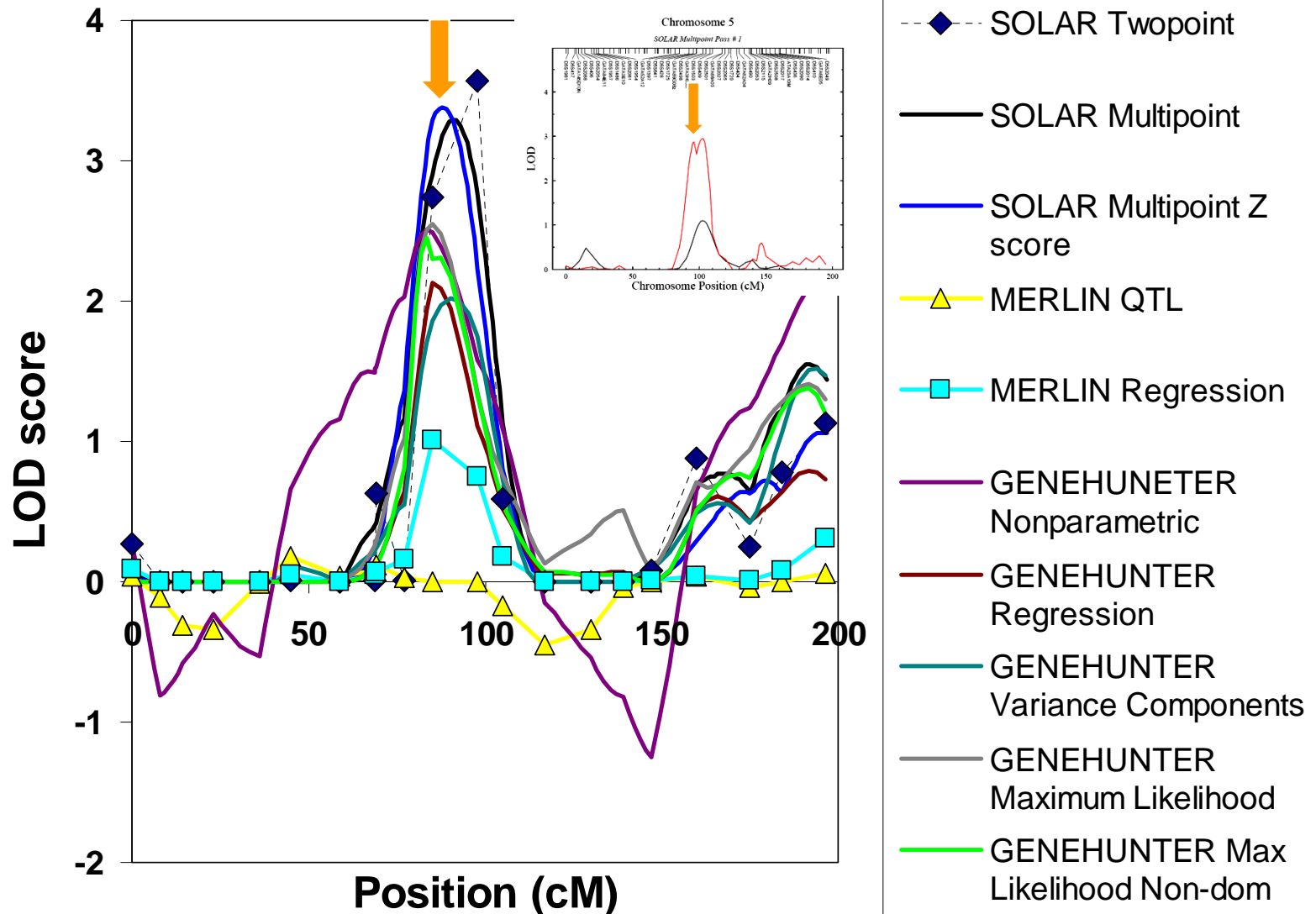


□ **Variants in CAST, the gene  
encoding Calpastatin  
associate with variation in  
Cystic Fibrosis lung function**

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# Linkage of lung function and BMI to chromosome 5



