Understanding the contribution of copy number polymorphisms to multigenic traits

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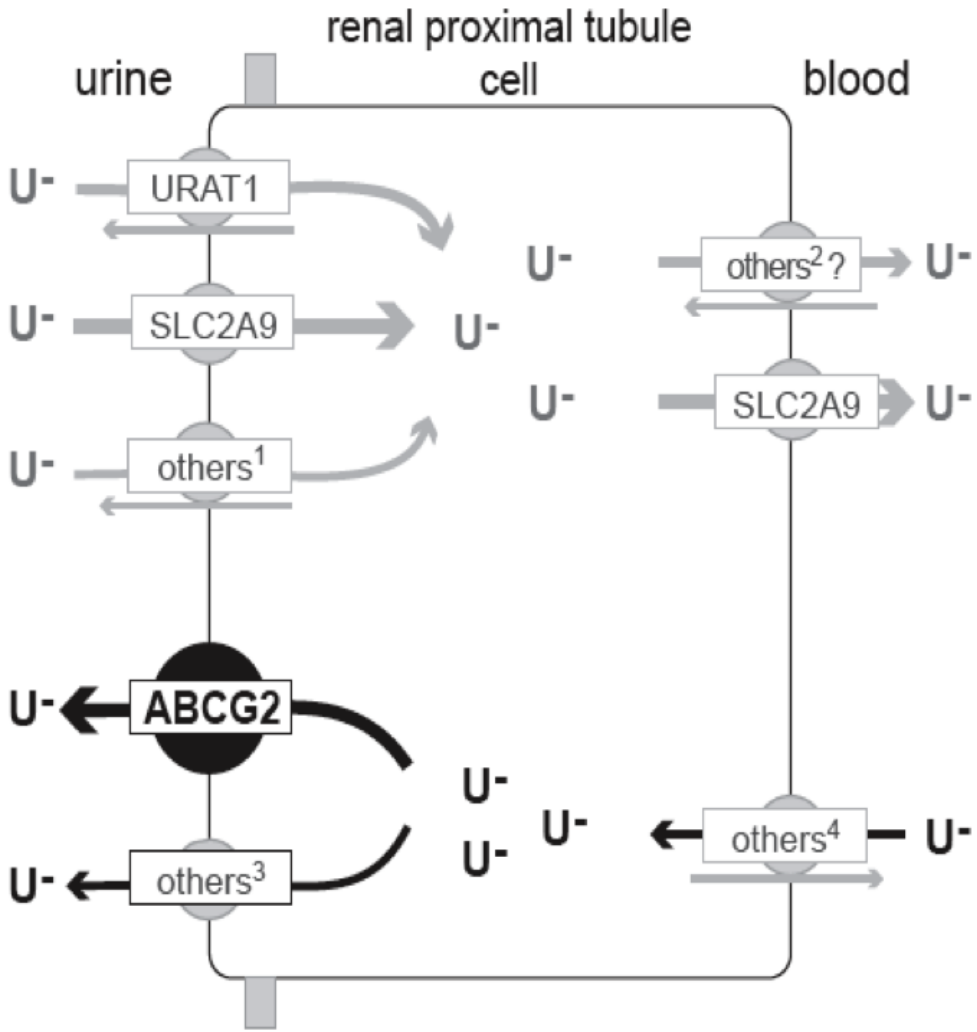
Serum uric acid concentrations

- serum uric acid concentrations are highly heritable
- high levels of serum uric acid (hyperuricemia) is associated with multiple diseases (gout, cardiovascular disease, and renal complications)
- SNPs in *SLC2A9* and *ABCG2* increase the risk for hyperuricemia and gout

Function of *SLC2A9* and *ABCG2* in the kidney



Woodward *et al.*, PNAS (2009)

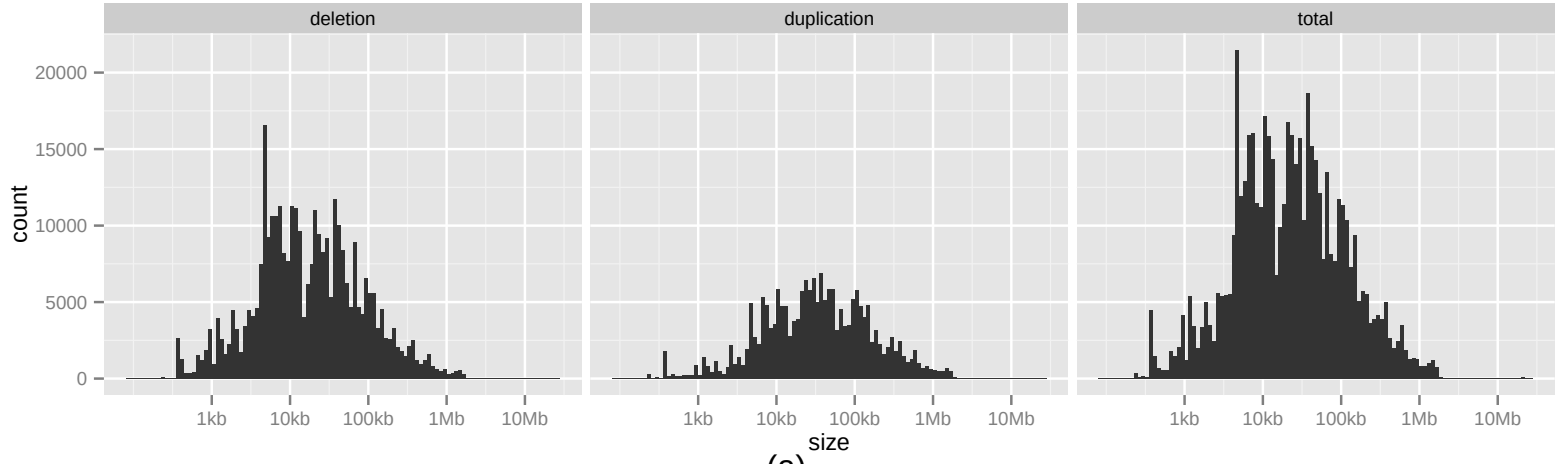
Atherosclerosis Risk in Communities Study

- 8,598 European ancestry (EA), 3,392 African Americans (AA) passing QC
- IQR age: 49-59 (median 54)
- 47% of EA and 38% of AA participants are male

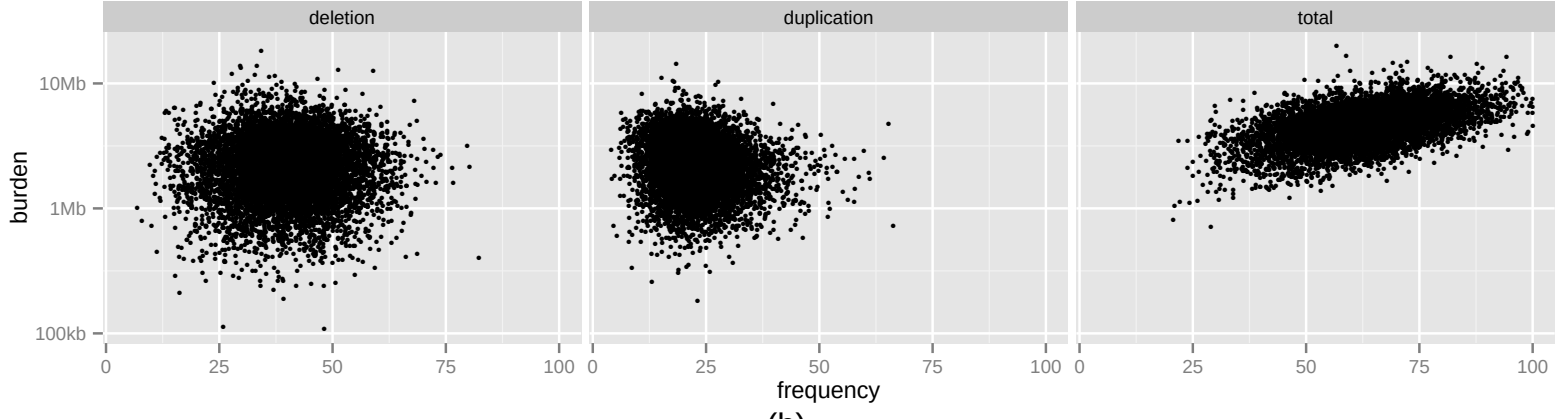
Copy number estimation

1. Preprocess Affy 6.0 CEL files (R package `crlmm`)
2. Fit 6-state HMM to each sample (R package `VanillaICE`)
   $\rightarrow$ genomic intervals with estimated copy number

CNVs (European ancestry)



(a)



