

# **Genetic Susceptibility to Childhood Leukemia**

ICNIRP/WHO/BfS International Workshop on  
Risk Factors for Childhood Leukemia  
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# Outline

- Background
- Review from literature
- Northern California Childhood Leukemia Study (NCCCLS) 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

Background

Review

NCCLS

Challenges

Future Directions



# Review

# Candidate pathways in published reports

Background

Review

NCCLS

Challenges

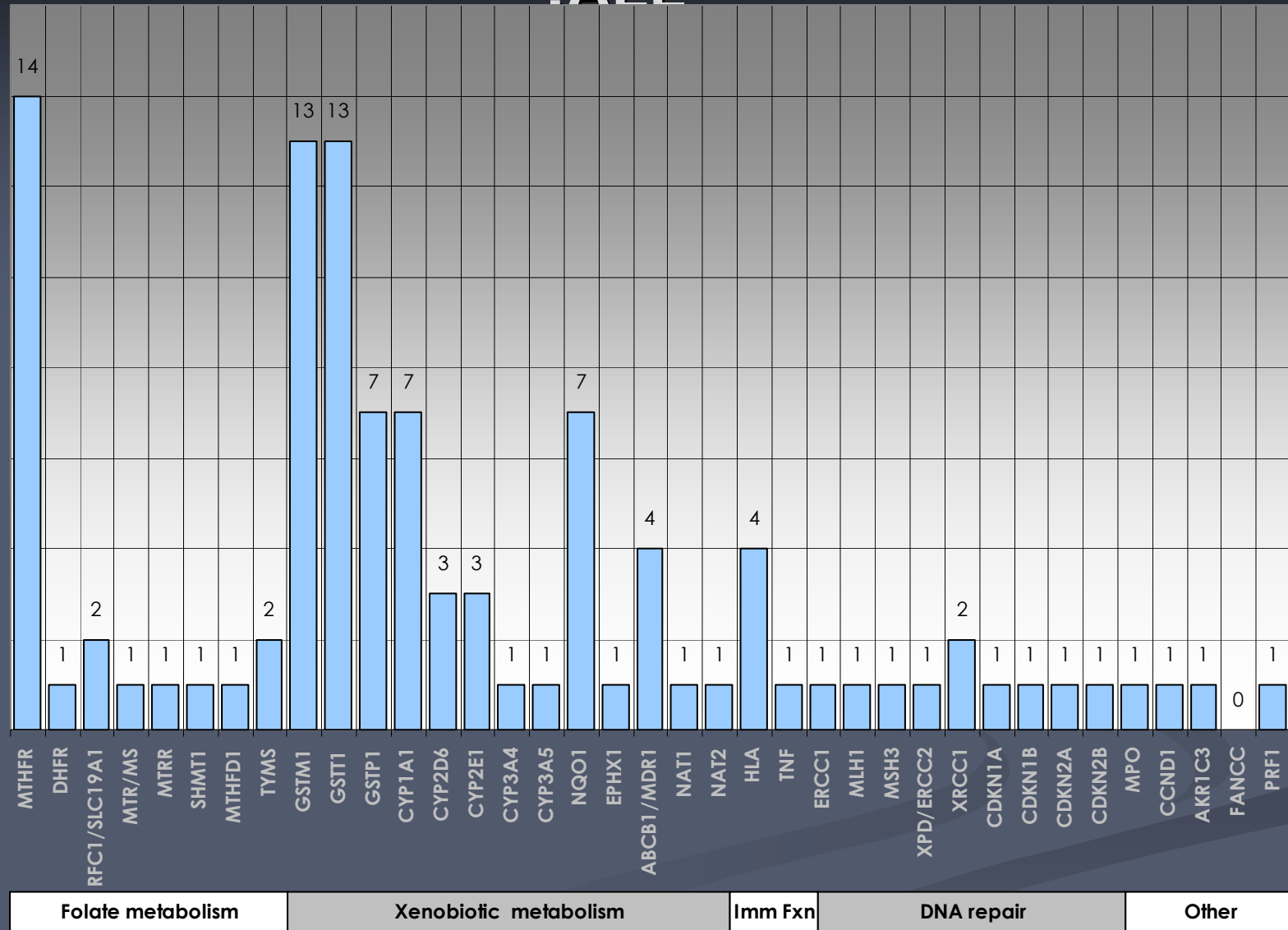
Future  
Directions

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



# Primary reports of gene main effects

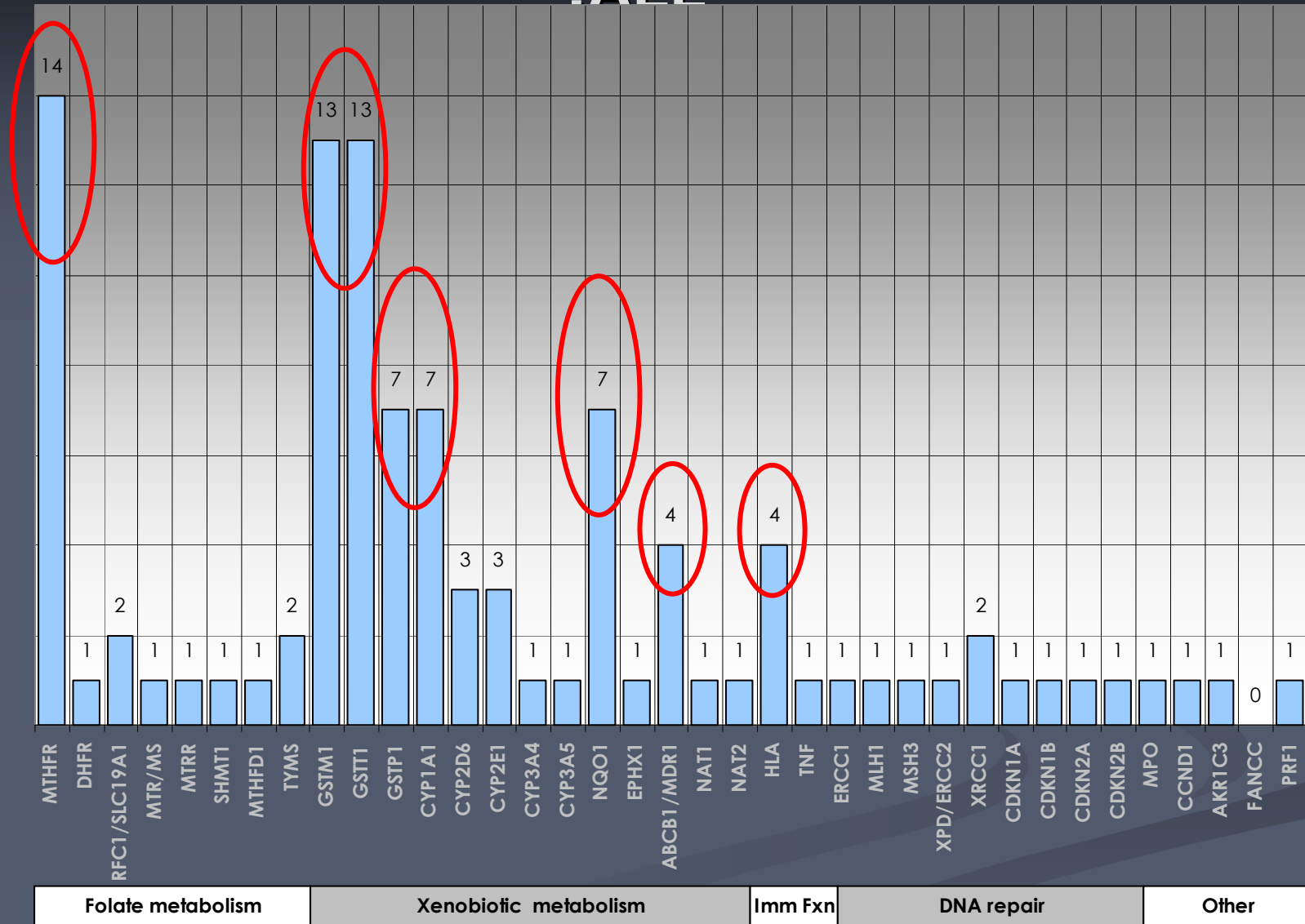
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# Primary reports of gene main effects

: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)