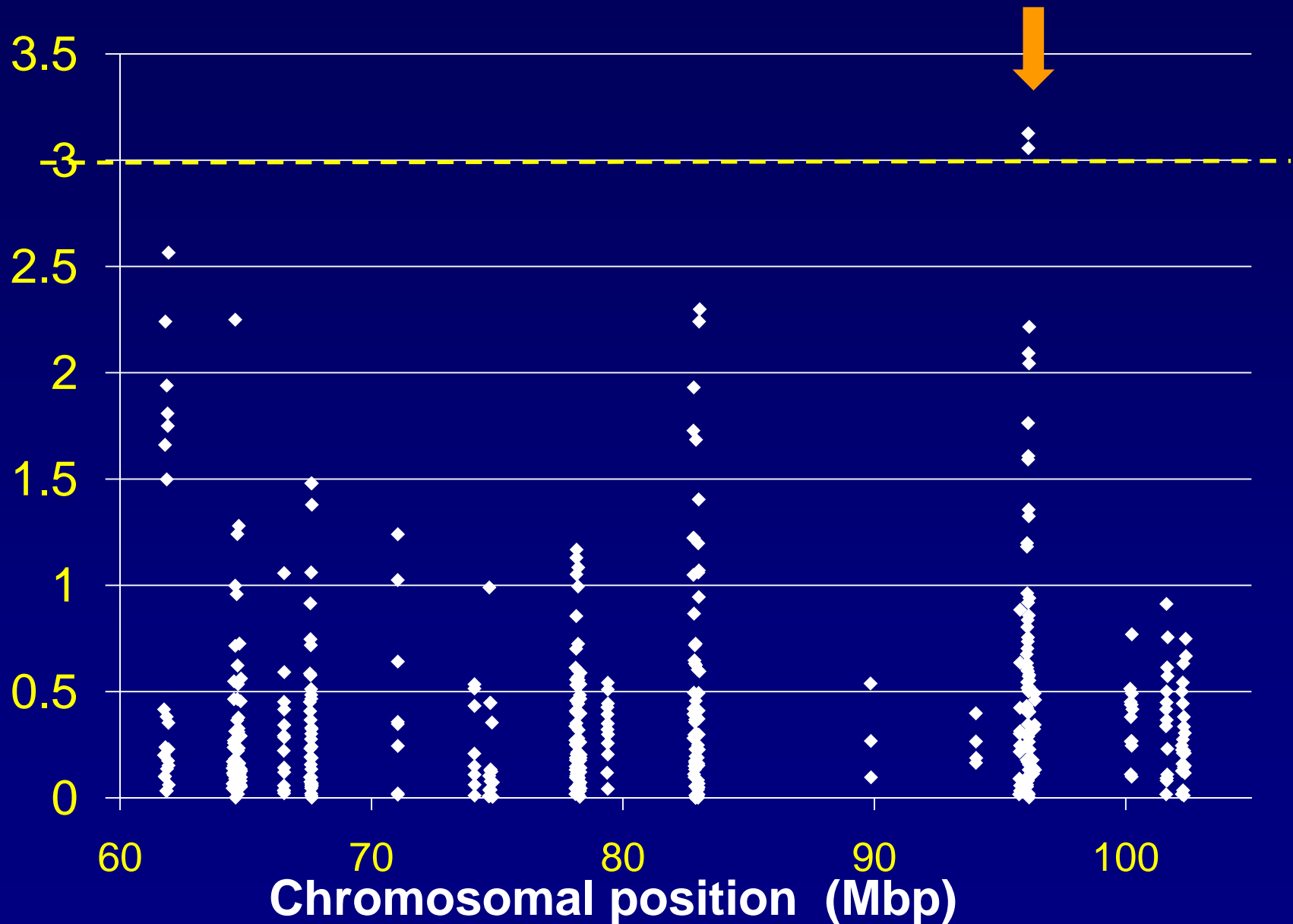
# Candidate Gene Analysis

- 22 genes within chromosome 5 linkage peak (65-108Mbp) selected on basis of putative role in lung function and/or nutritional status
- Selection of 480 Tagged SNPs with 'tagger' in haploview
  - Located within or upstream (5Kb) of gene
  - Minor allele freq > 0.05
  - Correlation ( $R^2$ ) >0.8
- SNP typing performed by NHLBI Resequencing and Genotyping service
- Association analyzed by Transmission Disequilibrium Testing(TDT) using FBAT and qTDT programs

