Genetic Risk Factors for Childhood Leukemia

GETA Symposium
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Outline

- Background
- Review from literature
- Northern California Childhood Leukemia Study (NCCLS) experience
- Challenges
- Future directions

Genetic susceptibility

- Q: What is genetic susceptibility?
- A: Heritable factors that increase risk of a given disease
- Usually one or more genes, or gene variations
- May work in concert with
  - Other genetic factors, AND/OR
  - Environmental and lifestyle factors
- Degree of involvement of other factors depends on penetrance

Penetrance

- Penetrance
  - High: rare, but high risk (e.g., BRCA1, RR~5)
    - Major part of short causal pathway to disease

  - Low: common, but low risk (RRs of ~1.3-1.8)
    - Minor part of long causal pathway to disease

- Low-penetrance genetic factors likely to comprise the bulk of inherited cancer risk

Rationale for genetic susceptibility to CL

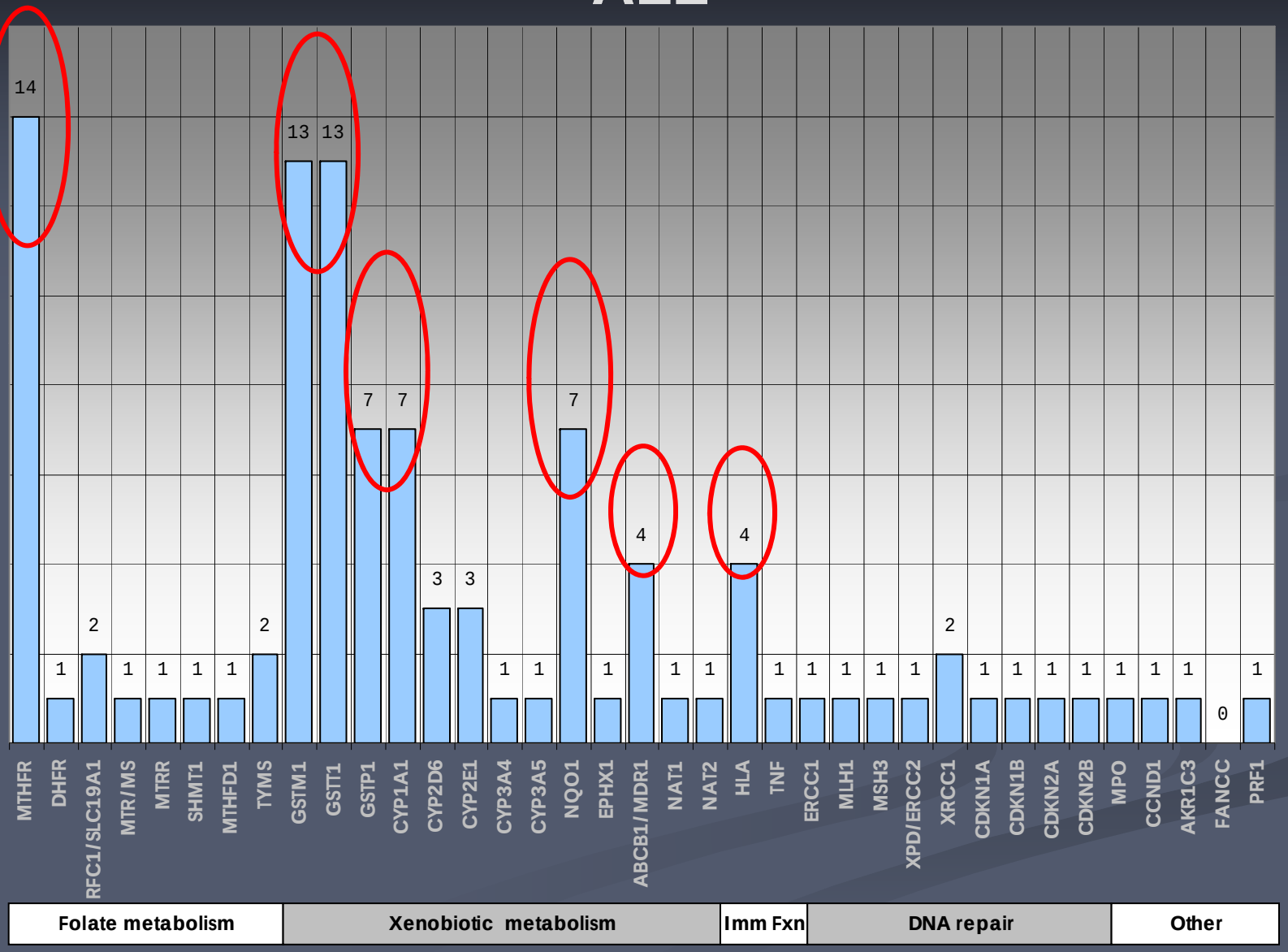
- Early age of onset
- Risk in twins
  - Mostly intraplacental metastasis, not highly penetrant risk allele
  - Suggests low penetrance susceptibility alleles

Review

Candidate pathways
in published reports

- Folate metabolism
- Xenobiotic (exogenous chemical) transport and metabolism
- Immune function
- DNA repair

Primary reports of gene main effects: ALL



Folate metabolism & ALL

- Folate
  - Essential micronutrient, modulates balance between accuracy of DNA synthesis and DNA methylation
  - Deficiency can induce chromosomal damage and fragile chromosomal sites → carcinogenesis
  - Maternal supplementation during pregnancy may reduce risk
- MTHFR, 2 loss-of-function variants: 14 reports
  - 677C>T: null effect or modest risk reduction (OR~0.9)
  - 1298A>C: null effect

