Looking for a genetic component to alcohol drinking

Luisa Zuccolo

Department of Social Medicine and
MRC Centre for Causal Analyses in Translational Epidemiology

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Outline

- Background and rationale
- Validation studies
  - Methods
  - Results
- Limitations and Conclusion

Rationale

What causes variation in alcohol drinking ?

- A multi-factorial complex trait
- 30-60% Heritability

Rationale

Who is looking for genetic variants and why ?

- Genetics of alcoholism
- Use variants with established links to alcohol behaviour to make robust inference on the effects of alcohol (avoiding confounding and reverse causation)

How large is the anticipated effect of genes ?

- Quite small for individual variants (European populations)
- There are exceptions, *ie* ALDH2 (North-East Asia)

Genetic association studies

Candidate gene studies

Functional candidates (incl. alcohol metabolism)

Evidence of functionality for SNP or gene

Strong prior

More inclusive

Genome-wide studies

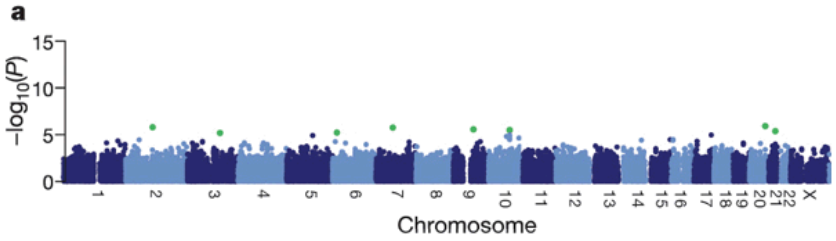
Associations with what can be measured (intake)

whole genome (incl. deserts, introns…)

Pathway knowledge?

More conservative

"True" hits



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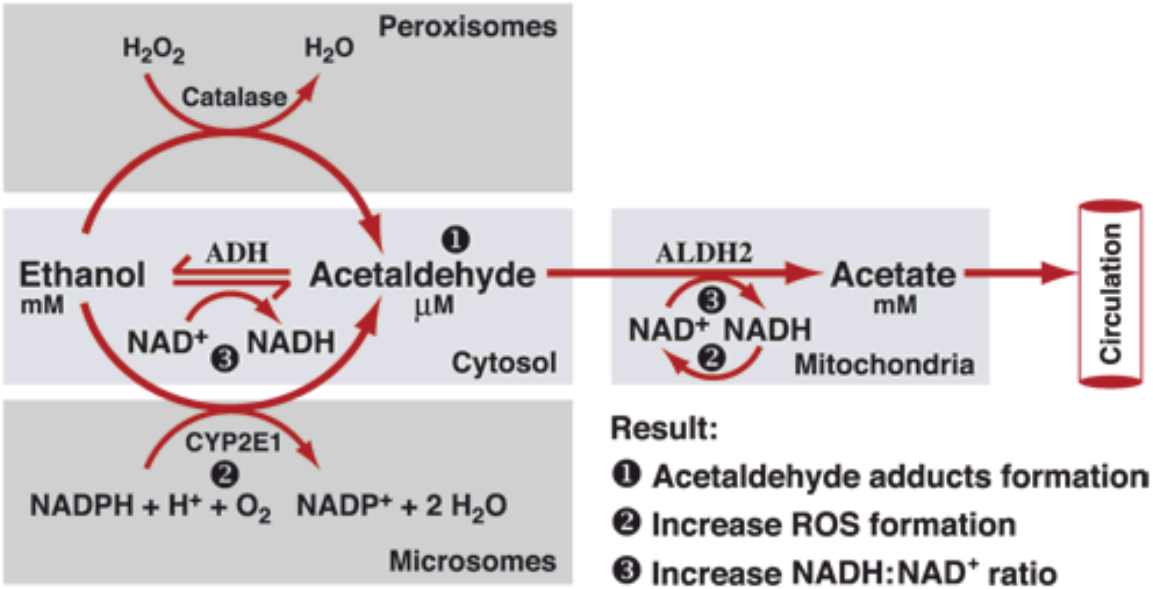
Alcohol metabolism



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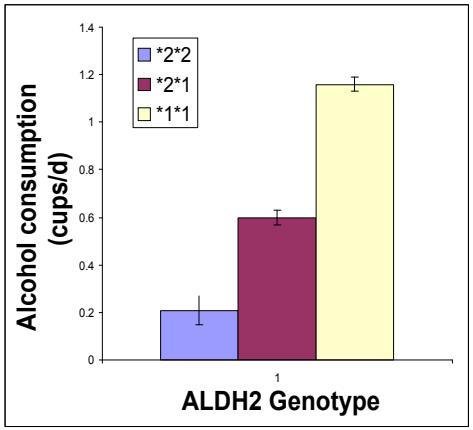
Zakhari S. Alcohol Research & Health 2006; 29(4): 245-54

ALDH2

199 publications on this gene (HuGE Navigator 1.3)

