NIH Workshop – Genomic Opportunities for Studying Sickle Cell Disease

"Phenotyping in SCD"

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Disclosures

- Adventrx – Honorarium and travel for advice related to a possible clinical trial

Major points

- Robust phenotypes can (must) be developed in SCD
- Lessons from other disease states may help guide the approach to phenotypes
- Endophenotypes and quantitative traits can be key
- Problems with phenotyping in SCD and possible solutions
- Stroke and possibly pain are good targets for further genetic analyses

Characteristics of a Good Phenotype for Genetic Exploration

- Common traits of importance, or uncommon traits of great importance
- Homogenous disease etiology
- Prior evidence of heritability
- Large sample sizes available
- Quantitative traits, when possible
- Definable variations in treatment responses to drugs or other therapies

Where have we been successful in establishing strong genotype-phenotype correlation?

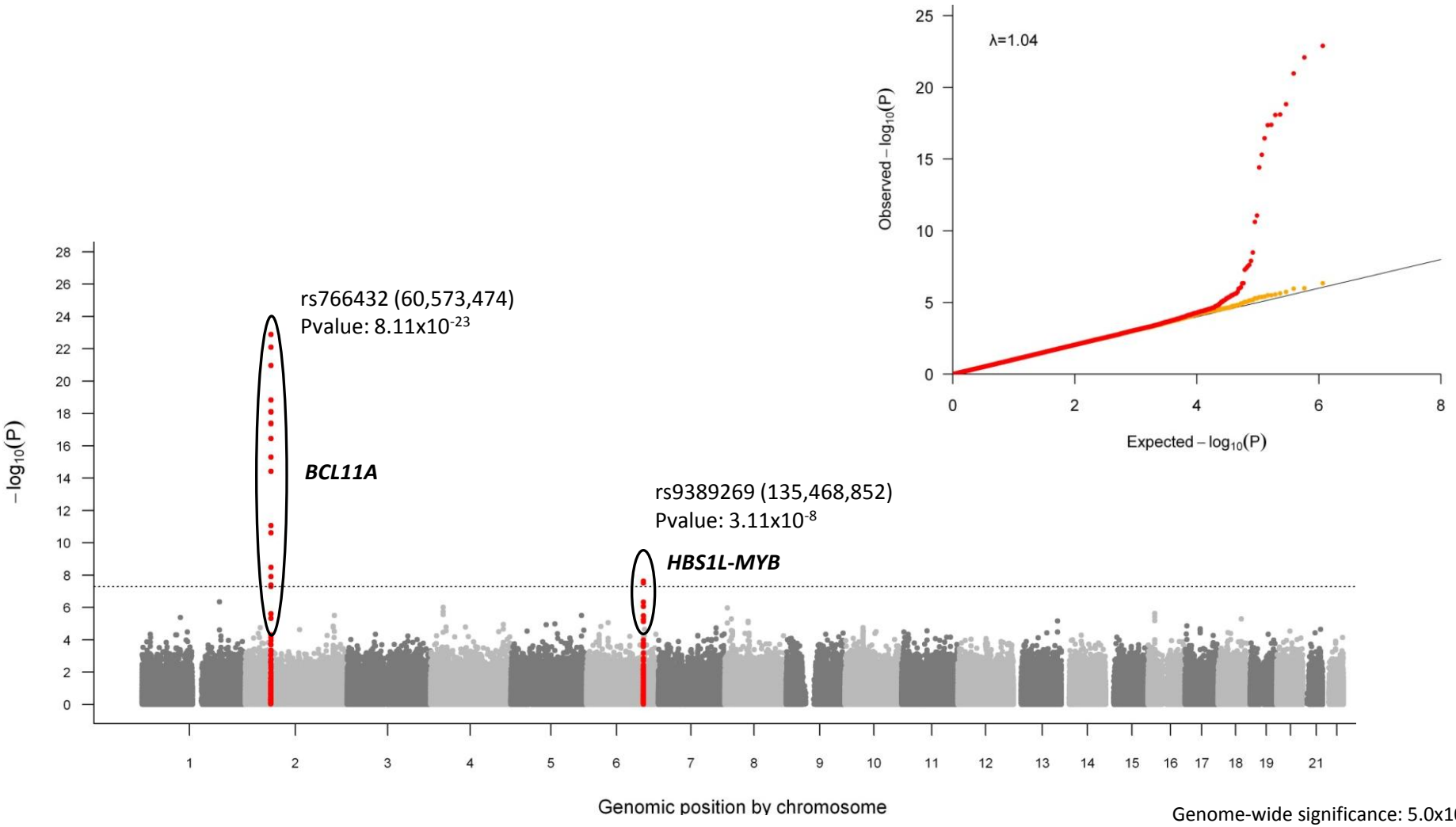
- Variations in the beta globin gene
- Haplotypes
- Alpha thal
- Hb F
- Bilirubin