# Xenobiotic metabolism & ALL

Background

Review

NCCLS

Challenges

Future Directions

- 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

Background

Review

NCCLS

Challenges

Future  
Directions

- 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

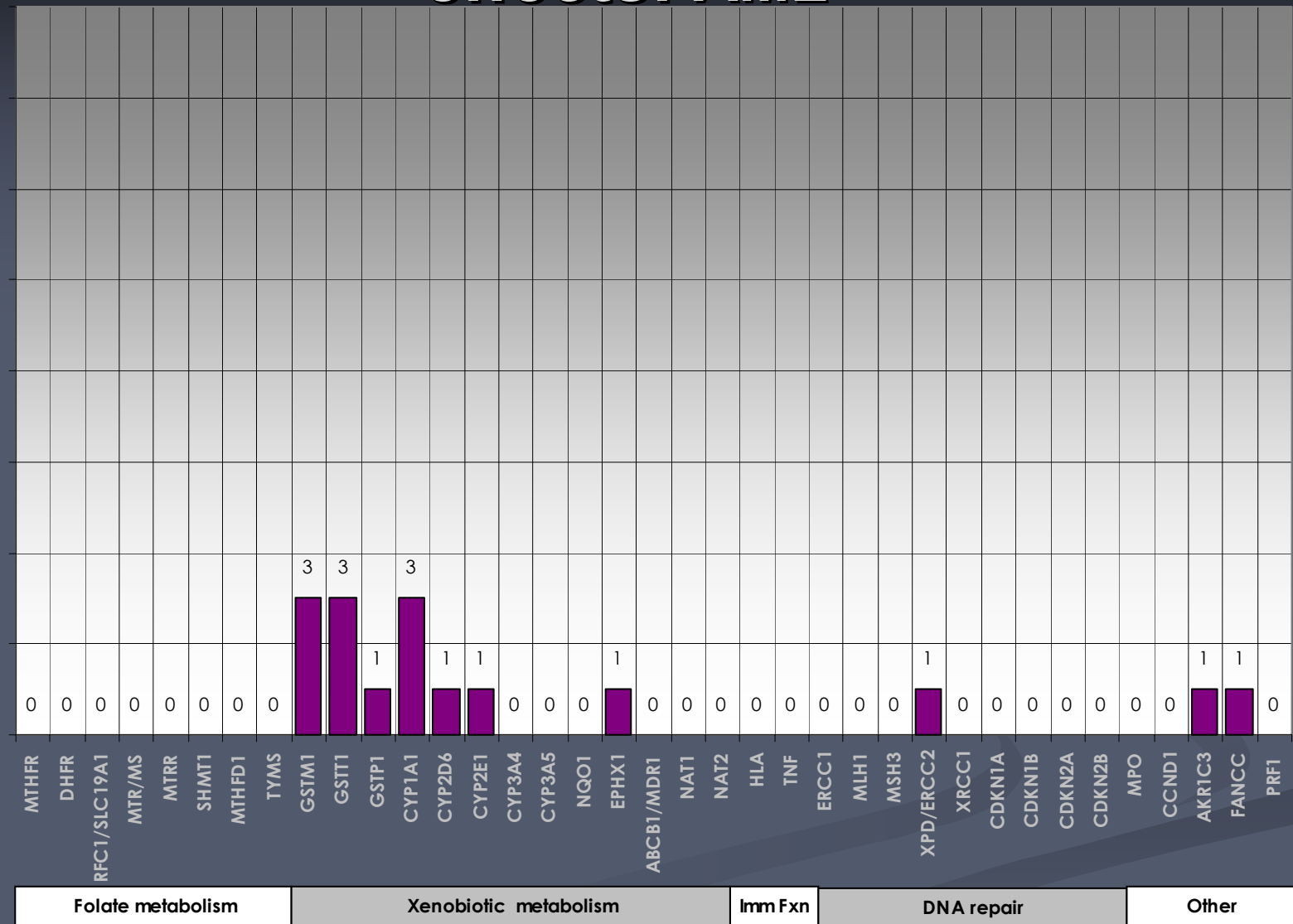
Background

Review

NCCLS

Challenges

Future Directions



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



# 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



The screenshot shows the International HapMap Project website. At the top, there is a banner with the project logo (a world map with a DNA double helix) and the title "International HapMap Project". Below the banner, there are navigation links: "Home | About the Project | Data | Publications | Tutorial".

On the left side, there is a sidebar with navigation links: "Instructions", "Search", "Overview", and "Details".

The main content area contains the following sections:

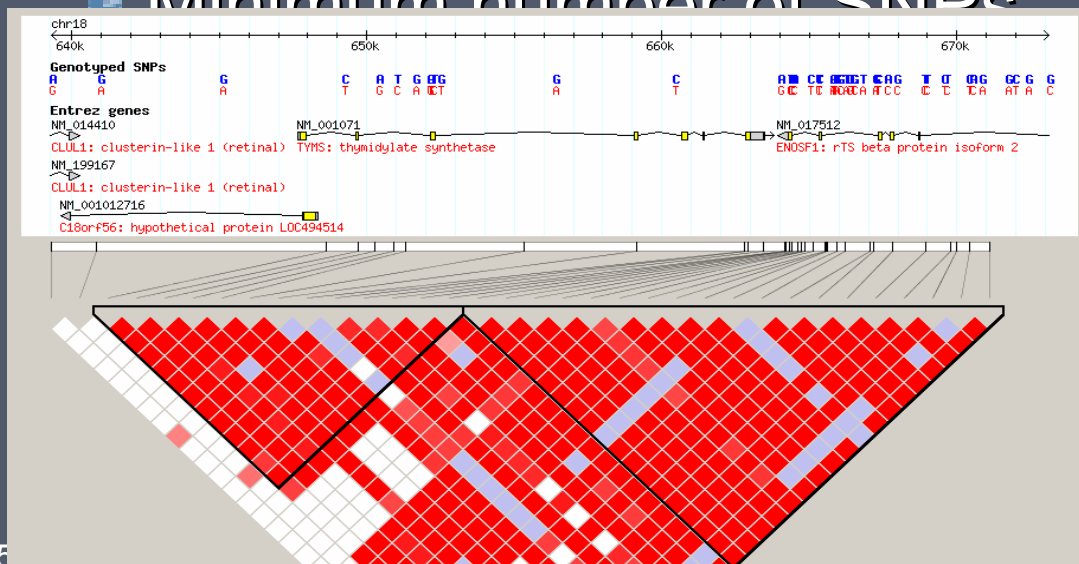
- Instructions:** Search using a sequence name, gene name, locus, or other landmark. The wildcard character \* is allowed. To center on a location, click the ruler. Use the Scroll/Zoom buttons to change magnification and position.
- Examples:** Chr20, Chr9:660,000..760,000, SNP:rs6870660, NM\_153254, BRCA2, ENM010.
- Search:** A search bar with a "Search" button. Below it, there is a "Data Source" dropdown menu set to "HapMap Data Rel#19/phaseII Oct05, on NCBI B34 assembly, dbSNP b124".
- Reports & Analysis:** A dropdown menu set to "Annotate LD Plot" with "Configure..." and "Go" buttons.
- Population descriptors:** YRI: Yoruba in Ibadan, Nigeria, JPT: Japanese in Tokyo, Japan, CHB: Han Chinese in Beijing, China, CEU: CEPH (Utah residents with ancestry from northern and western Europe).
- Footer:** For performing in depth LD and Haplotype analysis of genotype data install Haploview in your local machine. Haploview (ver3.12) is now available for download.