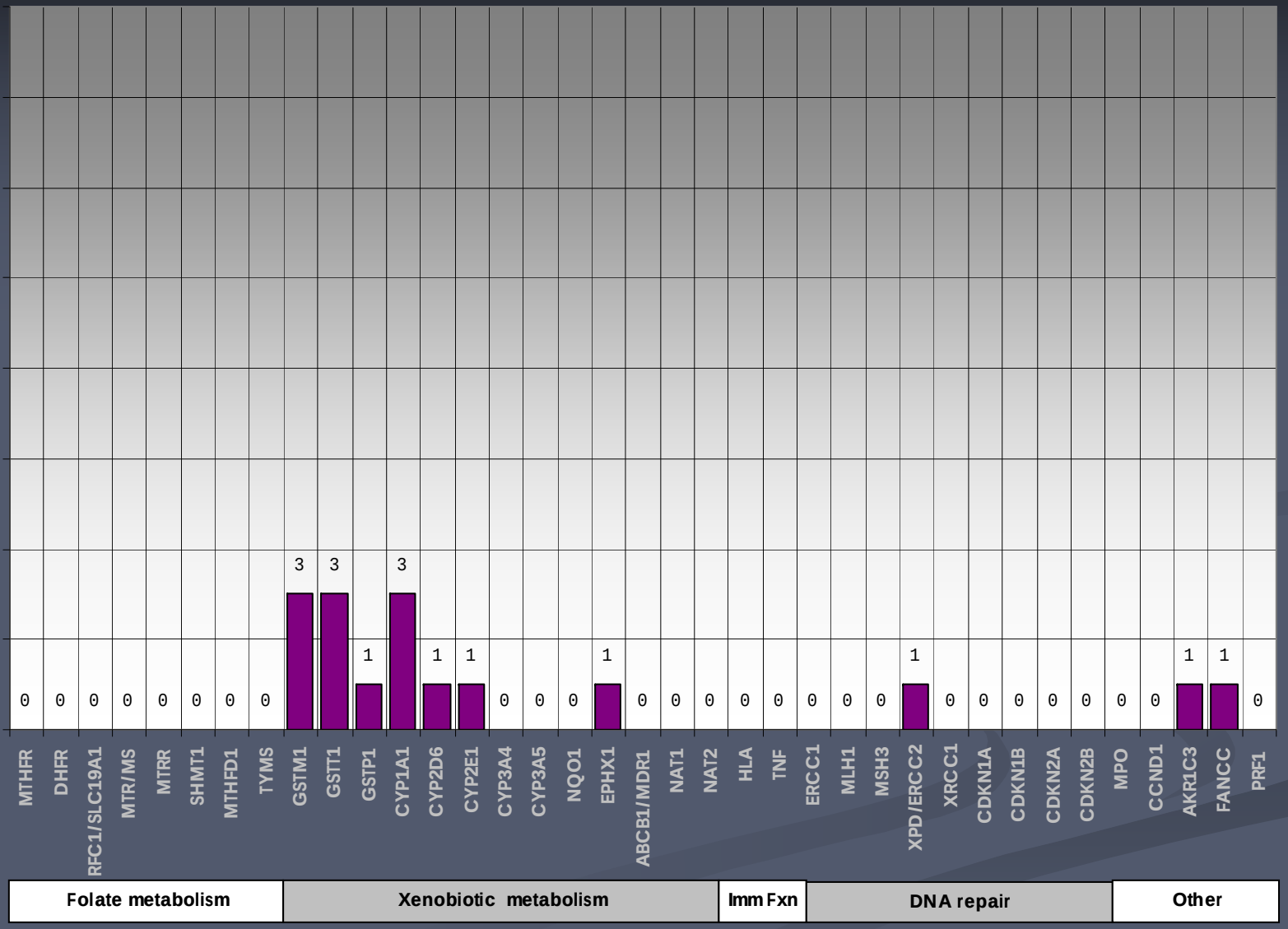
Xenobiotic metabolism & ALL

- To do harm, exogenous chemicals must
  - enter cells
    - Membrane transporters (e.g., MDR1)
  - be metabolized into harmful species
    - Phase 1, bioactivation enzymes (e.g., CYPs)
    - Phase 2, detoxification enzymes (e.g., GSTs, NQO1)
- Transporters
  - MDR1: 4 reports
    - 3435C>T: null risk
- Phase 1, bioactivation
  - CYP1A1: 7 repots
    - 6235T>C: inconsistent

Xenobiotic metabolism & ALL

- Phase 2, detoxification
  - GSTM1 (detoxifies PAHs): 13 reports
    - deletion: null to modestly increased risk
  - GSTT1 (detoxifies epoxides and halomethanes): 13 reports
    - deletion: null risk
  - GSTP1: 7 reports
    - I105V: null risk
  - NQO1 (anti-oxidant, detoxifies quinones): 7 reports
    - 609C>T: null risk
    - 465C>T: null risk (2 reports)

Primary reports of gene main effects: AML



Evaluating the evidence

- HuGENet – Human Genome Epidemiology Network
- HuGENet Encyclopedia: synopsis of evidence for genetic associations of complex disease
- CL as one of several prototype encyclopedia entries
- Venice meeting (2006): to develop criteria for rapid evaluation of evidence to facilitate encyclopedia effort
  - 3 criteria:
    - Amount of evidence
    - Replication
    - Protection from bias
  - Letter grades (A, B, C) for each – AAA is ideal

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Assessment of cumulative evidence on genetic associations: interim guidelines

John P A Ioannidis,[1–3]* Paolo Boffetta,[4] Julian Little,[5] Thomas R O'Brien,[6] Andre G Uitterlinden,[7] Paolo Vineis,[8] David J Balding,[8] Anand Chokkalingam,[9] Siobhan M Dolan,[10] W Dana Flanders,[11] Julian P T Higgins,[12] Mark I McCarthy,[13,14] David H McDermott,[15] Grier P Page,[16] Timothy R Rebbeck,[17] Daniela Seminara[18] and Muin J Khoury[19]