SITT GWAS – Illumina HumanHap650Y + Omni1m_Quad

Genome-wide significance of % fetal hemoglobin (cube root transformed)

Linear regression adjusted for age (age_regis), sex (patientgender) and top 10 Eigenvectors

1,160,145 SNPs and 547 samples (Males: 282; Females: 265)

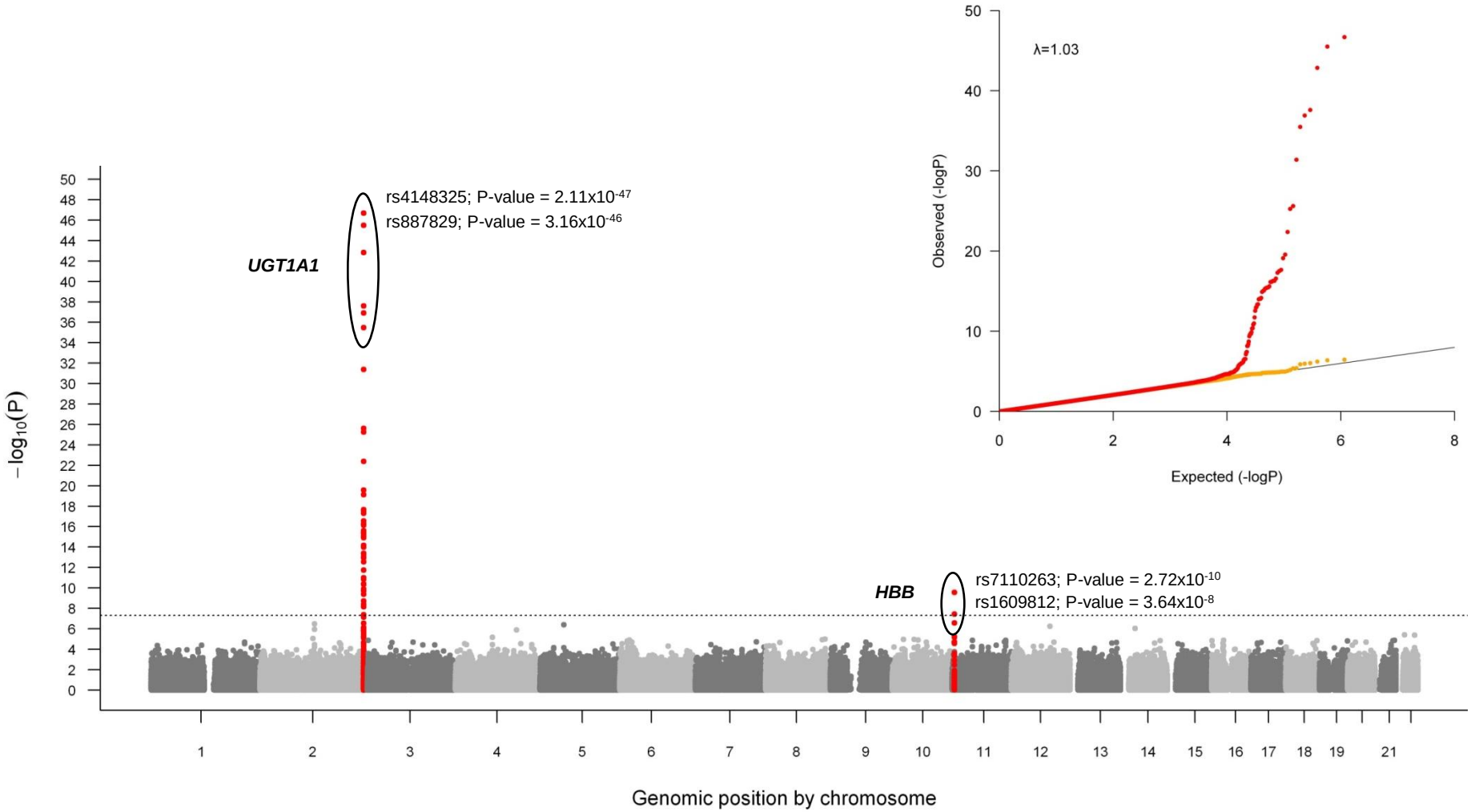


Genome-wide significance: 5.0×10^{-8}

Genome-wide Significance of Total Bilirubin (totbilirubin) in SITT Cohort

Linear regression with age (age_regis), sex (patientgender) and first 10 PCs adjusted model

1,160,145 SNPs and 905 samples (Male: 480; Females: 425)

