Design and Statistical Challenges for Personalized Medicine and How to Meet Them

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- Gene/Biomarker Restricted Clinical Trials: Why?
- Identifying Susceptibility and Intervention Response Markers: The GWAS Paradigm
- The Efficiency of Targeted or Restricted-Entry Clinical Trials
- The Ultimate Study Design for Personalizing Medicine: The 'N-of-1' Clinical Trial

SCRIPPS GENOMIC MEDICINE
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Gene/Biomarker Restricted Clinical Trials: Why?

‘**Predictive biomarker**:’ Static marker that can be used to predict therapeutic appropriateness

‘**Surrogate endpoint**:’ Dynamic marker that can be used to monitor disease progression or existence during prevention/treatment (not discussed here…)

For **preventive trials**: enrich the study participants for those most likely to *develop* the condition for which the intervention was designed to prevent

- Treat the marker as a ‘risk factor’
- Results in more outcomes in the placebo group
- If the intervention works, then the treatment/placebo difference will be large
- Necessary sample sizes will be reduced

For **treatment based-trials**: enrich the study subjects for those most likely to *respond* to the treatment

- Pharmacogenetic effect
- Results in more individuals in treatment group responding
- If the intervention works, then the treatment/placebo difference will be large
- Necessary sample sizes will be reduced

Ultimate Goal: Match Drugs with Marker Profiles

The New York Times — December 30, 2008

Pairing Drugs and Genes

Genetic screening can help determine which patients are best suited to certain drugs. Here is a sampling of drugs for which genetic markers can help identify the suitable patients. Screening tests range in price from a few hundred dollars for tests for drugs like the breast cancer medicine Tamoxifen or the painkiller Celebrex to $3,800 for a test to guide breast cancer chemotherapy. Because experts do not always agree on whether the genetic links are conclusive or whether the screening tests are reliable, the Food and Drug Administration does not always require that drug labels mention such testing.

Key: What drug's label says about testing:
(1) Testing required
(2) Testing recommended
(3) Genetic relationship only mentioned
(4) Test not mentioned

DRUG	USE	GENETIC MARKER	SUITABILITY
IDENTIFYING SUITABLE PATIENTS			
Herceptin (trastuzumab)	Breast cancer	Her2 in tumor	Patients whose tumors have overabundance of Her2 protein. (1)
Erbitux (cetuximab) and **Vectibix** (panitumumab)	Colon cancer	KRAS	Ineffective for patients whose tumors have a mutation in KRAS gene. (4)
Chemotherapy	Breast cancer	Activity of various genes in tumor	Tests help determine whether patient can safely forgo chemotherapy after surgery. (4)
Tamoxifen	Breast cancer	CYP2D6	Women with certain variants of CYP2D6 gene may not benefit from drug. (4)
AVOIDING SIDE EFFECTS			
Ziagen (abacavir)	H.I.V. and AIDS	HLA-B*5701	This variant of an immune system gene may pose risk of severe allergic reaction to drug. (2)
Camptosar (irinotecan)	Colon cancer	UGT1A1*28	This gene variant poses higher risk of white blood cell deficiency from drug. (2)
Tegretol (carbamazepine)	Epilepsy	HLA-B*1502	This variant of immune system gene, most prevalent in Asians, poses greater risk of life-threatening skin reactions to drug. (2)
Coumadin (warfarin)	Blood clot prevention	CYP2C9 and VKORC1	Variants of these two genes help determine safe and effective dosage. (2)
Celebrex (celecoxib)	Pain and arthritis	CYP2C9	Certain variants of gene might pose risk of building up unsafe levels of drug in blood. (3)
PROSPECTIVE MARKERS ON THE HORIZON			
Plavix (clopidogrel)	Blood clot prevention	CYP2C19	Variants of this drug-metabolizing gene may block drug's benefits. (4)
Iressa (gefitinib) and **Tarceva** (erlotinib)	Lung cancer	EGFR in tumor	Tumors with mutation in this gene may respond better to these drugs than to standard chemotherapy. (4)
Bucindolol	Heart failure	Alpha-2c and beta-1 adrenergic receptors	Company hopes genetic tests could predict who would benefit from the drug, which is not yet approved.
Prozac (fluoxetine) and other antidepressants	Depression	CYP2D6, CYP2C19, others	Variations in drug-metabolizing genes might help identify best antidepressant and avoid side effects. (4)
Avastin (bevacizumab)	Various cancers	VEGF	Breast cancer research suggests variants of this gene might help predict which patients benefit from drug and which might be most at risk of side effects. (4)

Sources: F.D.A., various researchers

Improving Specificity by Designing Drugs to Overcome Specific Protein Damage due to Mutations in Cancer

- Some FDA approved cancer drugs work (only or at least) better when used against certain genomic profile (Gleevec, Herceptin, Iressa, Tarceva)
- However, the drugs were found to work best in certain situations serendipitously
- Most drugs are designed to work against available (i.e., often wild type/normal) targets!