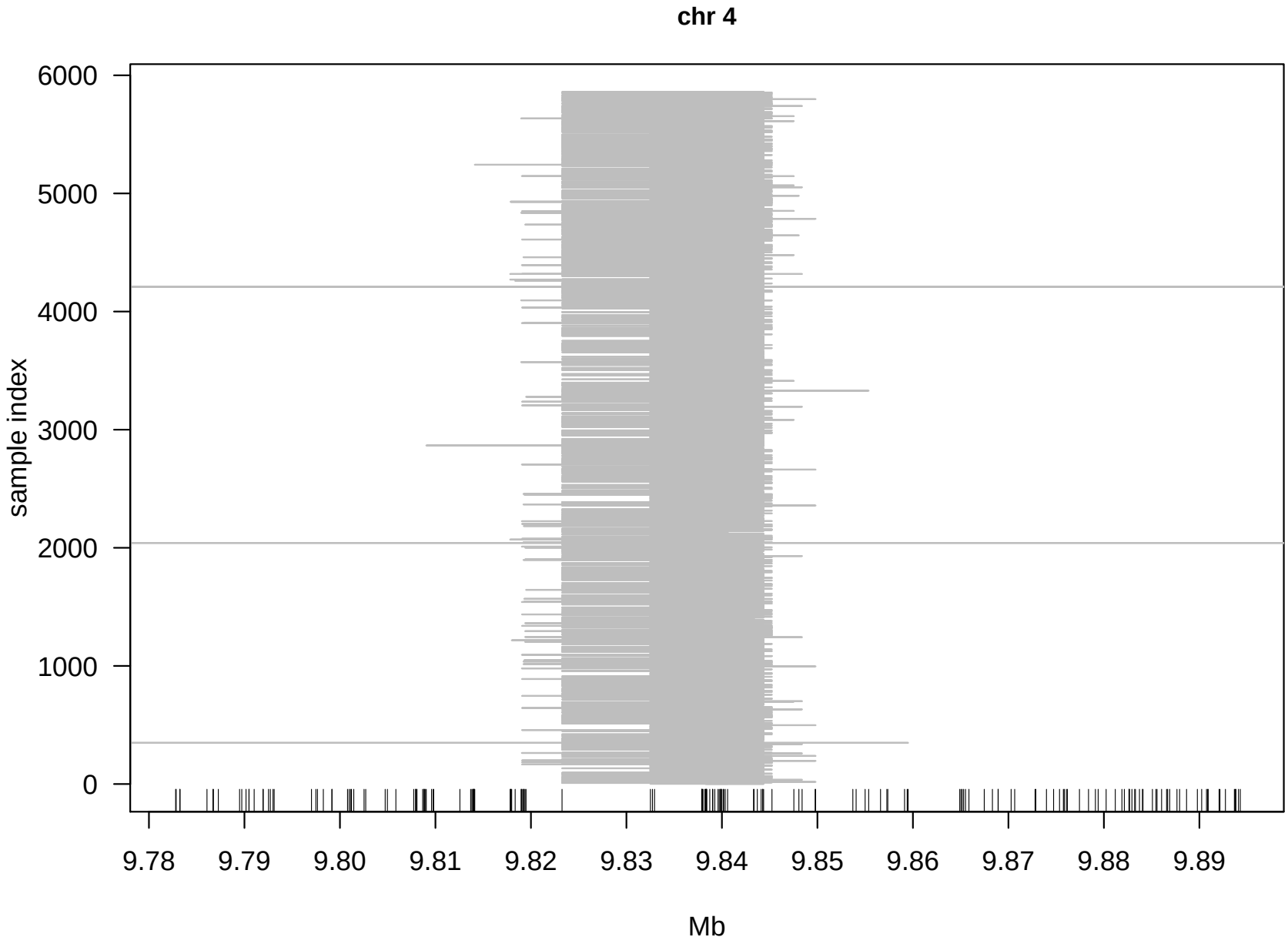
(b)

Copy number estimation

3. Translate list of CNVs to a rectangular matrix (rows are genomic intervals, columns are samples)

4. Define copy number polymorphisms (CNPs) as intervals for which at least 1% of ARIC participants have a CNV
    - 14,678 such intervals in ARIC corresponding to approximately 434 regions

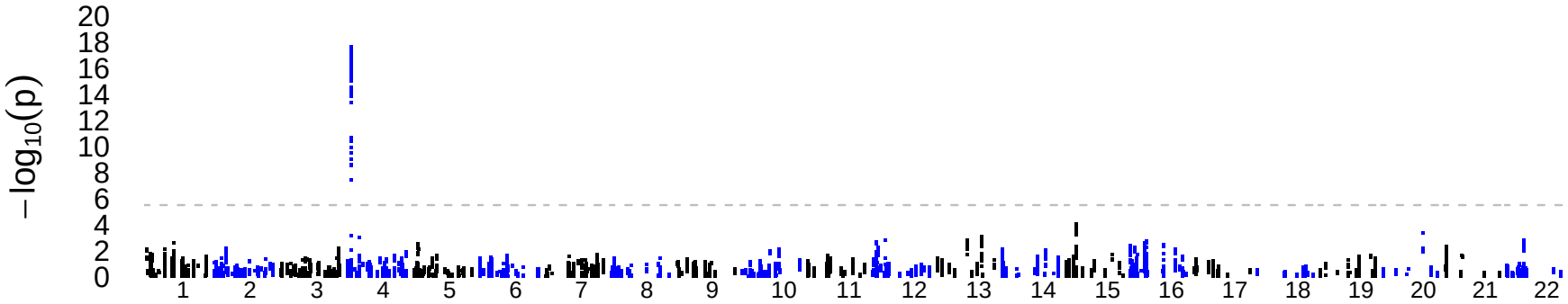
Many regions are complex



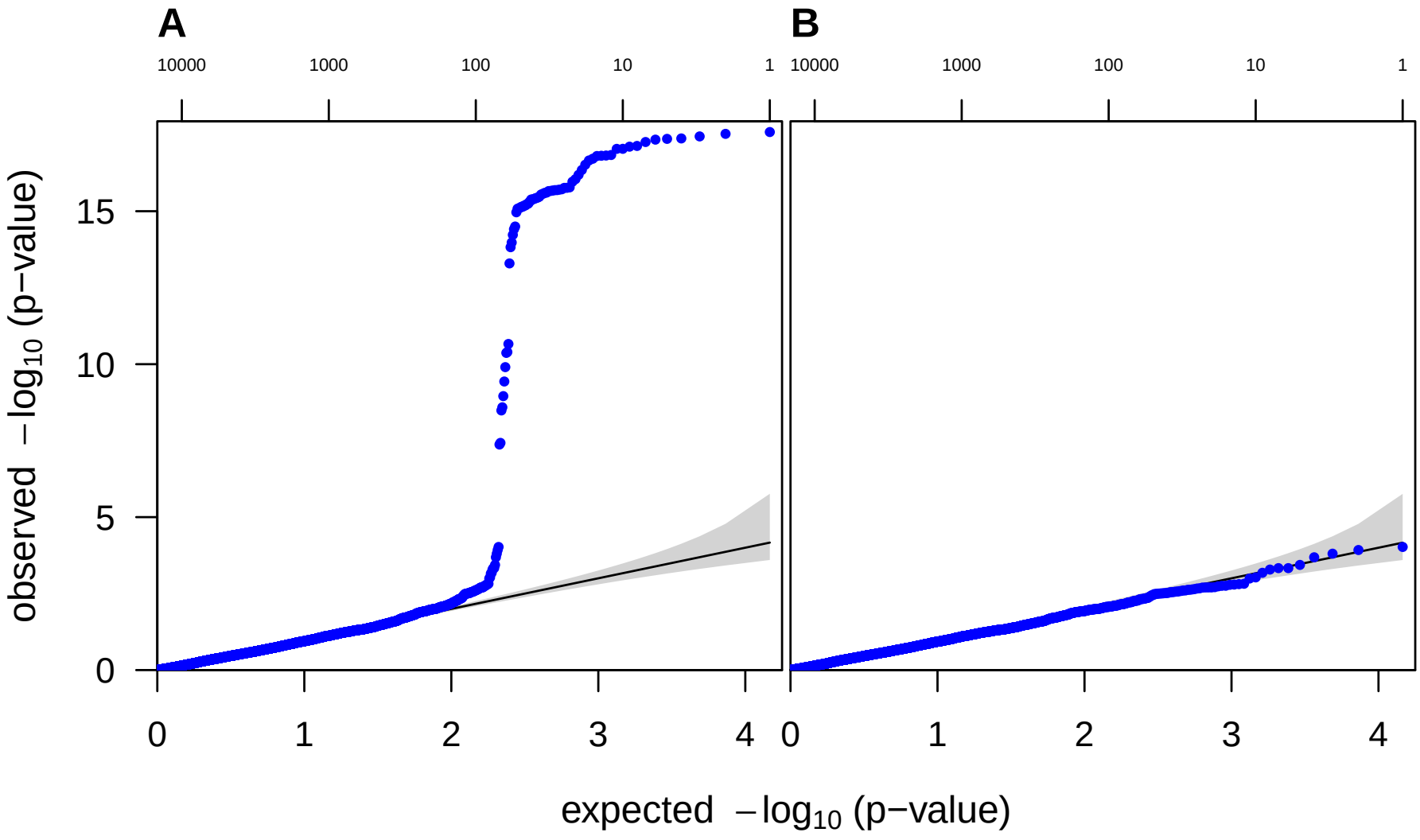
Association of copy number and uric acid