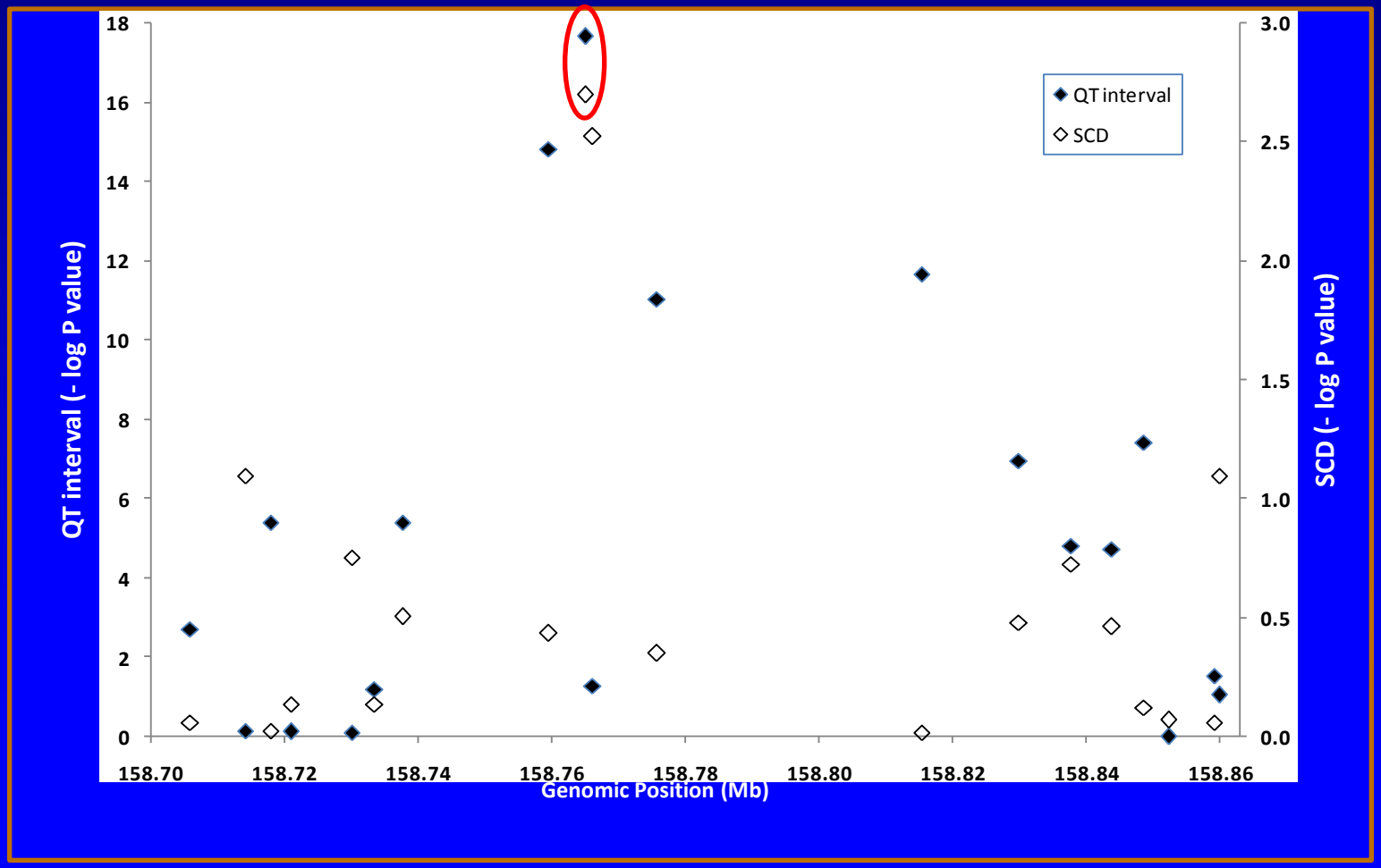


Genome-wide significance (P-value) < 5.0×10^{-8}

Where have others been successful?

- Target – Sudden Cardiac Death (SCD)
- Initial Study - Prolonged QT
  - Identification of *NOS1AP* as the major QT interval associated gene using a small GWAS
- Subsequent association studies of large cohorts
  - *NOS1AP* is also associated with both prolonged QT and risk for sudden cardiac death

Association of *NOS1AP* SNPS with QT Interval and SCD in the ARIC/CHS Whites



Where have others been successful?

