Understanding the Genetic Architecture of Common Disease: A Comparison of Genome Scans

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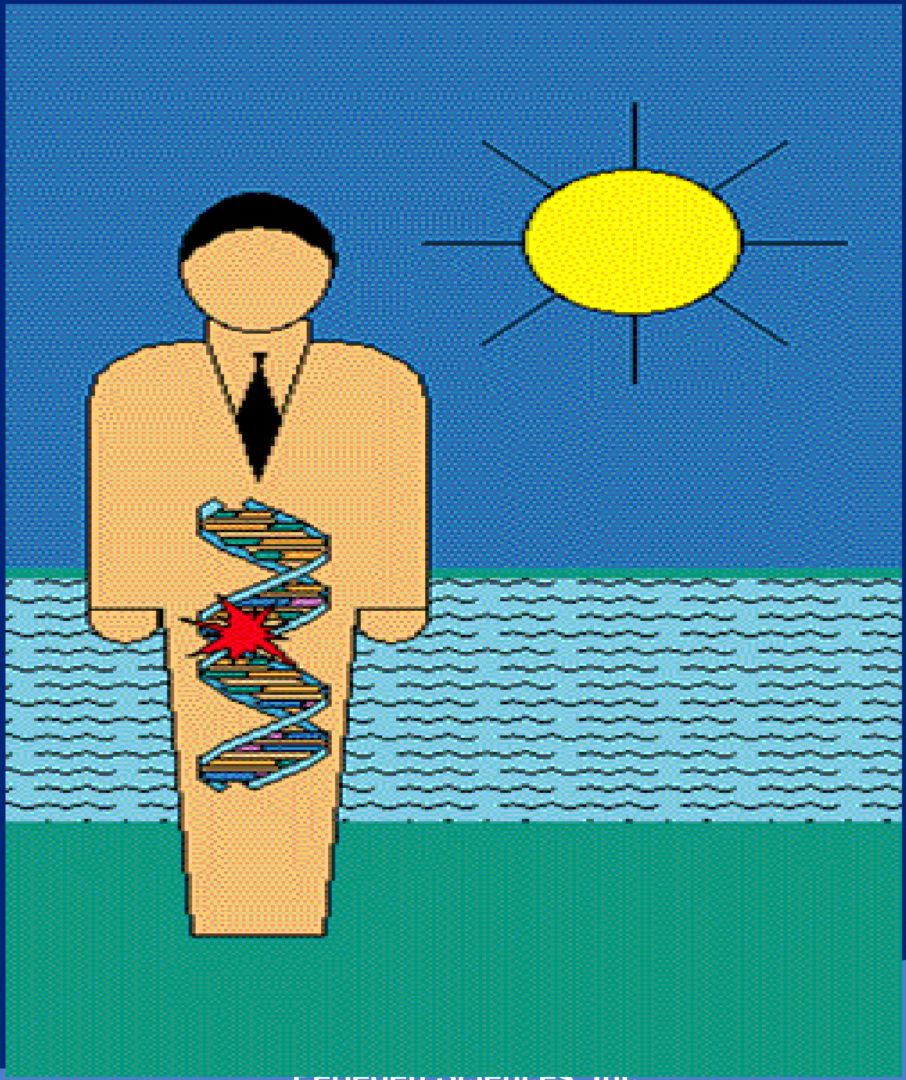
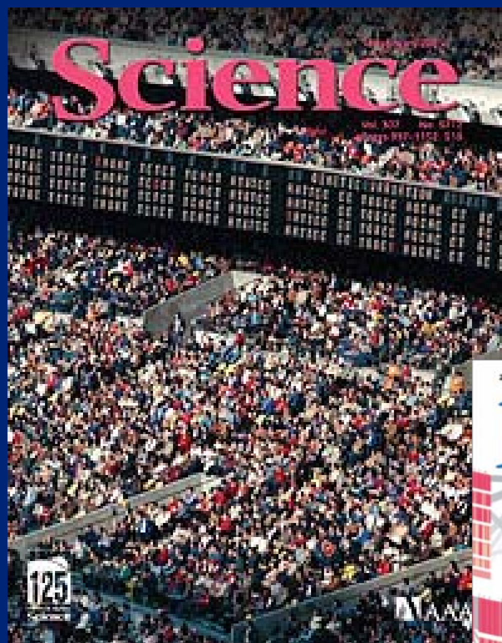
Genes, through the proteins they encode, interact with challenges from the environment



Whole-Genome Patterns of Common Human DNA Variation Have Recently Been Characterized



18 February 2005

........................February, 2005

27 October 2005

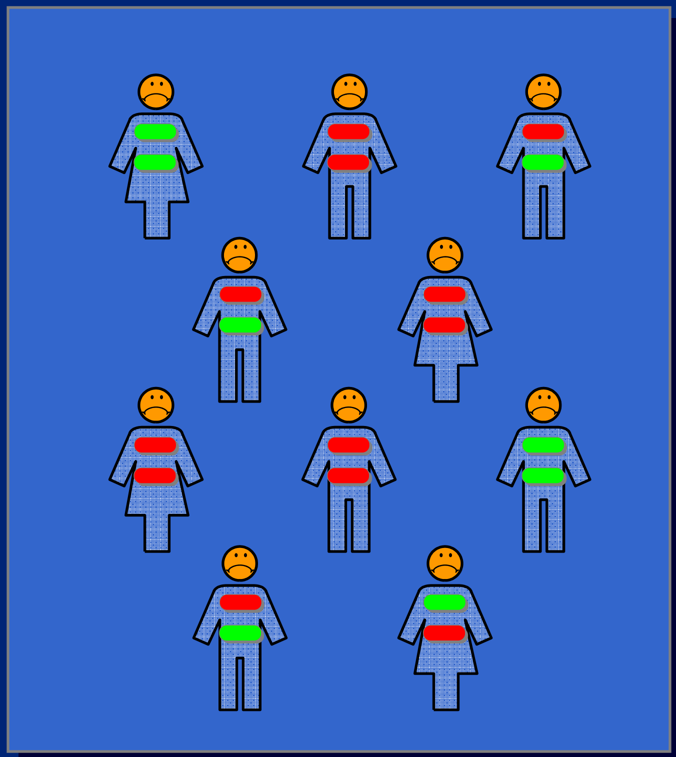
..............October, 2005

Common Human SNPs

- Robust assays have been developed for more than 2.5 million of the total 9-10 million common SNPs estimated to be present in the humans.
- Although the vast majority of these SNPs are present in all human ethnic groups and geographic locations, the allele frequencies are more variable between than within ethnicities and geographies
- The correlation structure of more than 2.5 million SNPs has been empirically determined using small population samples from diverse geographic locations.
- The observed correlations between common SNPs reveal that by genotyping a selected subset of 300,000- 400,000 SNP (ie. TAG SNPs) in an individual, one obtains nearly the same genetic information as would be obtained by interrogating all 9 to10 million common human SNPs.