Table 2 FDA approved anti-cancer drugs that target mutant genes

Drug (brand name; manufacturer)	Mechanism of action	Known target protein	Targeted mutation	Diagnostic test	Disease	Purpose
Imatinib mesylate (Gleevec; Novartis)	Tyrosine kinase inhibitor	BCR-ABL	Translocation	Cytogenetics, FISH and PCR	CML	Candidacy for therapy
		BCR-ABL	Missense mutations/ presence of RNA	Sequencing/PCR	CML	Monitor response to therapy; monitor drug resistance
		c-KIT, PDGFR-α	Mutations	Sequencing	GIST	Candidacy for therapy; monitor response; drug resistance in GIST
Trastuzumab (Herceptin; Genentech)	Antibody	ERBB2	Amplification	Immunohistochemistry, FISH	Breast cancer	Candidacy for therapy
Cetuximab (Erbitux; ImClone Systems)	Antibody	EGFR	Amplification	Immunohistochemistry, FISH	Colorectal cancer, head and neck cancer	Candidacy for therapy
Gefitinib (Iressa; Astra-Zeneca)	Tyrosine kinase inhibitor	EGFR	Activating mutations in the kinase domain	Sequencing	NSCLC	Candidacy for therapy
Erlotinib (Tarceva; OSI Pharmaceutical/Genentech)	Tyrosine kinase inhibitor	EGFR	Activating mutations in the kinase domain	Sequencing	NSCLC	Candidacy for therapy

BCR-ABL, Breakpoint cluster region–Abelson; PDGFR-α, platelet-derived growth factor receptor alpha; ERBB2, v-erb-b2 erythroblastic leukemia viral oncogene homolog 2; EGFR, epidermal growth factor receptor; CML, chronic myelogenous leukemia; GIST: gastrointestinal stromal tumors; NSCLC, non-small cell lung cancer; FISH, fluorescent *in situ* hybridization; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

Papadopoulos et al. Nature Biotechnology 24:985-995; 2006

Ultimate Goal: Match Drugs with Marker Profiles

Warfarin

FDA Approves Updated Warfarin (Coumadin) Prescribing Information Page 1 of 2

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FOR IMMEDIATE RELEASE
August 16, 2007

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FDA Approves Updated Warfarin (Coumadin) Prescribing Information

New Genetic Information May Help Providers Improve Initial Dosing Estimates of the Anticoagulant for Individual Patients

The U.S. Food and Drug Administration announced today the approval of updated labeling for the widely used blood-thinning drug, Coumadin, to explain that people's genetic makeup may influence how they respond to the drug.

Manufacturers of warfarin, the generic version of Coumadin, are to add similar information to their products' labeling, FDA said.

The labeling change highlights the opportunity for healthcare providers to use genetic tests to improve their initial estimate of what is a reasonable warfarin dose for individual patients. Testing may help optimize the use of warfarin and lower the risk of bleeding complications from the drug.

These labeling updates are based on an analysis of recent studies that found people respond to the drug differently based, in part, on whether they have variations of certain genes.

FDA estimates that 2 million persons start taking warfarin in the United States every year to prevent blood clots, heart attacks and stroke. Warfarin is a difficult drug to use because the optimal dose varies and depends on many risk factors including a patient's diet, age, and the use of other medications.

Patients who take a dose larger than they can tolerate are at risk of life-threatening bleeding. Those who receive too low a dose are at risk of equally dangerous blood clots. Dosing is particularly important at the beginning of therapy, when problems in adjusting the dose can lead to complications such as bleeding.

Warfarin is the second most common drug – after insulin –implicated in emergency room visits for adverse drug events.

Physicians and other health care professionals who prescribe warfarin regularly check to see if the drug is working properly by ordering a test called the PT or prothrombin time that evaluates the blood's ability to clot properly. The results are measured in seconds and compared with the expected value in healthy people, known as the International Normalized Ratio or INR.

"Today's approved labeling change is one step in our commitment to personalized medicine. By using modern science to get the right drug in the right dose for the right patient, FDA will further enhance the safety and effectiveness of the medicines Americans depend on," said Commissioner of Food and Drugs Andrew C. von Eschenbach, M.D.

The FDA's "personalized medicine" initiative makes use of pharmacogenomics—the science that predicts a response to drugs based upon a person's genetic makeup. This effort supports the personalized health program spearheaded by Health and Human Services Secretary Mike Leavitt.

A person's genes "encode" enzymes and differences in the sequence of a gene can cause differences in enzyme activity or sensitivity. That is why different people process the same drug differently.

One-third of patients receiving warfarin metabolize it quite differently than expected. Research has shown that some of the unexpected response to warfarin depends on a patient's variants of the genes CYP2C9 and VKORC1.