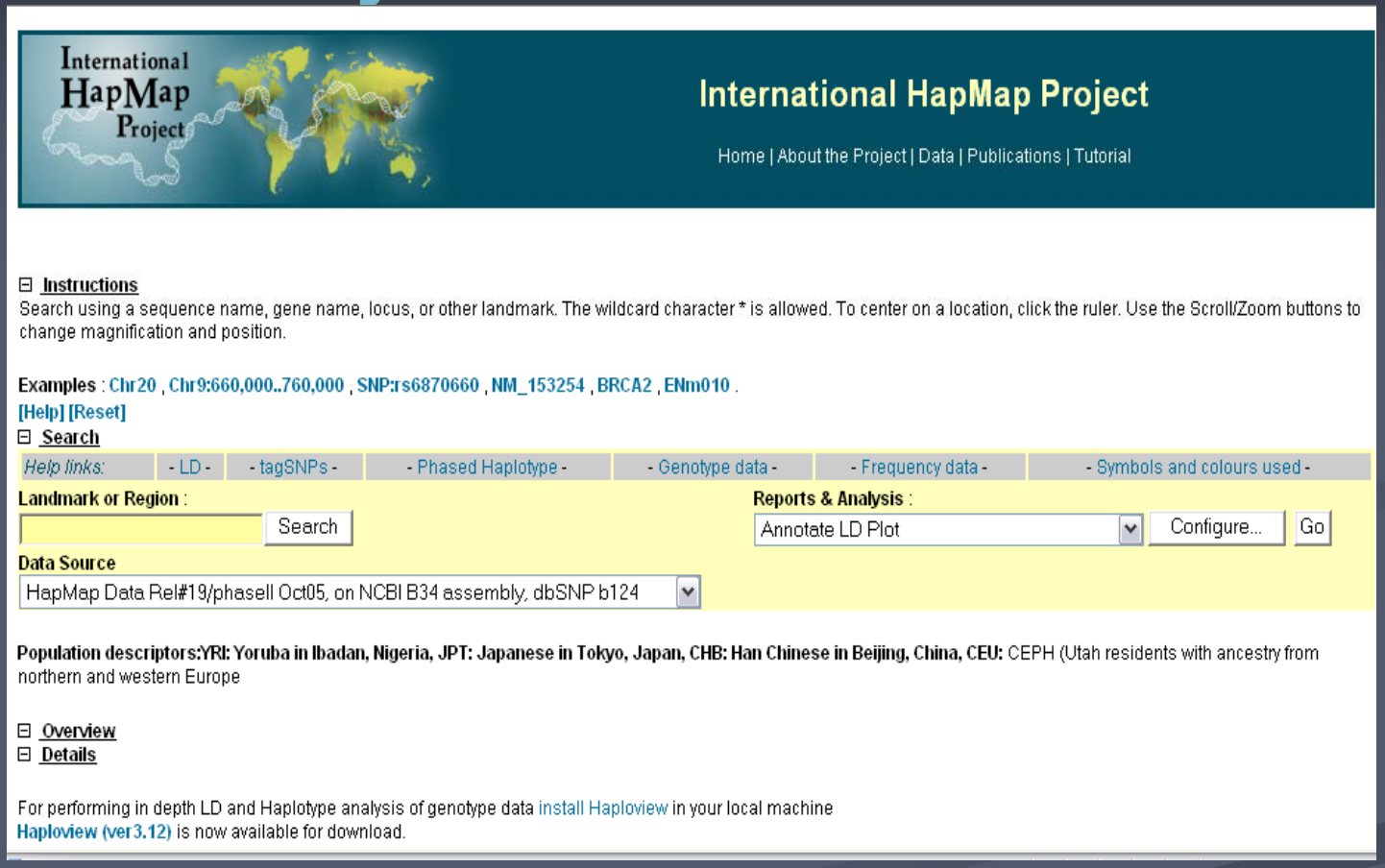
Venice criteria evaluation for ALL
Pilot Results

- Result of preliminary review
  - None have reached A status in any criterion
  - Only MTHFR and GSTM1 rank BBB
  - All else have a C in at least one criterion
- Evaluation of criteria in progress
- Next steps:
  - Refine criteria
  - Develop systems for
    - Consistent assignment and adjudication of grading
    - Updating as new evidence is published

Summary

- Few genes have been studied to date for ALL, even fewer for AML
- Many high-probability candidates remain unexamined or unreplicated
- Entire candidate pathways with very strong biological plausibility remain poorly studied (e.g., immune function, DNA repair)
- Reports to date do not ensure good coverage of variation within a gene

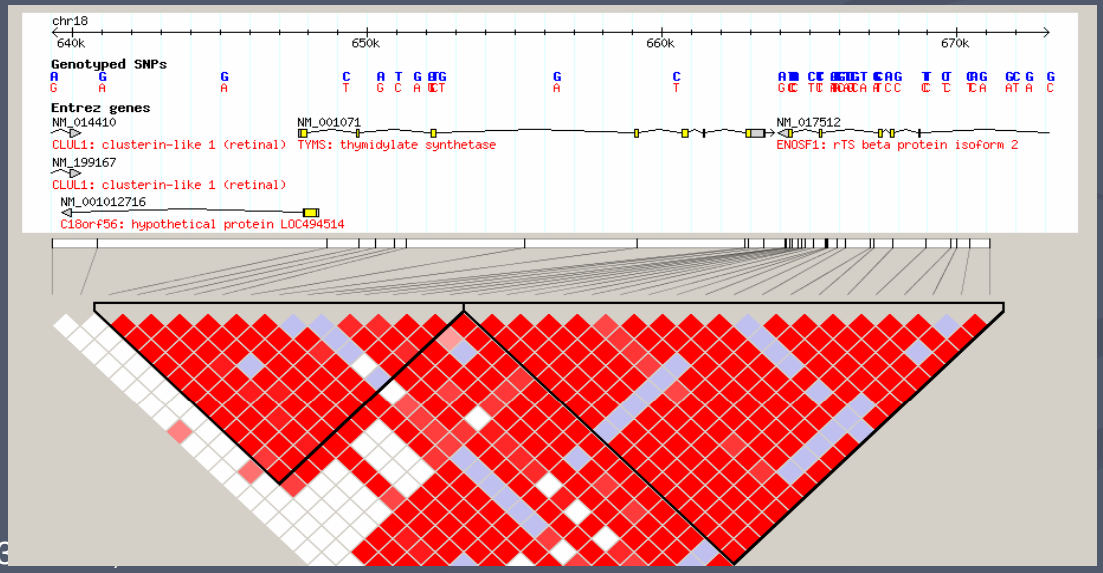
HapMap Project
Publicly available SNP data



- Data on linkage between SNPs in different populations

Haplotype-based analysis

- Uses HapMap and similar data
- Permits:
  - Maximal coverage of variation within genes
  - Minimum number of SNPs