- Data on linkage between SNPs in different populations



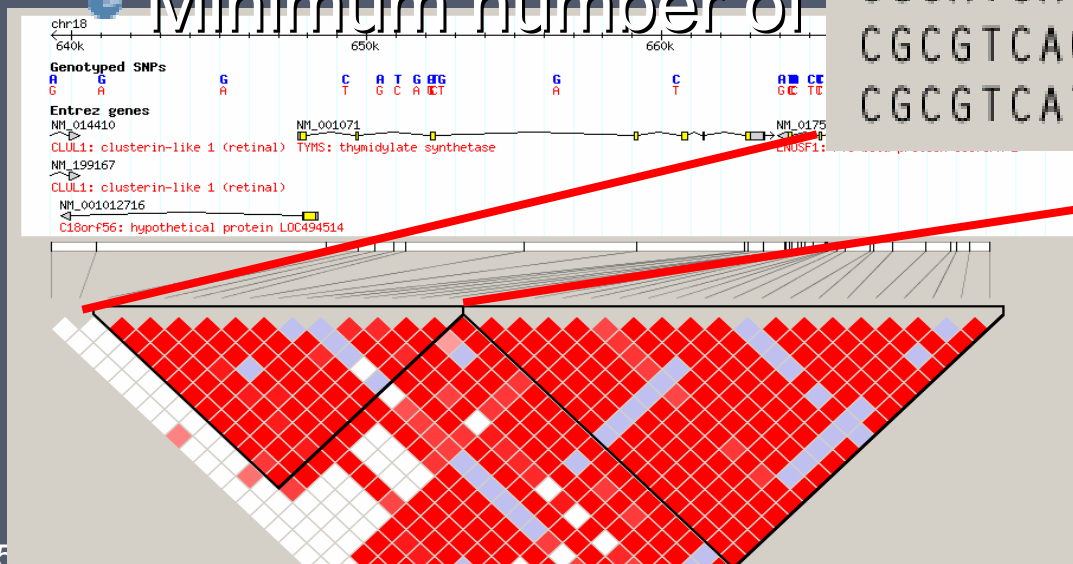
# Haplotype-based analysis

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



# Haplotype-based analysis

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Block 1

SNP	1	2	3	4	5	6	7	8	9	10	11	12
TATGCTGCGGCCGC	.508											
CGCATCATACTTAT	.200											
CGCGTCACGGCCGC	.175											
CGCGTCATACTTAT	.067											