A functional variant causes dramatic differences in alcohol intake



Takagi et al, Hypertens Res 2002;25:677-81

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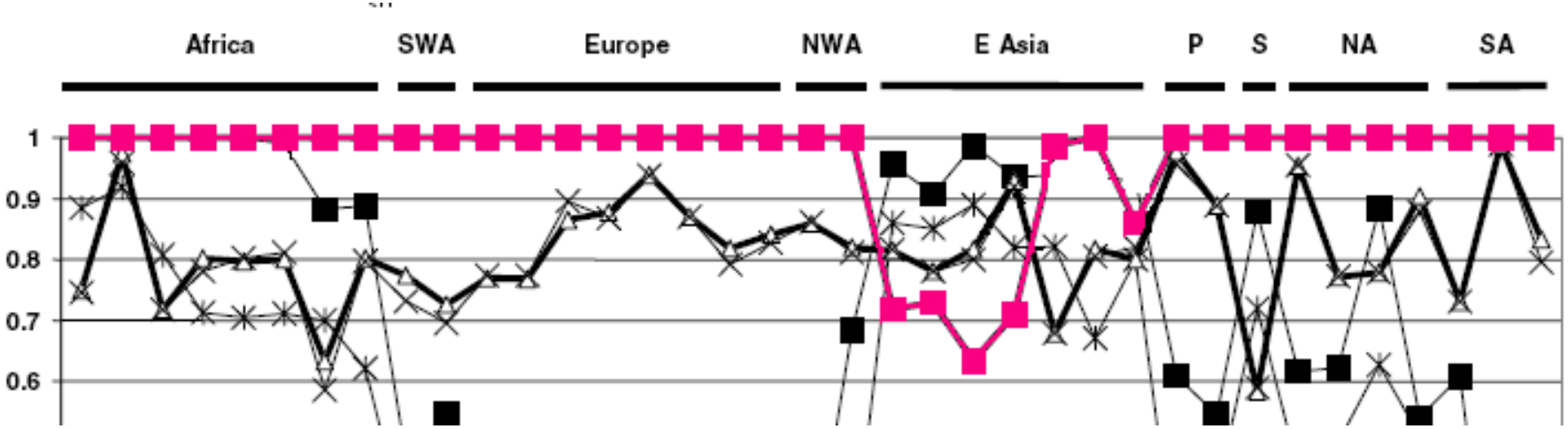
ALDH2

199 publications on this gene (HuGE Navigator 1.3)

A functional variant causes dramatic differences in alcohol intake

Prevalence varies worldwide :

variant absent in Caucasians

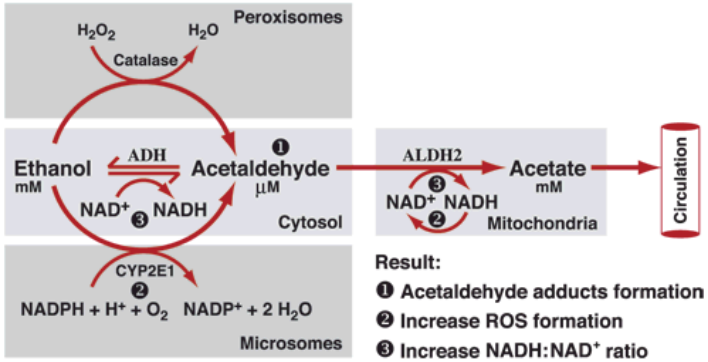


Oota H. et al. Annals of Human Genetics (2004) ***68****,93–109*

ADH – a review of the literature

Functional candidates

- Classical variants in ADH1B and ADH1C
- These are found in European populations



- Evidence for an increased activity of ADH1B variant *in vitro* (but not *in vivo*)

"Association" candidates

- ADH4 – key role at intoxicating levels of alcohol / associated with multidrug abuse
- ADH5 – some evidence for association with alcoholism
- ADH7 – Strong evidence of GxE with alcohol drinking for rs1573496 and upper digestive tract cancers
- ADH1A – more active in foetal liver / associated with alcohol dependence in some studies

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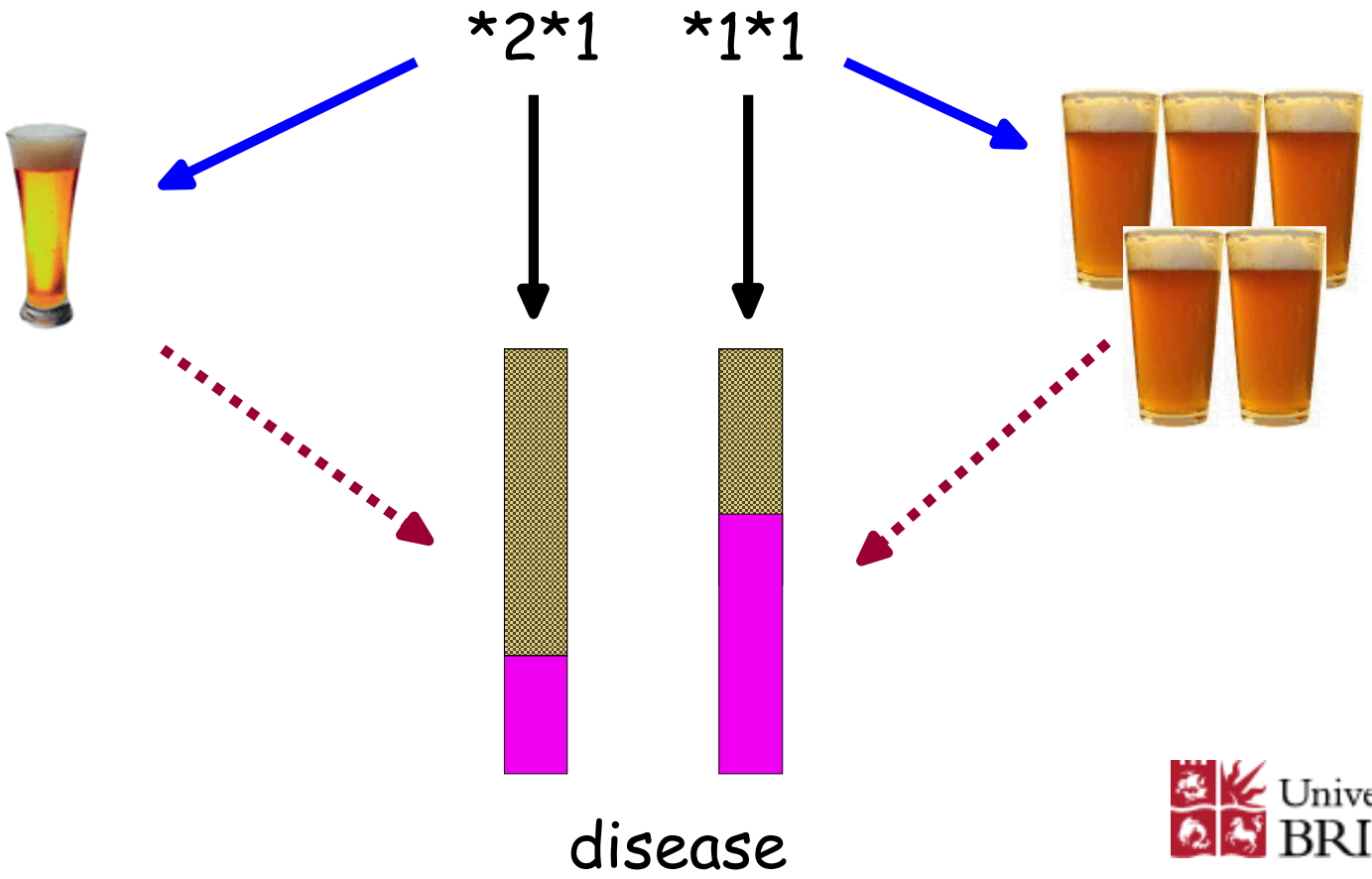
Methods

