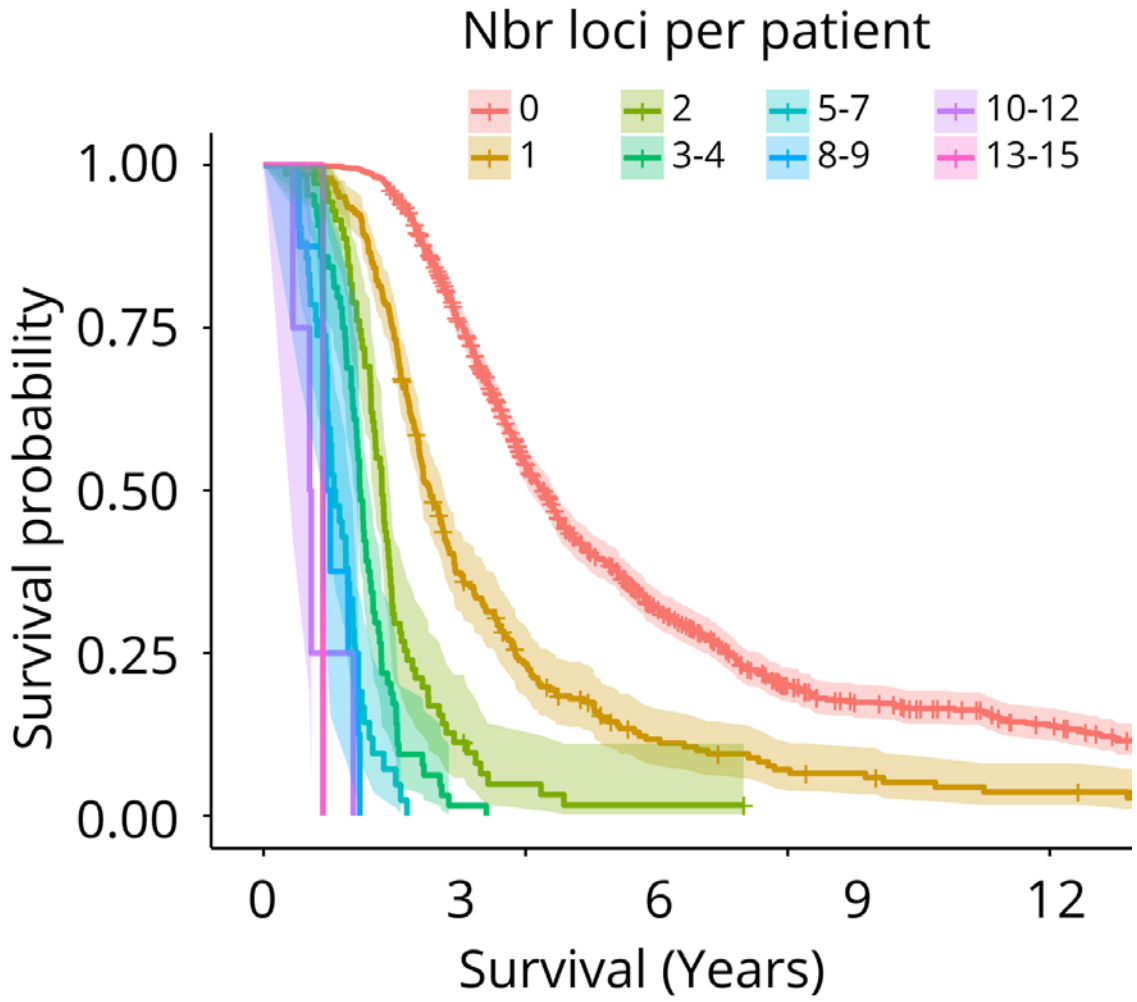
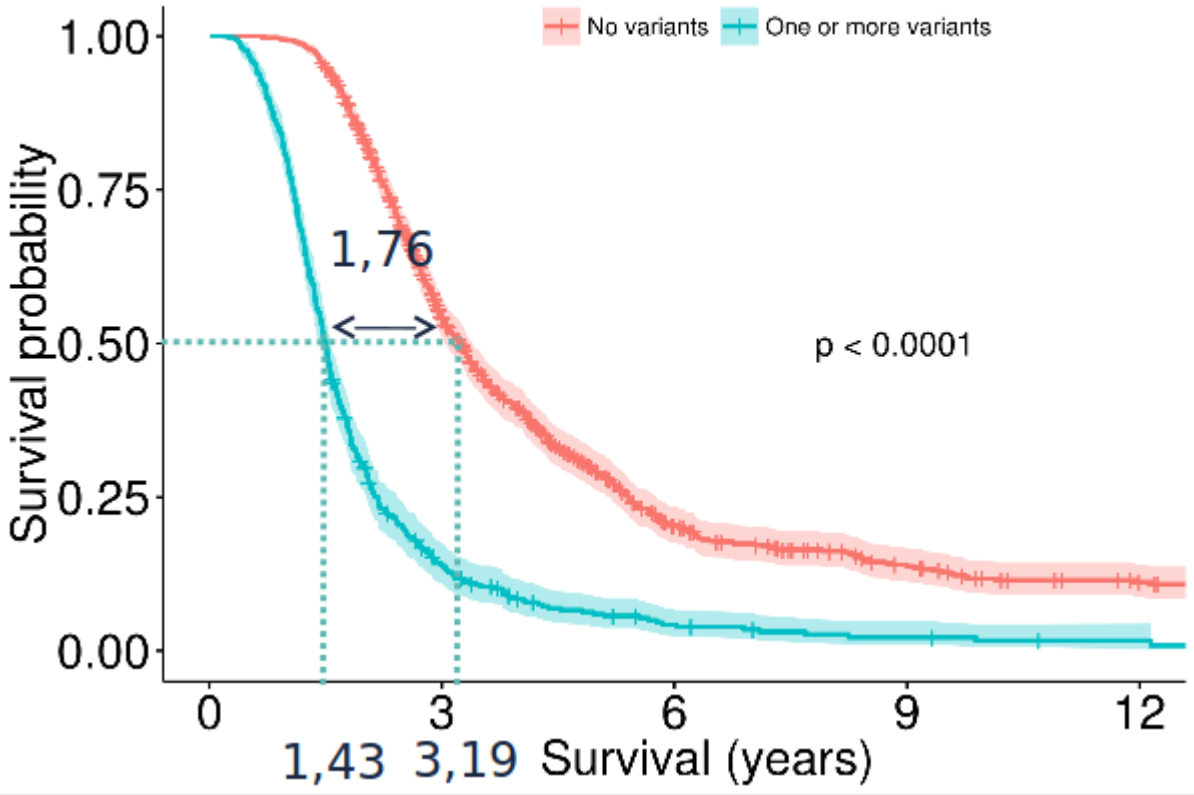
Additive effect





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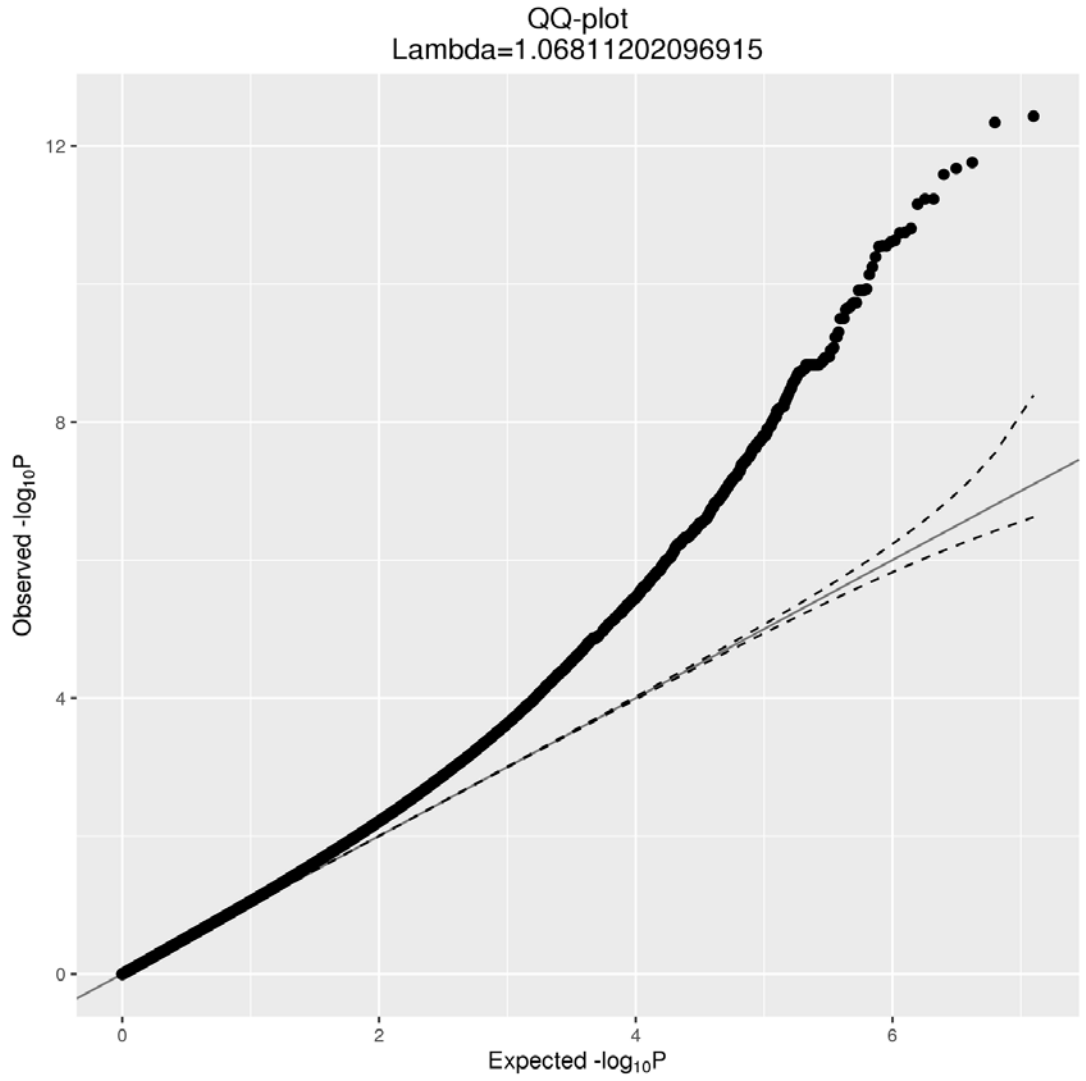
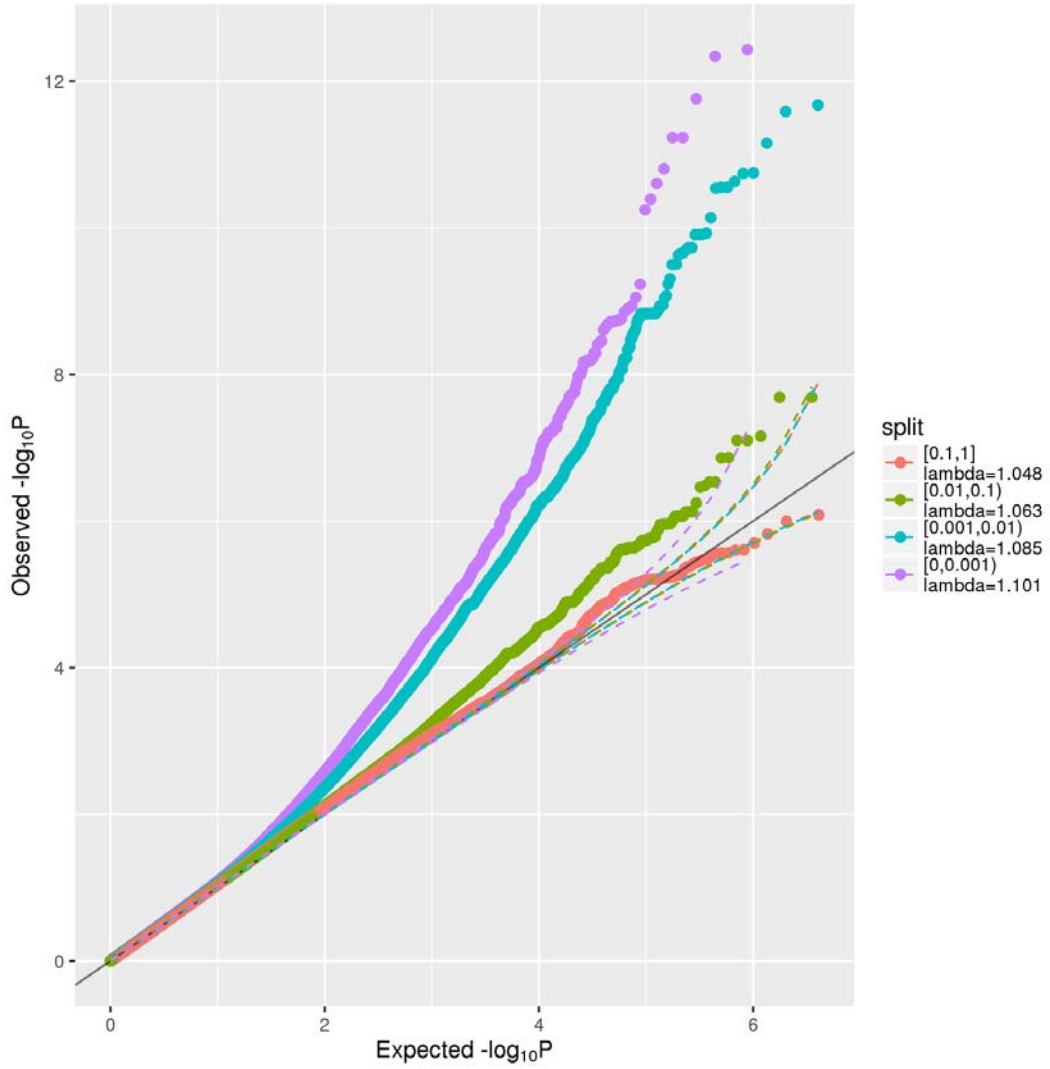
QQ plots reveal deviation from expected