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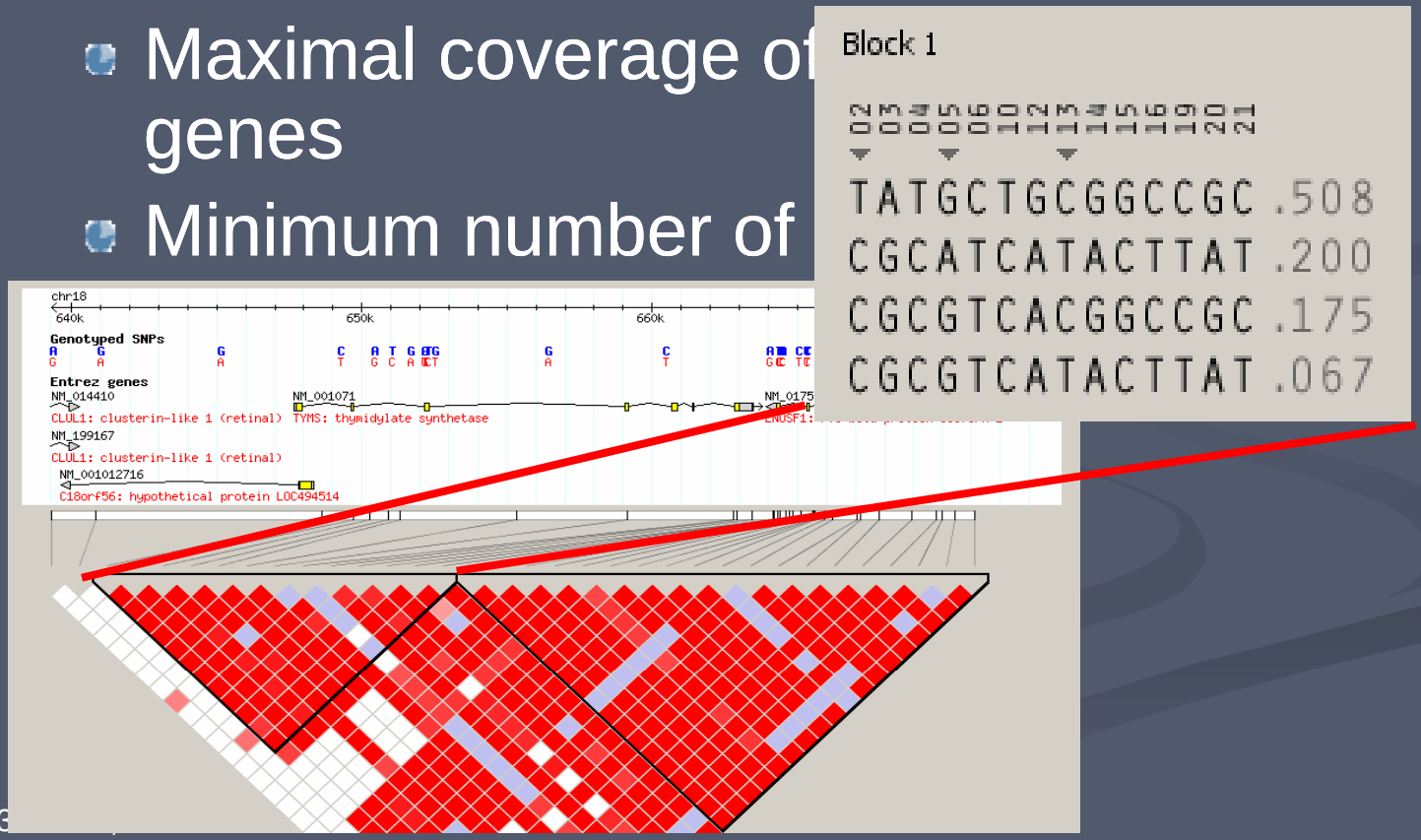
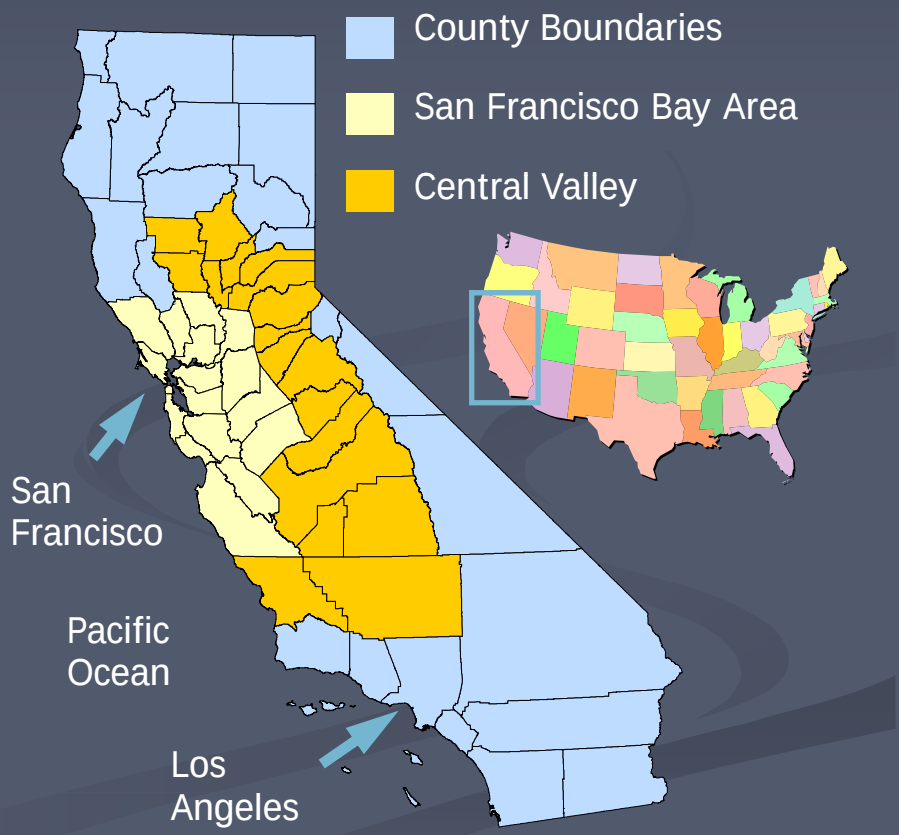