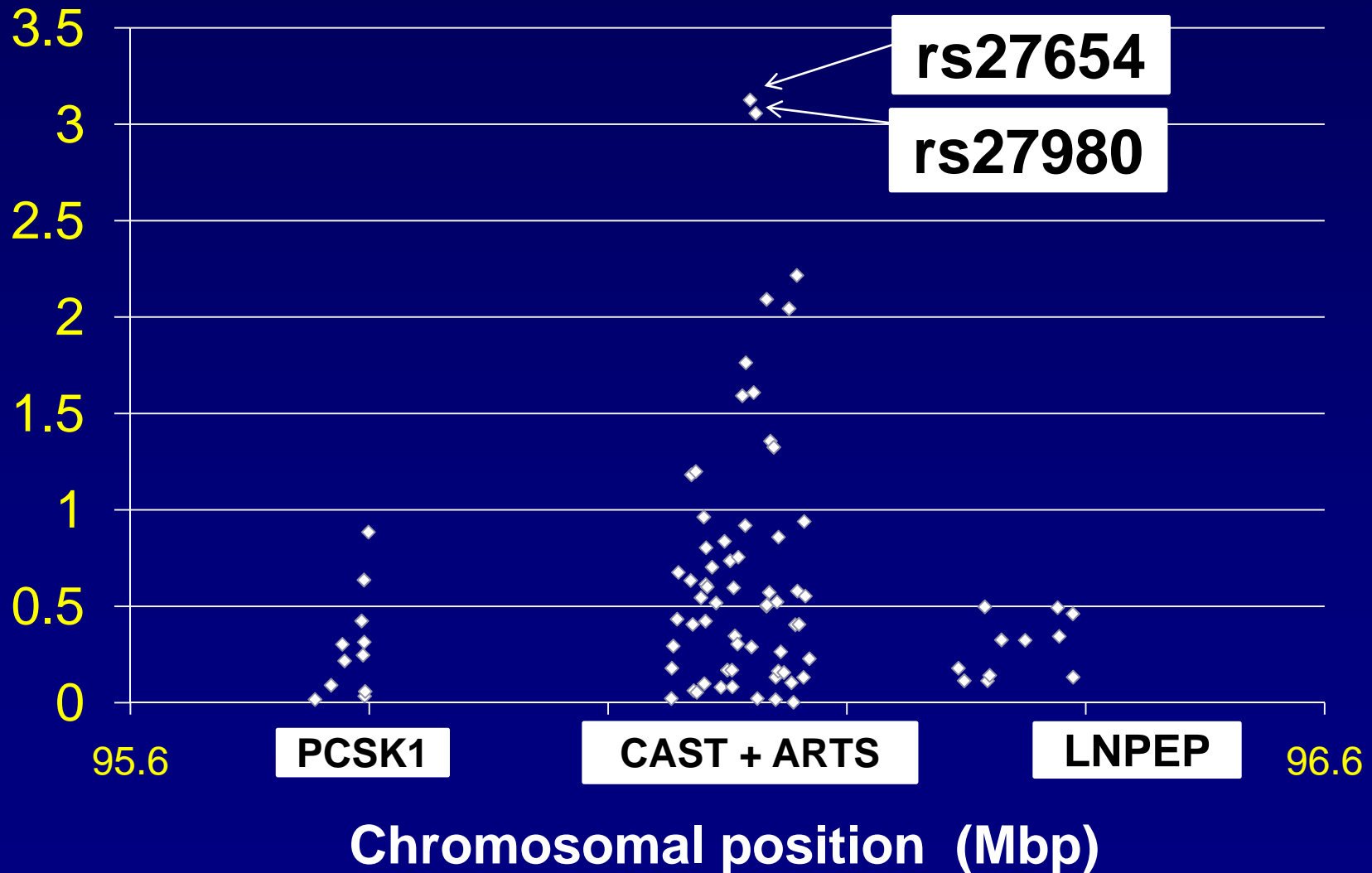
# Association study by TDT

- CF twins & sibling study based at JHU 800 patients from 408 families (563 trios)
- Trait: BMI and 3 lung function measures:
  - Cross sectional: **MaxFEV1CF%** is the best FEV1 percentile in the most recent year of data.
  - Longitudinal:
    - **AvgFEV1CF%**: average of all the CF specific percentiles using min 4yrs of PFT data
    - **BayesFEV1CF%**: estimate of FEV1 at age of 20years derived from at least 5 yrs of PFT data