QQ deviation due to rare variants



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Issues

- **Issue:** Hard from distinguish real an association from a random
- **Solution:** permutations
- Currently at 1,789,090

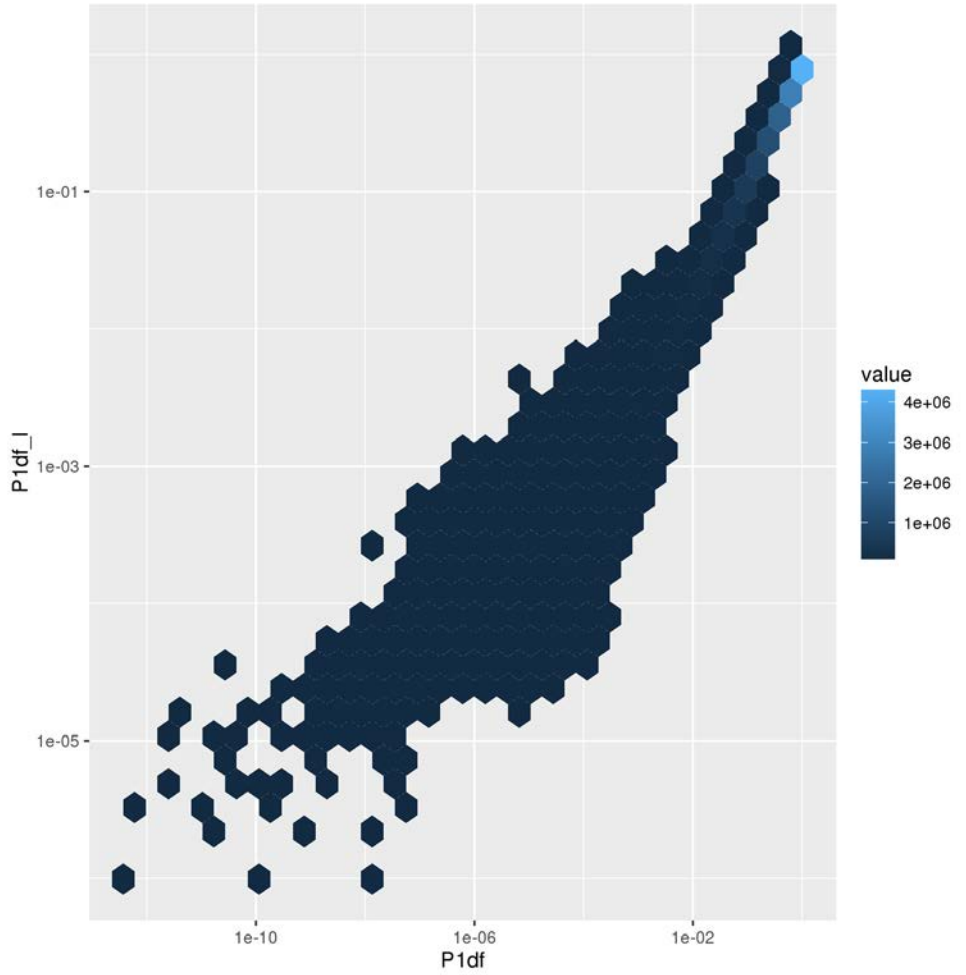
Table 1 Adaptive permutation recommendations

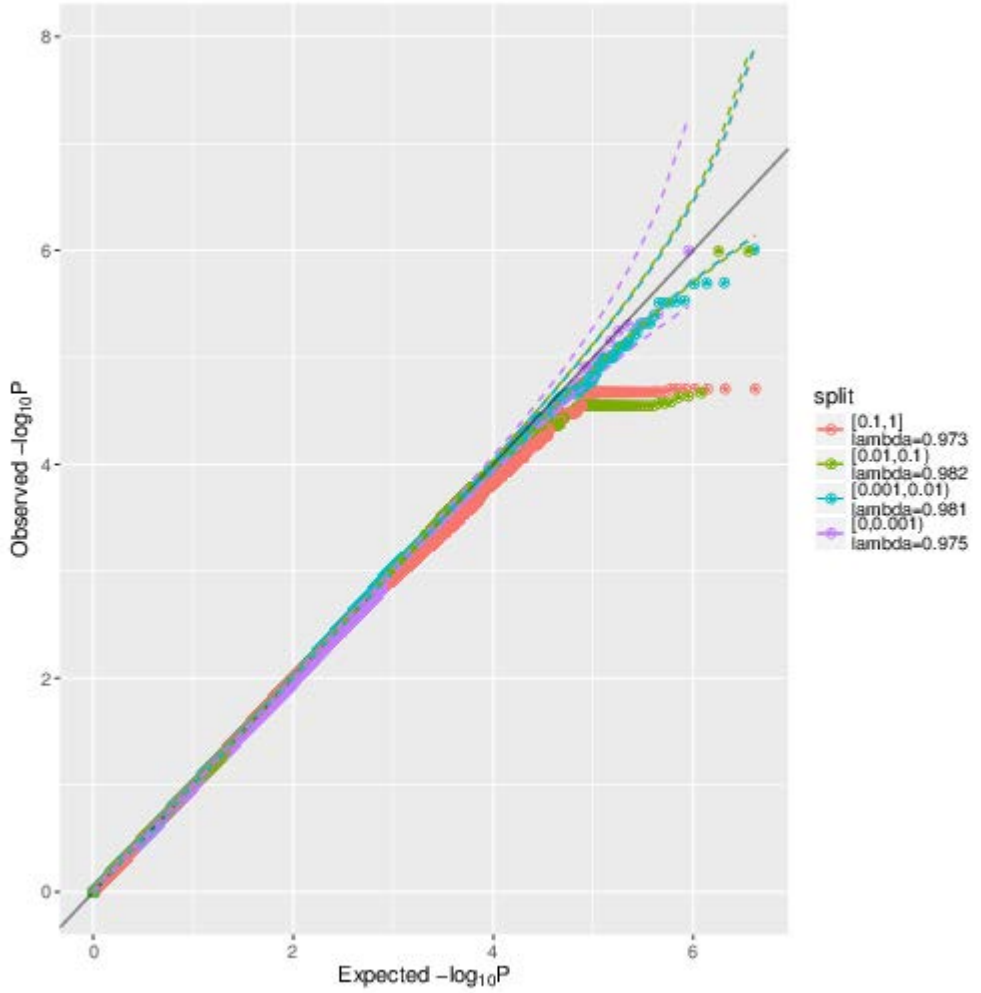
	c	0.1		0.2	
m	α_p	*b*	*r*	*b*	*r*
1	0.05	1,900	115	475	34
5	0.01	9,900	120	2,475	36
50	1e-3	99,900	121	24,975	36
500	1e-4	999,900	121	249,975	36
1,000	5e-5	1,999,900	121	499,975	36
10,000	5e-6	19,999,900	121	4,999,975	36
100,000	5e-7	199,999,900	121	49,999,975	36
1,000,000	5e-8	1,999,999,900	121	499,999,975	36

Recommendation of the number of permutations (*b*) and cut-off value (*r*) varying the number of SNPs (*m*), PWER (α_p) and precision level (*c*), with a fixed EWER (α_e = 0.05).

Che et al., BioData mining 2014

Permutations





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Validation cohort

- Cohort of 616 ALS patients
- Could decrease the p-value of 6 with a p-value < 5e-8

ALS and survival extremes

Single variants associated with extreme survival

- 613 cases from BE and NL origin with survival > 4 or < 1.5 years
- Linear-regression with the following cofactors:
  - Age
  - Gender
  - Sequencing technology
  - Population through pca1-10
  - c9orf72
  - Site of onset

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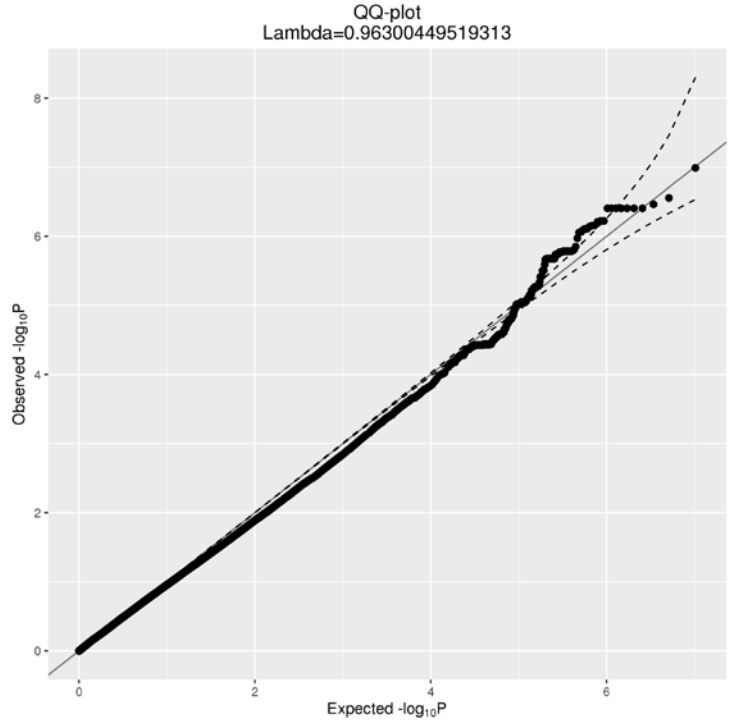
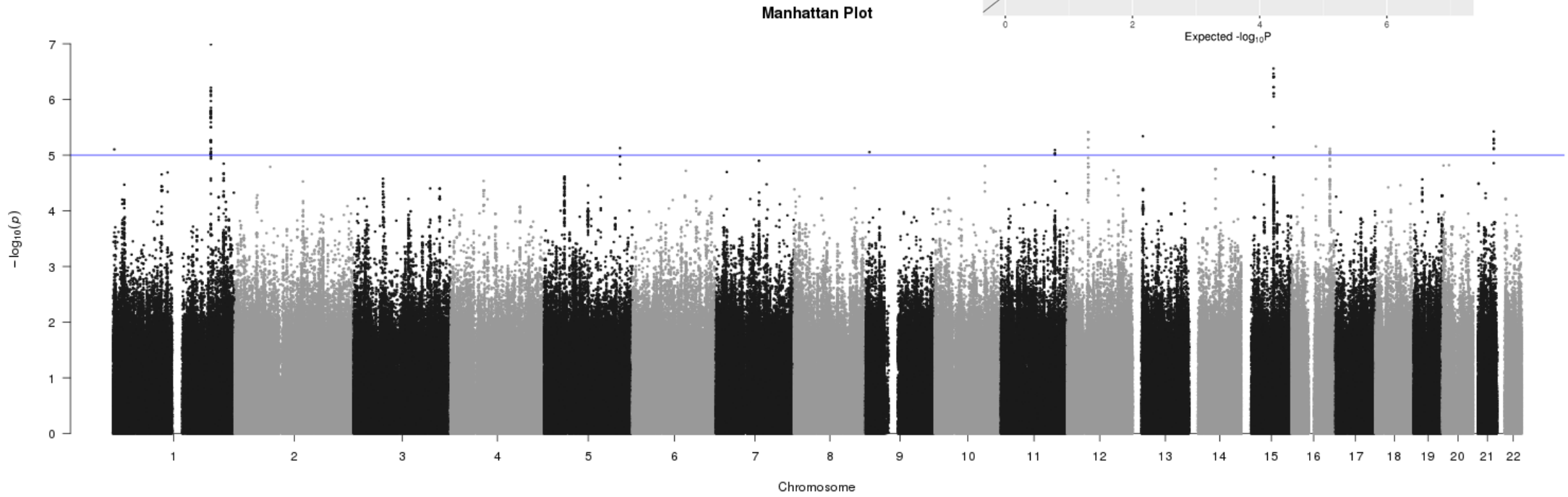
Extremes analysis