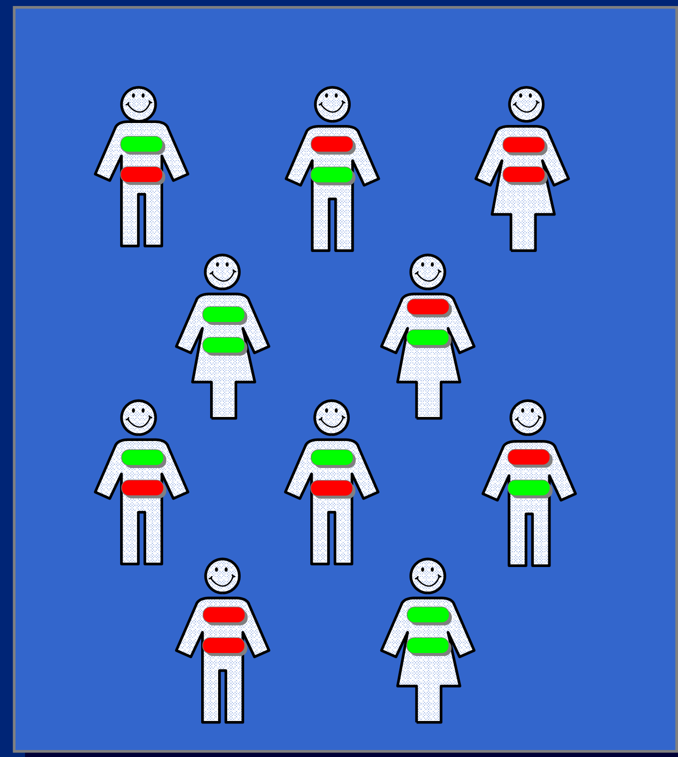
Genetic Association

Cases- drug toxicity



40% Green and 60% red

Controls- no toxicity



50% Green and 50% red

If a segment of the genome is “associated” with toxicity, cases will have a different SNP allele frequency than controls.

Number of LD Bins (>1 SNP) Spanning the Genome in the Perlegen Sciences Study vs. the HapMap Study

r^2 threshold	Afr-Am	Eur-Am	Han Chinese
r^2 >= 0.8	540,533	296,313	256,766

r^2 threshold	YRI	CEU	CHB +JPT
r^2 >= 0.8	474,409	293,835	259,779

Questions That Remain Unanswered

What is the relative role of common versus rare genetic variation in complex human traits?

What is the relative role of population specific versus global genetic variation in complex human traits?

Which segments of the human genome play the most important role in human phenotype variation?

What is the size of individual genetic effects?

Small Sample Size Results in Large Variation in Observed Allele Frequency

ALLELE FREQUENCY	0.10	0.20	0.30
SAMPLE SIZE	95% confidence interval of allele frequency		
10	+/- 0.13	+/- 0.18	+/- 0.20
20	+/- 0.09	+/- 0.12	+/- 0.14
50	+/- 0.06	+/- 0.08	+/- 0.09
100	+/- 0.04	+/- 0.06	+/- 0.06
200	+/- 0.03	+/- 0.04	+/- 0.04
400	+/- 0.02	+/- 0.03	+/- 0.03
600	+/- 0.02	+/- 0.02	+/- 0.03
800	+/- 0.01	+/- 0.02	+/- 0.02
1000	+/- 0.01	+/- 0.02	+/- 0.02

Case/Control Genetic Association Requires Hundreds of Samples to Detect Small Allele Frequency Differences

Case/Control Allele Frequency Difference

(3 Controls/Case, Control Allele Frequency = 0.20, Single SNP)

# of CASES	0.30	0.20	0.10	0.08	0.05
	Probability of observing allele frequency difference by chance				
10	0.019399	0.106478	0.3887	0.4827	0.651109
20	0.000912	0.022023	0.2214	0.3193	0.521173
50	1.49E-07	0.000286	0.0527	0.1147	0.309503
100	1.05E-13	2.85E-07	0.0061	0.0256	0.150402
200	7.26E-26	3.82E-13	0.0001	0.0016	0.041908
400	4.81E-50	9.49E-25	4E-08	8E-06	0.004007
600	3.67E-74	2.71E-36	2E-11	4E-08	0.000424
800	2.97E-98	8.18E-48	9E-15	3E-10	4.7E-05
1000	2.5E-122	2.55E-59	4E-18	2E-12	5.36E-06

Whole Genome Genetic Association Requires A Multiple Testing Penalty

Case/Control Allele Frequency Difference

(3 Controls/Case; Control Allele Frequency = 0.20; 325,000 SNPs)

	0.30	0.20	0.10	0.08	0.05
# of CASES	**Probability of observing allele frequency difference by chance**				
10	1	1	1	1	1
20	1	1	1	1	1
50	0.049	1	1	1	1
100	3.43E-08	0.093	1	1	1
200	2.36E-20	1.242E-07	1	1	1
400	1.56E-44	3.08E-19	0.013	1	1
600	1.19E-68	8.80E-31	5.98E-06	0.015	1
800	9.66E-93	2.66E-42	2.81E-09	8.64E-05	1
1000	8.1E-117	8.29E-54	1.36E-12	5.28E-07	1