# NCCLS experience

# Northern California Childhood Leukemia Study 1995-present

Background

Review

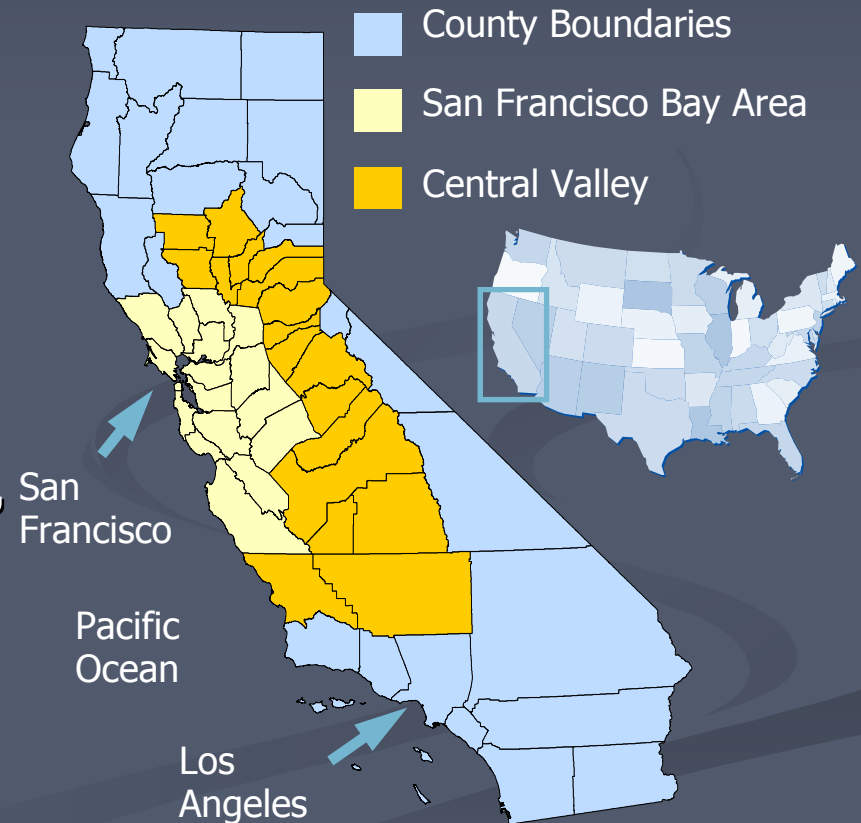
NCCLS

Challenges

Future  
Directions

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

Map of NCCLS Study Area