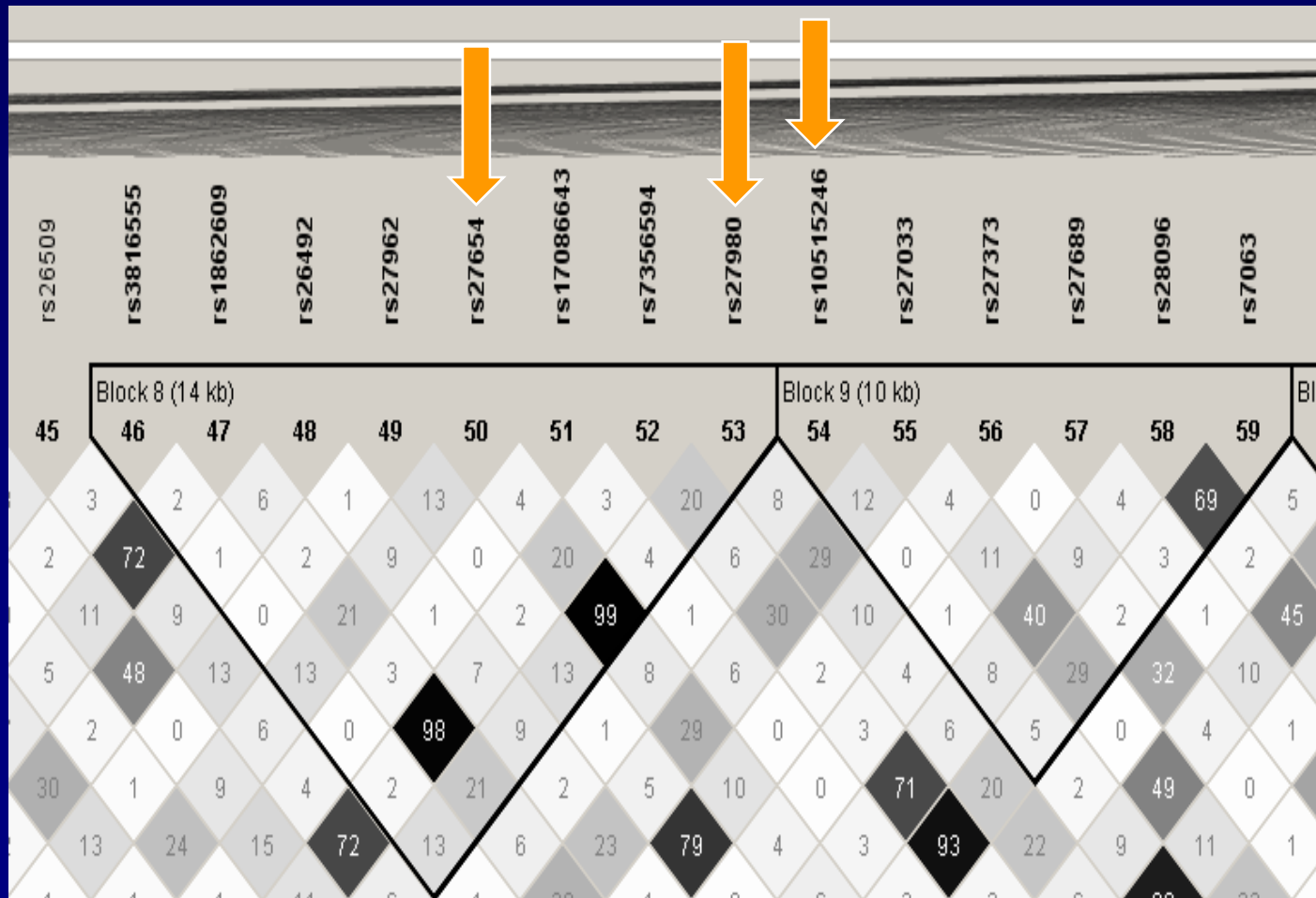
# Plot of $-\log p$ values for association of the tagSNPs with longitudinal lung function (BayesFEV1CF% shown)



# Two SNPs in CAST associated with variation in longitudinal lung function measures



# Haplotype: Third SNP (rs10515246) is not in linkage disequilibrium with two associated SNPs



# Analysis of all SNPs in CAST identifies a 3 SNP haplotype has highest association with variation in lung function

P-values (p-value after 100k permutation)

rs27654-rs27980- rs10515246	T- A- A (34%)	C- C -A (52%)	C- C -G (13%)
AvgFEV1CF%	0.002 (0.002)	-0.009 (0.04)	NS

# Calpastatin as a potential modifier of CF lung disease

- CAST gene encodes for Calpastatin,
  - inhibitory component of the Calpain system:  $\text{Ca}^{++}$  regulator
- Possible modifier mechanisms
  - Modulating  $\text{Ca}^{++}$  dependent  $\text{Cl}^-$  current, an alternate conductance pathway for  $\text{Cl}^-$  in pulmonary epithelia
  - Modulating apoptosis of neutrophils through Bax pathway, thereby varying inflammation and destruction in lungs
  - Modulating repair of skeletal and cardiac muscles under stress by regulating of proteolysis

# Conclusions

- A candidate gene within a region of linkage on chromosome 5 appears associated with variation in lung function
- CAST associates with longitudinal lung function variation but not with cross-sectional measures or BMI
- rs10515246 is not significant at SNP level but important in differentiating haplotype association
- Replication of calpastatin association could implicate calcium regulation as an important mechanism for modifying CF lung function

# Thank you

- CF patients & families and CF centers participating in CF twin and sib study
  - RS&G at NHLBI
  - Scott Blackman
  - Lindsay Bremer
- Kathleen Naughton
  - Sarah Ritter
  - Darci Ferrer
  - Garry Cutting