"Although genetic testing can currently identify who has these genetic variants, more studies are needed to explore the precise starting dose for these patients," said Larry Lesko, Ph.D., director of the FDA's Office of Clinical Pharmacology. "FDA has been working with other government agencies and organizations to develop such studies under the auspices of our three-year-old Critical Path Initiative, which addresses the challenges of moving promising medical products from discovery to patient use."

FDA's Critical Path Initiative has funded a research project with the University of Utah and the Critical Path Institute of Tucson, Ariz., to develop genetically based instructions for warfarin dosing. The initiative has also facilitated meetings and planning with the National Heart, Lung and Blood Institute for a clinical trial that will study warfarin dosing based on genetic test information and is helping to pay for another clinical study being conducted by Harvard Partners that will derive personalized warfarin dosing algorithms for patients new to the drug.

The dosage and administration of warfarin must be individualized for each patient according to the particular patient's PT/INR response to the drug. The specific dose recommendations are described in the warfarin product labeling, along with the new information regarding the impact of genetic information upon the initial dose and the response to warfarin. Ongoing warfarin therapy should be guided by continued INR monitoring.

http://www.fda.gov/bbs/topics/NEWS/2007/NEW01684.html 12/27/2007

Carbamazepine

Carbamazepine Prescribing Information to Include Recommendation of Genetic Test for Patients with A... Page 1 of 1

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FDA News

FOR IMMEDIATE RELEASE
December 12, 2007

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Carbamazepine Prescribing Information to Include Recommendation of Genetic Test for Patients with Asian Ancestry

Connection of genetic information with medication use can improve safe use of product

The U.S. Food and Drug Administration today announced that the manufacturers of drugs containing the active ingredient carbamazepine have agreed to add to the drugs' labeling a recommendation that, before starting therapy with the drugs, patients with Asian ancestry get a genetic blood test that can identify a significantly increased risk of developing a rare, but serious, skin reaction.

Carbamazepine is a drug used for treatment of epilepsy, bipolar disorder, and neuropathic pain. It is sold under the brand names Carbatrol, Equetro and Tegretol.

"Science is now letting us individually treat patients based on how their body might react to a drug," said Janet Woodcock, M.D., FDA's deputy commissioner for scientific and medical programs, chief medical officer, and acting director of the Center for Drug Evaluation and Research. "When being considered for treatment with carbamazepine, genetically high-risk patients can be given a test that will help their health care providers make personalized drug treatment decisions and help avoid potentially serious skin reactions."

The prescribing information for these drugs already includes a warning that for all patients starting carbamazepine therapy, regardless of ethnicity, rare but severe and sometimes life-threatening skin reactions can occur. These life-threatening skin reactions include toxic epidermal necrolysis and Stevens-Johnson syndrome, characterized by multiple skin lesions, blisters, fever, itching and other symptoms.

The risk of these reactions is estimated to be about 1 to 6 per 10,000 new users of the drug in countries with mainly white populations. However, the risk is estimated to be about 10 times higher in some Asian countries.

The skin reaction warnings will be moved to the current boxed warning section of the labeling. The new recommendation that health care providers give patients with Asian ancestry a genetic test before starting treatment will also be added to the boxed warning section.

To screen for this genetic marker, a patient's blood can be drawn by a health care provider and the test administered at a laboratory. It is estimated that about 5 percent of patients being considered for treatment with carbamazepine are of Asian ancestry and would need to have this test.

Studies have found a strong association between certain serious skin reactions and an inherited variant of a gene, HLA-B* 1502, an immune system gene, found almost exclusively in people with Asian ancestry. Patients testing positive for this gene should not be treated with carbamazepine unless the benefit clearly outweighs the increased risk of these serious skin reactions.

Patients who have taken carbamazepine for more than a few months and not experienced any skin reactions are unlikely to ever experience these reactions, regardless of ancestry or genetic test results. Patients currently taking carbamazepine who are concerned about these skin reactions should not stop taking the drug without first consulting their health care provider.

Carbatrol is manufactured by Shire Pharmaceuticals, Wayne, Penn.; Equetro is manufactured by Validus Pharmaceuticals Inc., Parsippany, N.J.; and Tegretol is manufactured by Novartis, East Hanover, N.J. Generic versions of carbamazepine are available.