- 0 loci have a p-value < 5e-8
- 59 variants have a p-value < 5e-6
- HR between 0.34 and 2.6
- MAF from 14% up to 50%





Onset analysis

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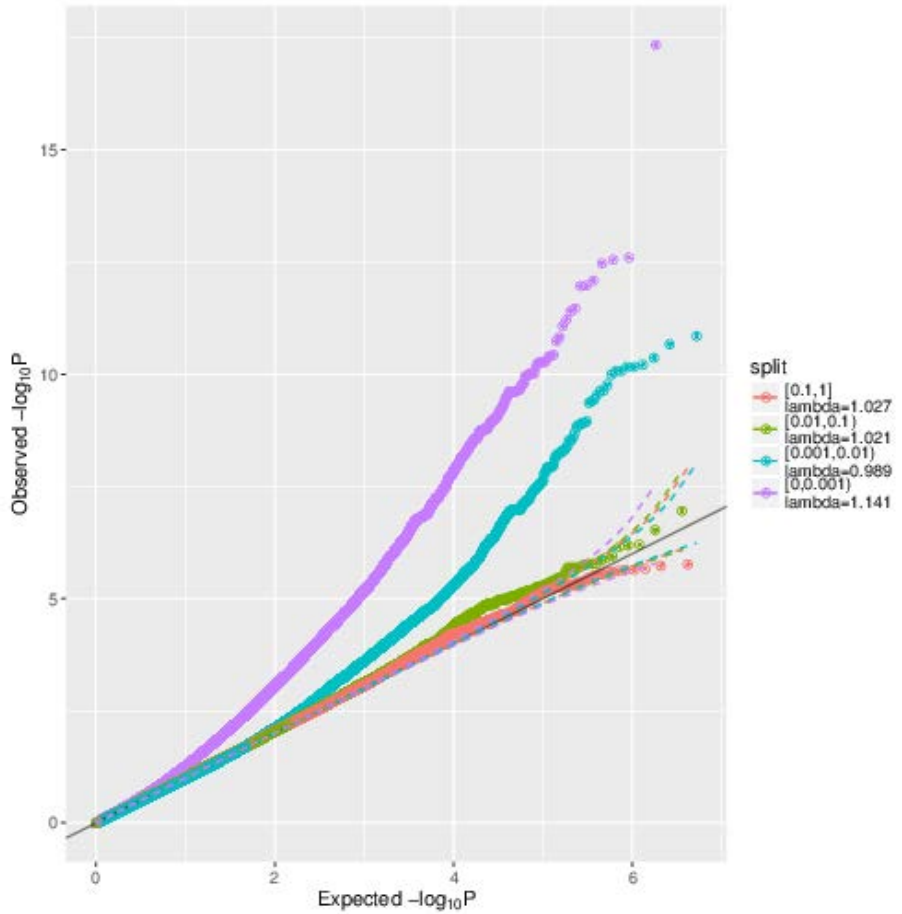
ALS and onset

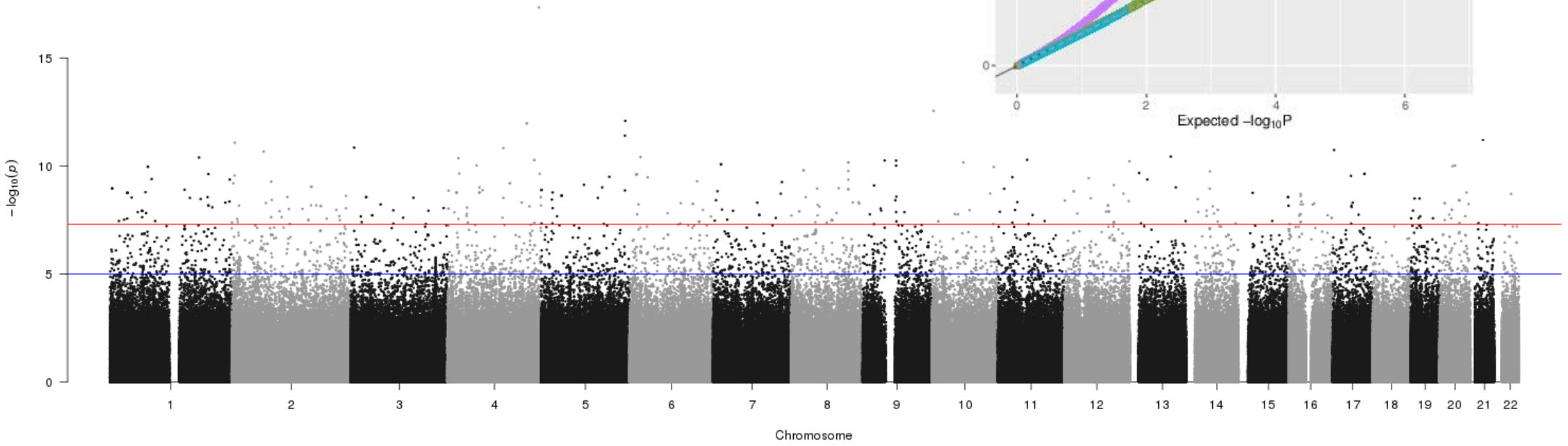
Single variants associated with onset

- 1552 cases and 881 controls from BE and NL origin had the required clinical info and used in the analysis
- Cox-regression with the following cofactors:
  - Gender
  - Seq technology
  - Population through pca1-10

Findings

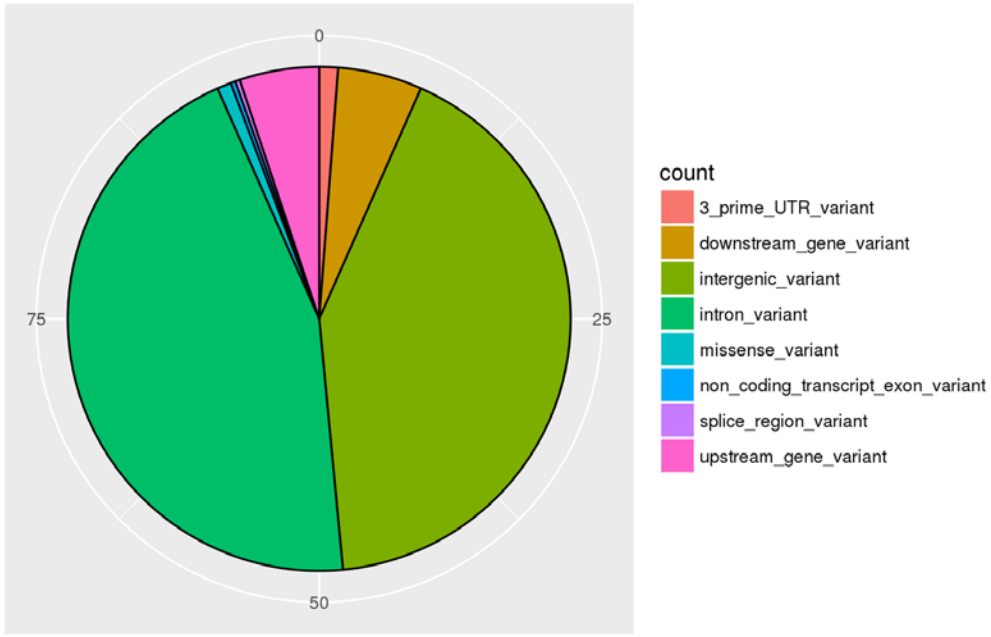
- 337 loci have a p-value < 5e-8
- Smallest p-value 4.6e-18
- HR between 2.3 to 93
- MAF between 0.05% and 1.0%