- Cystic Fibrosis (CF)
  - Severity of lung disease not explained by allelic variation or candidate gene studies
  - Small samples sizes – similar to SCD
  - FEV1 chosen as a quantitative marker of severity – known to be >50% heritable
  - Design included GWAS (using extremes of phenotype) followed by linkage studies

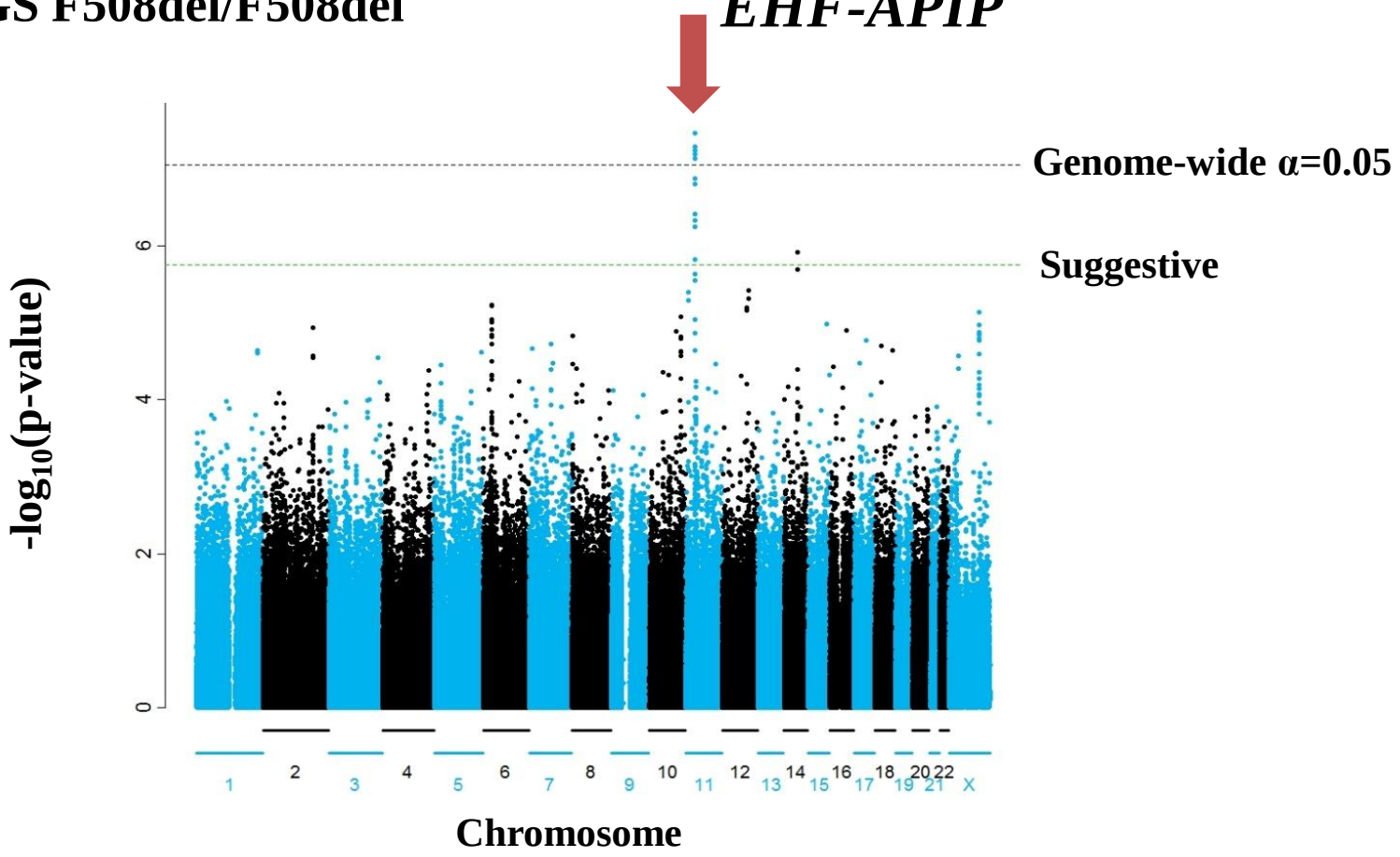
Study populations for genome-wide association and linkage

	Genetic Modifier Study (GMS)		Canadian Consortium for Genetic Studies (CGS)	Twins & Sibs Study (TSS)
Lead Institution(s)	Univ. of North Carolina/Case Western		Hosp. Sick Children	Johns Hopkins
Design	Extremes-of-Phenotype Unrelated		Population-Based Unrelated	Family-Based
Type of Evidence	Association		Association	Linkage and association
Number of patients	1,137		1,357	973 [a] (486 sibling pairs)
	Severe (*n* = 406)	Mild (*n* = 731)		

Wright F et al Nat. Genetics, 2011

Genome-wide association results for the lung function phenotype

GMS & CGS F508del/F508del (n=1,978)



Replication in F508del homozygotes (TSS) *P*= 6X10^{-3}

Joint analysis (GMS,CGS and TSS) in F508del homozygotes: *P* = 1.49X10^{-9}

Where have others been successful?