5. Regress uric acid on copy number estimates
    - model copy number as continuous

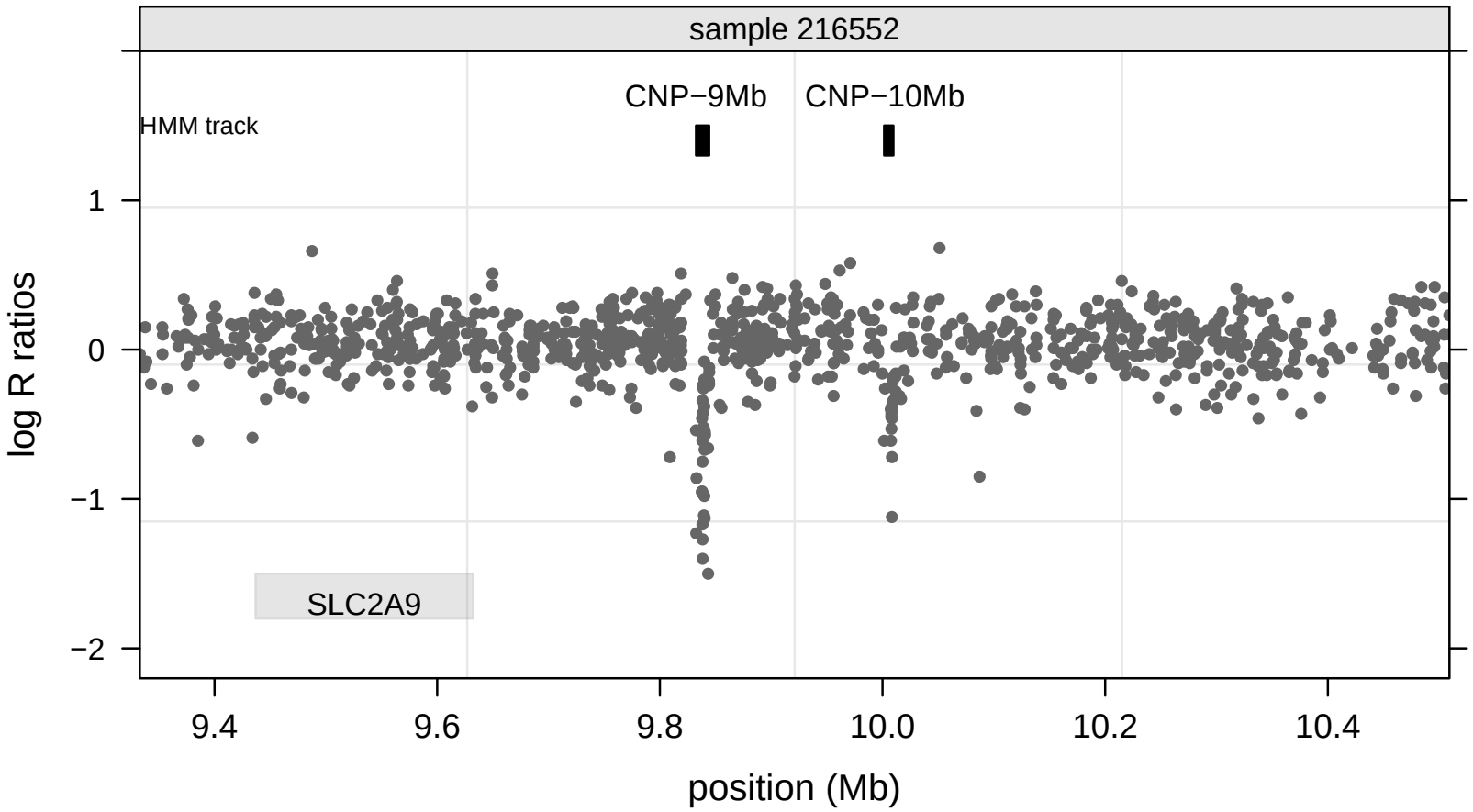
Copy number GWAS



Copy number GWAS

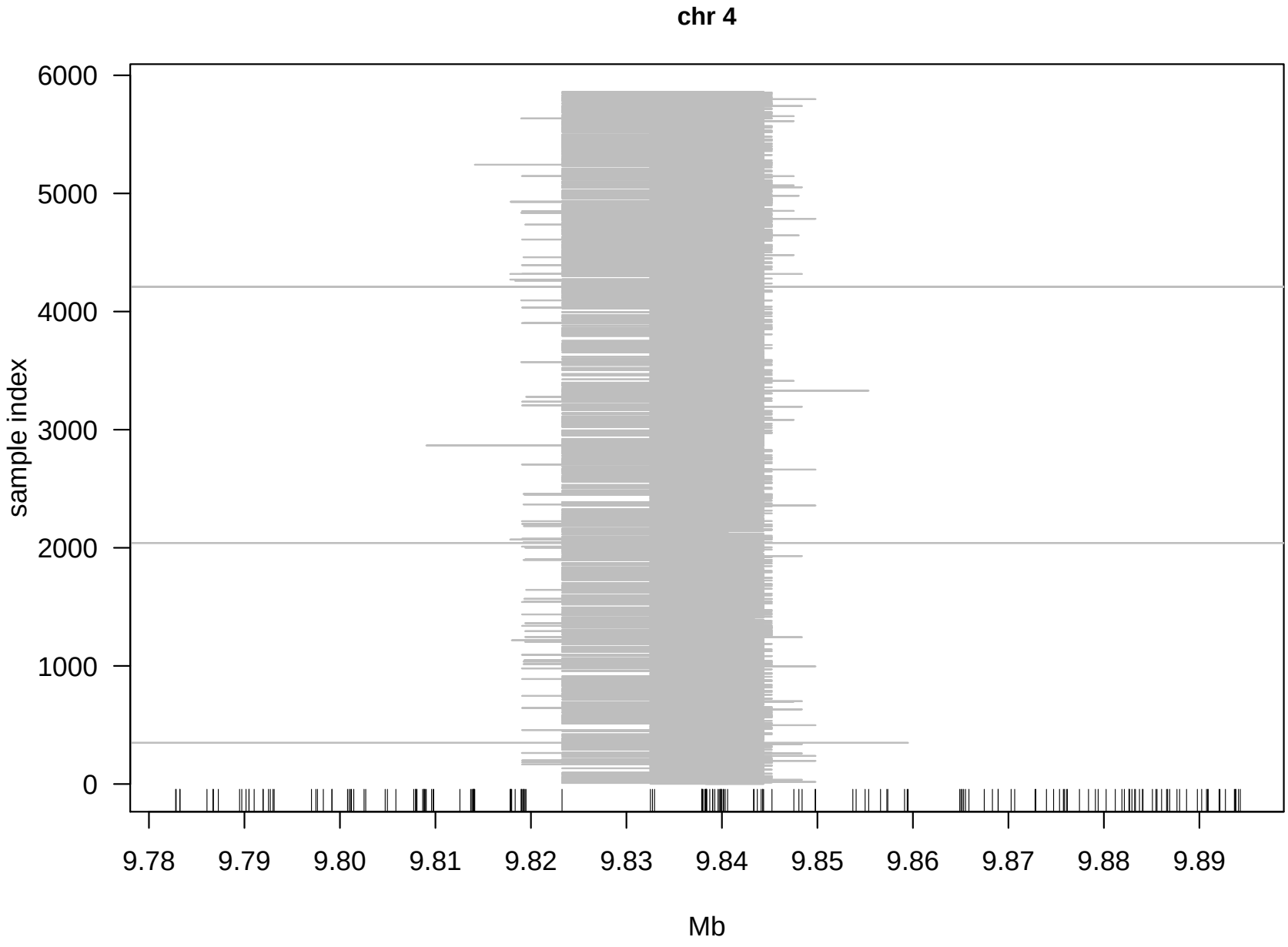


Signal is from two non-overlapping CNPs

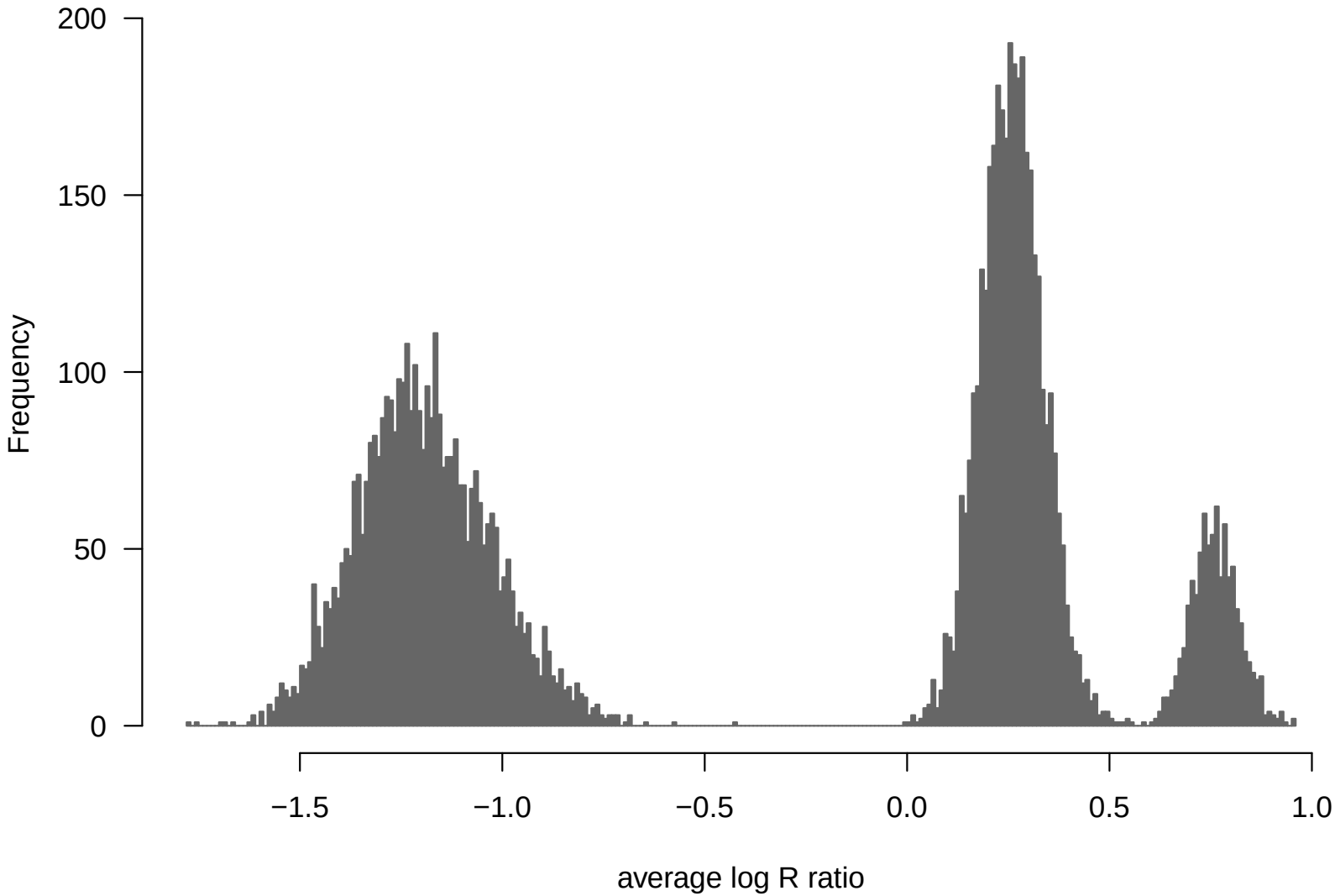


- CNP regions are small and data is noisy, but CNPs are near *SLC2A9*
- *SLC2A9* is transcribed in the reverse direction