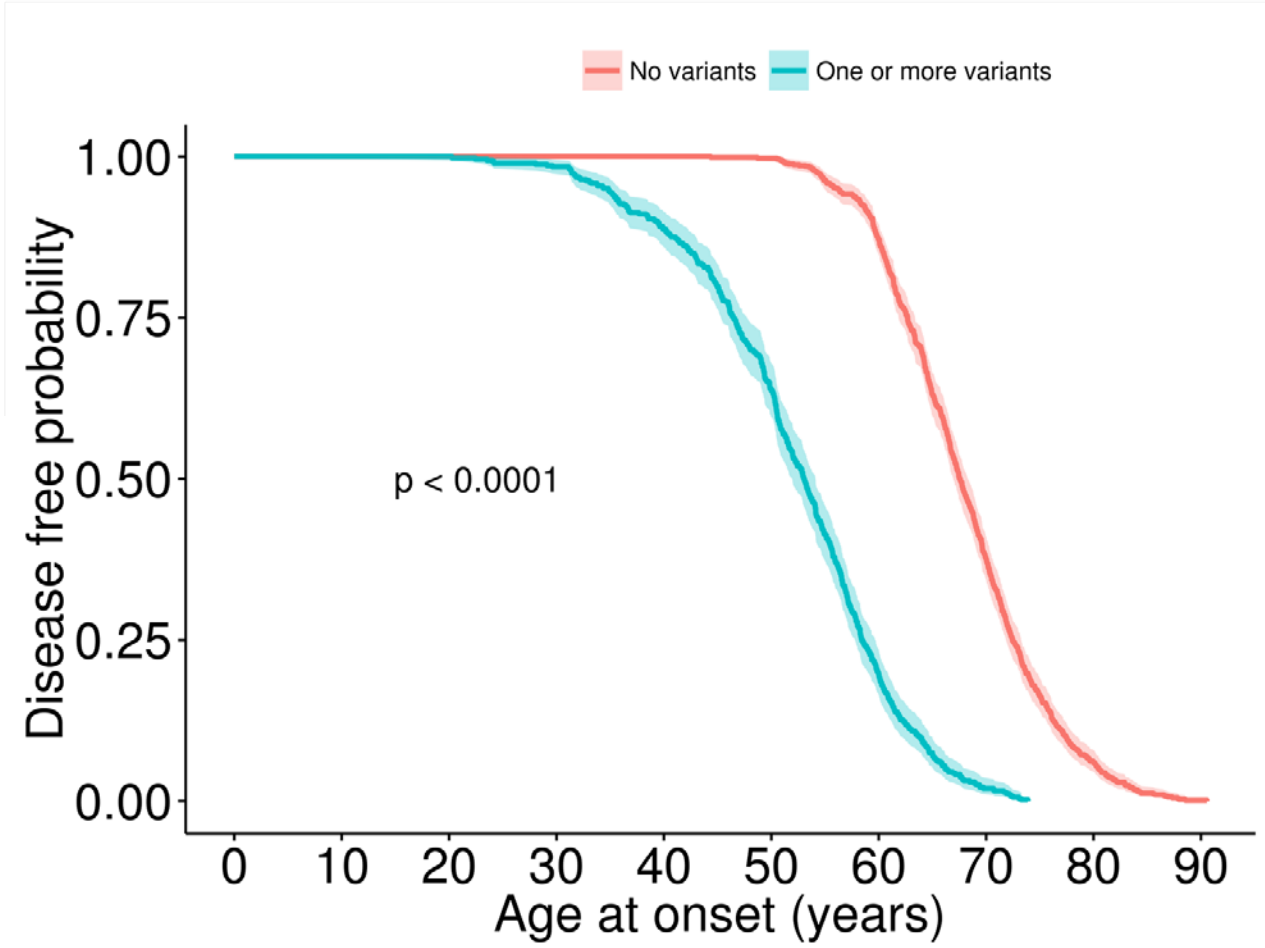




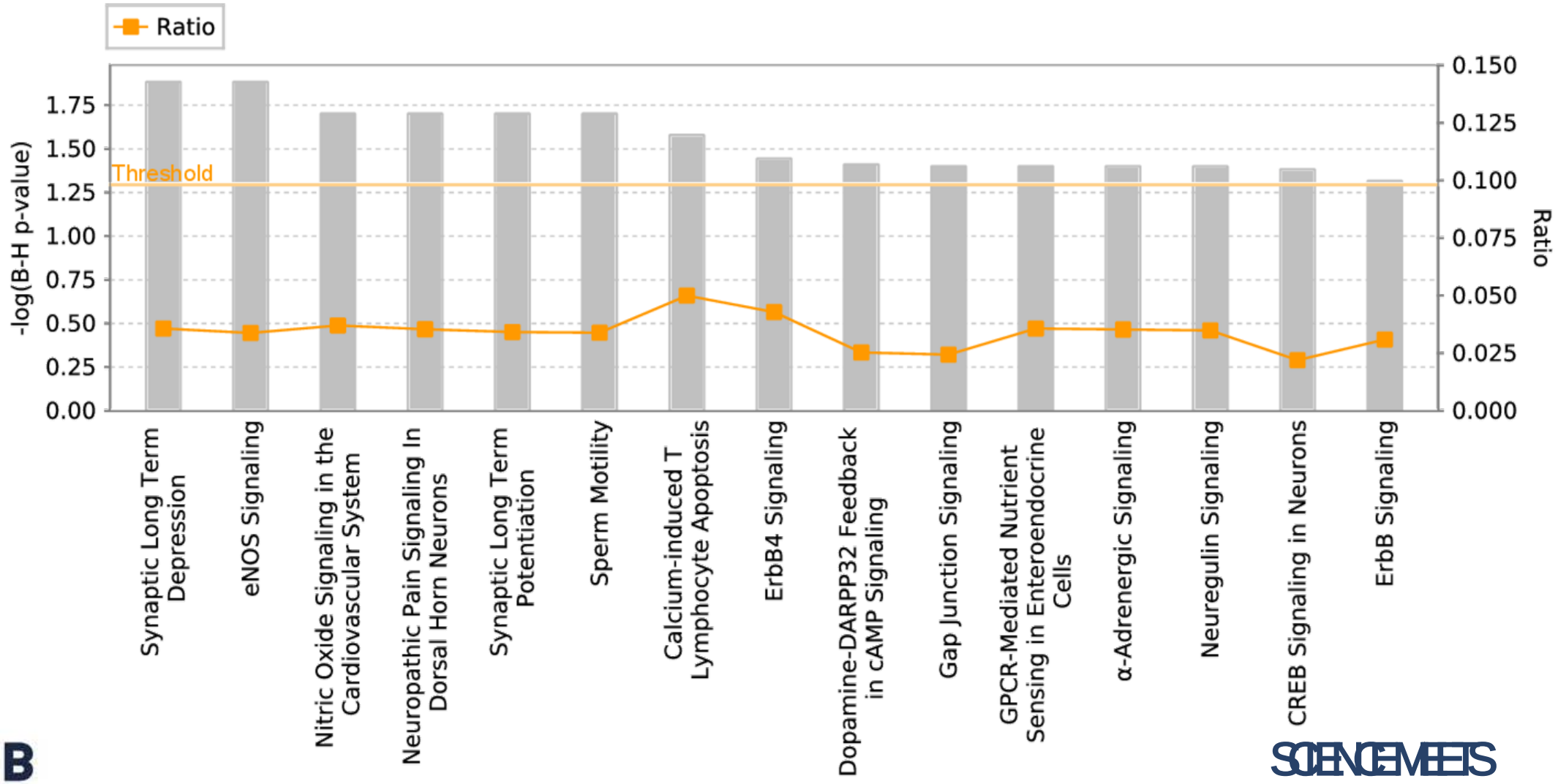
Findings

- 337 loci have a p-value < 5e-8
- Smallest p-value 4.6e-18
- HR between 2.3 to 93
- MAF between 0.05% and 1.0%
- 51 loci validated





Pathway analysis



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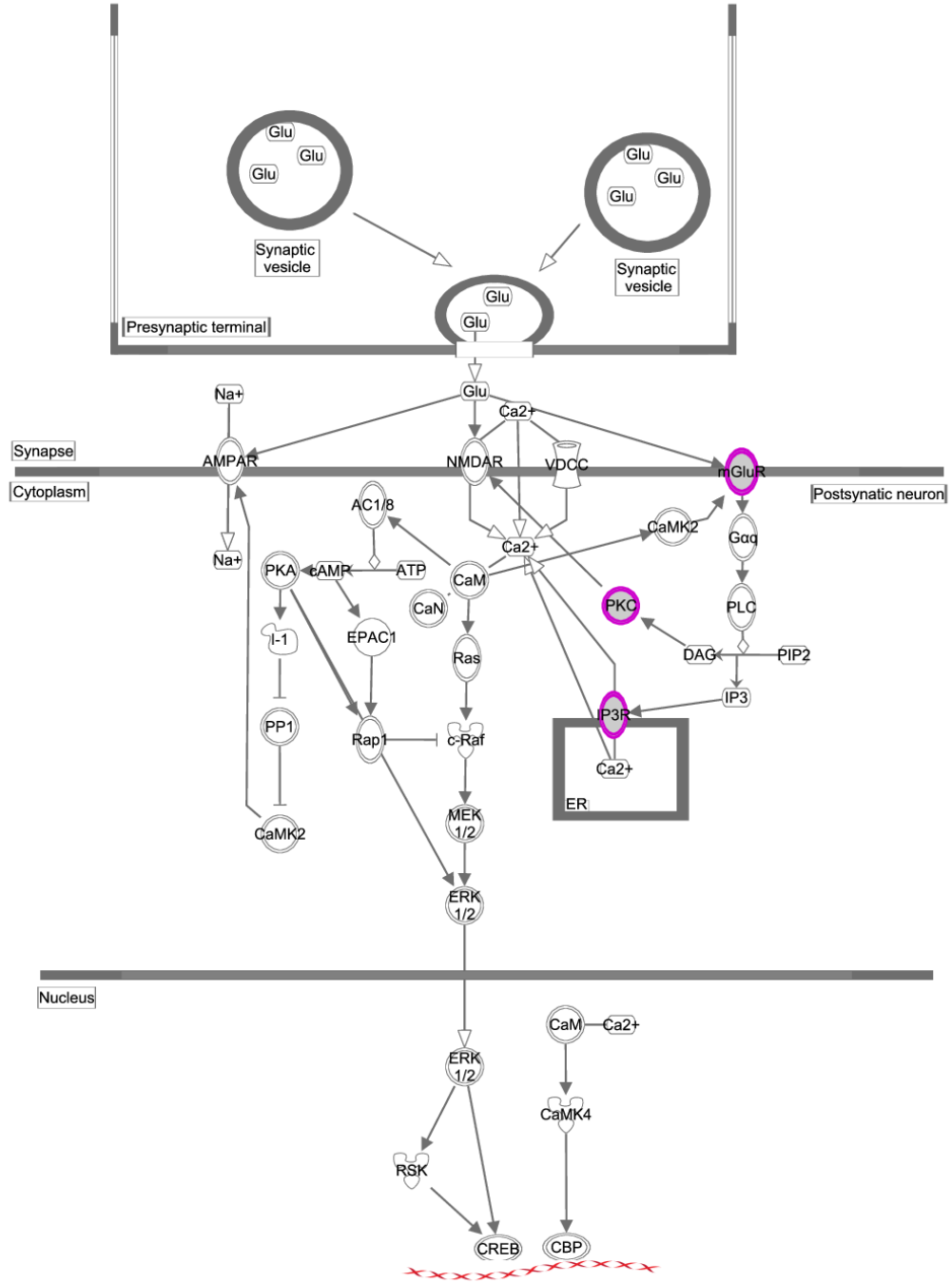
Path Designer Synaptic Long Term Potentiation



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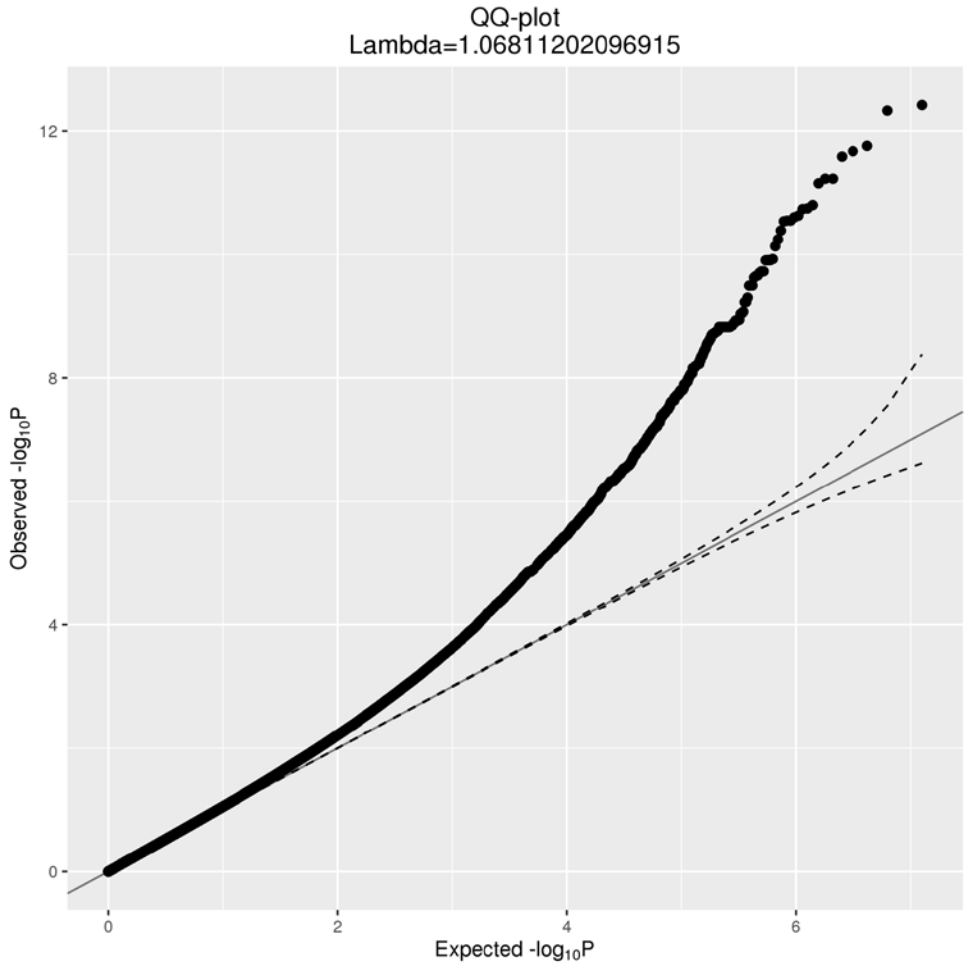
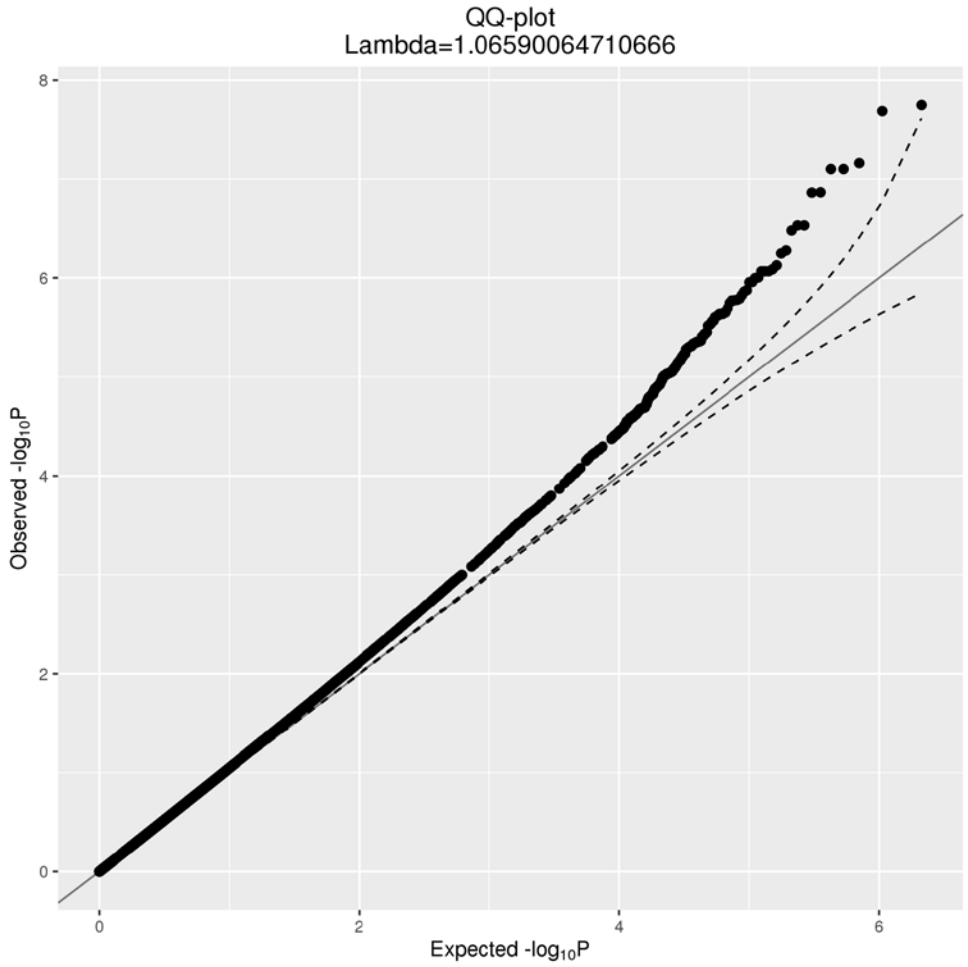
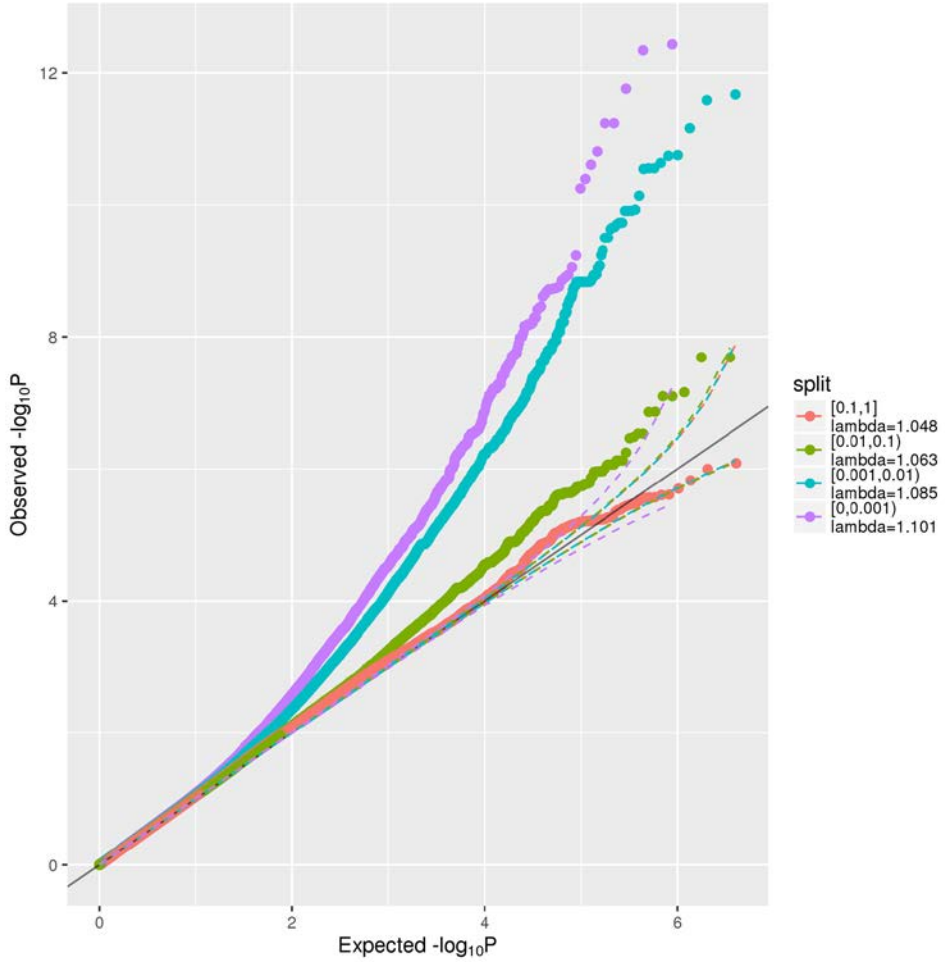
QQ plots reveal deviation from expected