# NCCLS Biospecimen collection

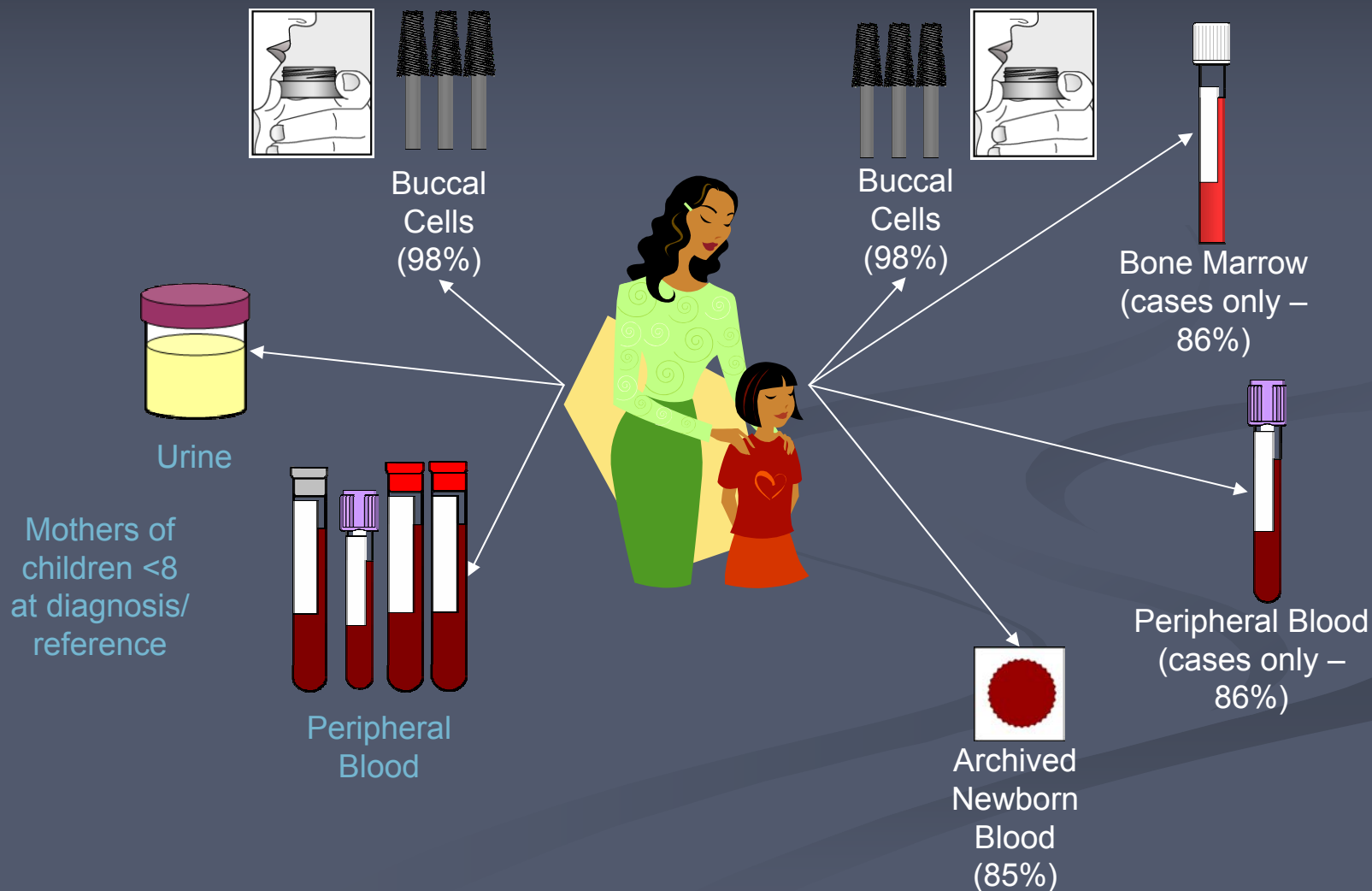
Background

Review

NCCLS

Challenges

Future Directions



# NCCLS Biospecimen collection

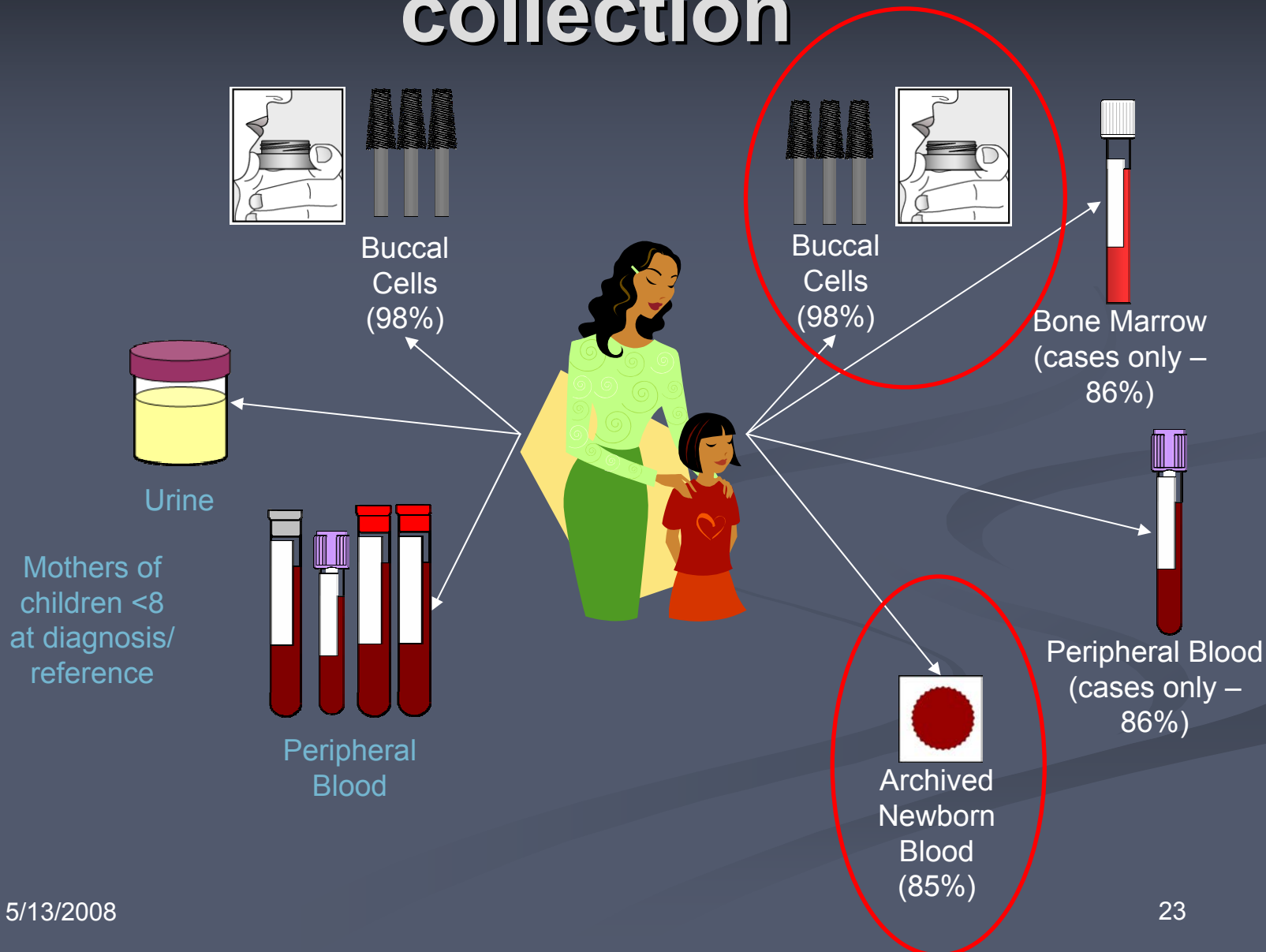
Background

Review

NCCLS

Challenges

Future Directions