NCCLS experience

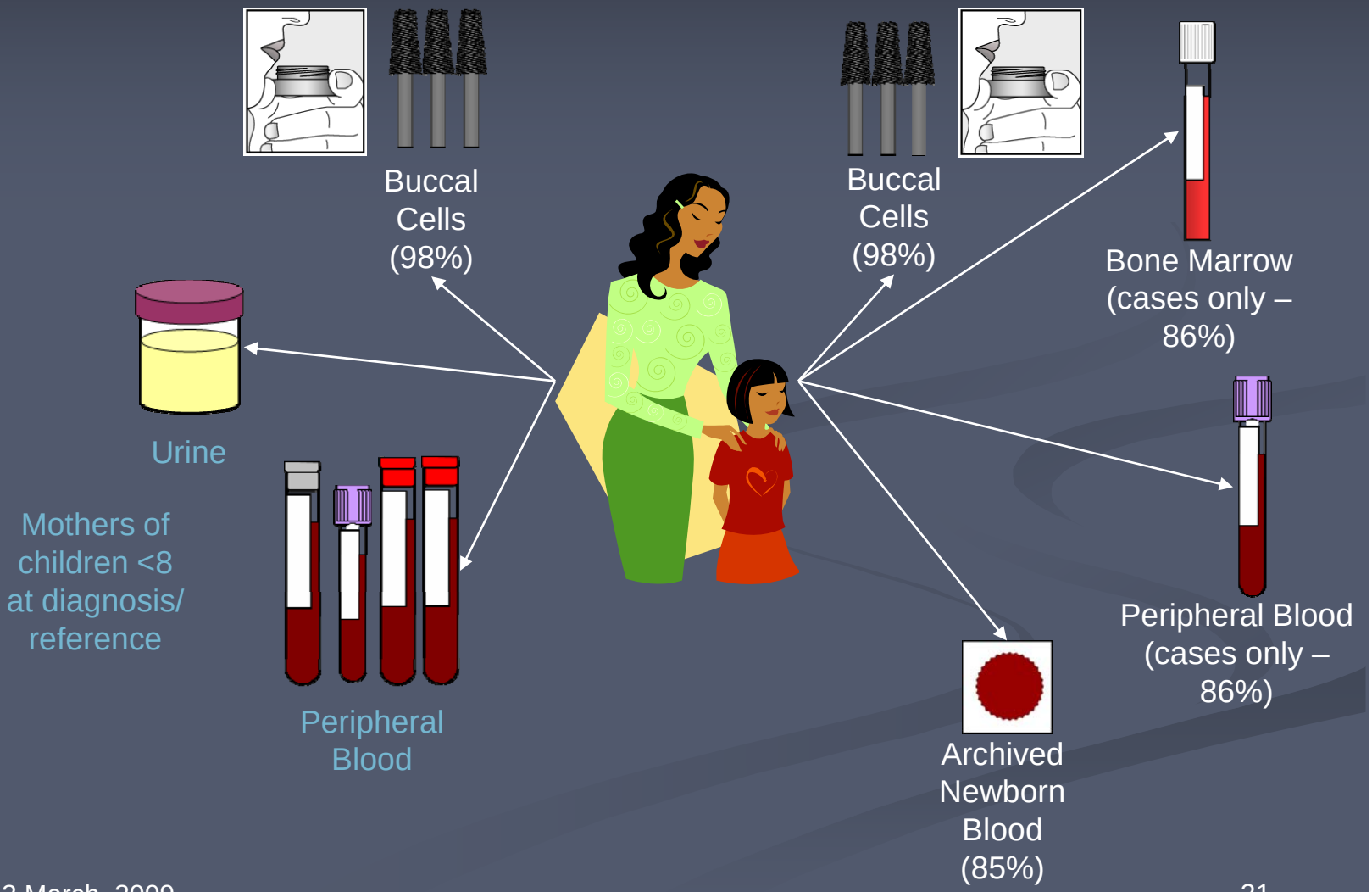
Northern California Childhood Leukemia Study

1995-present

- Population-based case-control study
- Incident cases ascertained from 9 pediatric hospitals in N. & C. California
- Controls individually matched (DOB, sex, Hispanic status, and maternal race)
- 42% Hispanic