Mendelian randomization with ALDH2

Comparison by genotype (*2 = inactive allele)



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Selection of candidate ADH variants

- Identify polymorphisms related to alcohol intake and/or metabolism - through bioinformatics and reviews
- Functional candidates - alcohol metabolism : alcohol dehydrogenases (ADH family)
- Criteria for selection of genetic variants (hierarchical order)
  - Evidence of association with alcohol consumption / metabolism / diseases
  - Full coverage of variation in gene region for genes with substantial evidence (eg ADH1B, ADH4, ALDH2)
- Exclusion criteria
  - Rare variant (minor allele frequency <5%) in Europeans
  - Strong correlation with another variant already included
- Limiting factors : current evidence, funding
  - n = 15 SNPs in study 1
  - n = 9+1 SNPs in study 2
  - n = 8 SNPs in common

Samples from 2 MR studies

Study 1 : a case-control nested in ProtecT (NIHR HTA Programme-funded)

- "Alcohol and prostate cancer: identifying potentially modifiable lifestyle-related causes of cancer through Mendelian randomization" (MRC-funded training fellowship)
- UK men aged 50-69 from 65,000 men attended PSA testing Jun2001-May2008
- Derived variables (q.aire):
  - Habitual bingeing (5+ pints or 5+ drinks of wine or spirits)
  - Drinking most days of the week
  - Weekly drinking in 3 categories - <7 units/wk (37%) , 7-20 units/wk (34%), 21+ units/wk (29%)
- Validation of genes-alcohol association in ~ 2,000 **controls** of white ethnic background

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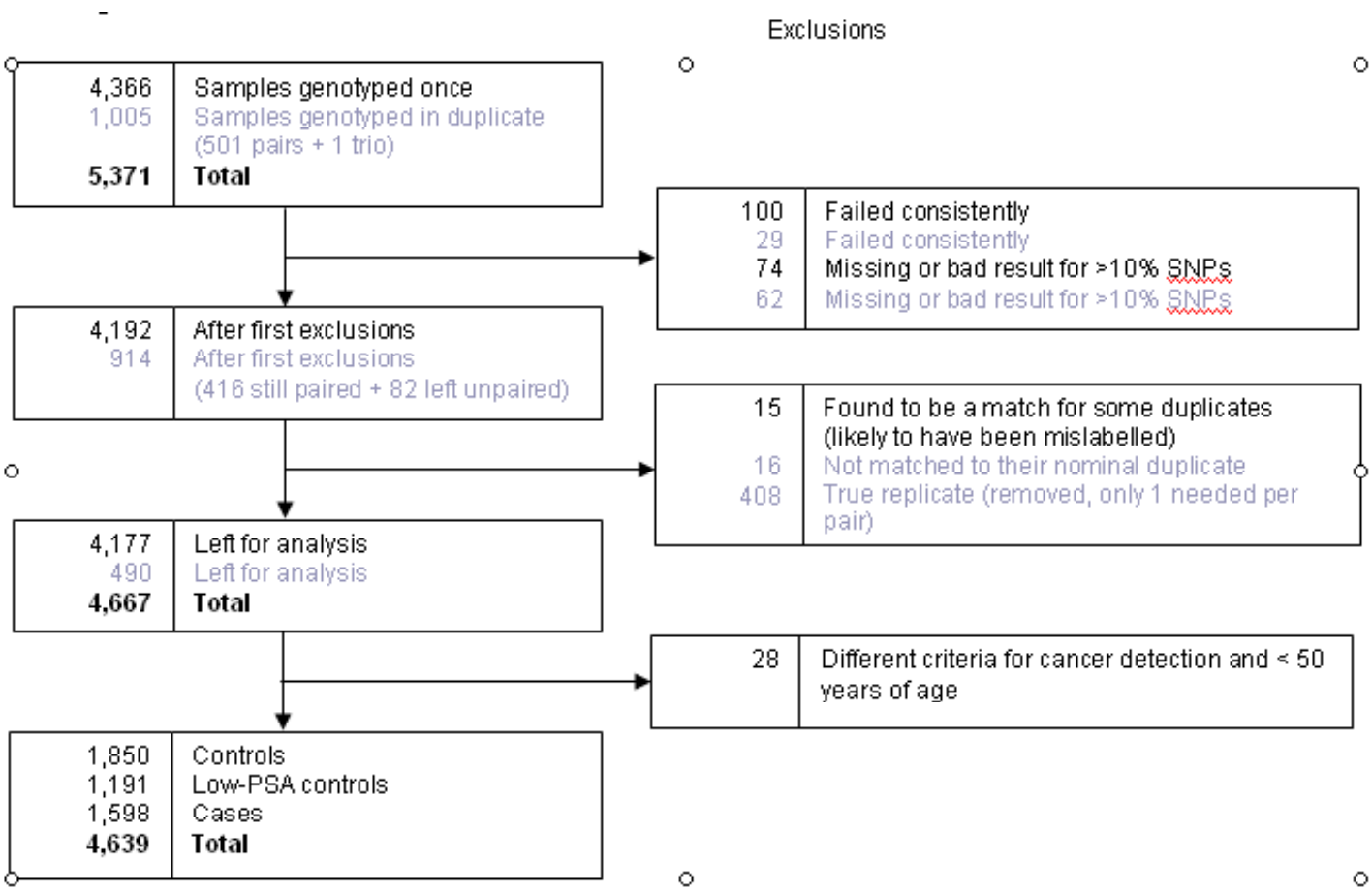
Samples from 2 MR studies