CNP-9Mb breakpoints

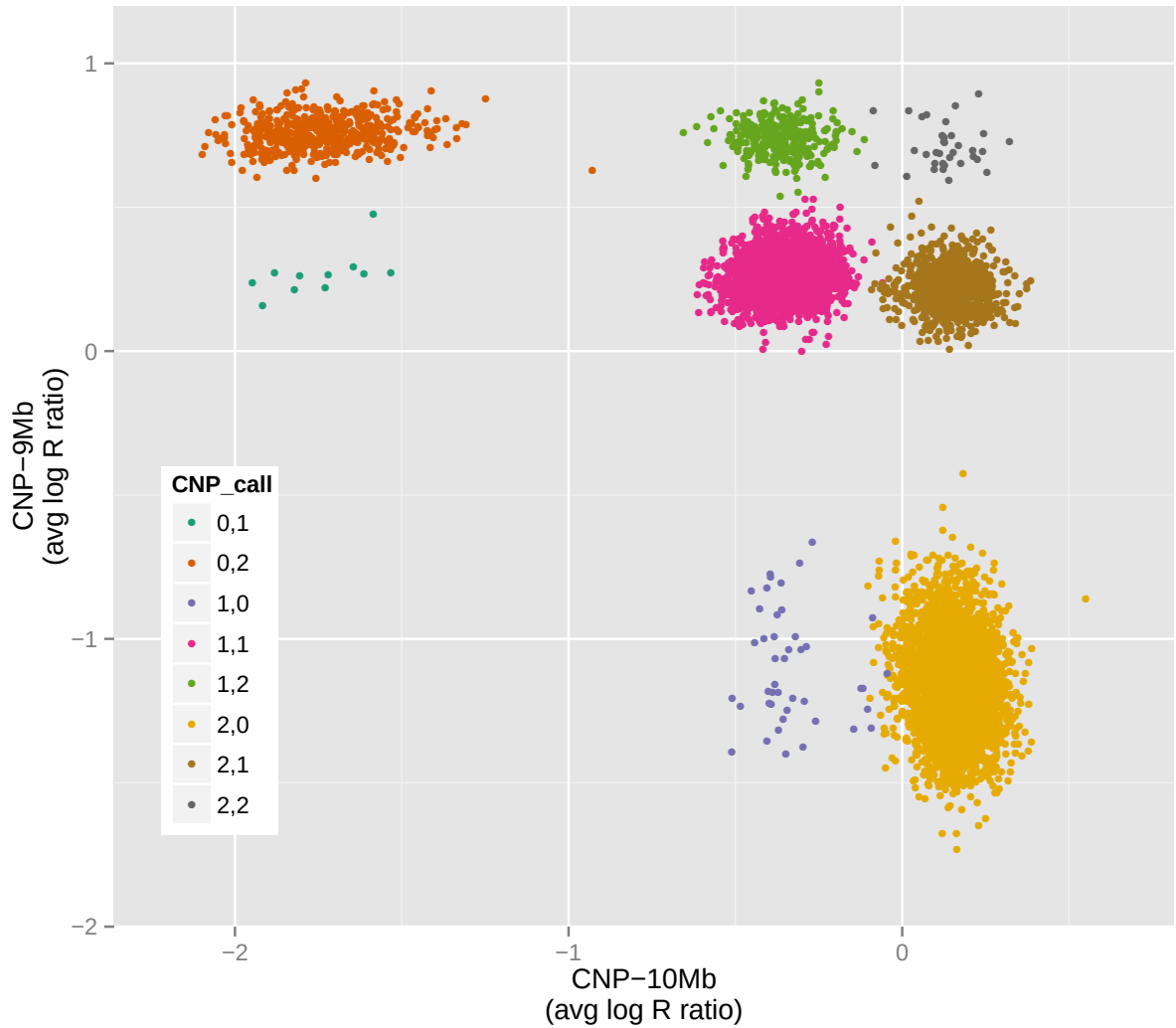


CNP-9Mb average log R ratios

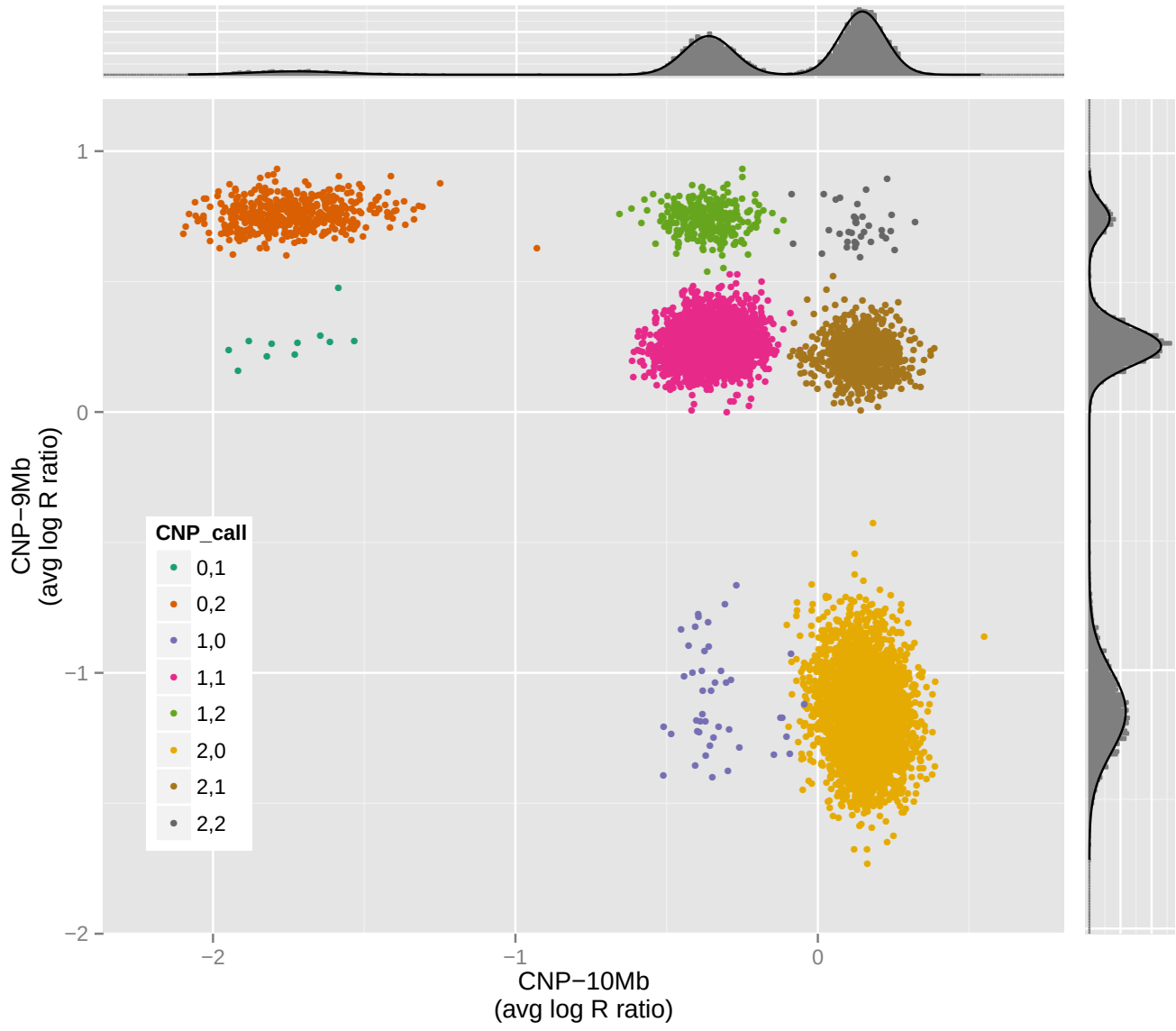


Marginal distribution **across samples** – peaks are well separated

CNP-10Mb vs CNP-9Mb log R ratios



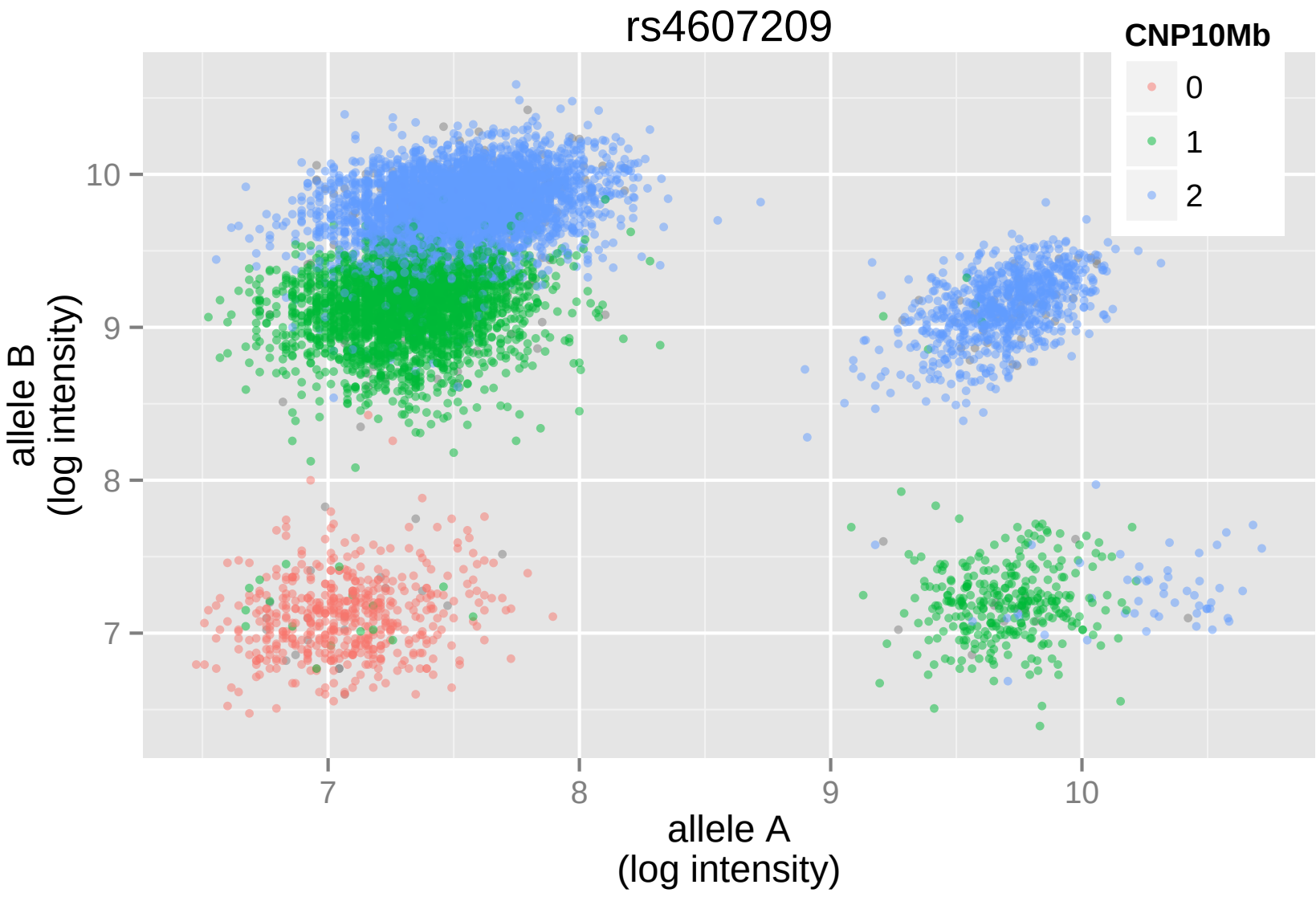
Marginal distributions are a mixture of normals



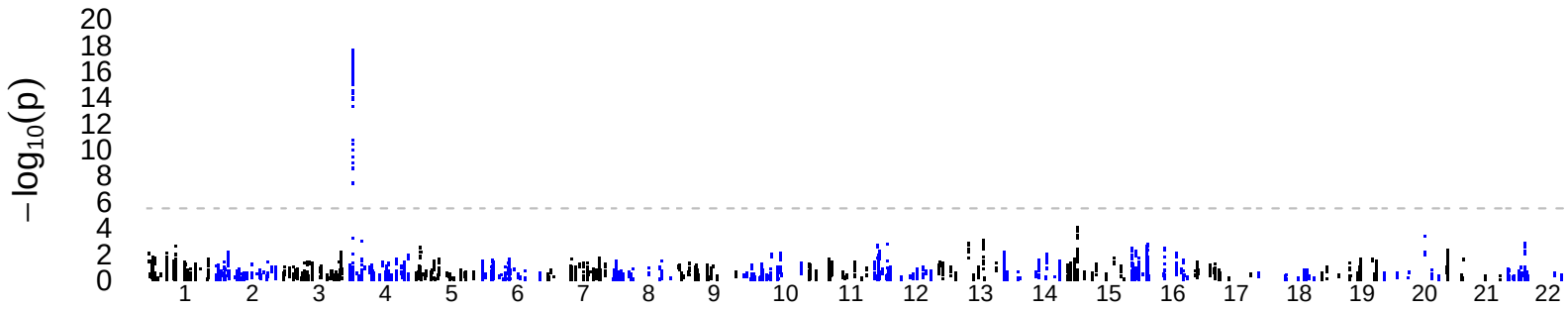
Marginal distributions are a mixture of normals

- we implemented a Gibbs' sampler to approximate the posterior distribution and classify copy number
- different implementations have been developed by others (Korn *et al.*, Nature Genetics (2008))