NCCLS Biospecimen collection



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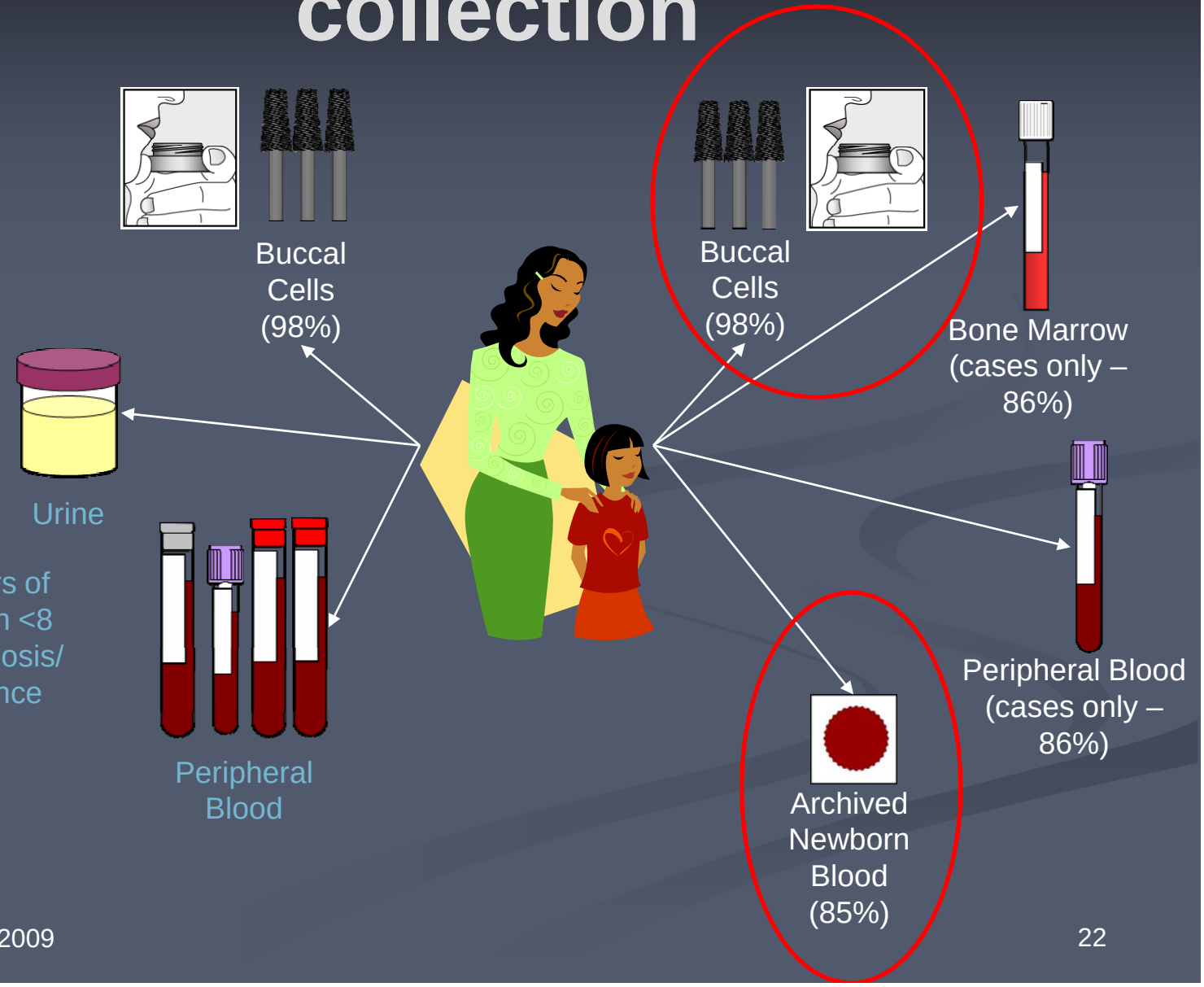
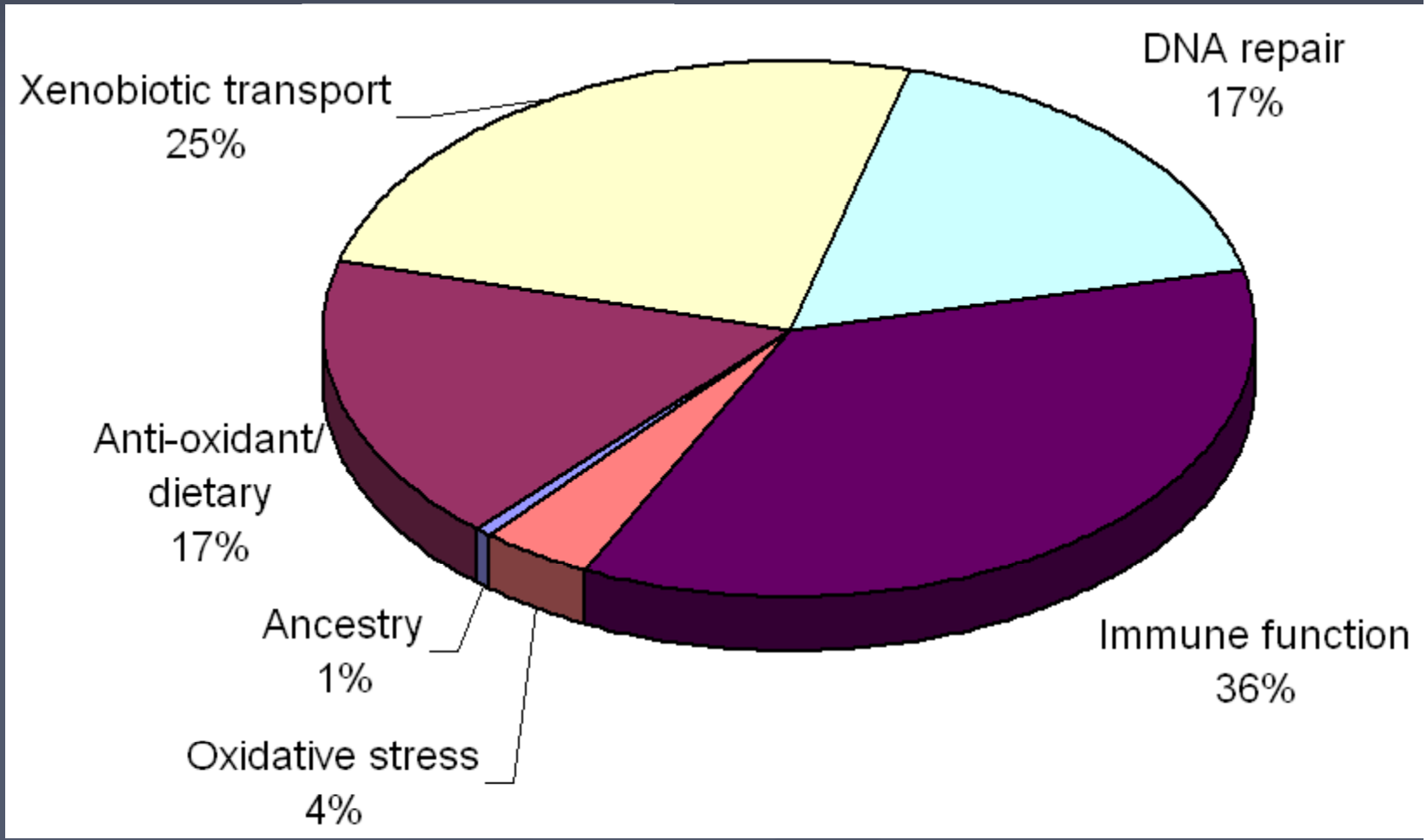