Study 2 : a cohort study based on data from ALSPAC

- "Effects of prenatal alcohol consumption and alcohol metabolising genes on child growth and neurodevelopment in the ALSPAC study" (Wellcome Trust funded)
- 14,000 pregnant women enrolled April 1991 - December 1992)
- Derived variables (q.aires):
  - Bingeing at any point during pregnancy (2+ pints or 4+ wine glasses or pub measures of spirits)
  - Bingeing after pregnancy, when child is 8 months old (same as above)
  - Weekly drinking in 3 categories - <1 units/wk (41%) , 1-6 units/wk (47%), 7+ units/wk (12%)
- Validation of genes-alcohol association in ~ 7,000 mothers of white ethnic background

Results

Quality control and exclusions – Study 1



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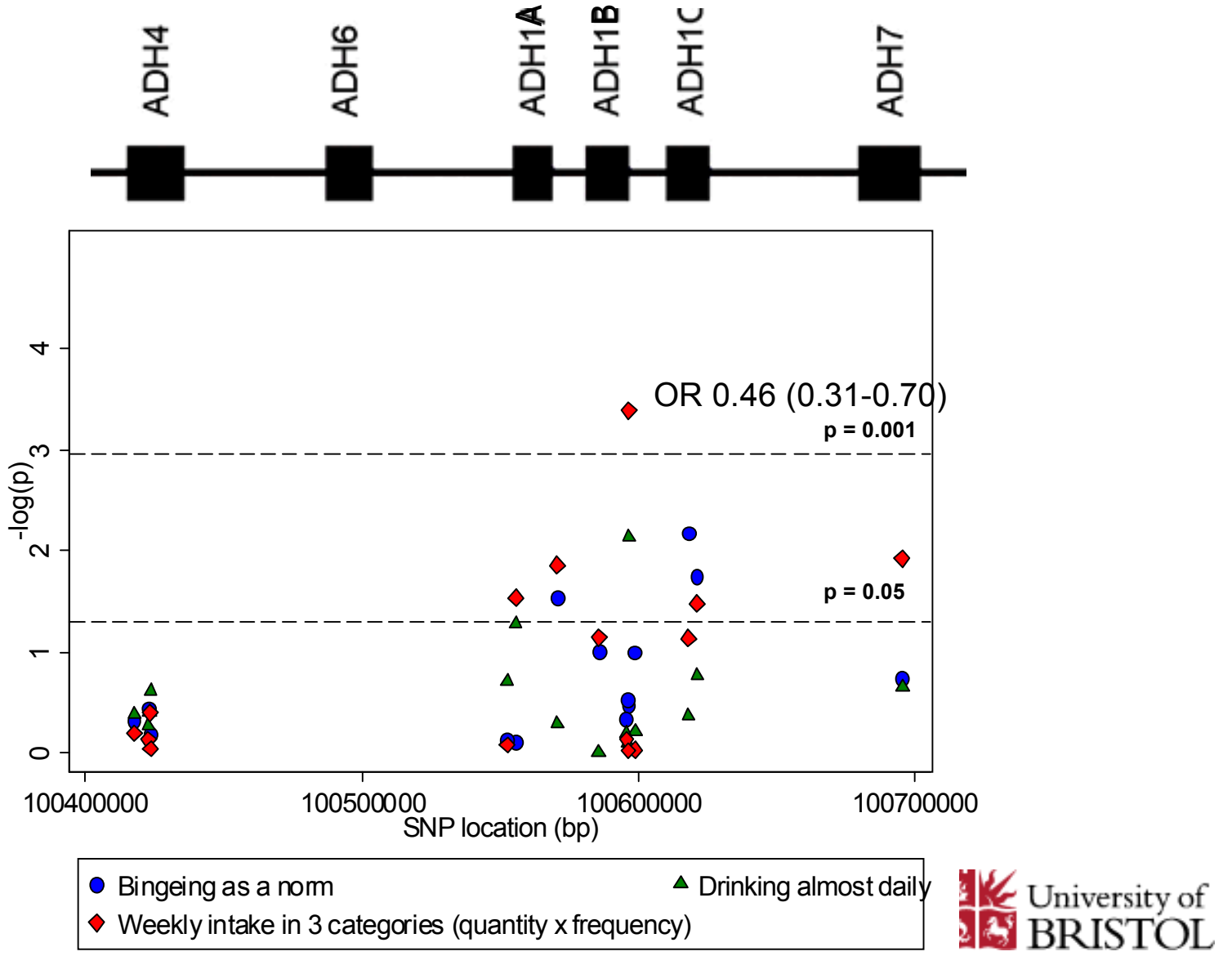
Univariate association of SNPs across the ADH gene cluster with alcohol consumption in UK men aged 50-69 (N = 2,000) - ProtecT controls