Definitions of SNP Association

Exploratory SNP association – non-significant association of an allele with the trait, when correctly adjusting for multiple tests

Valid SNP association - significant association of an allele with the trait in a single sample corrected for multiple testing

Replicated SNP association – significant association of an allele with the trait in multiple independent samples from a population of interest

Am. J. Hum. Genet. 77:000–000, 2005

High-Resolution Whole-Genome Association Study of Parkinson Disease

Demetrius M. Maraganore,[1] Mariza de Andrade,[2] Timothy G. Lesnick,[2] Kari J. Strain,[2] Matthew J. Farrer,[3] Walter A. Rocca,[1,2] P. V. Krishna Pant,[4] Kelly A. Frazer,[4] David R. Cox,[4] and Dennis G. Ballinger[4]

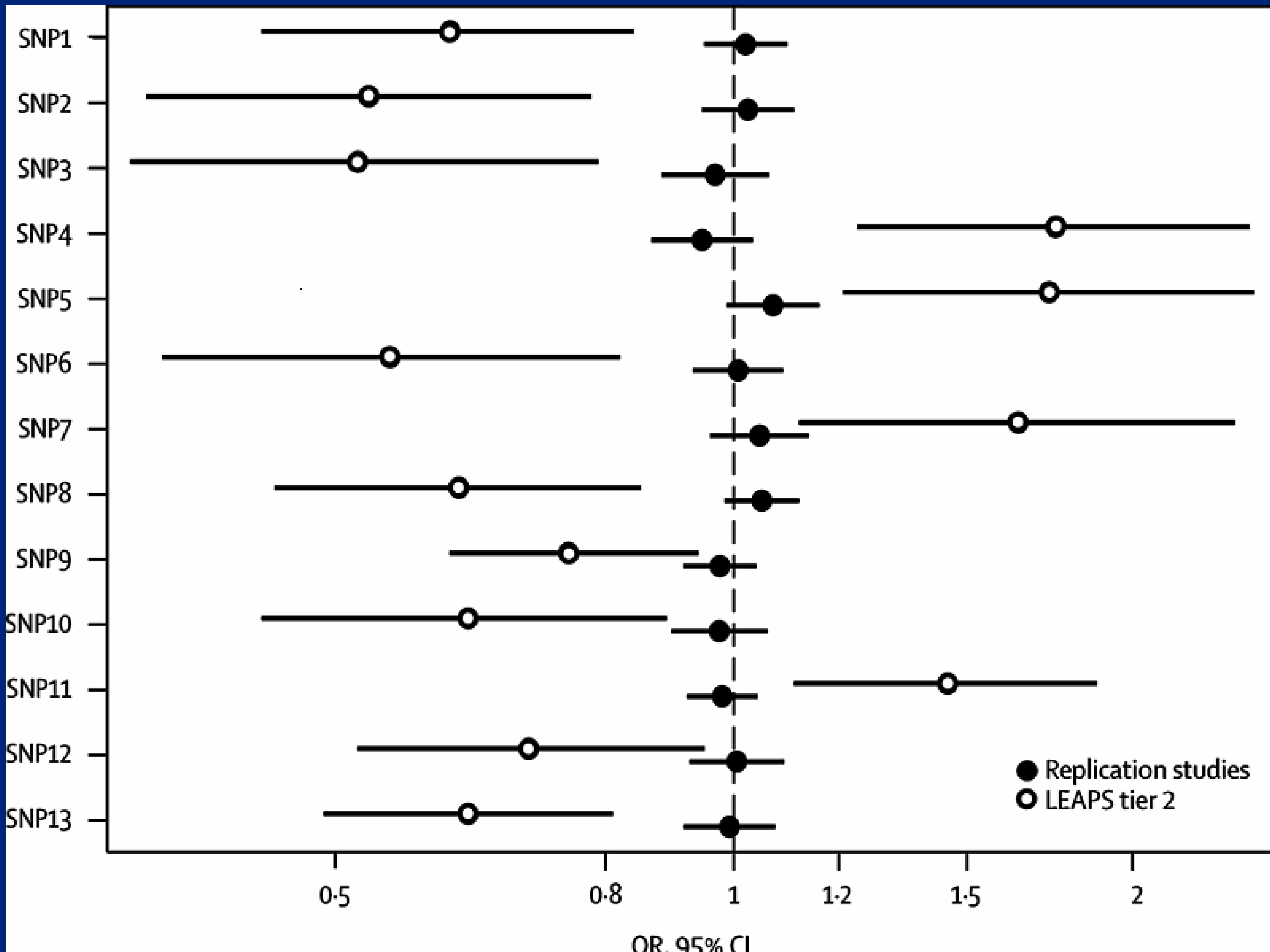
Departments of [1]Neurology and [2]Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN; [3]Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL; and [4]Perlegen Sciences, Mountain View, CA

The top 10 SNPs from joint analysis of all data

		Maraganore et al. (Tier 1 and Tier 2)					Meta-analysis, replication studies				
Marker	Alleles (high-risk allele)	cases	controls	allele frequency (controls)	Odds Ratio (95% CI)	*p*	cases	controls	allele frequency (controls)	Odds Ratio (95% CI)	*p*
rs10200894	C/G (C)	772	772	0.88	1.84 (1.38–2.45)	1.70E-05	1566	1546	0.89	1.14 (0.96 - 1.35)	0.125
rs11737074	G/A (A)	764	764	0.19	1.50 (1.21–1.86)	1.55E-04	1563	1542	0.21	1.02 (0.9 - 1.15)	0.77
rs16851009	C/T (T)	741	741	0.08	1.84 (1.36–2.49)	4.17E-05	1539	1544	0.1	0.98 (0.83 - 1.16)	0.853
rs17329669	A/G (G)	768	768	0.12	1.71 (1.33–2.21)	2.30E-05	1554	1525	0.12	1.13 (0.97 - 1.32)	0.102
rs2245218	A/G (G)	770	770	0.12	1.67 (1.29–2.14)	4.61E-05	1571	1563	0.16	0.94 (0.82 - 1.08)	0.369
rs2313982	C/T (T)	740	740	0.07	2.01 (1.44–2.79)	1.79E-05	1562	1554	0.09	0.88 (0.73 - 1.04)	0.138
rs7520966	C/T (C)	769	769	0.7	0.67 (0.55–0.81)	2.96E-05	1563	1550	0.72	1.07 (0.96 - 1.2)	0.242
rs7702187	T/A (T)	761	761	0.81	1.74 (1.36–2.24)	7.62E-06	1541	1541	0.83	1.07 (0.93 - 1.22)	0.334
rs7723605	T/C (C)	773	773	0.11	1.78 (1.35–2.35)	3.30E-05	1567	1571	0.13	1.03 (0.89 - 1.19)	0.684
ss46548856	G/C (G)	765	765	0.9	1.88 (1.38–2.57)	3.65E-05	1551	1528	0.9	1.12 (0.94 - 1.33)	0.196