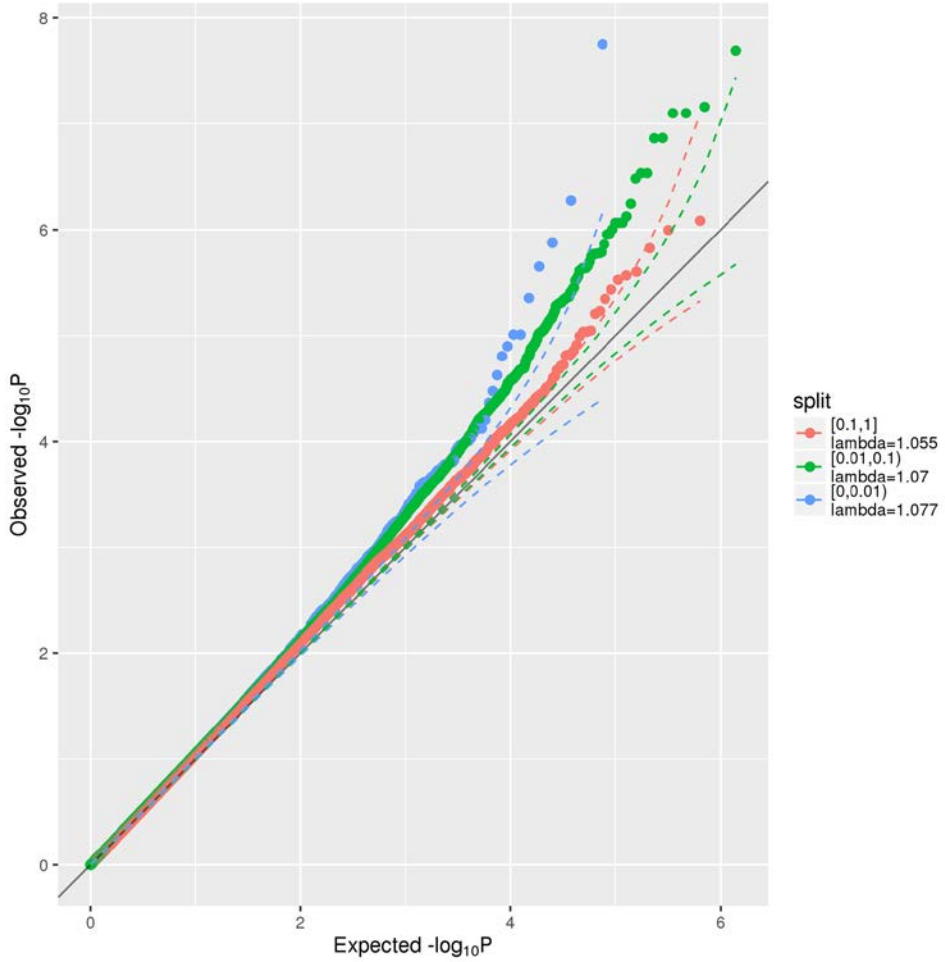




Pruned

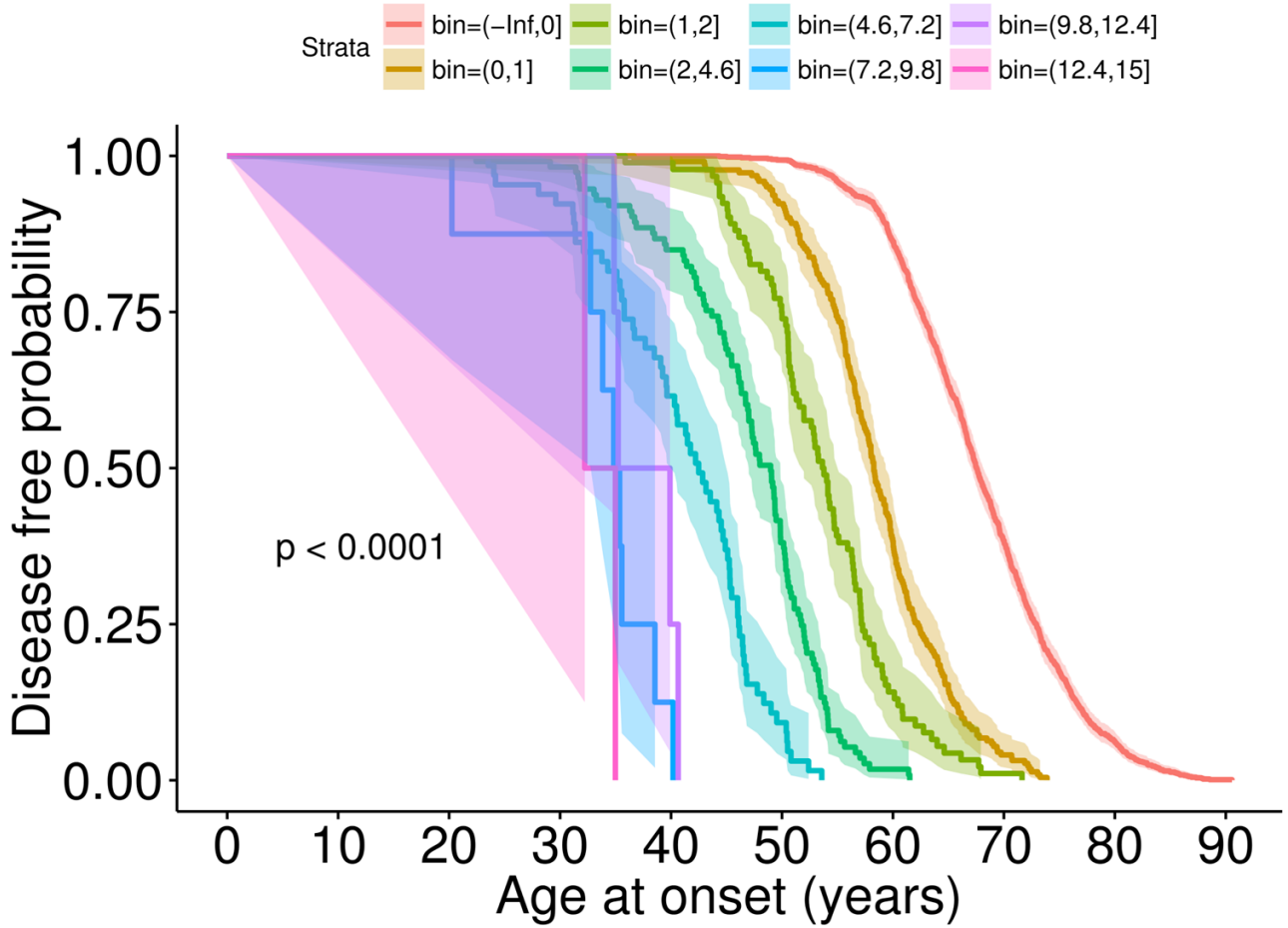
QQ deviation due to rare variants

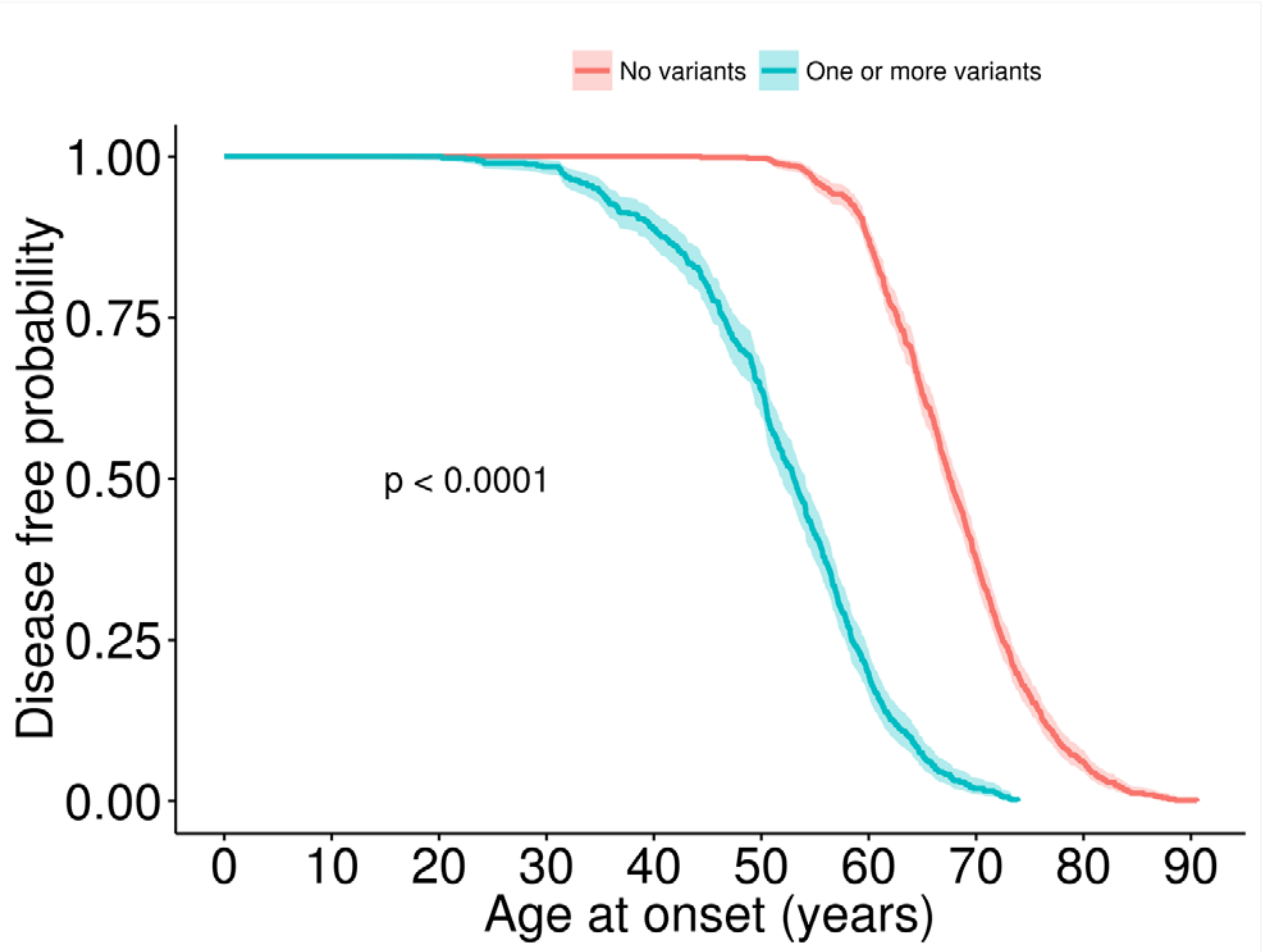




Pruned

Additive effect





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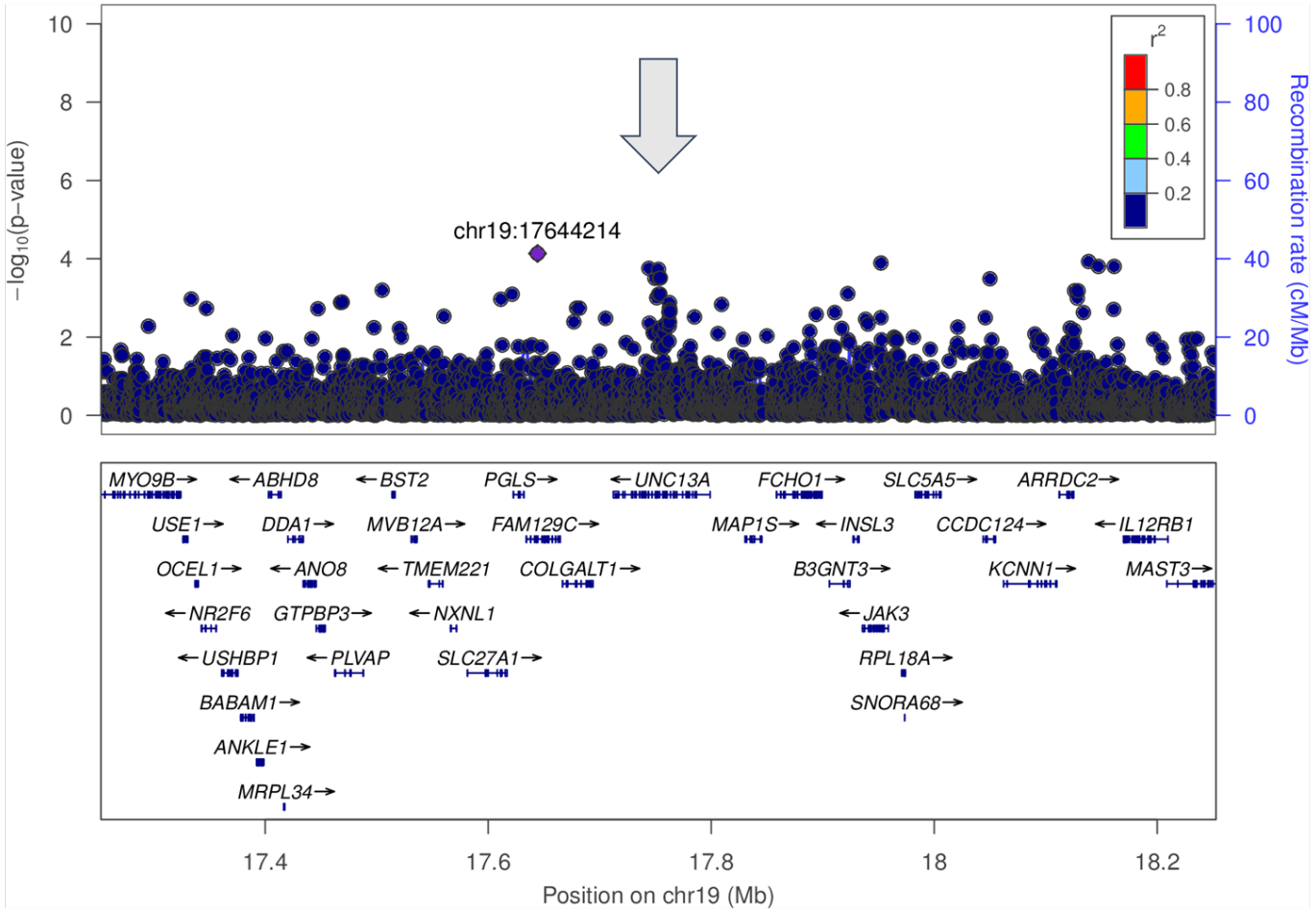