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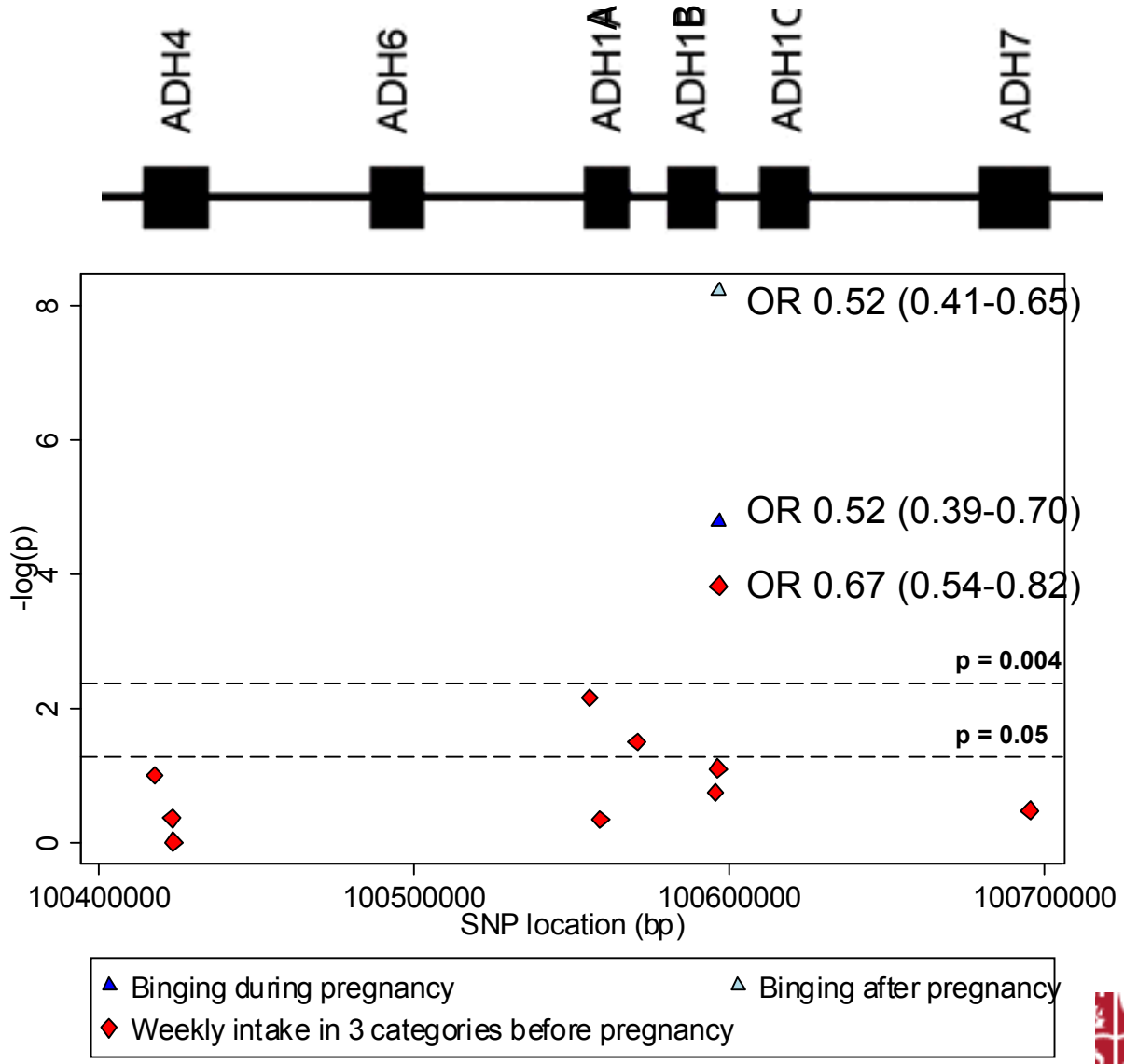
Univariate association of SNPs across the ADH gene cluster with alcohol consumption in UK women of reproductive age (N = 7,000) – ALSPAC mothers



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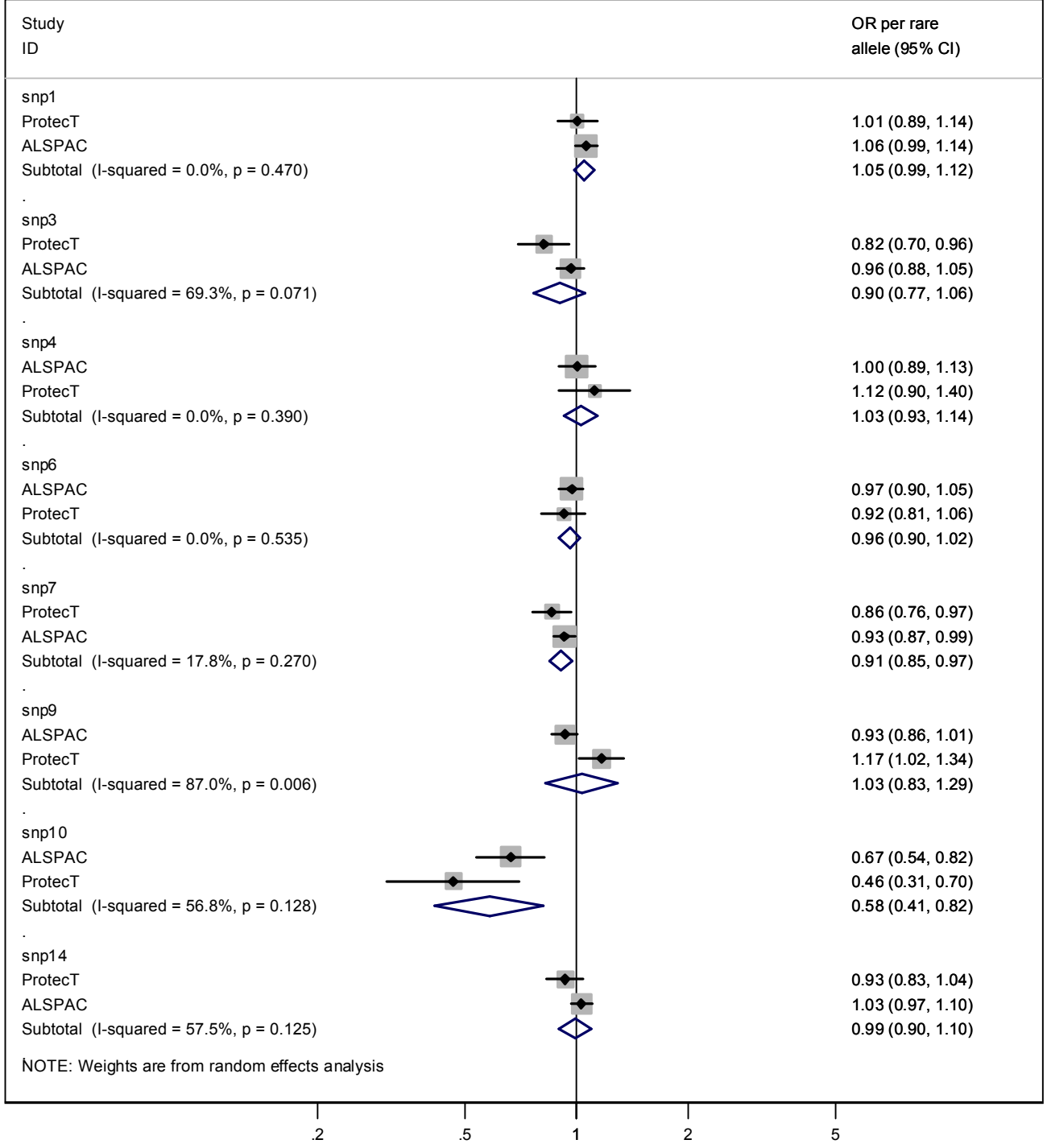