Four replication studies published in AJHG August 2006

Large-scale replication study in ~5,000 cases and controls



Elbaz, A. *et al.* Lancet Neurology Early Online Publication, 27 September 2006

SNP Associations with HDL levels

Original Population- 345 Low HDL, 321 High HDL

4 SNPs, all of which are in the same gene (CETP), have significantly different allele frequencies between high and low HDL individuals

“Replication” Population- 83 Low HDL, 78 High HDL

All four CETP SNPs significantly associated with HDL level in the original population are also significantly associated with HDL level in the “replication population

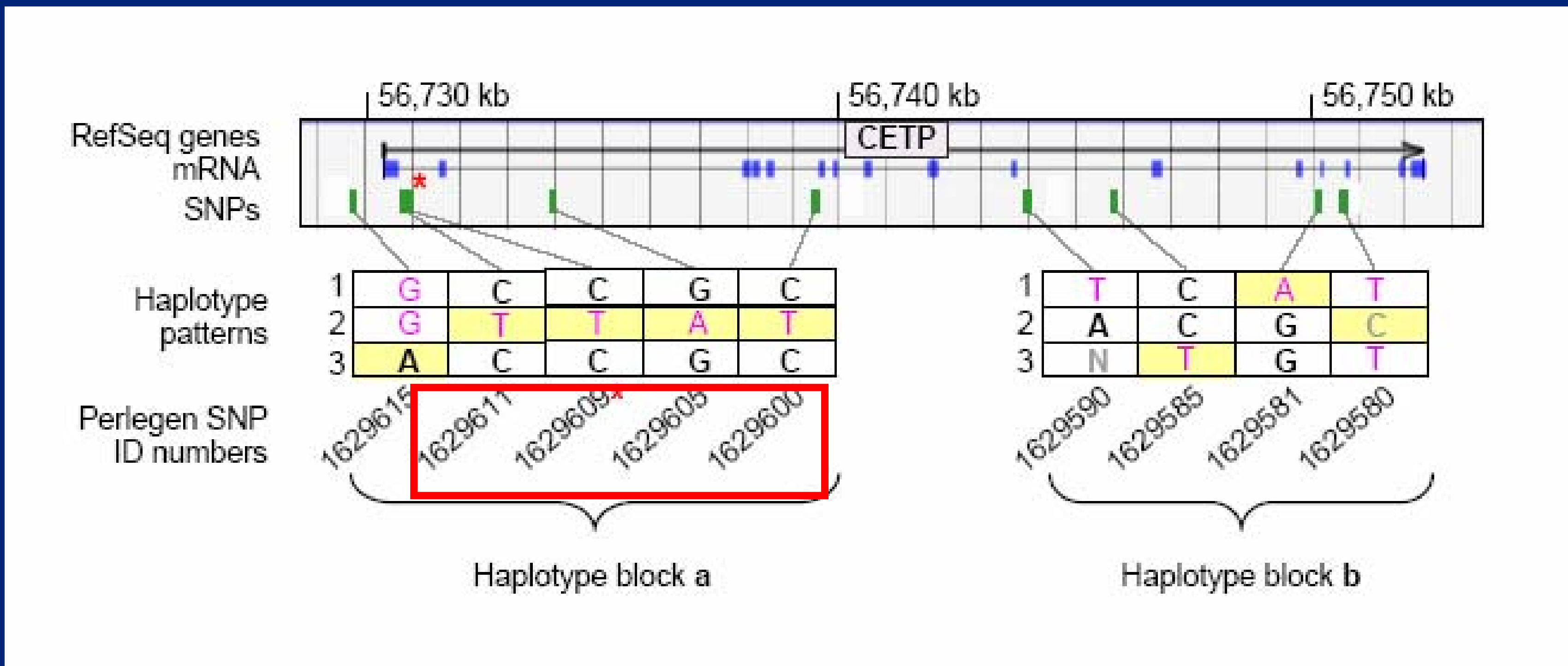
Hinds et. al., Human Genomics 1:421-434, 2004

Four SNPs in the CETP gene are very significantly associated with HDL levels in both original and replicate study populations

Table 7. Genotyping results for 14 *CETP* gene SNPs in the test and replicate samples