Previously reported associated loci

UNC13A



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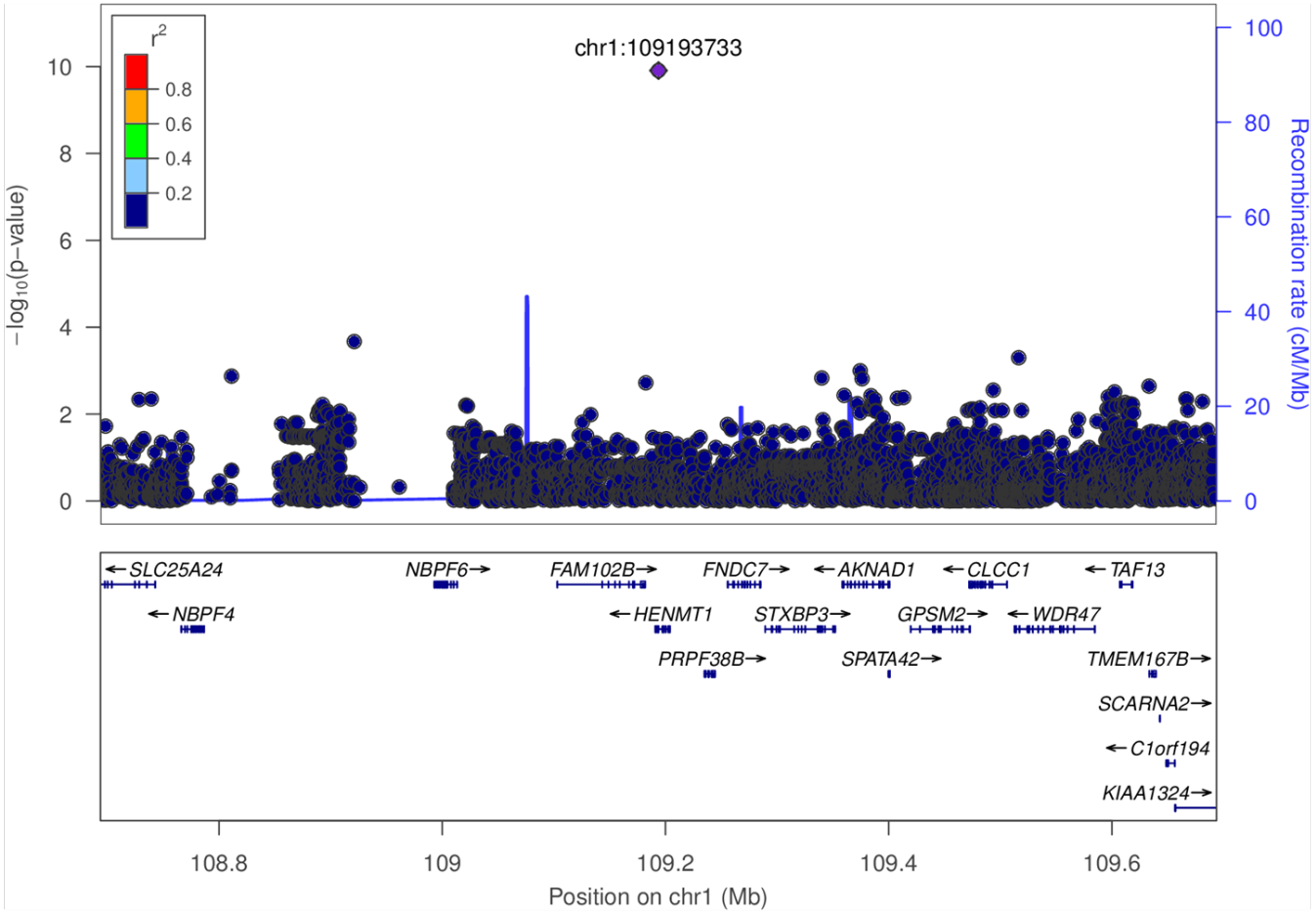
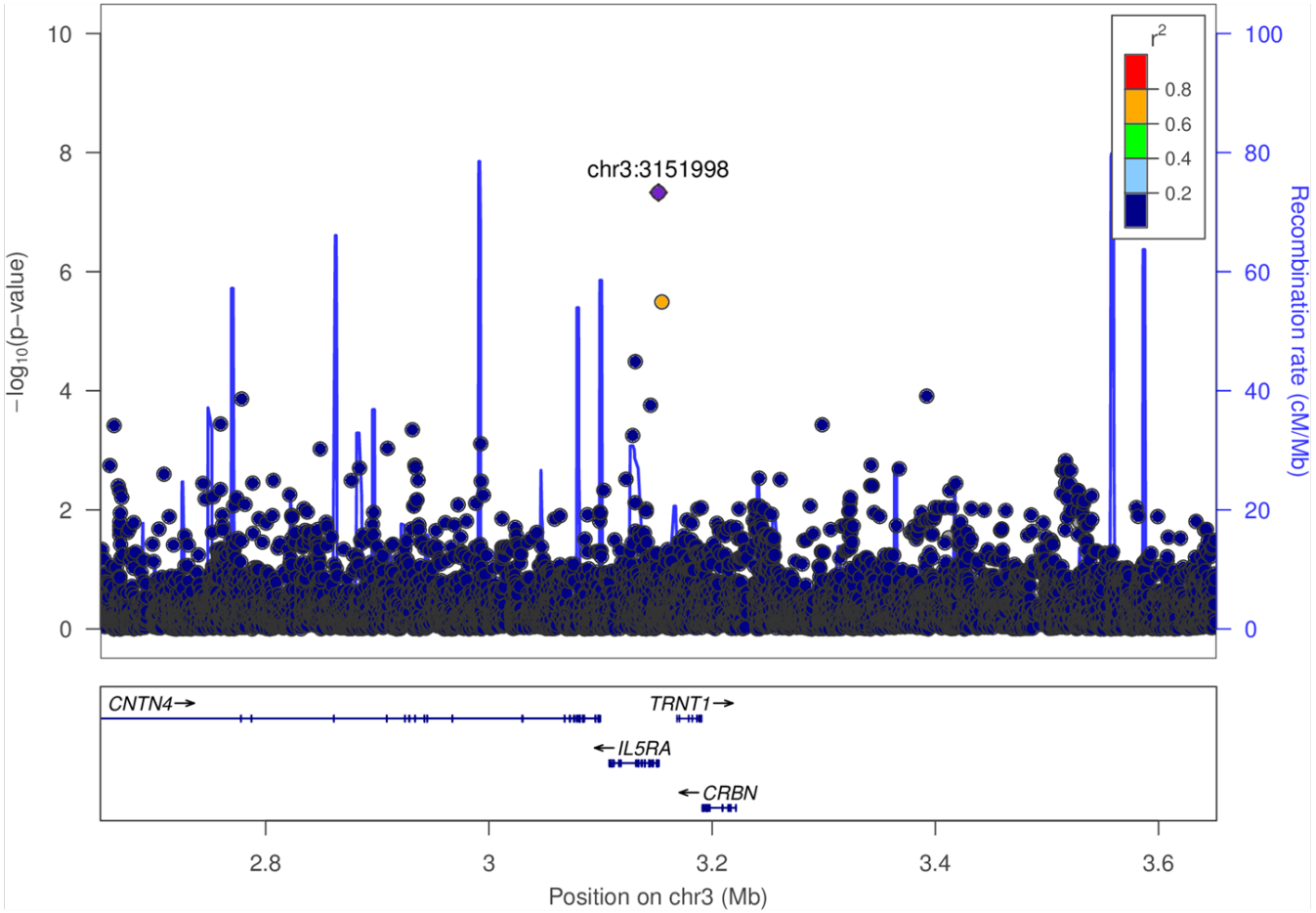
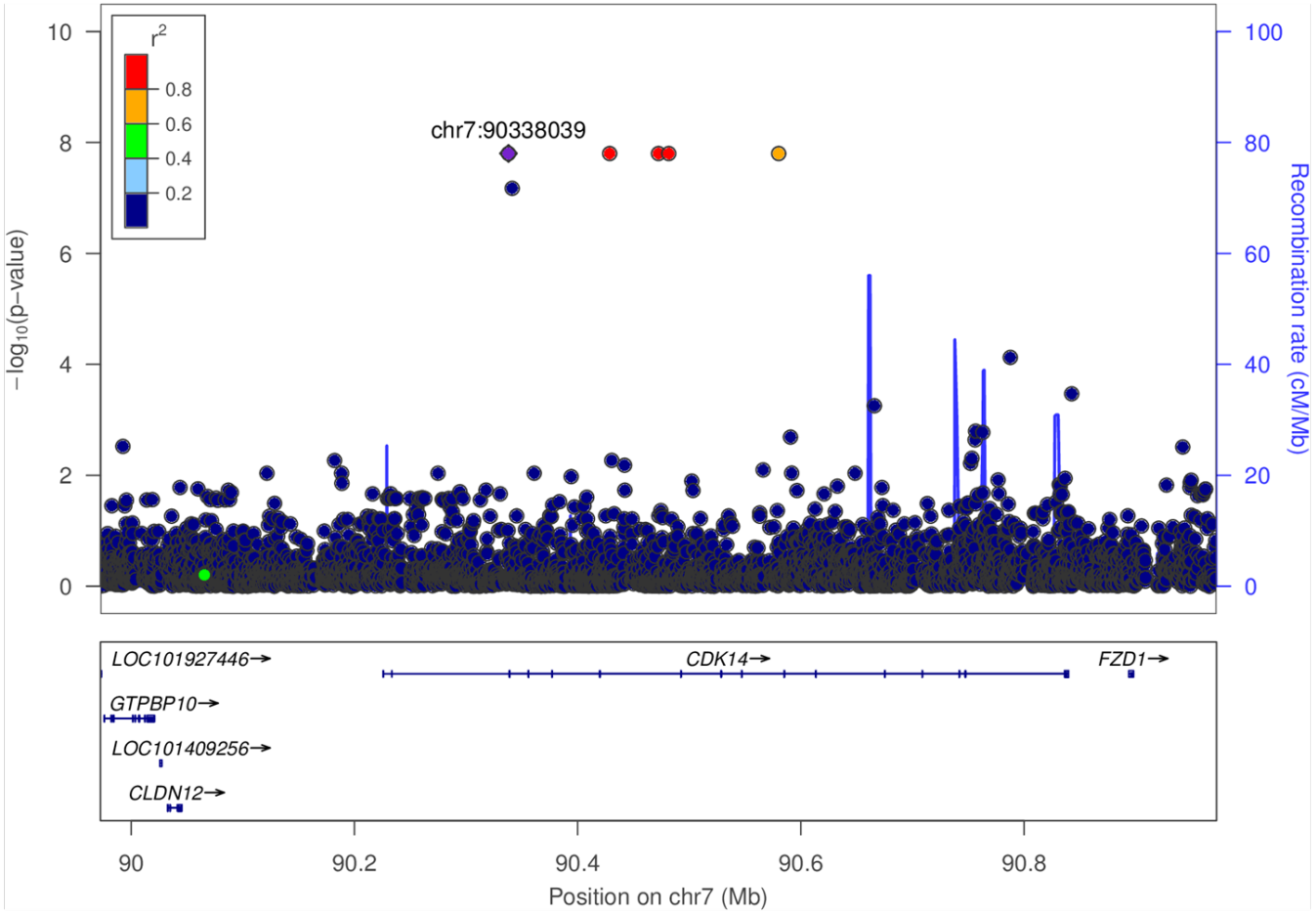
Exonic loci in HENMT1