Random-effects meta-analysis of ADH variants - alcohol intake associations.

ALSPAC mothers (N = 7,000) + ProtecT controls (N=2,000)

Outcome: weekly alcohol intake in 3 categories

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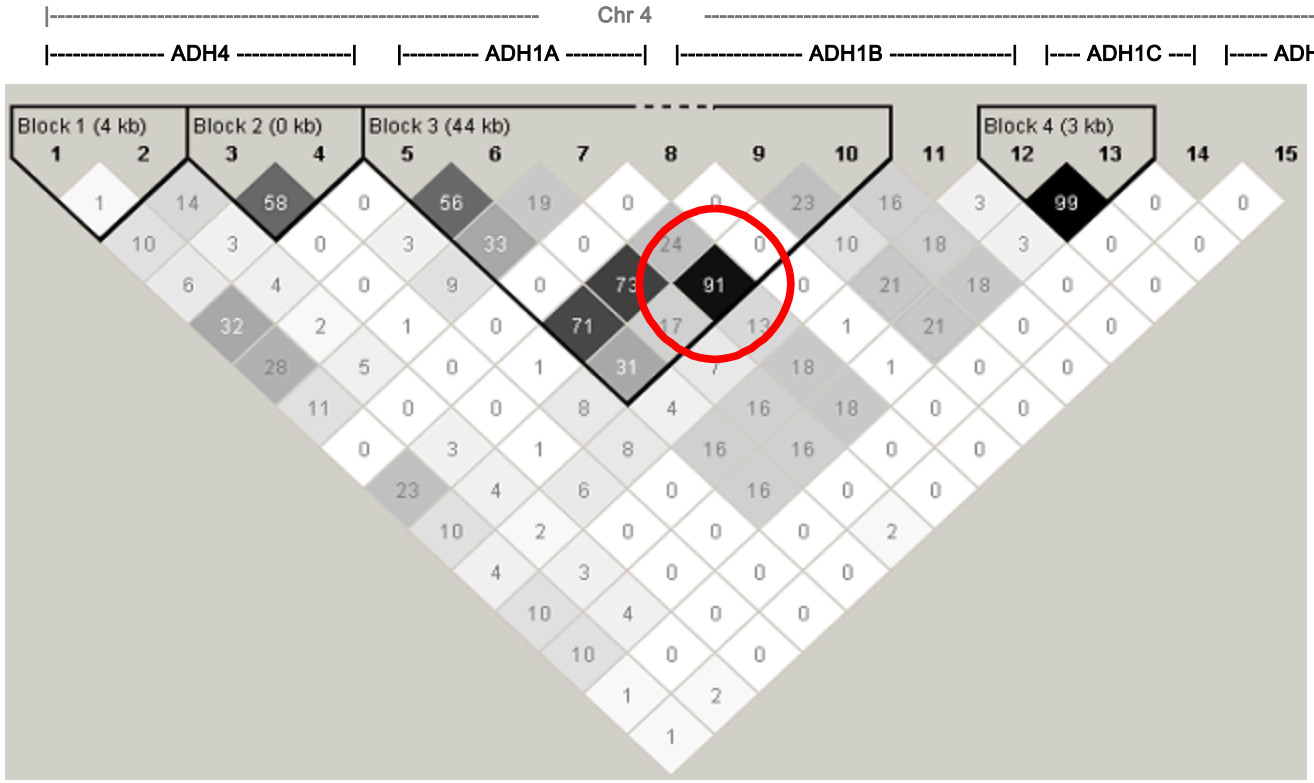
Linkage Disequilibrium across 15 typed markers in the ADH region in ProtecT (r^2)



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ProtecT controls only

Variable selection with reversible jumps (Bayesian model)

Model	Posterior probability	snp1	snp2	snp3	snp4	snp5	snp6	snp7	snp8	snp9	snp10	snp11	snp12	snp13	snp14	snp15
Null	67%															
Model 1	26%										+					
Model 2	2.6%									+						
Model 3	1.6%										+				+	