- Pain Phenotyping – orofacial pain (Oppera)
  - Established and followed prospective cohort of 3263 patients without orofacial pain (204 cases expected)
  - Case control of 185 patients with oral pain
  - Measure predictors of risk
    - non-causal and etiologic factors
      - Analyze individual and joint effects
    - Correlate with models and genetic factors

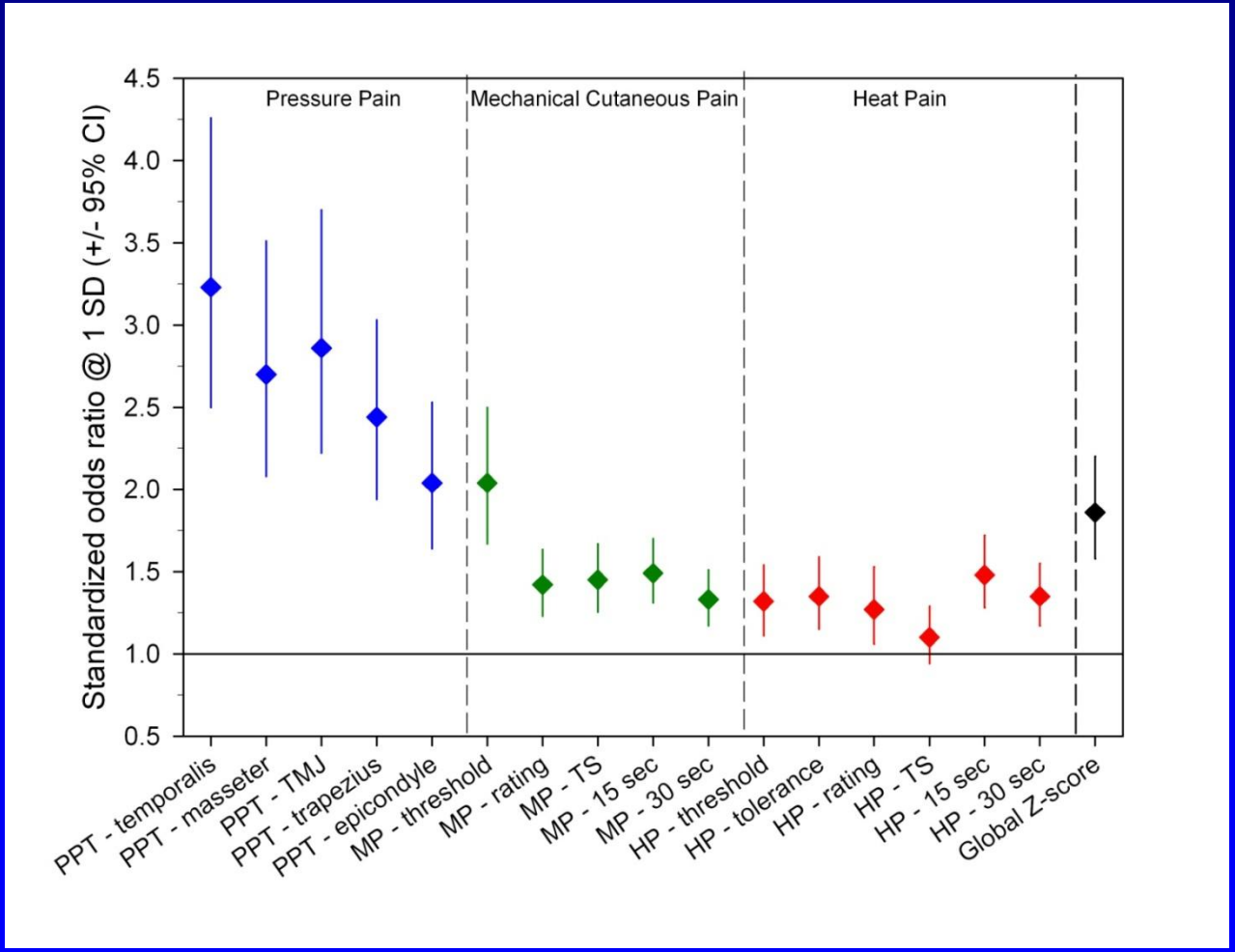
Oppera Study

- Intermediate Phenotypes
  - High psychological distress
  - High state of pain amplification
- Measure predictors
  - Clinical and sociodemographic characteristics
  - Heightened responsiveness to noxious stimuli
  - Pre-existing psychosocial profiles
  - Autonomic risk factors
  - Genetic variations that influence intermediate phenotypes