For Information

FDA Information for Healthcare Professionals: Carbamazepine
www.fda.gov/cder/drug/InfoSheets/HCP/carbamazepineHCP.htm

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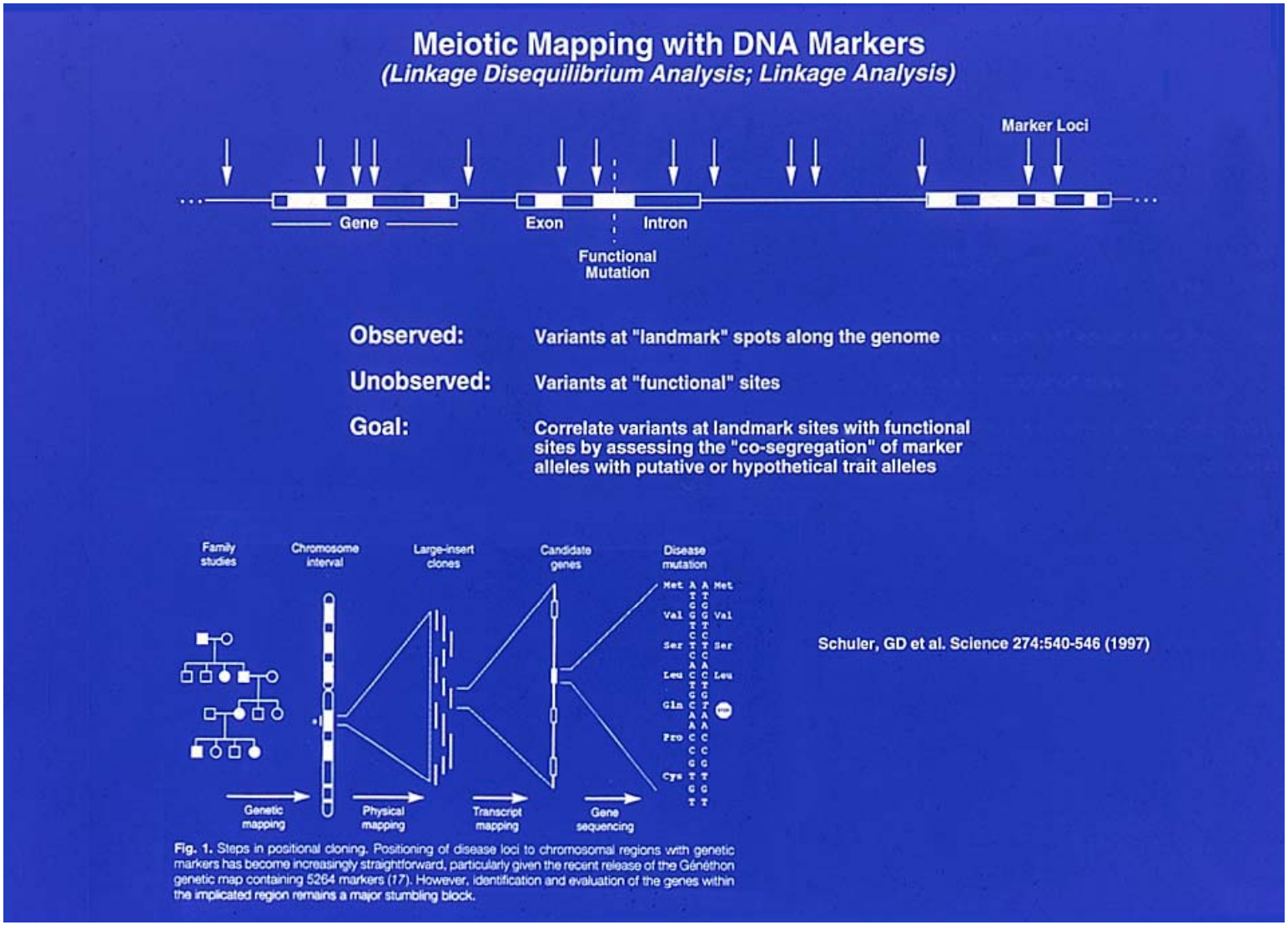
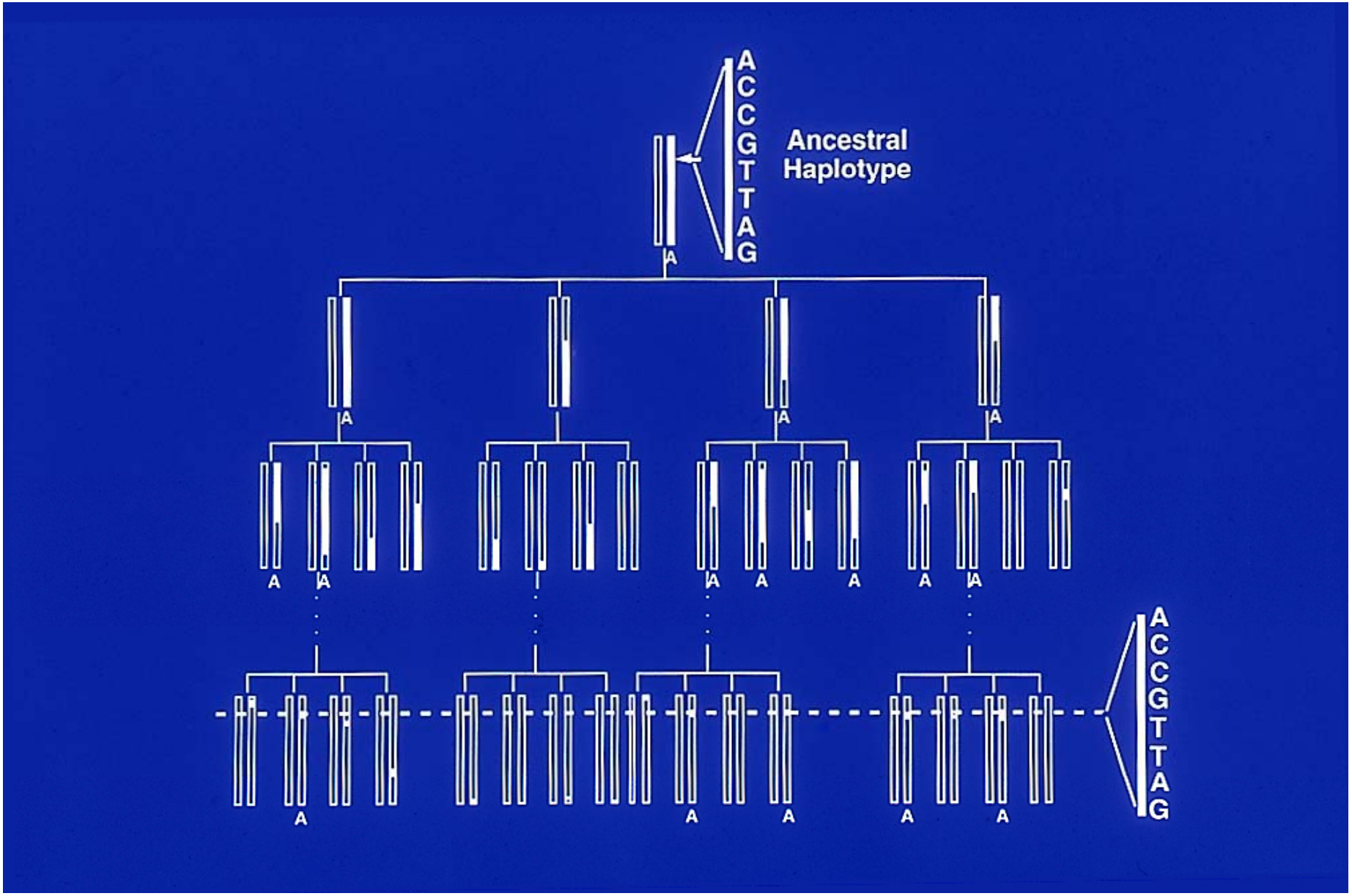
http://www.fda.gov/bbs/topics/NEWS/2007/NEW01755.html 12/27/2007