Large-scale Genotyping

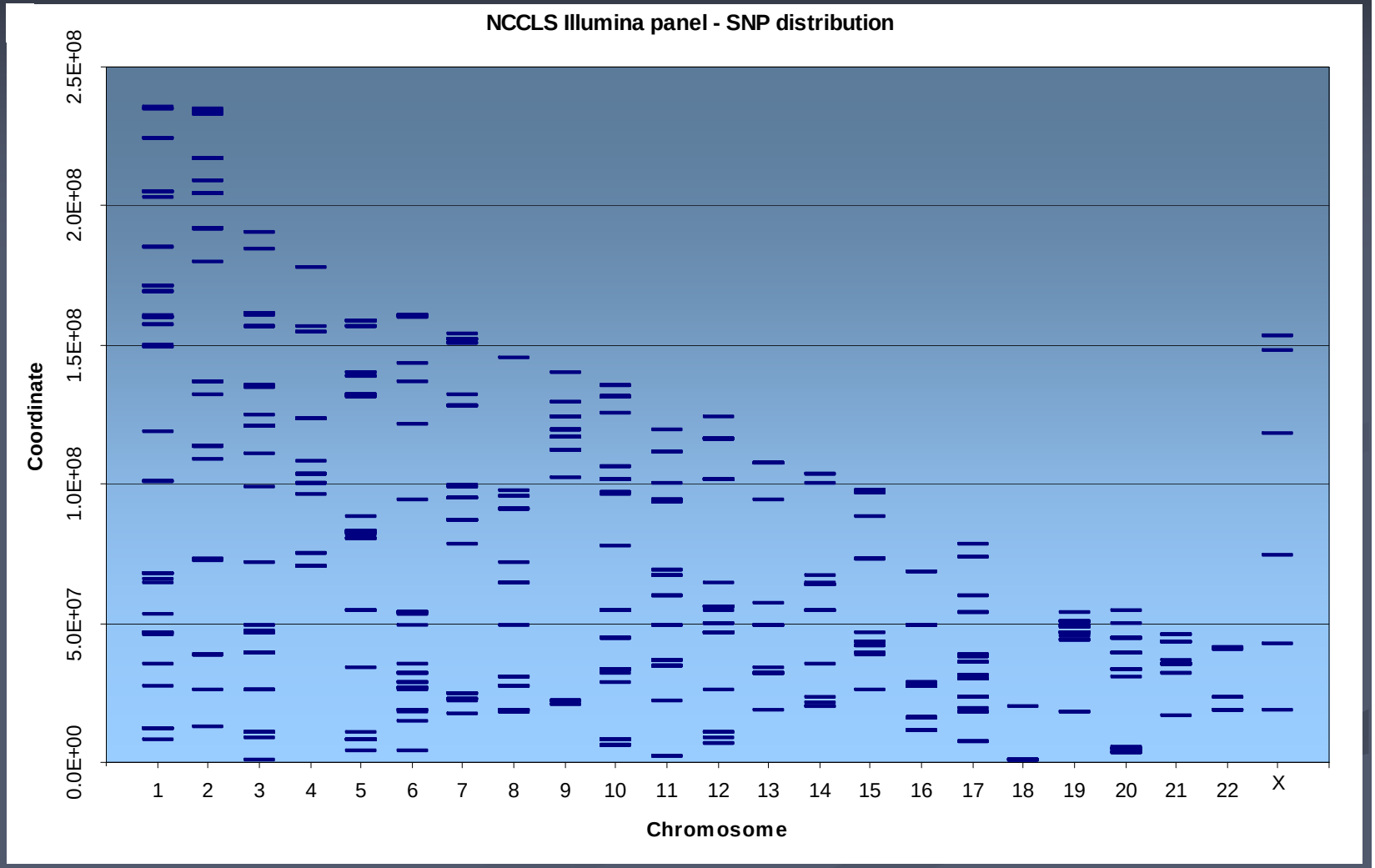
- Objective: to comprehensively examine ~200 candidate genes in subset of available children (464 cases, 464 controls)
- Custom Illumina 1536-plex
  - 183 genes
    - Haplotype tagging SNPs
    - Literature SNPs
  - Ancestry Informative Markers
    - Adjust for genetic ancestry

Candidate Pathways



Percent of 183 included genes

NCCLS panel coverage



NCCLS genotyping

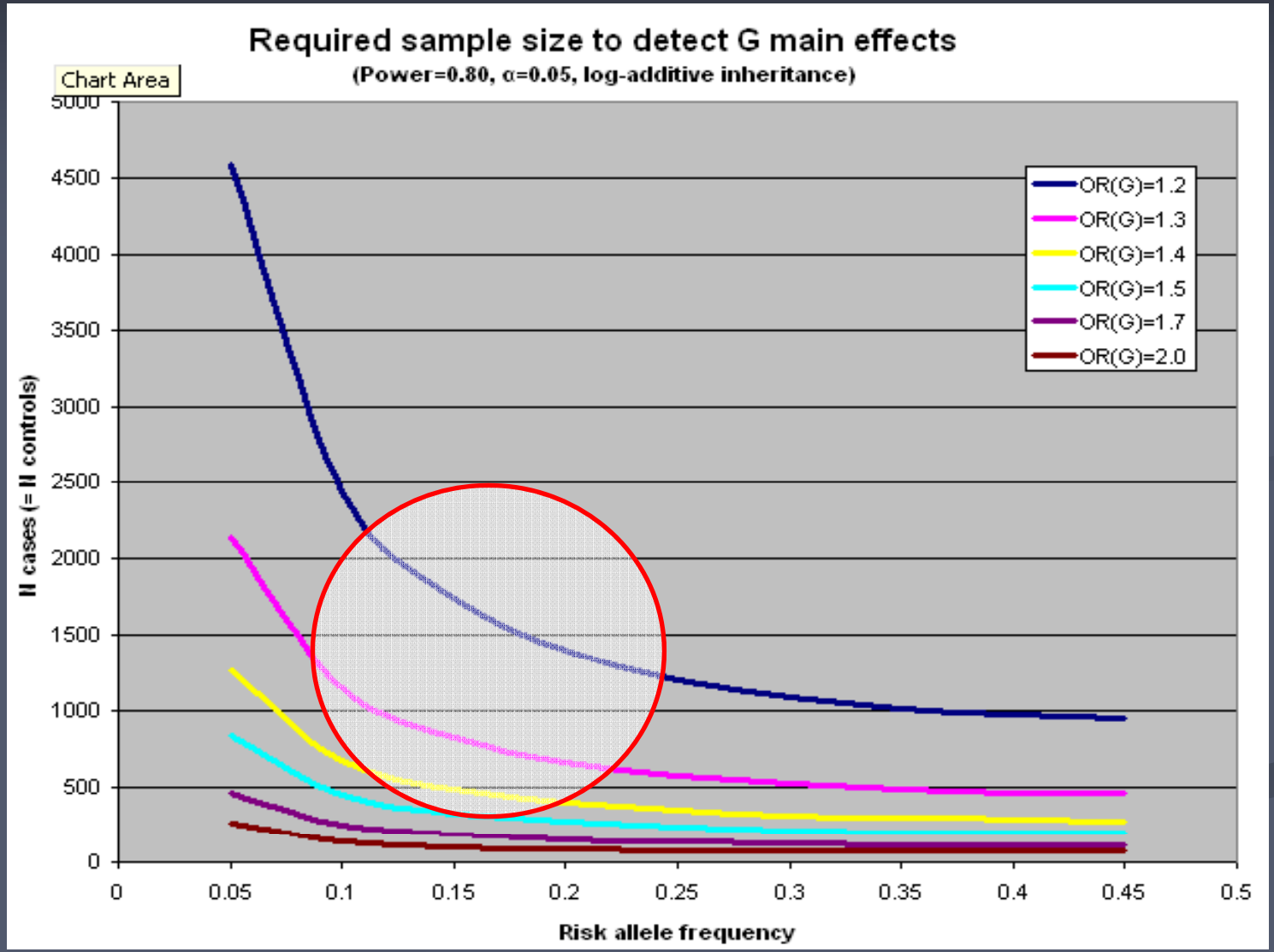
Current status – Preliminary analyses

- Adaptive immunity and ALL (208 SNPs in 29 genes)
  - 19 SNPs with nominal p<0.05
  - Single *IL12A* SNP (p<0.00001)
  - Haplotype sliding window analyses: regions in *CD28, FCGR2A, GATA3, IL2RA, IL12A, STAT4, and STAT6*
- Xenobiotic transport and metabolism and ALL (251 SNPs in 42 genes)
  - Individual SNP associations for *ABCB1, ABCC1, and CYP2C8* genes.
  - Haplotype sliding window analyses: regions in *ABCC1, ABCB1, CYP1A1, CYP1A2, CYP2B6, CYP2C8, IDH1, UGT1A1, UGT1A7, and UGT1A9*.
  - Potential interaction: *CYP2C8* risk haplotype and self-reported exposure to paints and solvents