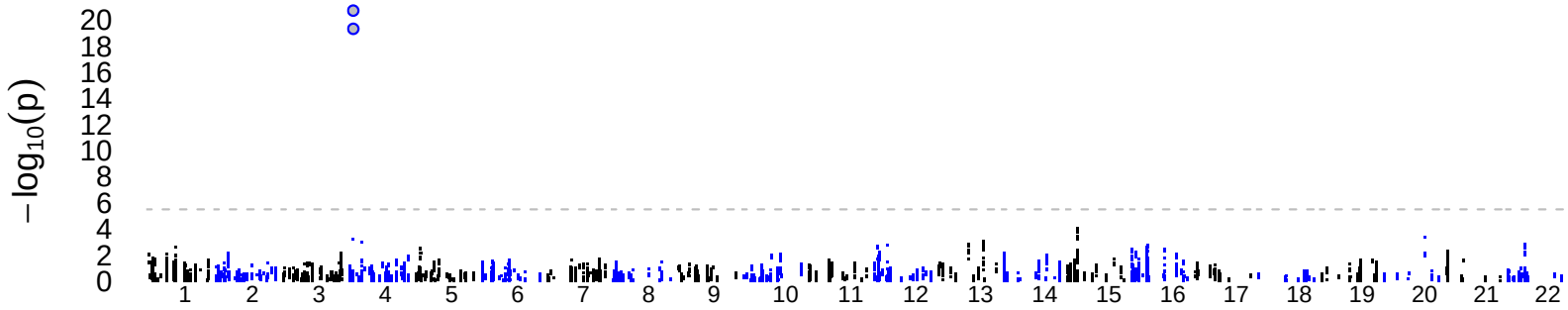
SNP in CNP-10Mb locus



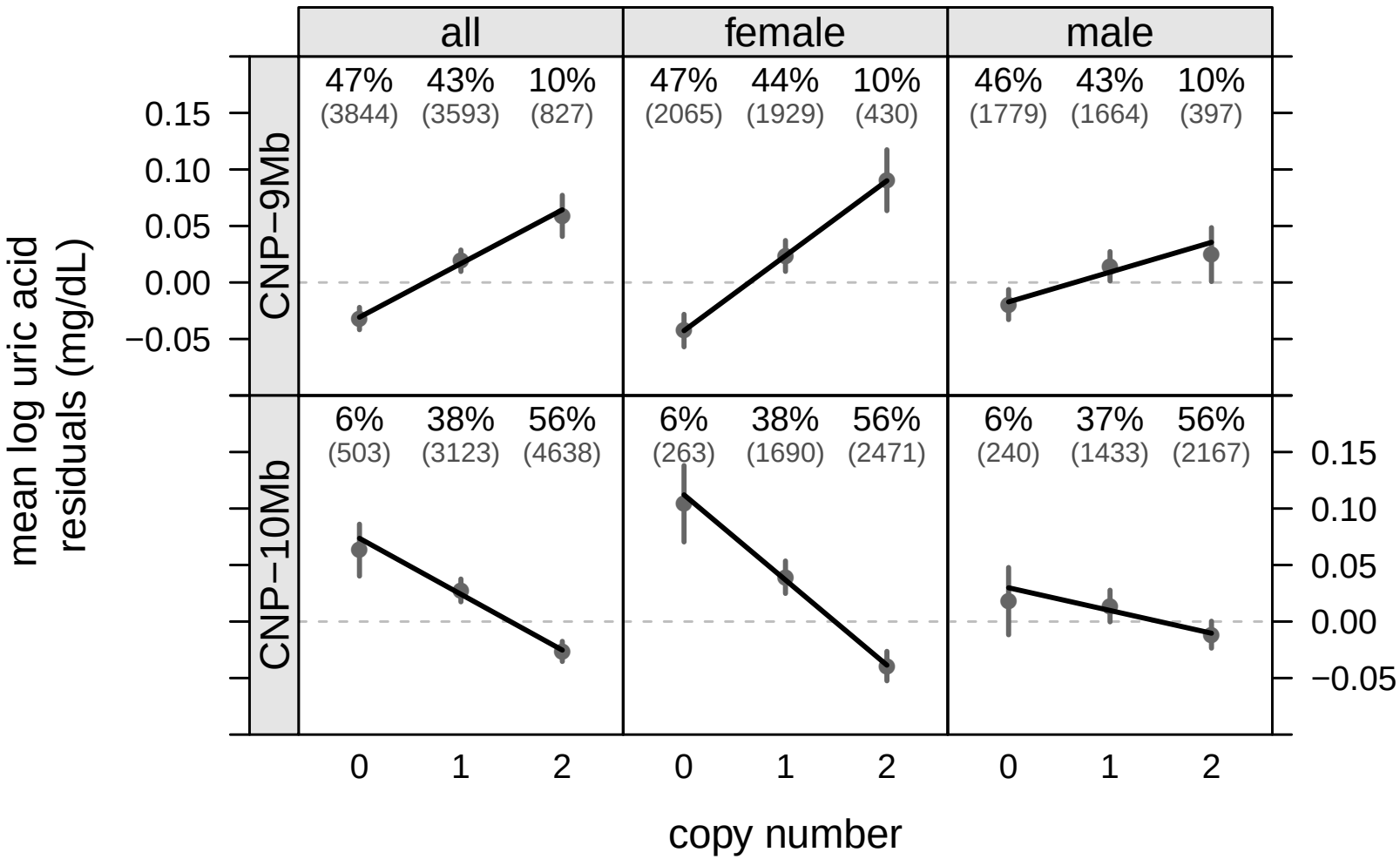
Association from HMM estimates



Association from mixture model



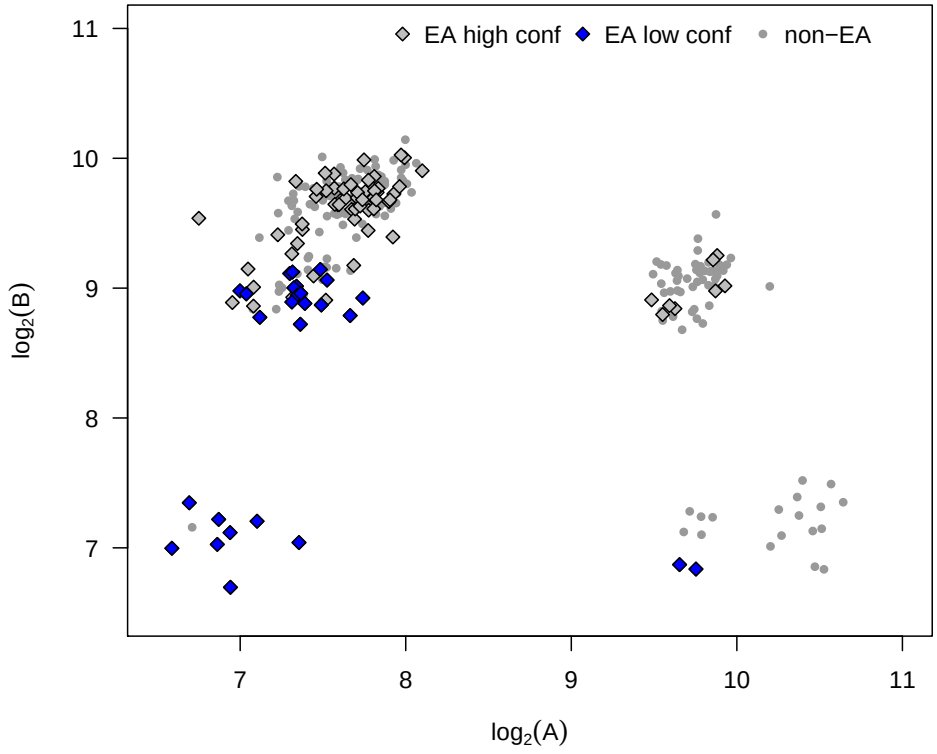
Gender-specific slopes



- Copy number estimates are negatively correlated
- Relationship between log (uric acid) and copy number is approximately linear