Position[a]	dbSNP	Study samples			Replicate samples			Overall
		Δp	*P* value	Odds ratio[b]	Δp	*P* value	Odds ratio	*P* value[c]
56729658	rs4783962	0.060	1.2×10^{-2}	1.38	0.100	2.8×10^{-2}	1.87	1.4×10^{-3}
56730831	**rs711752**	**0.165**	$\mathbf{2.4\times10^{-8}}$	**1.88**	**0.208**	$\mathbf{5.0\times10^{-4}}$	**2.21**	$\mathbf{5.9\times10^{-11}}$
56730908	**rs708272**	**0.151**	$\mathbf{7.2\times10^{-9}}$	**1.92**	**0.202**	$\mathbf{3.2\times10^{-4}}$	**2.25**	$\mathbf{1.2\times10^{-11}}$
56733948	**ss15723184**	**0.167**	$\mathbf{9.0\times10^{-8}}$	**1.85**	**0.186**	$\mathbf{1.9\times10^{-3}}$	**2.06**	$\mathbf{7.1\times10^{-10}}$
56739509	**rs7205804**	**0.146**	$\mathbf{9.7\times10^{-8}}$	**1.82**	**0.142**	$\mathbf{5.6\times10^{-3}}$	**1.89**	$\mathbf{1.9\times10^{-9}}$
56743996	rs289716	-0.060	1.6×10^{-2}	0.75	-0.039	3.9×10^{-1}	0.81	1.1×10^{-2}
56745805	rs4784744	-0.075	5.0×10^{-3}	0.72	-0.157	8.7×10^{-3}	0.53	2.4×10^{-4}
56750165	rs1800774	0.009	7.5×10^{-1}	1.04	0.102	5.8×10^{-2}	1.53	2.5×10^{-1}
56750712	rs5882	-0.073	4.4×10^{-3}	0.72	-0.017	8.0×10^{-1}	0.94	7.7×10^{-3}
56751939	rs1800777	-0.041	1.1×10^{-3}	0.33	-0.009	2.9×10^{-1}	1.06	4.2×10^{-3}
56752282	rs1801706	0.029	2.0×10^{-1}	1.20	-0.022	2.9×10^{-1}	0.73	5.0×10^{-1}
56752382	rs289742	-0.023	1.2×10^{-1}	0.76	-0.053	4.6×10^{-2}	0.40	3.0×10^{-1}

Associations of SNPs in the *CETP* gene with HDL levels



1	G	C	C	G	C
2	G	T	T	A	T
3	A	C	C	G	C

1	T	C	A	T
2	A	C	G	C
3	N	T	G	T

Haplotype pattern 2 in Haplotype block a is present at a 14% increased frequency in individuals with high HDL versus low HDL

A genome-wide association study in breast cancer

Douglas F Easton, Alison M Dunning, Karen Pooley, Paul DP Pharoah, David R Cox, Dennis G Ballinger, Deborah Thompson, D Gareth Evans, Diana Eccles, Nazneen Rahman, Michael R Stratton, Julian Peto, Olivia Fletcher, Bruce AJ Ponder, The Breast Cancer Association Consortium

CANCER RESEARCH UK

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Breast Cancer Study Design

Phase I: 266,722 tagging SNP set

"High-risk" breast cancer cases (n=440) ↔ Female controls EPIC (age>50) (n=400)

Compare genotype frequencies P<.05?

↓

Phase II: ~12,000 SNPs

4,600 breast cancers (ABC) ↔ 4,600 controls (EPIC)

P<.0001?

112 excess p values <0.05

↓

Phase III: 12-100 SNPs

- Prospective meta-analysis (n=20,000+, BCAC)
- Detailed evaluation of loci

SNP Associations with Breast Cancer

Locus	Heterozygote OR (95%CI)	Homozygote OR (95% CI)	P-trend (1df)	P-het (2df)
A	1.24 (1.12-1.36)	1.56 (1.36-1.77)	$5x10^{-17}$	$4.7x10^{-16}$
B	1.30 (1.14-1.47)	1.38 (1.21-1.58)	$2x10^{-6}$	$3.7x10^{-8}$
C	1.19 (1.05-1.36)	1.45 (1.26-1.66)	$4.1x10^{-8}$	$1.4x10^{-7}$
D	1.18 (1.06-1.31)	1.33 (1.17-1.50)	$8.5x10^{-8}$	$1.9x10^{-7}$
E	1.25 (1.12-1.39)	1.55 (1.18-2.04)	$1.0x10^{-7}$	$6.4x10^{-7}$
F	1.13 (1.03-1.24)	1.42 (1.22-1.66)	$1.9x10^{-7}$	$4.7x10^{-7}$
G	1.07 (0.97-1.18)	1.38 (1.21-1.57)	$6.7x10^{-7}$	$3.8x10^{-7}$
H	1.13 (1.03-1.23)	1.27 (1.08-1.49)	$3.9x10^{-7}$	$2.5x10^{-6}$
I	1.07 (0.97-1.18)	1.35 (1.19-1.53)	$6.2x10^{-7}$	$7.0x10^{-7}$
J	1.19 (1.08-1.32)	1.43 (1.20-1.72)	$8.3x10^{-7}$	$4.9x10^{-6}$