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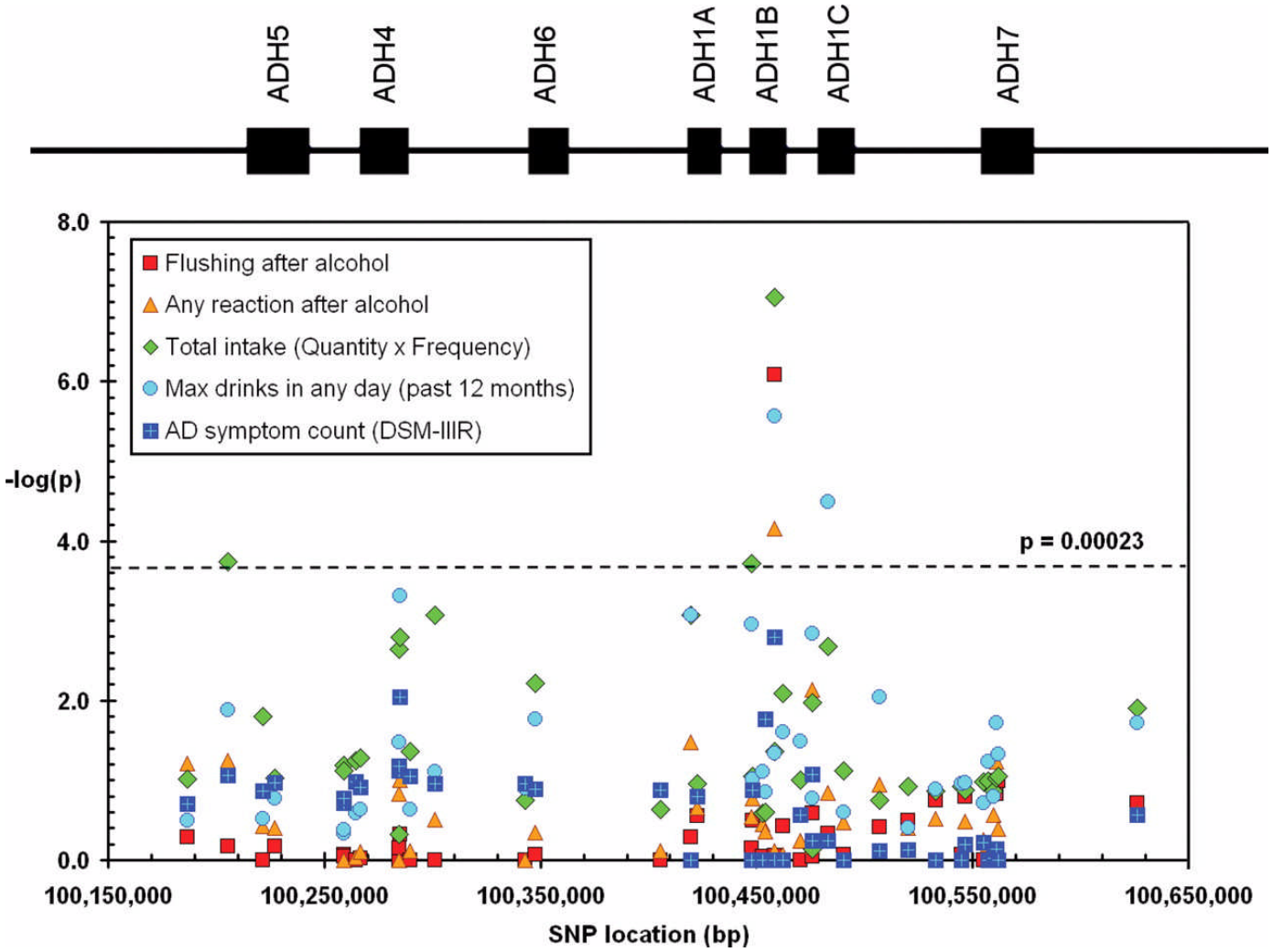
Univariate association of SNPs across the ADH gene cluster with quantitative AD variables, alcohol consumption variables and self-reported alcohol reactions



Macgregor, S. et al. Hum. Mol. Genet. 2009 18:580-593

Human Molecular Genetics

Different pathways

Further candidate genes

Gene	Chr	Pathway	Associations
CHRM2	7	Externalizing behaviour	Alcohol+substance dependence Bipolar disorder IQ
TAS2R38	7	Bitter taste receptors	Alcohol intake (differences across alcohol drinks)
GABRA2	4	Externalizing behaviour	Alcohol dependence Substance abuse Conduct disorder
CRHR1	17	Alcohol seeking (after stressful events)	Binge drinking Lifetime heavy alcohol consumption

Translation ?

- J-shaped curve for alcohol consumption and dementia / cognitive decline
- J-shaped curve for many CVD outcomes
- J-shaped curve for diabetes
- …
- But : Confounding, reverse-causation (non-drinkers?)

Problems and limitations

- Instruments (choice of SNPs)
  - Alcohol metabolism gene variants as instruments for metabolism Vs behaviour
  - Role of some variants on alcohol behaviour to be clarified – work in progress

- Mendelian Randomization
  - Limited evidence on dose-response trends (3-points trends Vs “J” shaped curve)
  - Pleiotropy → [illegible]
  - Weak instruments (see above)
  - Large samples needed

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Conclusions

- ALDH2 variant predicts alcohol intake , but is only present in East-Asia
- Need something similar for Caucasians
- The best candidate so far is a variant in ADH1B, others in ADH show some potential
- More candidates proposed not fully evaluated
- GWAS ? Pooling different measures of alcohol consumption ?