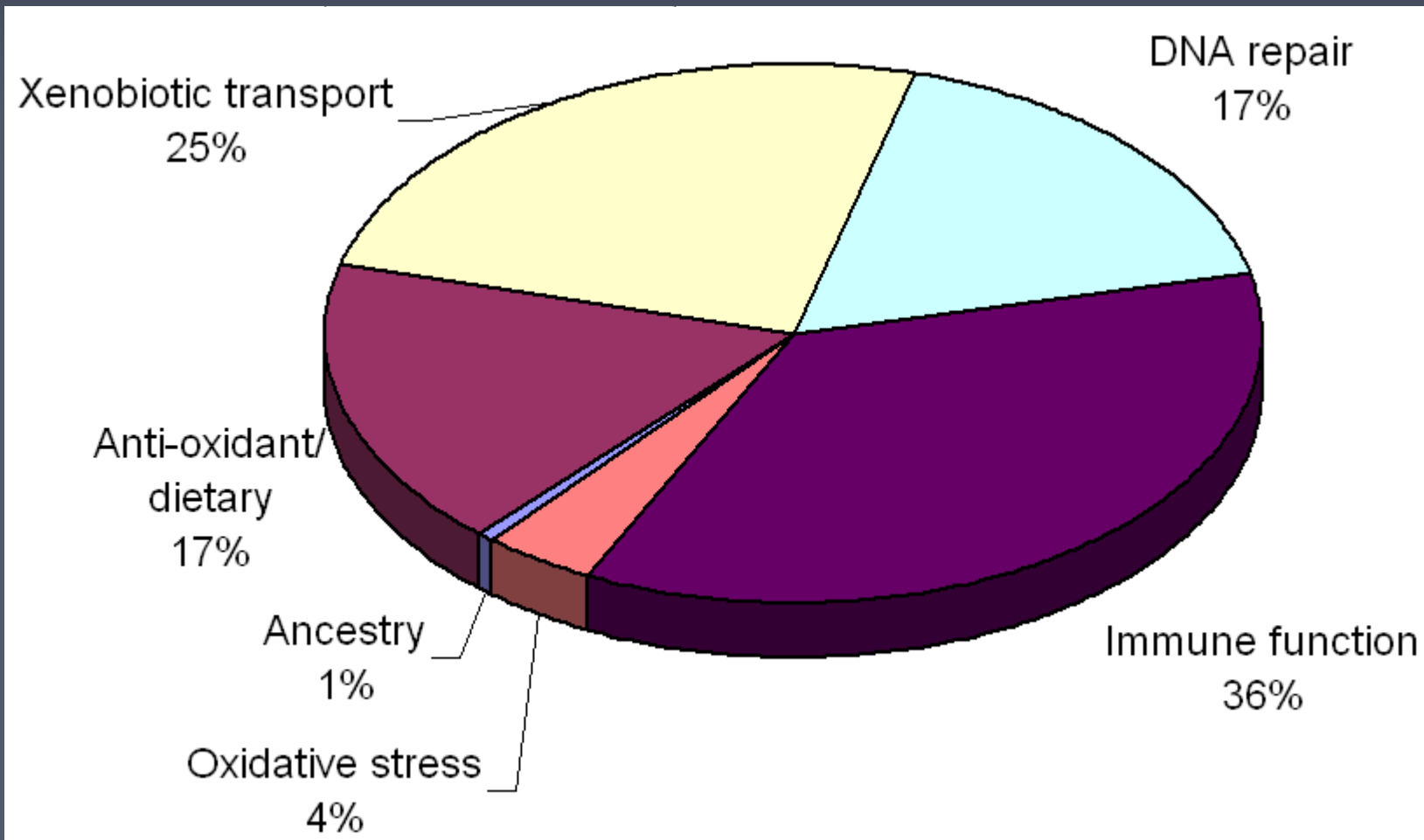
# Large-scale Genotyping

- **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

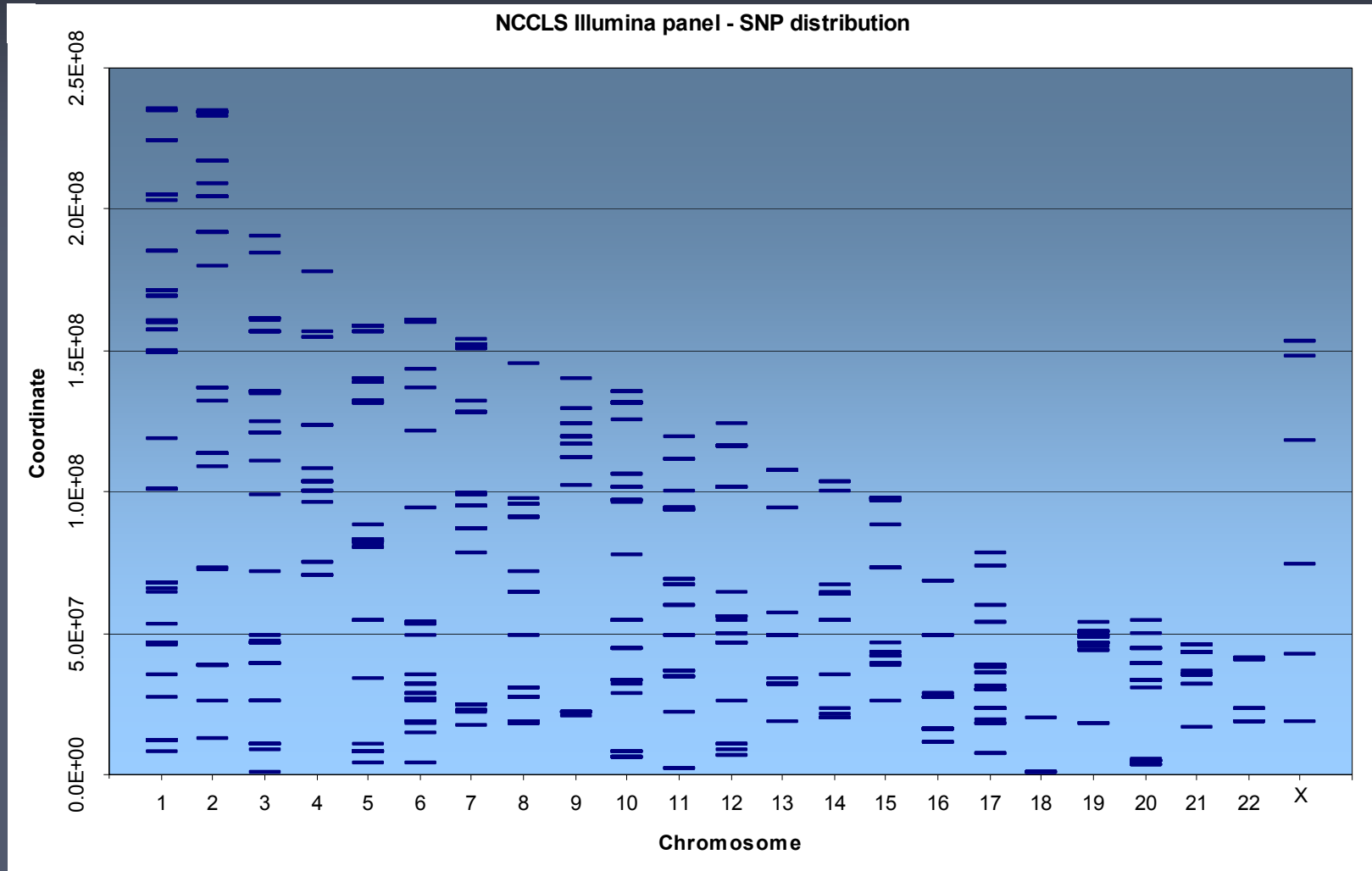




# Candidate Pathways



# NCCLS panel coverage



# NCCLS genotyping

## Current status

- Data available Feb 2008