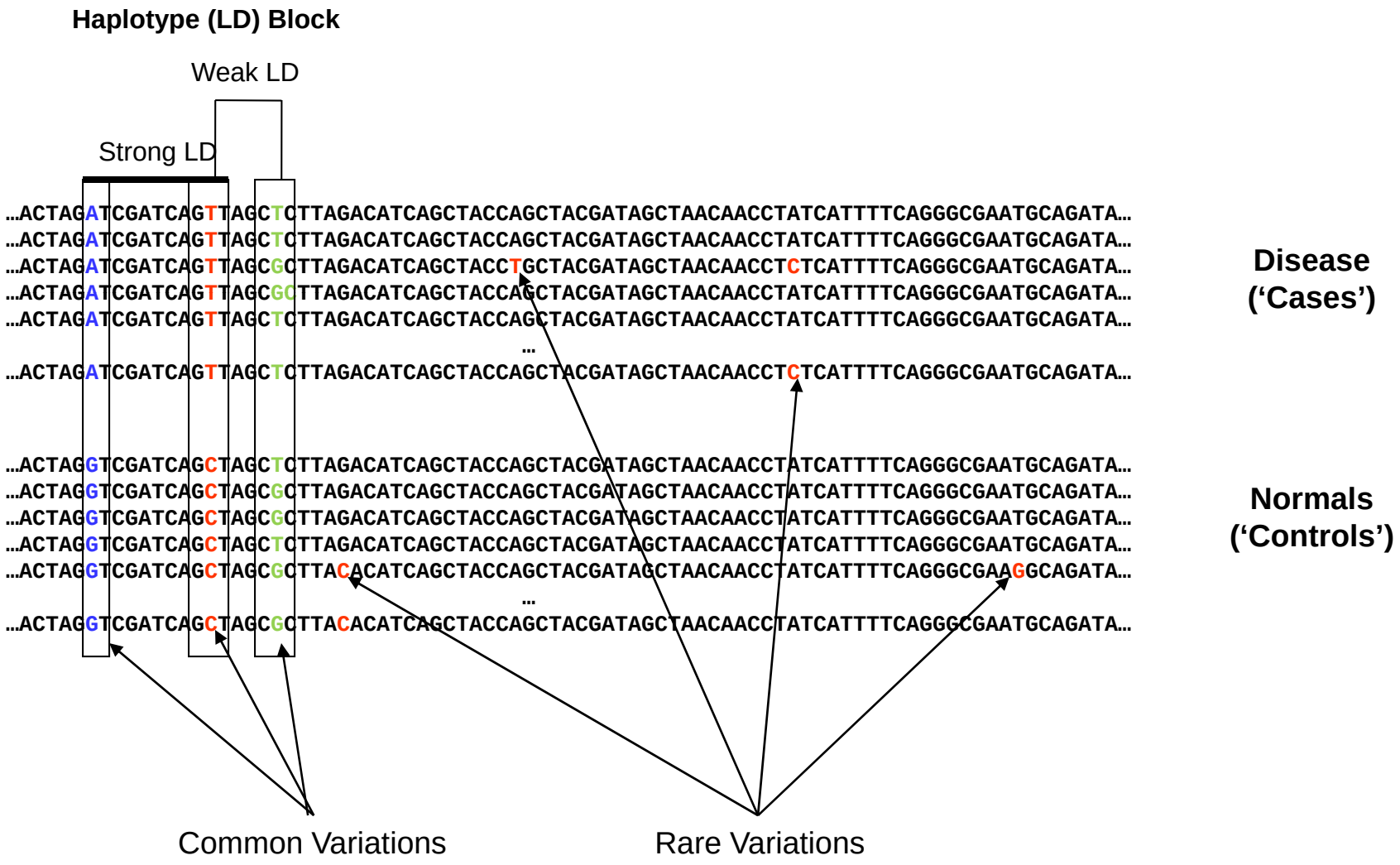
Identifying Susceptibility and Intervention Response Markers: The GWA Study Paradigm