  Different mechanisms
  - Metabolism → ♌♓■♑♏♓■♑
  - Impulsivity addiction

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Acknowledgments

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Financial support: MRC training fellowship programme

Data sources: ProtecT participants, PIs, data managers

ALSPAC participants, PIs, data managers

Unconfounded genes, confounded alcohol

Observed to expected* number of statistically significant associations (alpha=5%)

a) self-reported alcohol dimensions and potential confounders

b) genetic variants in ADH, ALDH2 and CNR1 and potential confounders

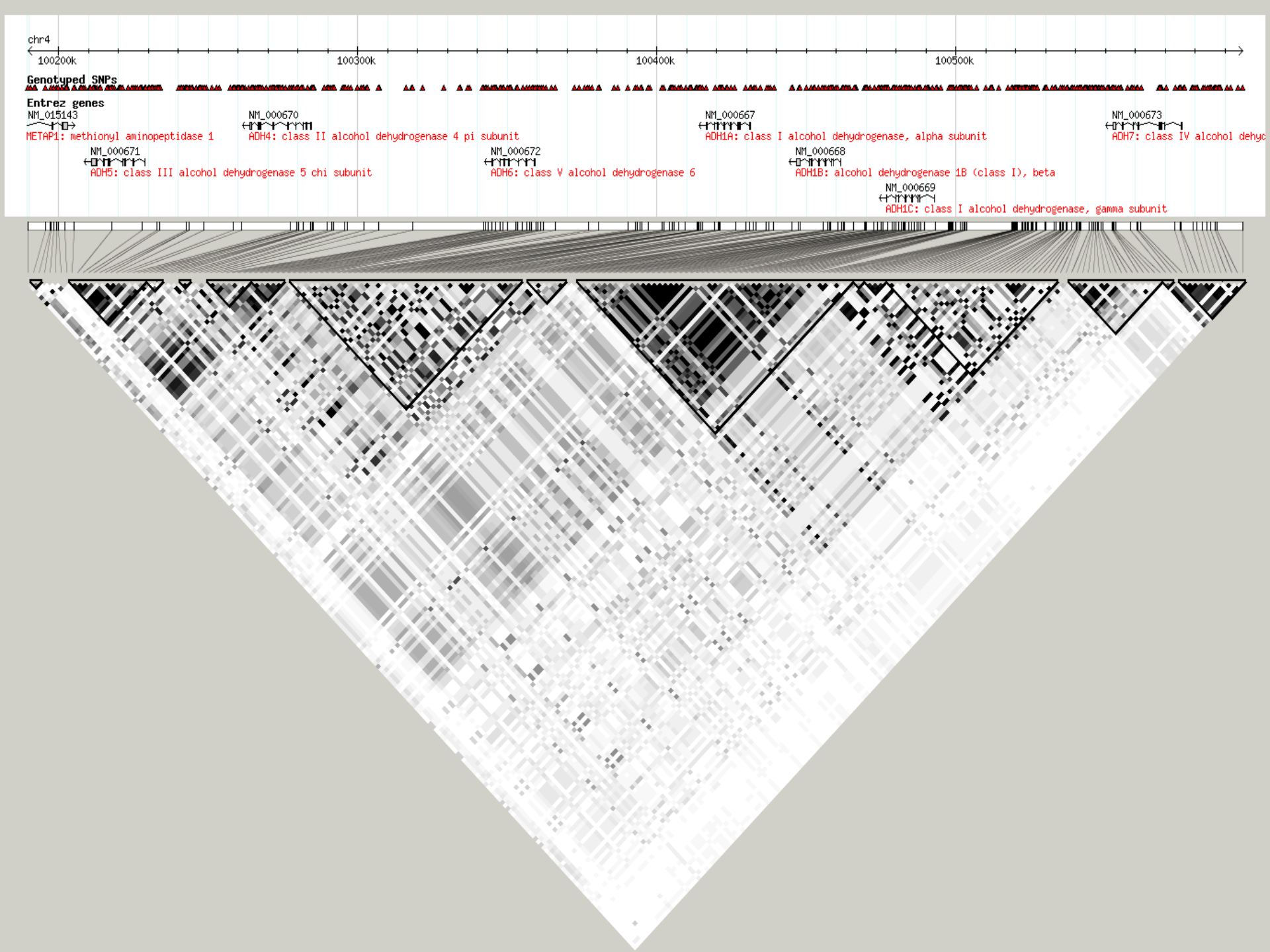
a)

SNP	N	E	O	p
Thirtiles of consumption	50	2.5	10	**1.59E-04**
Binging (5+ drinks/occasion)	50	2.5	27	**2.59E-22**
Drinking most days	50	2.5	14	**1.03E-07**

b)

SNP	N	E	O	p
rs971074	50	2.5	3	0.46
rs284779	50	2.5	0	1
rs1693482	50	2.5	0	1
rs698	50	2.5	2	0.72
rs1353621	50	2.5	0	1
rs1229984	25	1.25	0	1
rs4147536	50	2.5	1	0.92
rs1042026	50	2.5	3	0.46
rs1229966	50	2.5	3	0.46
rs975833	50	2.5	6	**0.04**
rs6837311	50	2.5	0	1
rs4148884	50	2.5	2	0.72
rs3762894	50	2.5	4	0.24
rs1800759	50	2.5	1	0.92
rs4699714	50	2.5	1	0.92
rs1049353	50	2.5	2	0.72
rs441	50	2.5	0	1
rs2238151	50	2.5	3	0.46
rs886205	50	2.5	1	0.92

*Os and Es obtained separately for standard and low-PSA controls, then added together


chr4
100200k
100300k
100400k
100500k
Genotyped SNPs
Entrez genes
NM_015143
METAP1: methionyl aminopeptidase 1
NM_000670
ADH4: class II alcohol dehydrogenase 4 pi subunit
NM_000667
ADH1A: class I alcohol dehydrogenase, alpha subunit
NM_000673
ADH7: class IV alcohol dehyc
NM_000671
ADH5: class III alcohol dehydrogenase 5 chi subunit
NM_000672
ADH6: class V alcohol dehydrogenase 6
NM_000668
ADH1B: alcohol dehydrogenase 1B (class I), beta
NM_000669
ADH1C: class I alcohol dehydrogenase, gamma subunit