Challenges to genetic susceptibility research in childhood leukemia

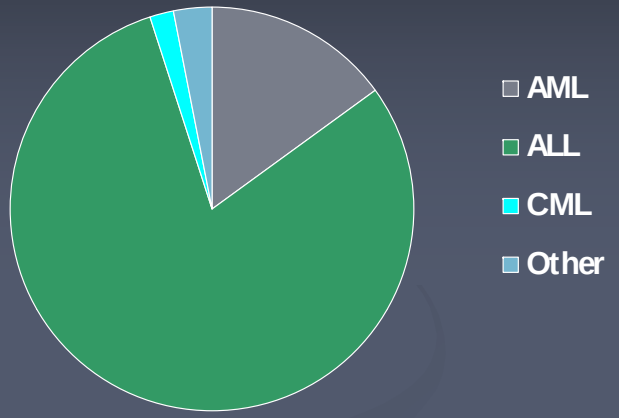
Statistical power and sample size issues
Maternal genes
Replication
Publication Bias

Statistical power



Sample size

- Sample sizes limited by
  - Low incidence
    - 4.5/100,00 person-years
  - Disease heterogeneity
    - Subtype groups
    - “Lumping vs. splitting”
  - Availability of high-quality DNA
- Published genetic studies typically 100-300 cases
  - Larger studies expected



Interactions

- Small effect sizes (OR~1.2-1.5) point to effects of GxE interactions
- Critical to understanding susceptibility
- Requires
  - High-quality exposure data
  - Even larger sample sizes for sufficient power

Maternal genes

Other major challenges

- Replication of results
  - Multiple studies
  - Multiple populations
- Publication bias
  - When null results are not presented or published

Future directions

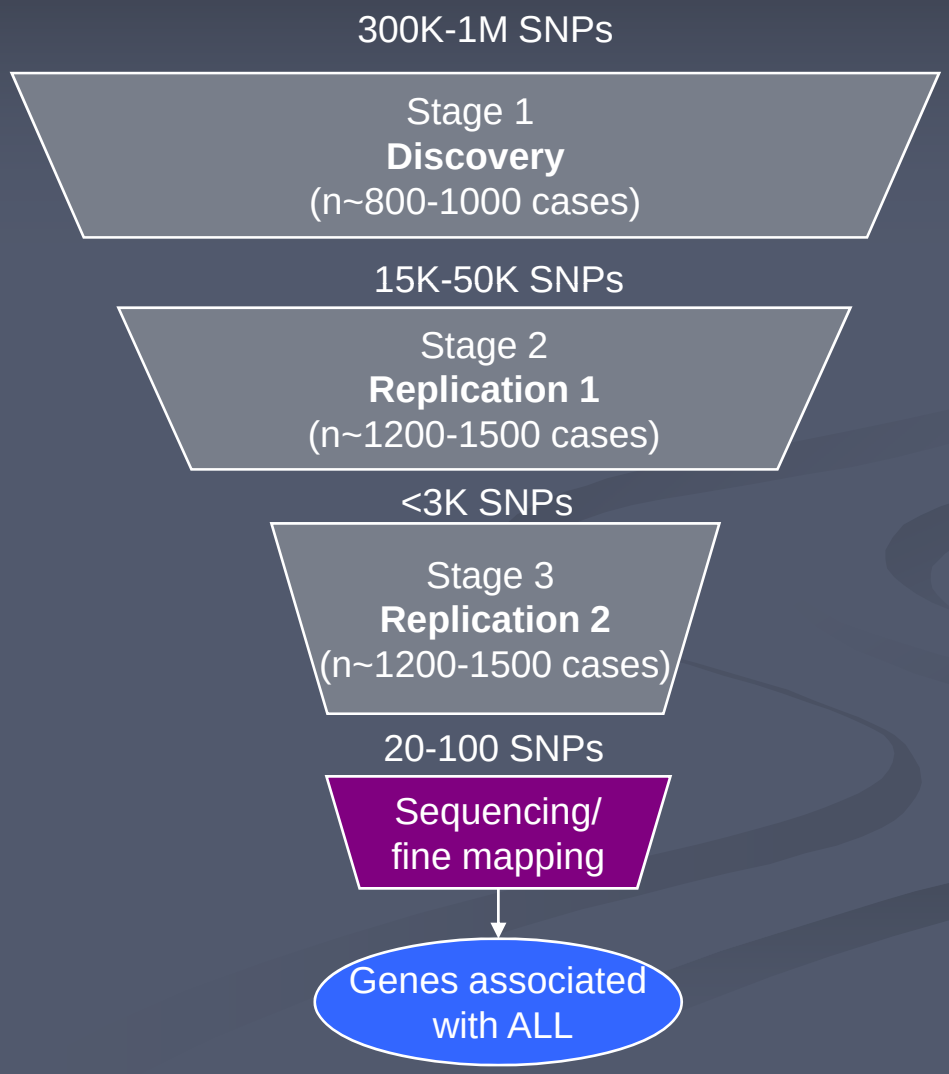
CLIC

- Childhood Leukemia International Consortium
  - www.clic.berkeley.edu
  - To date: 14 case-control studies in 10 countries
  - Over 9,000 cases
- Purpose
  - Replication of findings
  - Coordinated publication (address publication bias)
  - Data pooling (improve statistical power)
  - Collaborative research

GWAS

- **G**enome-**W**ide **A**ssociation **S**tudies
  - Allows exploration of genome beyond candidate genes
  - 300K-1M variants across the genome
- Issues
  - Sample size
  - Replication strategy
  - Technical requirements
    - DNA quality (unamplified, genomic DNA)
    - DNA quantity (~1 microgram)
  - Cost

GWAS:
Tiered replication approach



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