- Analyses ongoing

Background

Review

NCCLS

Challenges

Future  
Directions



# Challenges to genetic susceptibility research in childhood leukemia

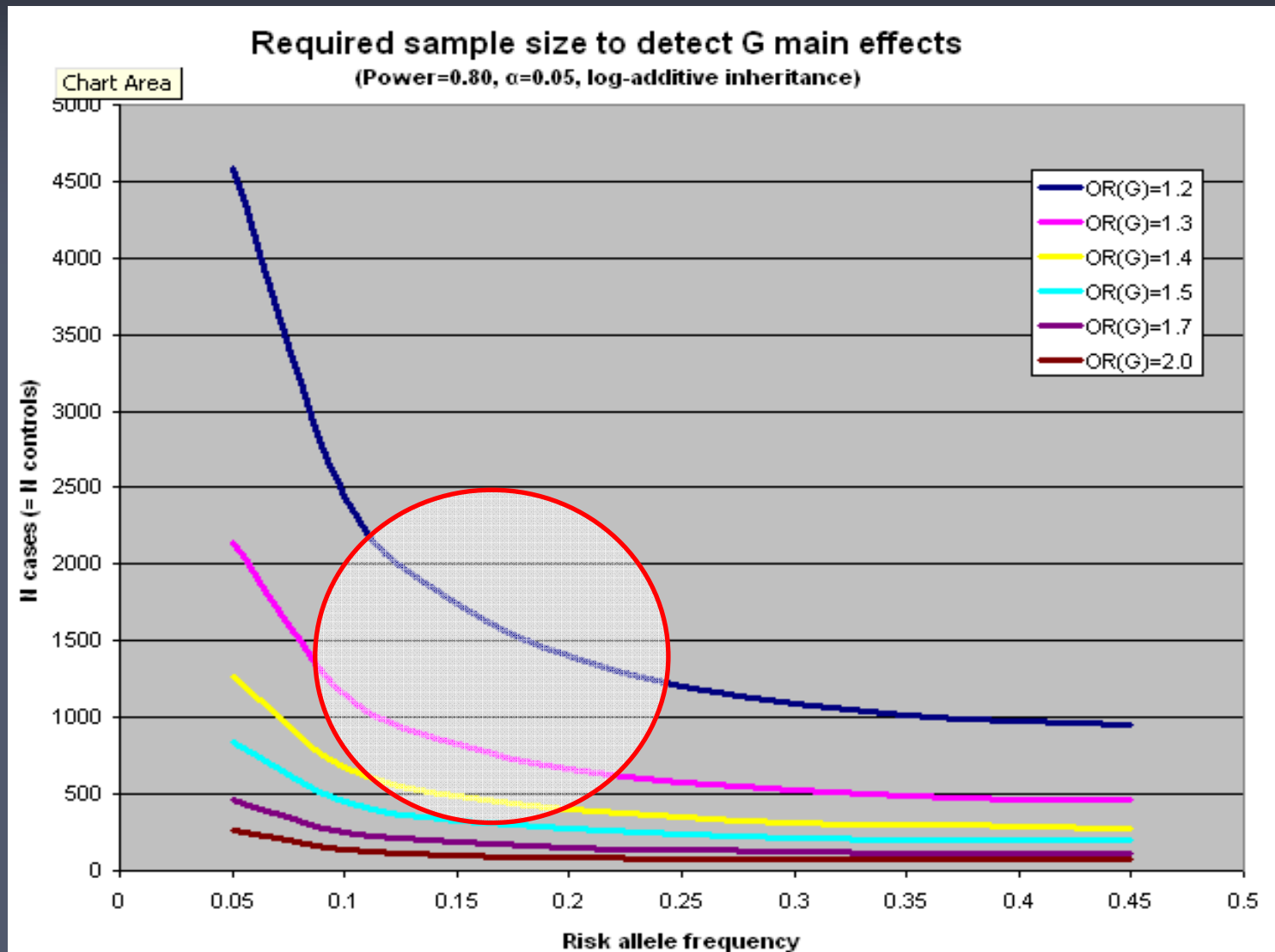
Statistical power and sample size  
issues