0ppera

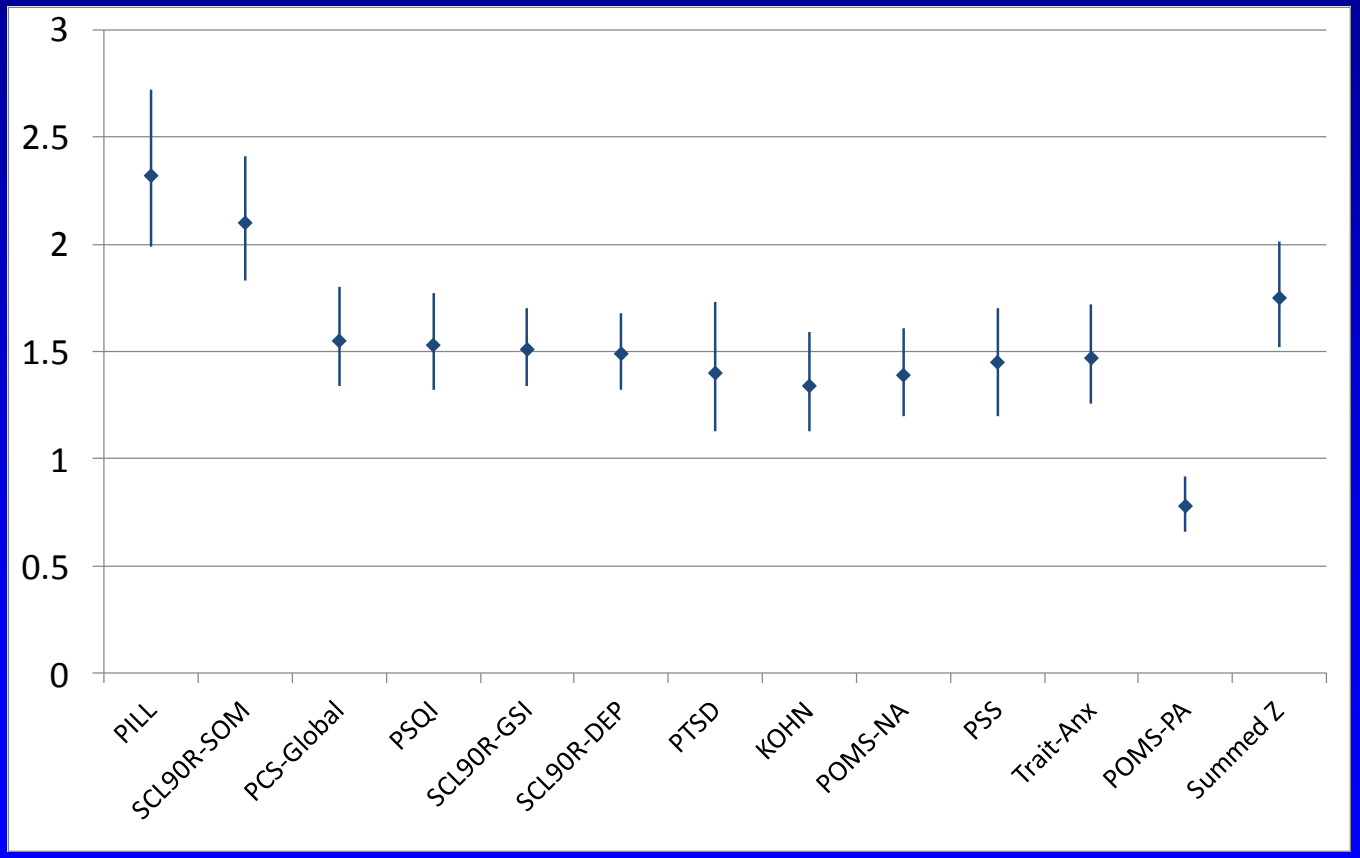
- Quantitative measures:
  - Pain sensitivity
    - Pressure Pain Thresholds (PPT):
    - Cutaneous Mechanical Pain Threshold and Suprathreshold Ratings
    - Heat Pain Threshold, Tolerance, and Suprathreshold Ratings:

Odds Ratios for Pain Sensitivity Measures - TMJD



N.B.: For threshold and tolerance measures, the original metric was reverse-coded, so the odds ratio represents the relative increase in odds of having TMJD with greater pain sensitivity for all measures.