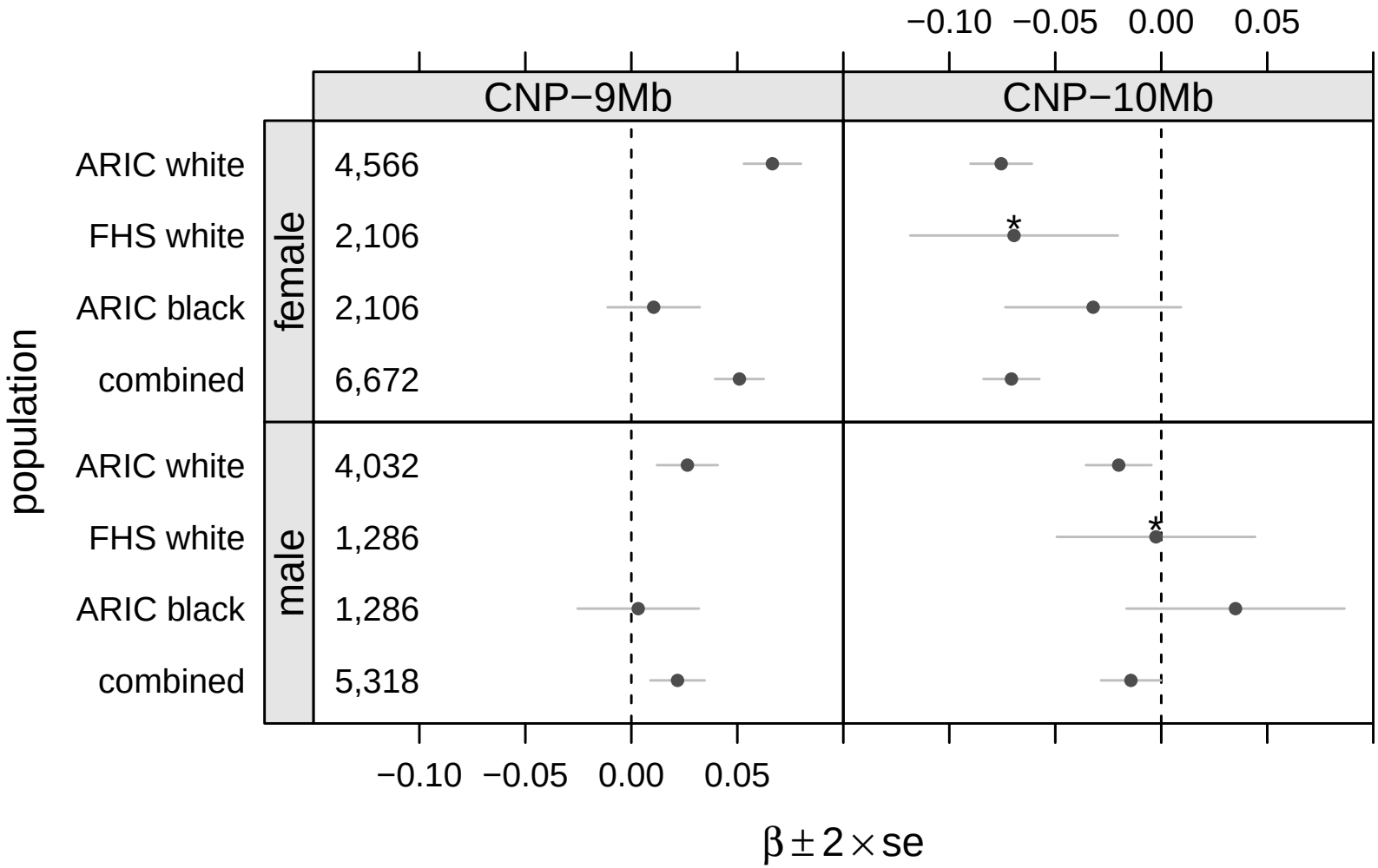
Replication in Framingham Heart Study

- Older array technology in Framingham (250k chips)
- No markers in the CNP-9Mb locus
- One SNP in the CNP-10Mb locus
- Only genotype calls available from our Framingham collaborators