5’UTR IL5RA


chr3:3151998
r²
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
CNTN4
TRNT1
IL5RA
CRBN
Position on chr3 (Mb)

CDK14 - gene with the most hits.



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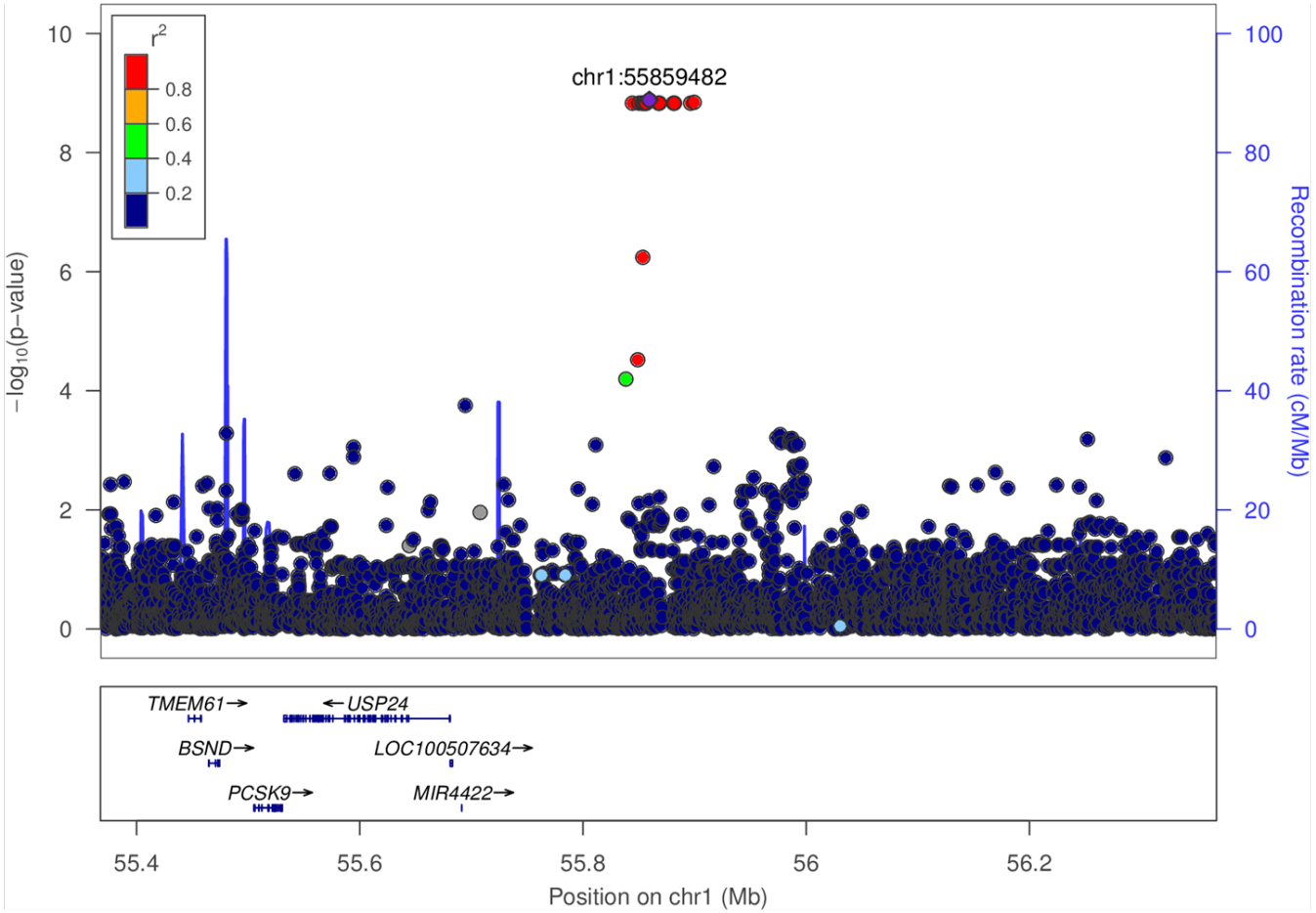
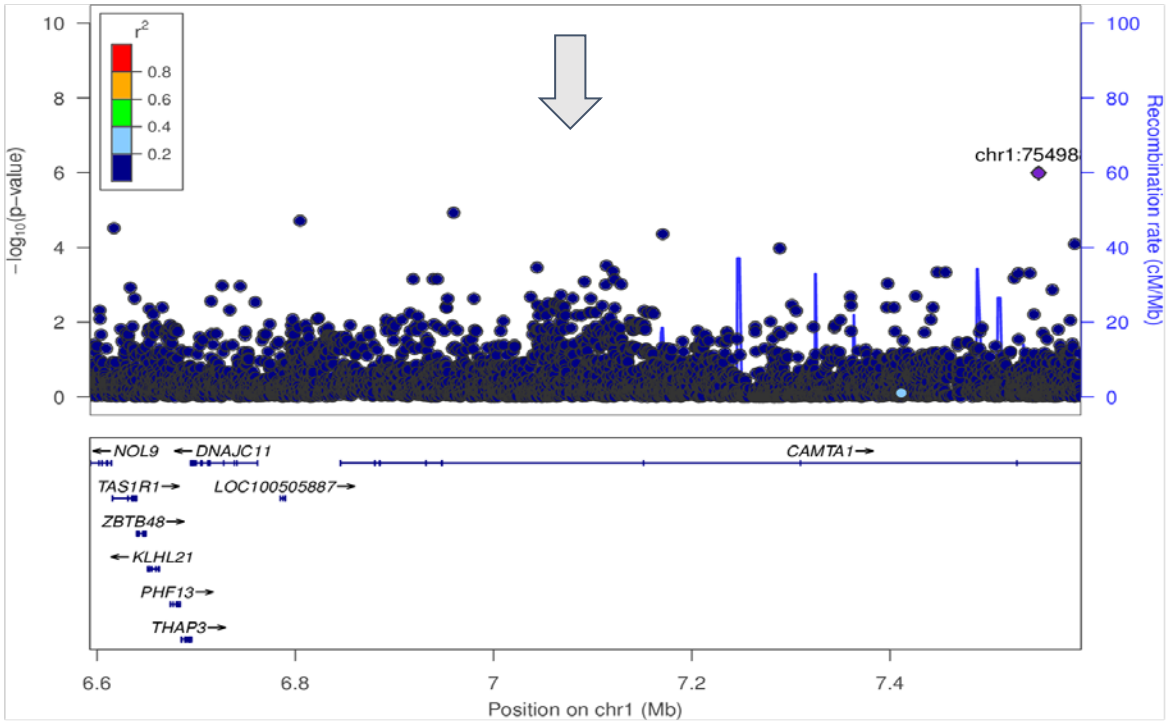
… followed by RNU6-830P