Odd ratios (adjusted) for Psychosocial Variables



Standardized odds ratios were adjusted for study site, sex, and race/ethnicity

Approaches to Variable Reduction

- Aggregation (summed Z-score)
- Factor analytic approaches and principal component analysis
  - Identify underlying dimensions based on association among the variables, reducing to a smaller set of factors
- Clustering

Genetics of TMD

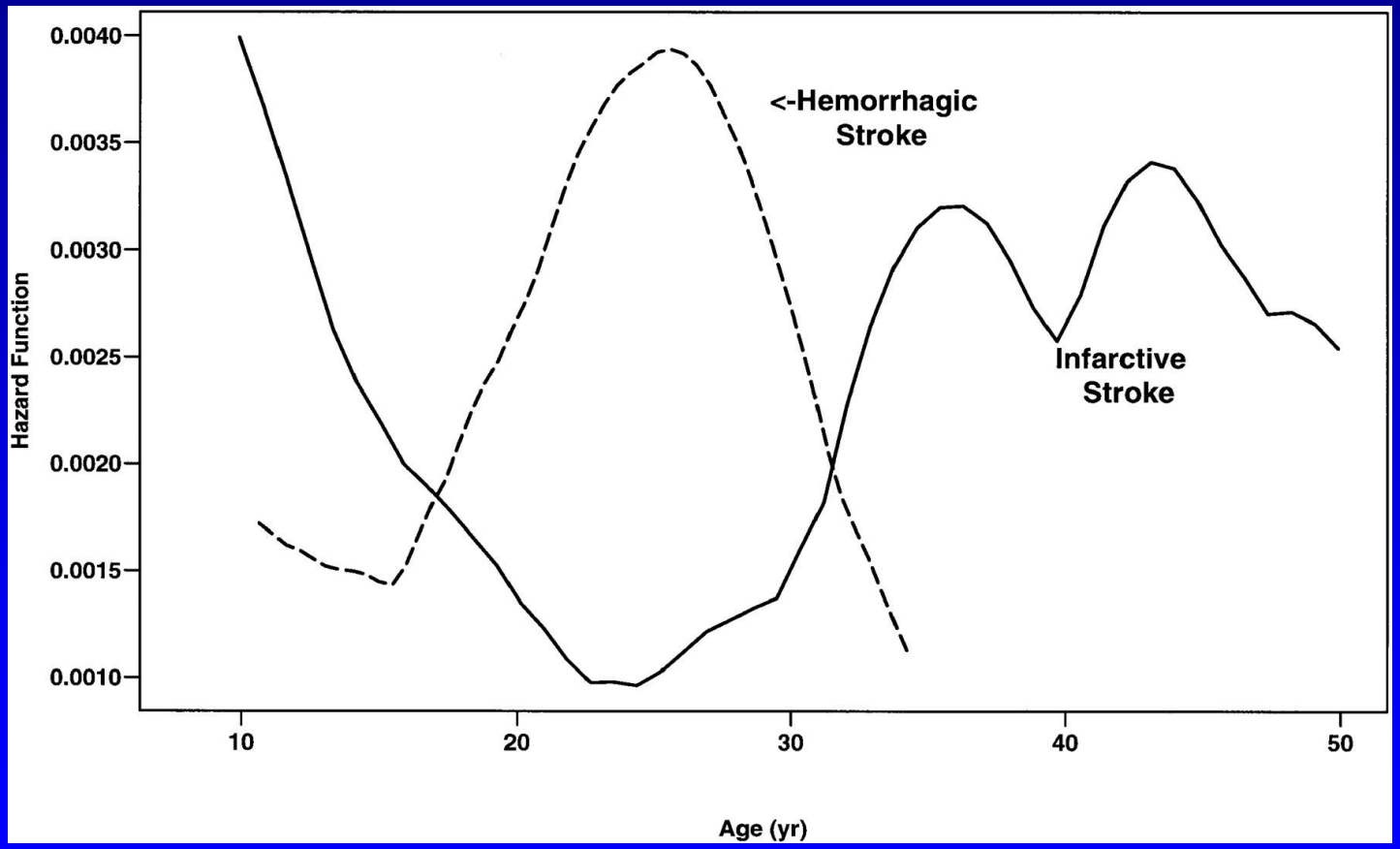
- Results provide evidence supporting previous association of COMT high pain phenotype and HTR2A (serotonin 2A receptor)
- Suggestive evidence for:
  - OPRD1 and GRIN2A genes (involved in pain regulatory pathways)
  - IL10 (anti-inflammatory)
  - Glucocorticoid receptors

What Phenotypes Should We Study?