Mapping for Gene Discovery via Linkage Disequilibrium



Linkage Disequilibrium Mapping and Haplotype ‘Blocks’



The International HapMap and Genetic Mapping Panels

NATURE|Vol 437|27 October 2005
Vol 437|27 October 2005|doi:10.1038/nature04226
nature

ARTICLES

A haplotype map of the human genome

The International HapMap Consortium*

Inherited genetic variation has a critical but as yet largely uncharacterized role in human disease. Here we report a public database of common variation in the human genome: more than one million single nucleotide polymorphisms (SNPs) for which accurate and complete genotypes have been obtained in 269 DNA samples from four populations, including ten 500-kilobase regions in which essentially all information about common DNA variation has been extracted. These data document the generality of recombination hotspots, a block-like structure of linkage disequilibrium and low haplotype diversity, leading to substantial correlations of SNPs with many of their neighbours. We show how the HapMap resource can guide the design and analysis of genetic association studies, shed light on structural variation and recombination, and identify loci that may have been subject to natural selection during human evolution.

Table 7 | Number of selected tag SNPs to capture all observed common SNPs in the Phase I HapMap

r^2 threshold*	YRI	CEU	CHB + JPT
$r^2 \geq 0.5$	324,865	178,501	159,029
$r^2 \geq 0.8$	474,409	293,835	259,779
$r^2 = 1.0$	604,886	447,579	434,476

Tag SNPs were picked to capture common SNPs in HapMap release 16c1 using the software program Haploview.
* Pairwise tagging at different r^2 thresholds.