K, L and T also replicate in phase 3

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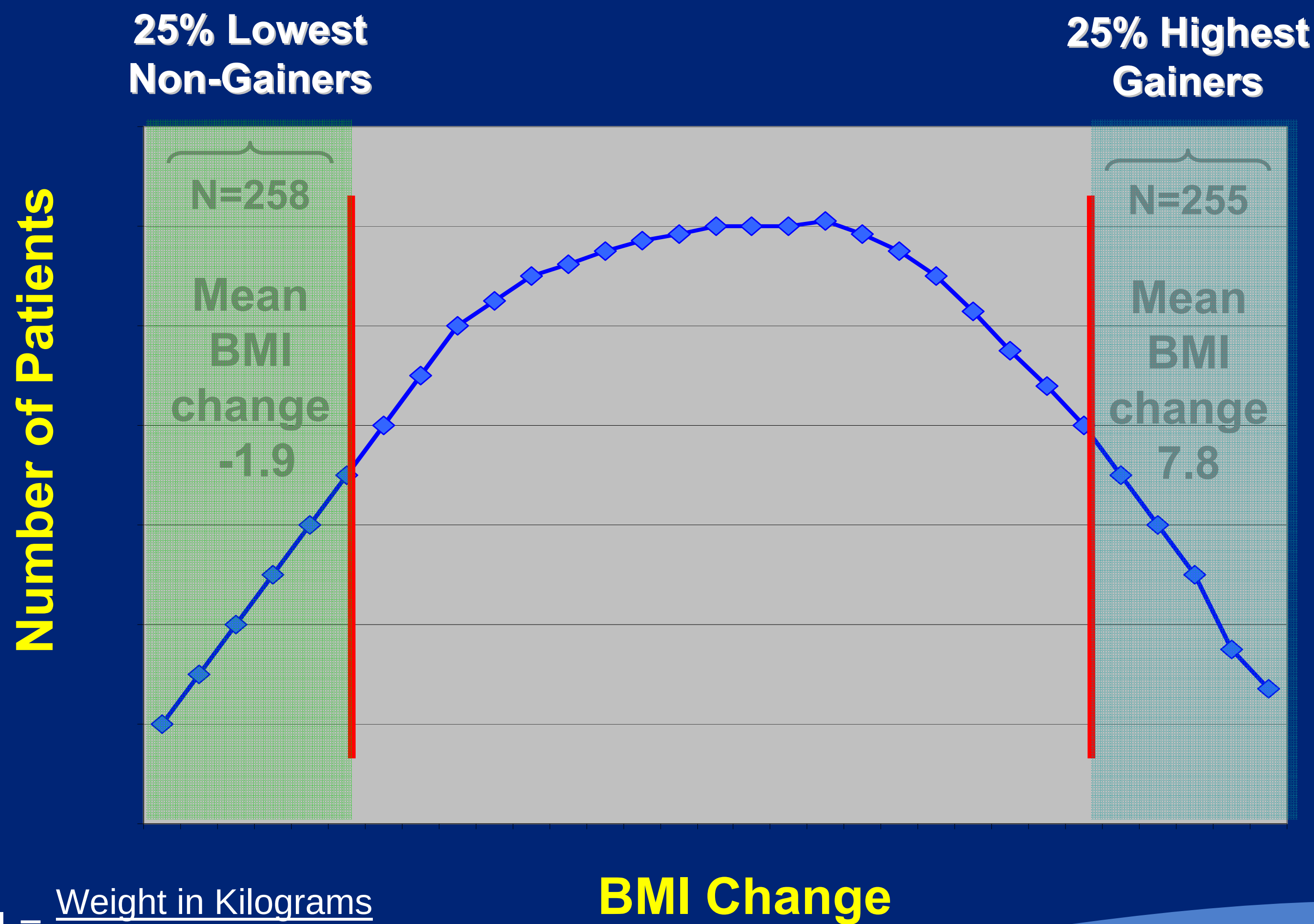
Estimated Contribution to Breast Cancer

Locus	Minor allele frequency	Attributable risk	Genetic variance explained (%)
A	38%	16%	2.0%
B	59%	22%	0.7%
C	54%	18%	1.4%
D	46%	14%	0.8%
E	16%	7%	1.3%
F	30%	8%	0.9%
G	41%	9%	1.0%
H	28%	7%	0.5%
I	44%	9%	0.9%
J	29%	10%	1.2%

Conclusions

- Genome-wide study successful at identifying novel breast cancer predisposition loci
- No loci of major effect (RR>2)
- Many loci of small effect (10s-100s)
  and many probably missed
- Little overlap with previously suggested candidate loci
- Little evidence of dominance
- Little evidence of age-dependent relative risks
- No evidence yet of (non-multiplicative) interactions

Olanzapine Treatment Emergent Weight Gain



BMI = $\frac{\text{Weight in Kilograms}}{(\text{Height in Meters})^2}$

Genetic Association with Treatment Emergent Weight Gain

SNP	P-VALUE	ALLELE FREQ. DIFFERENCE
1	1.4E-06	0.15
2	5.4E-06	0.10
3	5.4E-06	0.12
4	5.8E-06	0.10
5	6.5E-06	0.11
6	6.9E-06	0.10
7	1.2E-05	0.14
8	1.3E-05	0.14
9	1.6E-05	0.13
10	2.1E-05	0.13
11	3.3E-05	0.13

Follow-up of whole genome association results

Test the hypothesis that some genes associated with olanzapine emergent weight gain are also associated with familial obesity

Perform a genetic association study using samples from an independent set of individuals with familial obesity and 11 SNPs from the eight genes that had the strongest evidence for association with treatment-emergent weight gain in the genome-wide study

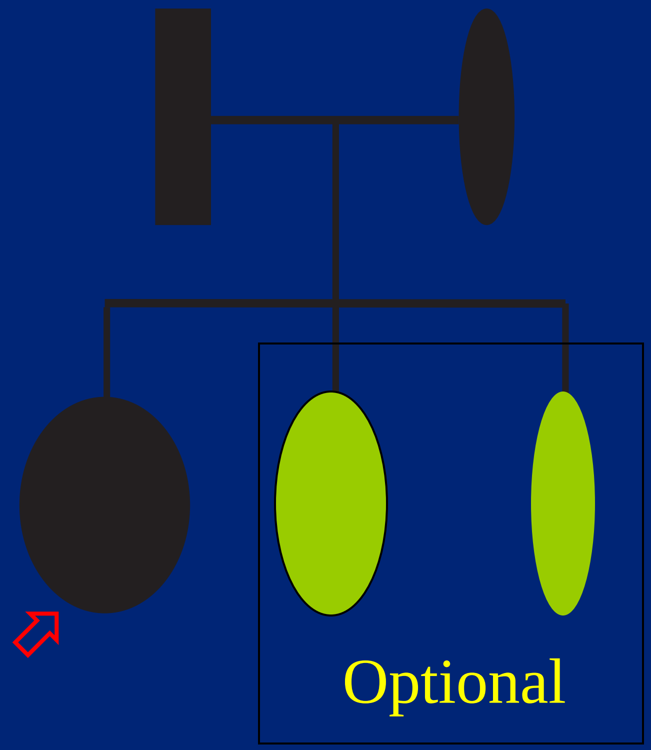
Ascertainment of Familial Obesity

Extreme Obesity:
Proband: BMI≥35

Normal Weight:
Parent: BMI<27

Optional:
Obese Sibling (BMI>30)
Thin Sibling (BMI<25)

348 Independent Families



PKHD1 Genetic Association with Obesity

Frequency of SNP allele predisposing to weight gain = 0.52

50% of individuals in population have one predisposing allele
27% of individuals in population have two predisposing alleles

Relative risk of obesity with one copy of allele = 1.30 (0.93 - 1.81)