- Stroke
  - Common phenotype
    - 11% of children, 24% overall
  - Heritable
    - Driscoll et al., 2003
  - Endophenotypes available
    - Silent cerebral infarction (heritable)
    - Volumetric analysis
    - Neuropsychology measurements
    - TCD velocities

Epidemiology of Overt Strokes

Ohene-Frempong 1998



Challenges for Genetic Studies in Sickle Cell Disease

- Adequate sample sizes are often difficult to obtain
- Phenotypes of interest are not always stable
  - Stroke, priapism, ACS
- Restricted ethnicity
  - Replication in different ethnic groups difficult
- Family studies often difficult due to family structure

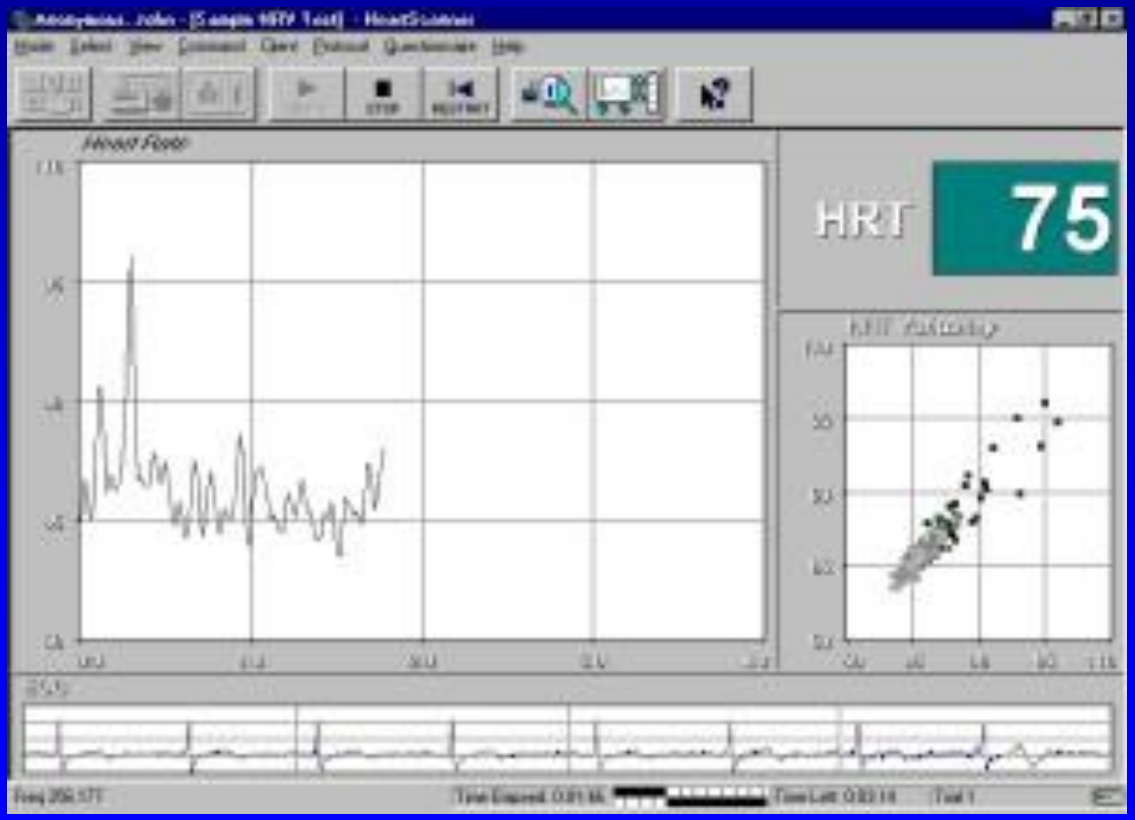
Challenges for Genetic Studies in Sickle Cell Disease

- Adequate sample sizes are often difficult to obtain
  - Consortium studies
  - Endophenotypes
  - Leverage clinical trials
  - Designed cohorts for phenotyping (ala Oppera)
- Phenotypes of interest are not always stable
  - Stroke, priapism, ACS
    - Explore epidemiology of events
    - Appropriate statistical models

Challenges for Genetic Studies in Sickle Cell Disease

- Restricted ethnicity
  - Replication in different ethnic groups challenging
    - Same genetic functional variant
    - Same gene, but different functional variants (gene-based tests)
- Family studies often difficult due to family structure
  - Pursue sib/twin studies

Blood Pressure Monitoring
Heart Rate Monitoring



Genetics of TMJ

- Heritability for fibromyalgia (51%) headache and neck pain (34-58%)
- Based on candidate gene studies:
  - 23 genes studied in the catecholamine, serotonin, opioid and cytokine pathways
  - Discovery panel of 350 pain related genes
  - Genotyped using 3295 SNP Affimetrix Pain Research Panel, including domains of:
    - 1) Pain perception
    - 2) Inflammatory markers
    - 3) Mood and affective states associate with pain
    - 4) Pharmacokinetics of analgesia