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nature

ARTICLES

A second generation human haplotype map of over 3.1 million SNPs

The International HapMap Consortium*

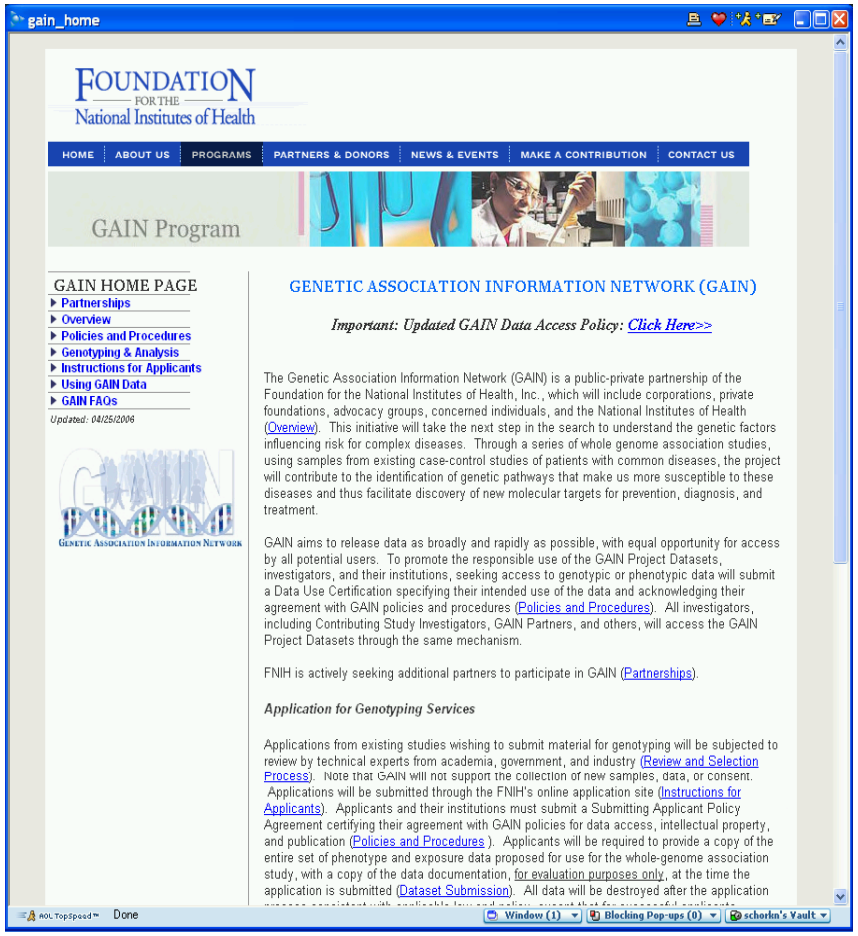
We describe the Phase II HapMap, which characterizes over 3.1 million human single nucleotide polymorphisms (SNPs) genotyped in 270 individuals from four geographically diverse populations and includes 25–35% of common SNP variation in the populations surveyed. The map is estimated to capture untyped common variation with an average maximum r^2 of between 0.9 and 0.96 depending on population. We demonstrate that the current generation of commercial genome-wide genotyping products captures common Phase II SNPs with an average maximum r^2 of up to 0.8 in African and up to 0.95 in non-African populations, and that potential gains in power in association studies can be obtained through imputation. These data also reveal novel aspects of the structure of linkage disequilibrium. We show that 10–30% of pairs of individuals within a population share at least one region of extended genetic identity arising from recent ancestry and that up to 1% of all common variants are untaggable, primarily because they lie within recombination hotspots. We show that recombination rates vary systematically around genes and between genes of different function. Finally, we demonstrate increased differentiation at non-synonymous, compared to synonymous, SNPs, resulting from systematic differences in the strength or efficacy of natural selection between populations.

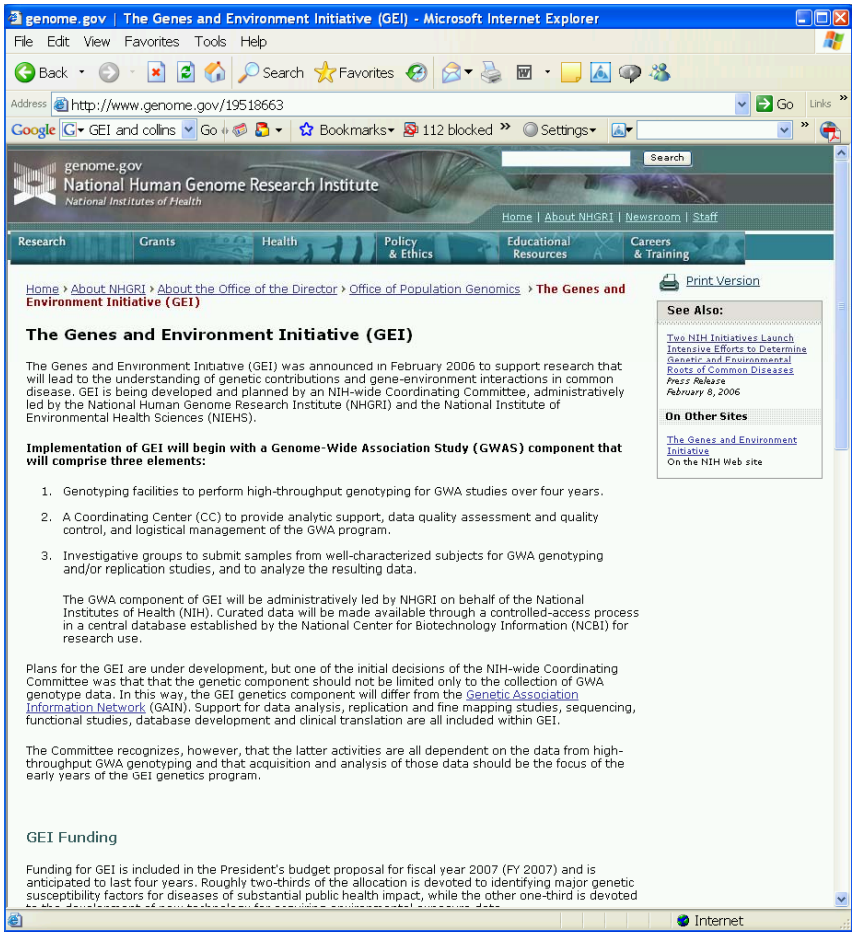
Table 3 | Number of tag SNPs required to capture common (MAF ≥ 0.05) Phase II SNPs