Relative risk of obesity with two copies of allele = 1.62 (1.05 - 2.51)

Fraction of obesity attributable to SNP allele = 0.24 (0.027- 0.453)

PKHD1 Mutant Mouse Model
(Greg Germino, JHU School of Medicine)

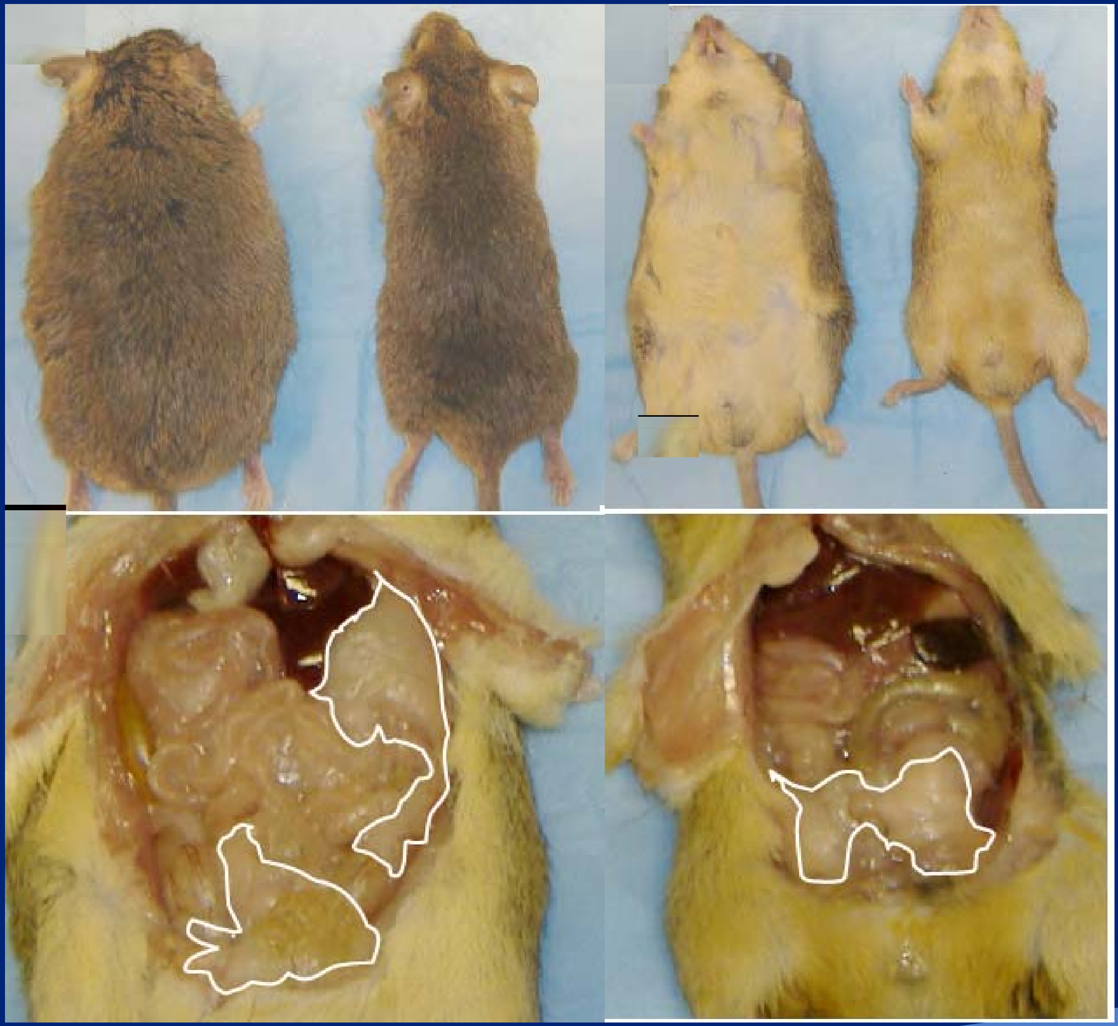
The mutant mouse PKHD1 gene lacks exons 3 and 4

Homozygote mutants develop kidney and pancreatic cysts

Heterozygous male mutants are obese, 50.7 +/- 2.9 g versus age and strain matched controls, 36.3 +/- 1.3 g (ANCOVA $p = 0.0001$)

Heterozygous mutants have increased abdominal and retroperitoneal fat deposits

Mice heterozygous for a partial deletion of PKHD1 have obesity and marked accumulation of visceral fat



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Novel pathway for drug development?

The PKHD1 gene product is a ciliary protein

Recent studies suggest that a primary cilium, present on most vertebrate cells, plays an important role in intracellular signaling by regulating the transport of transcription factors to the nucleus

Several human single gene disorders, including Alstrom syndrome and Bardet-Biedl Syndrome, characterized by multiple abnormalities including obesity, are caused by genetic defects in ciliary proteins