Maternal genes

Replication

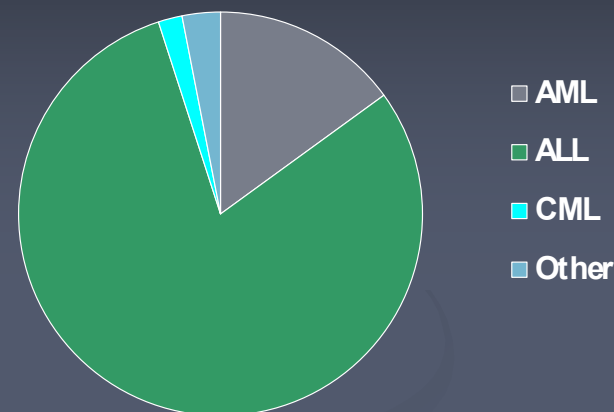
Publication Bias

# 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 needed
  - Even larger sample sizes for sufficient power



Background

Review

NCCLS

Challenges

Future  
Directions

# 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](http://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)
- Please join!
  - Data pooling (improve statistical power)
  - Collaborative research



# GWAS

- Genome-Wide Association Studies
  - 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 (1microgram)
  - Cost



Background

Review

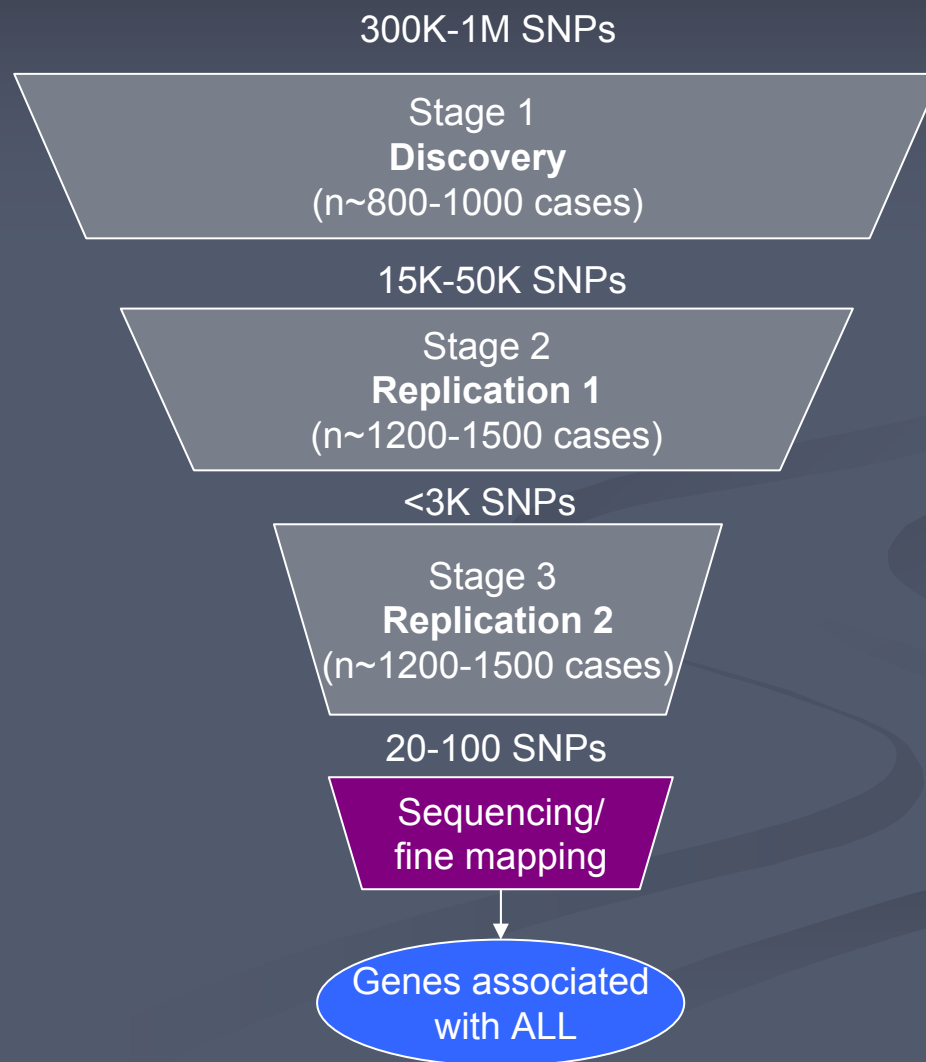
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Challenges

Future  
Directions

# GWAS:

## Tiered replication approach



# Acknowledgments

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