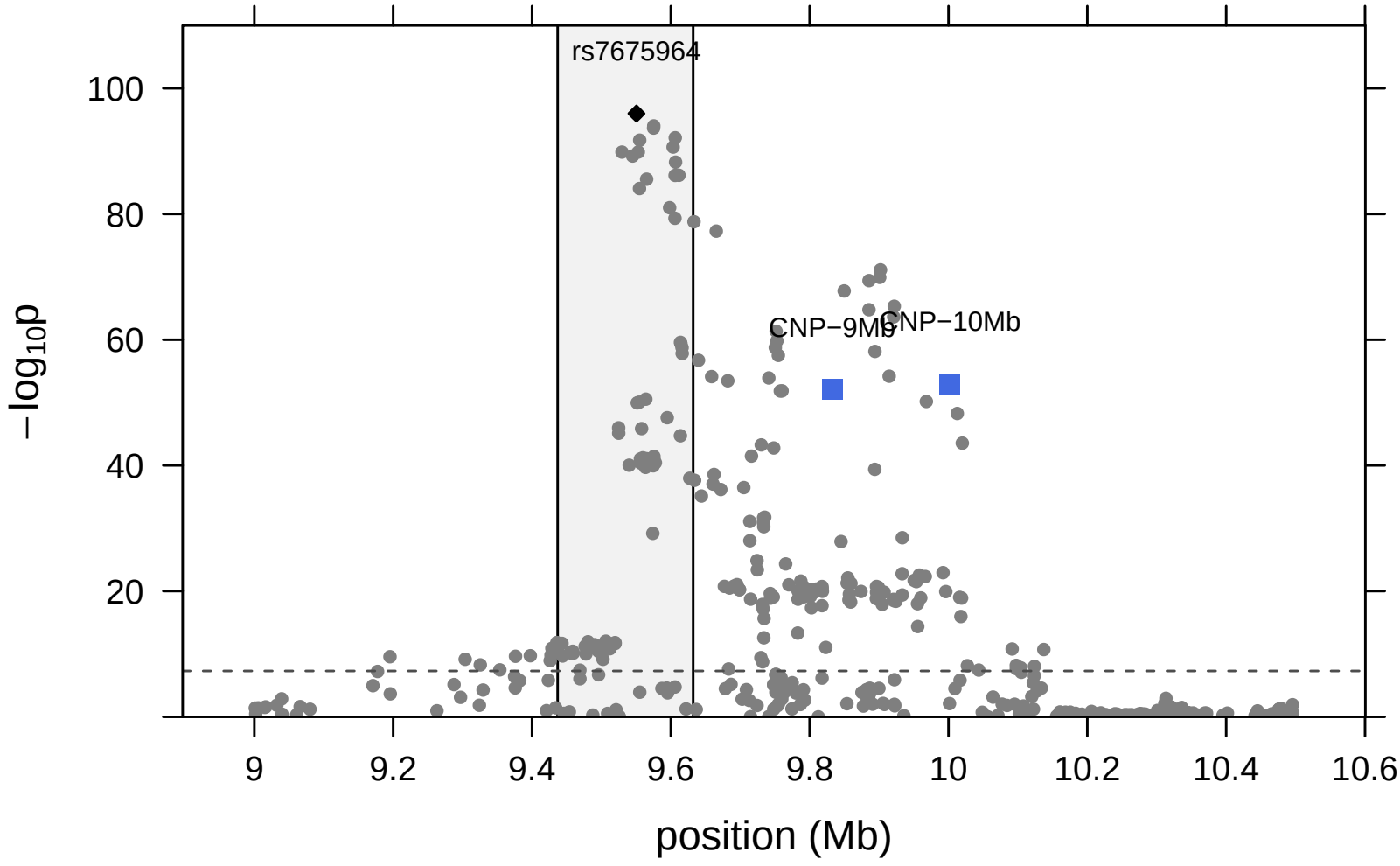
HapMap data for 250k chip

Replication in Framingham Heart Study

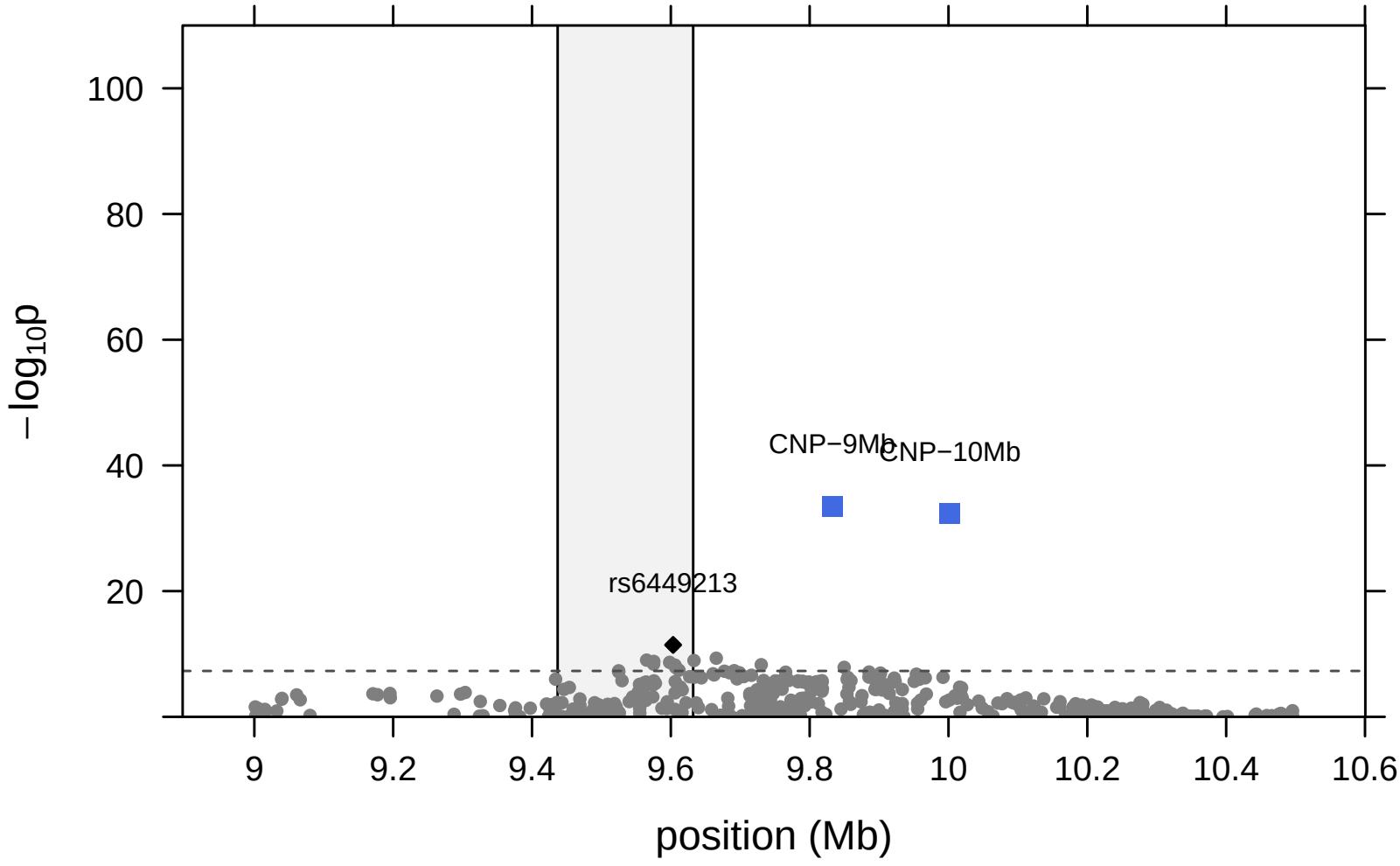


* Missing genotype calls used as a surrogate for deletion genotypes

SNPs in *SLC2A9* increase risk of hyperuricemia



CNP association is independent of SNPs in *SLC2A9*



Remarks regarding independence

- Reverse is also true: adjusted for CNP estimates, SNP rs7675964 remains genome-wide significant
- Some SNPs remain genome-wide significant in the rs7675964-adjusted model (i.e., rs6449213)

Phasing SNP allelic haplotypes with copy number

- We phased copy number estimates at CNP-9Mb and CNP-10Mb with the rs7675964 and rs6449213 genotypes
- Example haplotype

Haplotype 1 : $--a--b--0--1--$
Haplotype 2: $--b--b--1--1--$

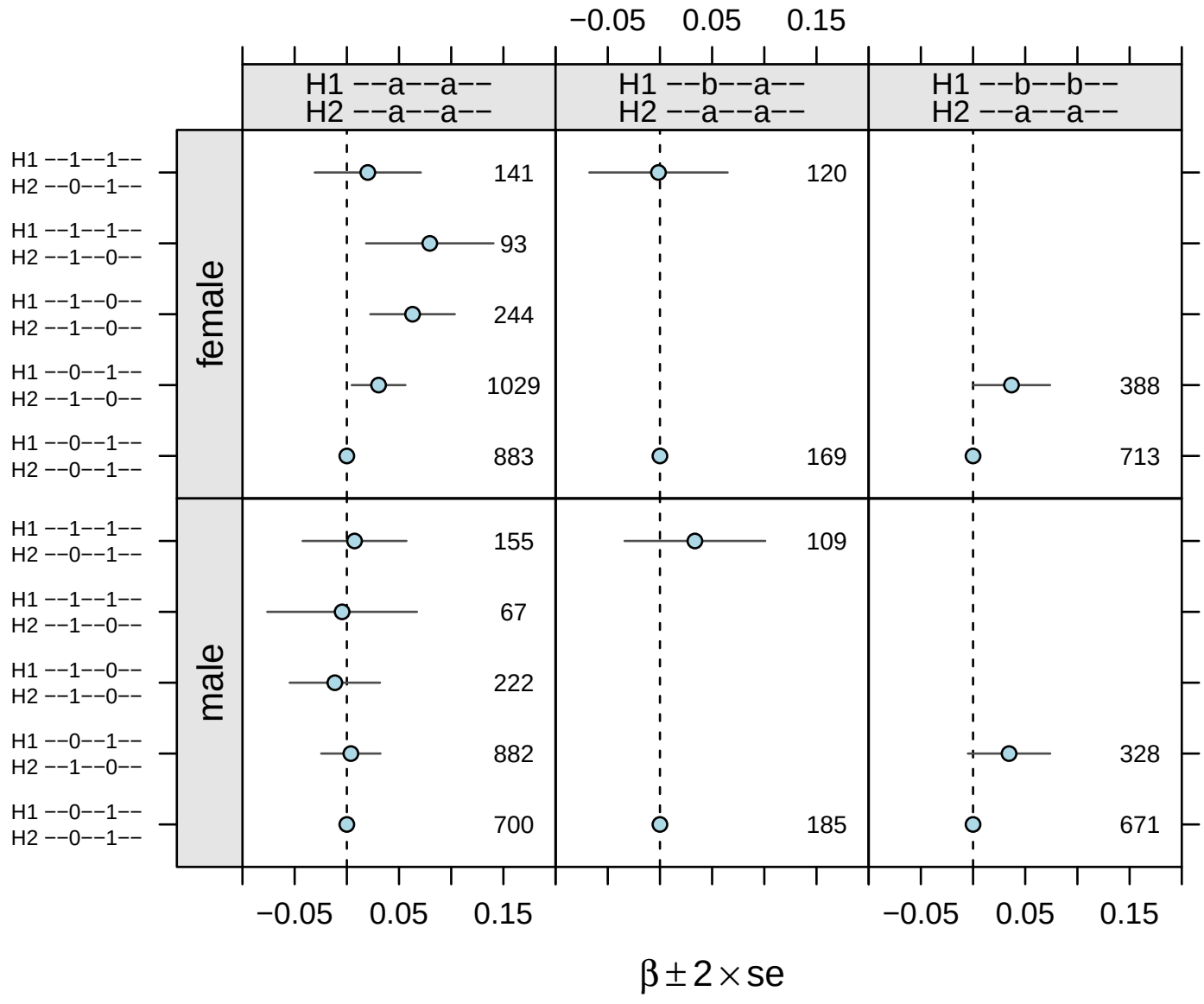
(Haplotype for subect that is heterozygous at SNP rs7675964, homozygous for the minor allele at SNP rs6449213, hemizygous deletion at CNP-9Mb, and diploid at CNP-10Mb)

- Phasing performed by fastPHASE: Scheet and Stephens, PLoS Genetics (2008)
- Thanks to Dan Arking for the suggestion of phasing

Phasing *SLC2A9* SNPs and CNPs

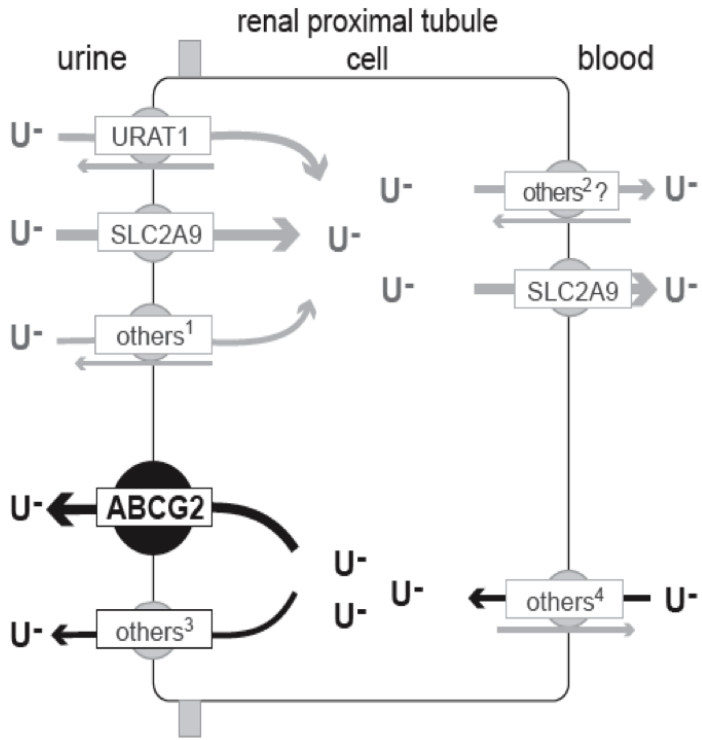
- Only three allelic haplotypes exhibit variation in the corresponding copy number haplotypes:
  1. H1:$--a--a--$
H2:$--a--a--$
  2. H1:$--b--a--$
H2:$--a--a--$
  3. H1:$--b--b--$
H2:$--a--a--$
- Among subjects with the same allelic haplotype, do we see variation in uric acid levels associated with copy number haplotypes?

Association of CNP haplotypes



Discussion and Summary

- Fewer copies of CNP-9Mb
  $\Rightarrow$ reduced *SLC2A9* expression
  $\Rightarrow$ less reabsorption of uric acid from urine
  $\Rightarrow$ decreased levels of serum uric acid concentrations



Discussion and Summary

- Deletions at the CNP-10Mb span a locus identified by ENCODE with strong evidence of DNase hypersensitivity and histone makers in several normal tissues, but nothing yet reported in kidney
- To date, no promoters have been reported at the CNP-9Mb locus
- Gene expression data on the same set of individuals in target kidney and liver tissues is needed to evaluate whether deletions effect transcription of *SLC2A9* as hypothesized, and to evaluate gender differences in *SLC2A9* expression
- Relationship of CNP-9Mb and CNP-10Mb with gout has not been evaluated

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