Lessons Learned From SNP Association Studies To Date

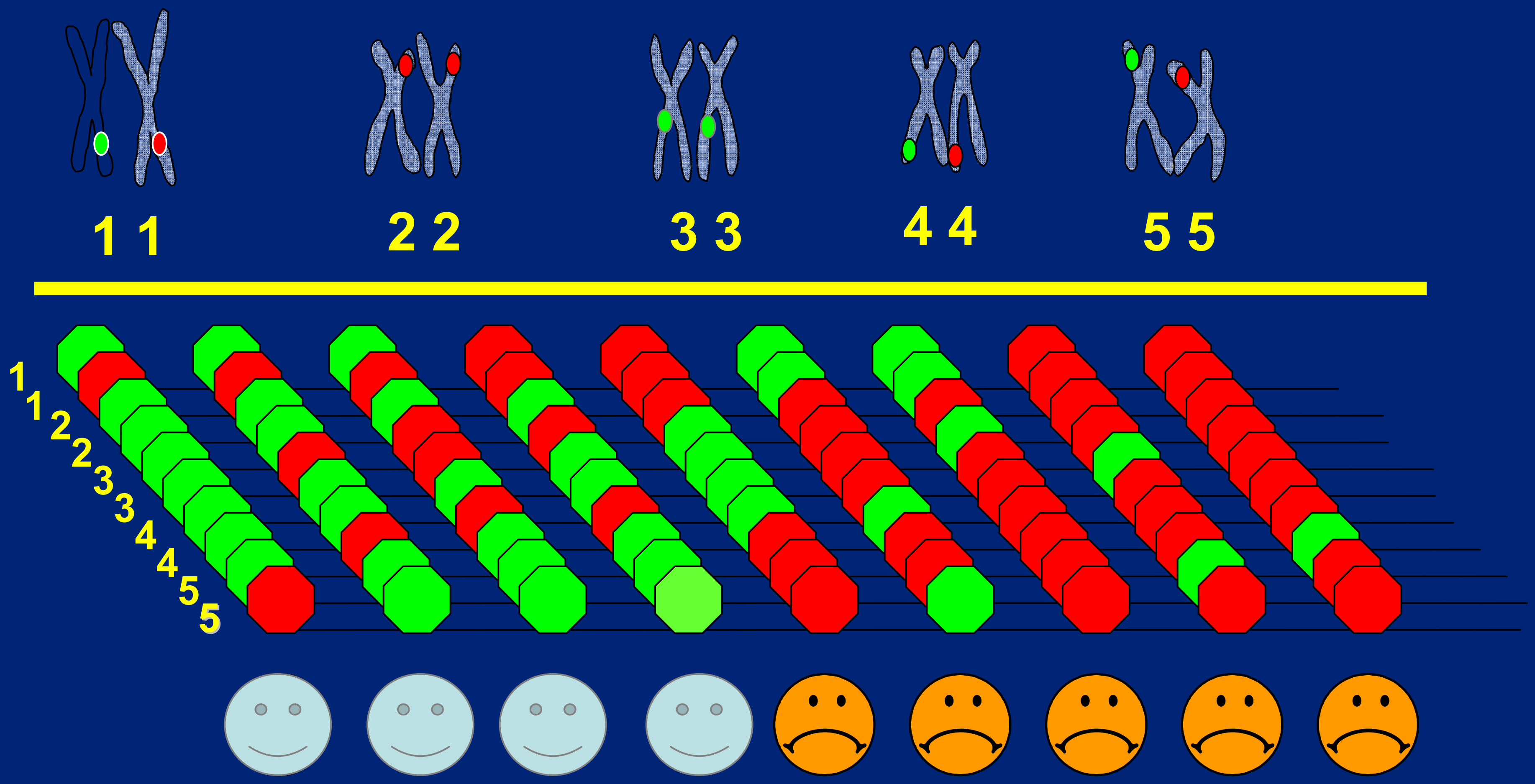
SNP associations can lead to novel biological insights

It is not possible to predict the fraction of variation of a complex trait determined by a SNP prior to performing an association study

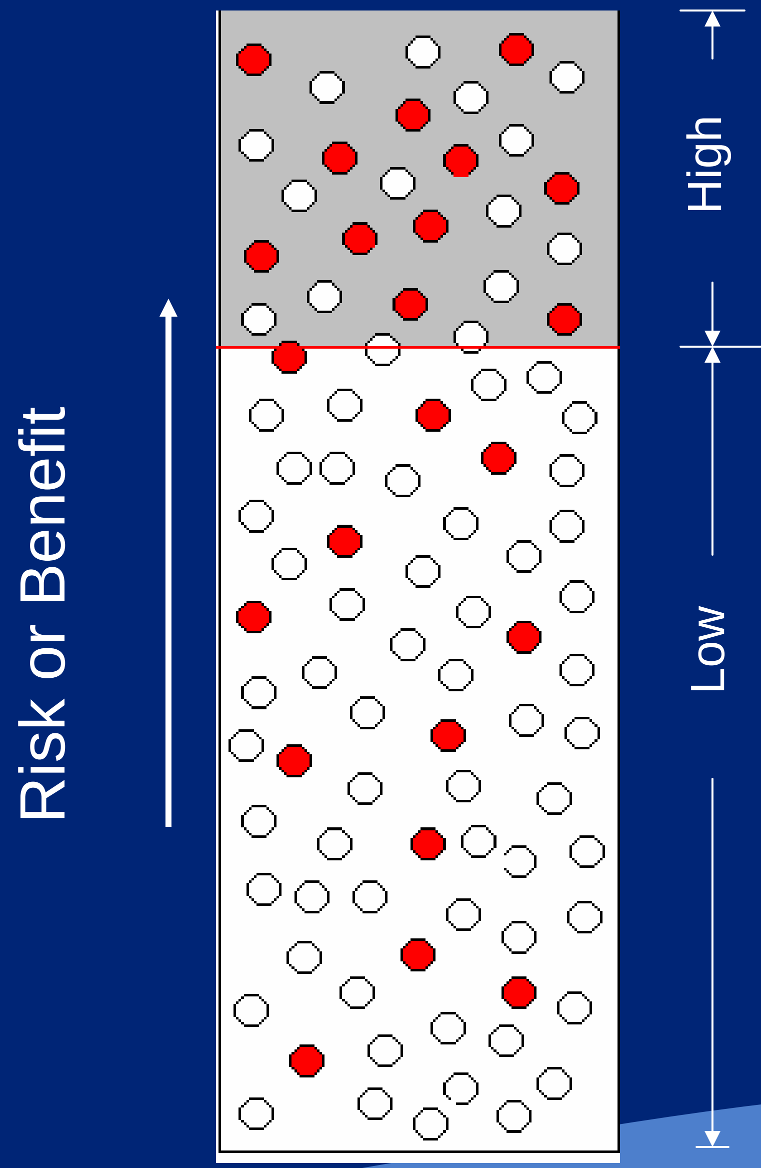
The majority of SNP associations account for a small fraction trait variability

Independent SNP associations, each accounting for a small fraction of trait variability, can be used in combination to provide clinically useful information

Additive Genetic Variance Predicts A Subset of the Population At Increased Risk For An Adverse Response To Treatment



Stratifier



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