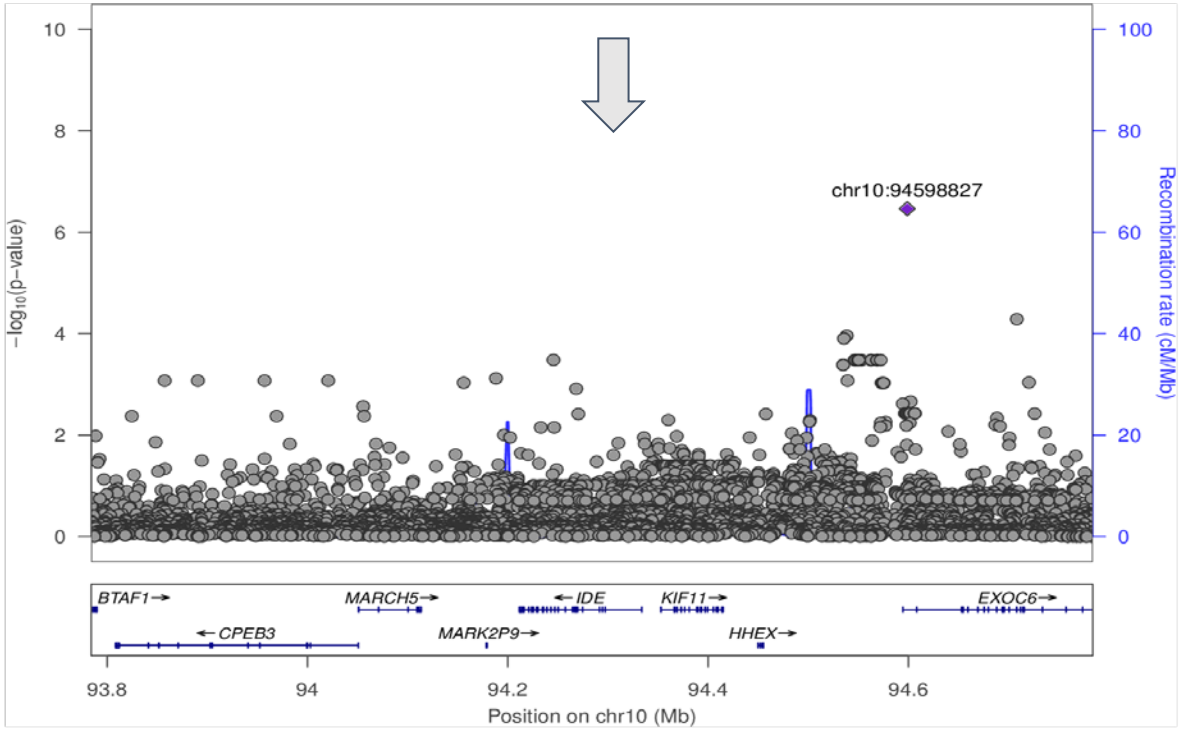


Previously reported associated loci

CAMTA1

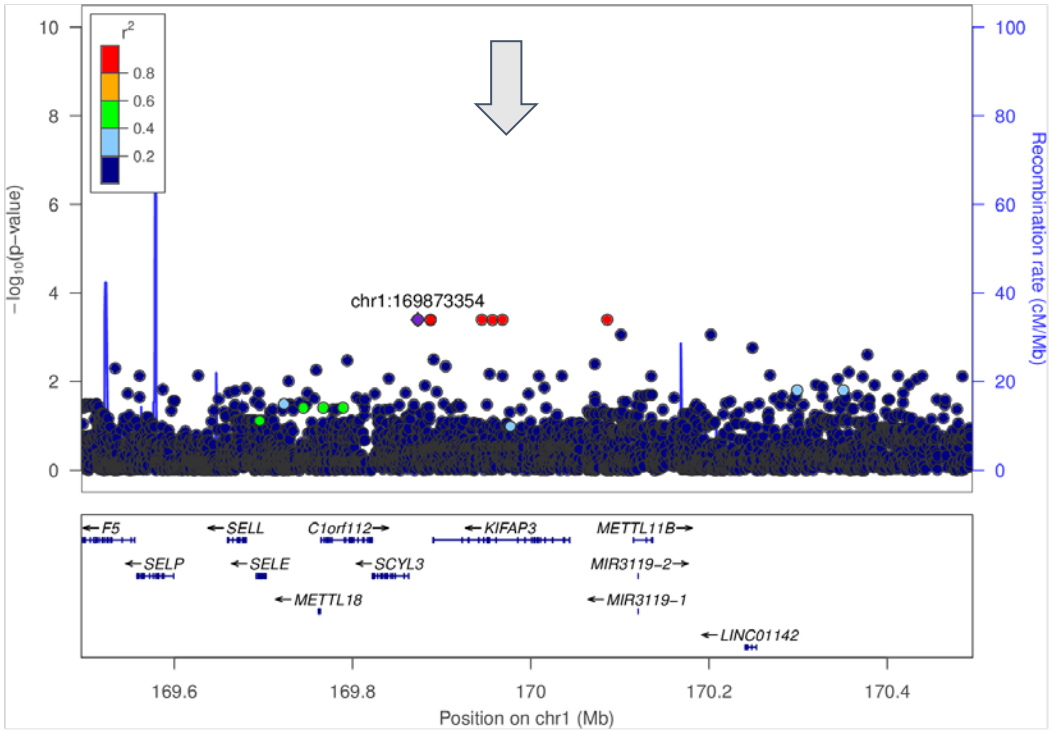


IDE

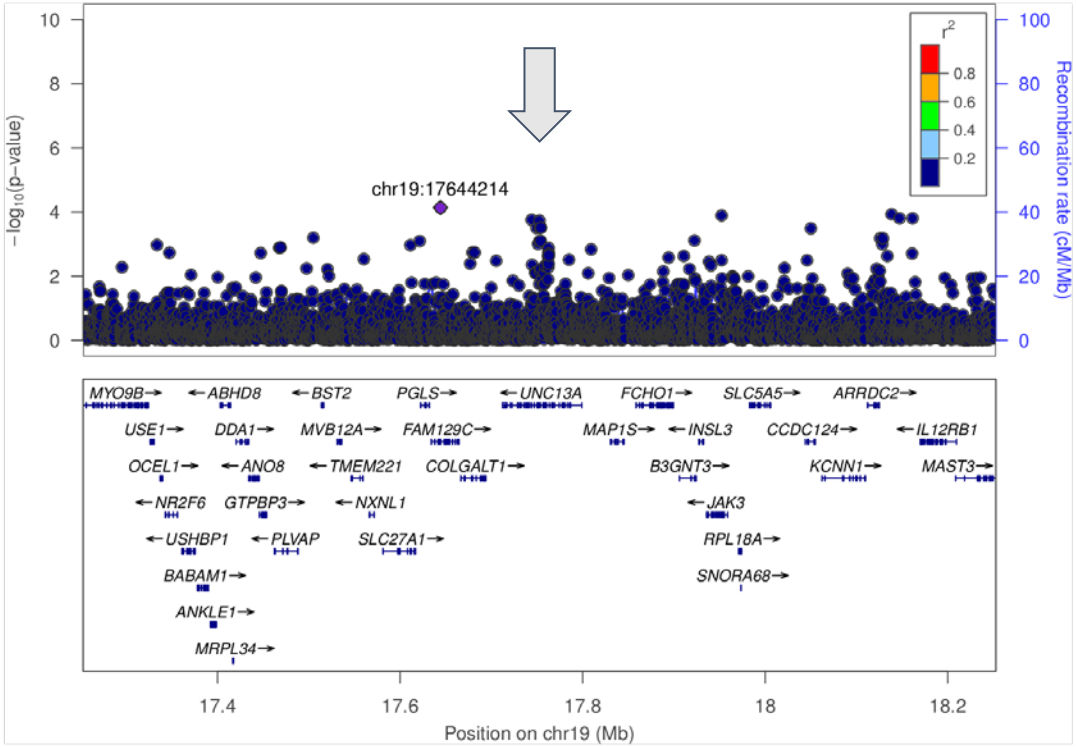


Previously reported associated loci

KIFAP3



UNC13A



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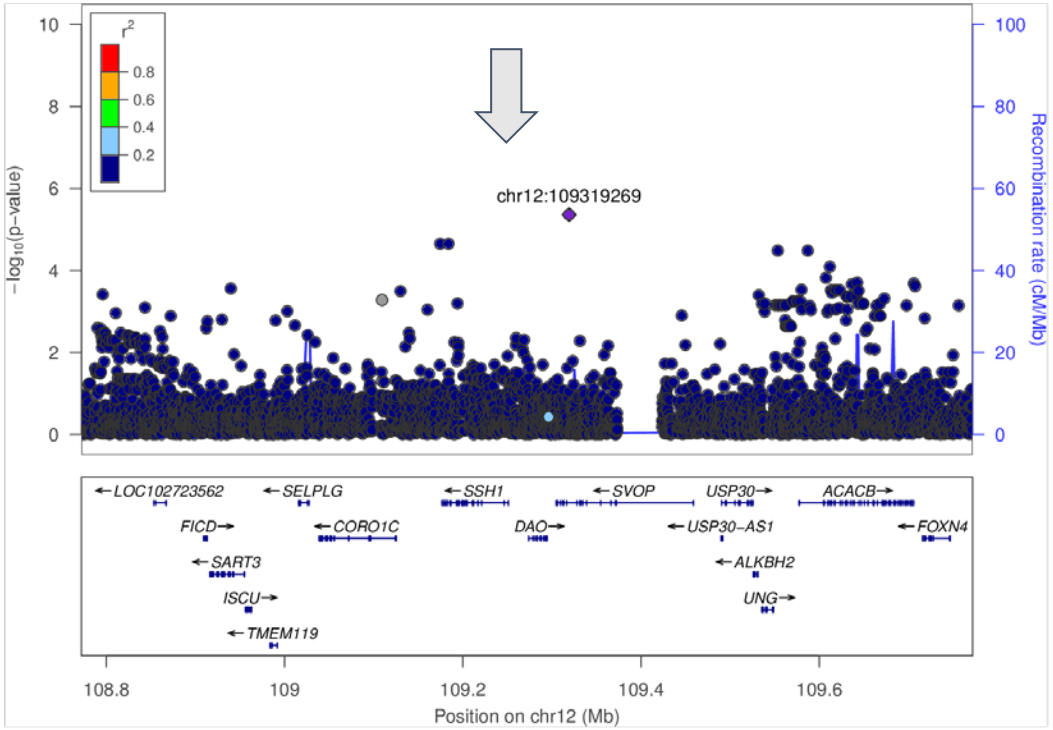
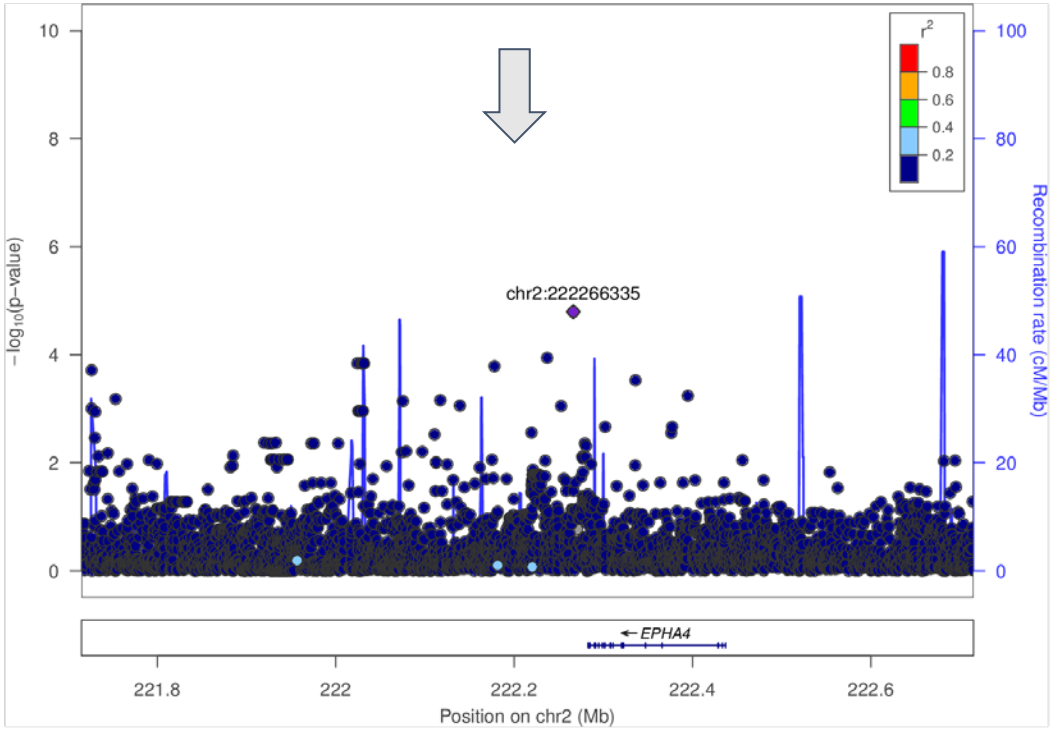
Previously reported associated loci

DOA



EPHA4



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