Threshold	YRI	CEU	CHB+JPT
$r^2 \geq 0.5$	627,458	290,969	277,831
$r^2 \geq 0.8$	1,093,422	552,353	520,111
$r^2 = 1$	1,616,739	1,024,665	1,078,959

Efficient Genotyping Technologies and GWAS Initiatives

- Single SNP genotyping costs have dropped ~1000 fold in the last 5 years
- Affymetrix, Illumina, Sequenom, outsourcing, etc. make large-scale studies feasible
- Recent initiatives emphasize Genome Wide Association (GWA) Studies





Closing the Net on Common Disease Genes

Huge data sets and lower cost analytical methods are speeding up the search for DNA variations that confer an increased risk for diabetes, heart disease, cancer, and other common ailments

CREDIT: GETTY IMAGES

AFTER YEARS OF CHASING FALSE LEADS, gene hunters feel that they have finally cornered their prey. They are experiencing a rush this spring as they find, time after time, that a new strategy is enabling them to identify genetic variations that likely lie behind common diseases. By scanning the genomes of thousands of people and comparing the sick with the healthy, biologists are uncovering markers for DNA sequences they believe clearly increase the risk of type 2 diabetes, cancer, heart disease, inflammatory bowel disease, and other debilitating ailments.

Their new tool, known as the genome-wide association (GWA) study, derives its power from the Human Genome Project and the more recent Haplotype Map that catalogs human genetic variation. The hunt has been sped along as well by the plummeting cost of gene scanning and by efficient gene-chip technologies available only in the past 2 years.

What sets these studies apart from earlier gene discoveries claimed for the same diseases is that the new associations are statistically far more powerful and highly unlikely to be due to chance. Researchers are also confident about a flurry of new results because they've been recorded again and again in populations studied by independent teams. Fueling the excitement is a sense of surprise: "Most of these genes were not on anybody's candidate gene list," says David Cox, chief scientific officer of Perlegen Sciences in Mountain View, California, which uses whole-genome scanning to identify drug targets. Cox recently co-authored a paper identifying a new genetic variant that raises heart disease risk and has another in the pipeline on breast cancer. He and many others expect the discoveries to point toward novel biology worth exploring.

At the same time, this wave of GWA studies is studded with caveats. Although many agree that the findings are real, few scientists believe that they should be quickly put to clinical use—for example, to evaluate a person's risk of having a heart attack. Scientists haven't sorted out how these genes might interact with the environment, or how lifestyle changes might modulate the risk they confer. "There's going to be some scrambling to catch up on the clinical side," says Nancy Cox, a human geneticist at the University of Chicago in Illinois.

Furthermore, these first studies may have identified only the strongest associations, with many more genes still to be dug up. Finding them will likely require an unusual degree of cooperation in this intensely competitive field.

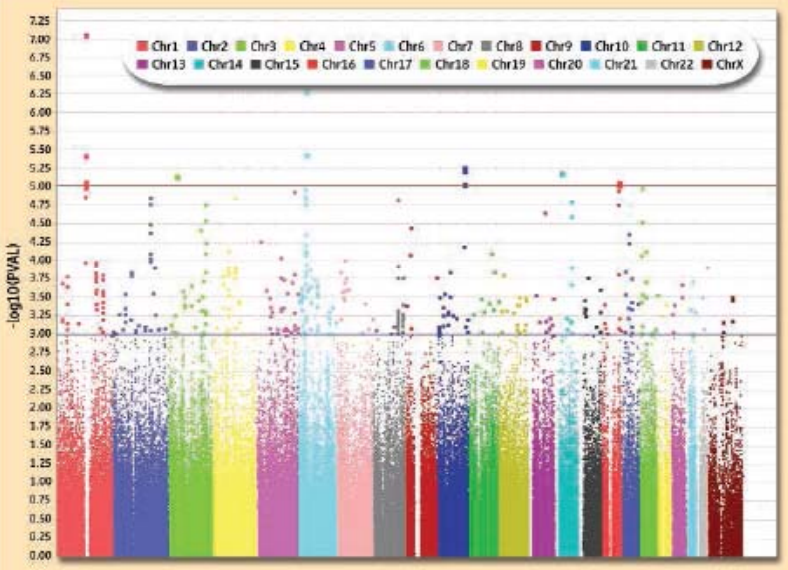
Uncommon beginnings

The new discoveries mark a major break with the past in part because their sweep is so broad. Traditionally, geneticists focused on single genes with potent effects, typically looking at large families riddled with rare diseases, such as cystic fibrosis or Huntington's disease or inherited forms of cancer. By tracking a small number of genetic markers that were linked to disease in such families, researchers successfully homed in on the culpable gene that causes disease.

These family "linkage" studies lacked the power to pick up genetic variants that have a modest effect or that may interact with environmental exposures, however. And yet it is these variants, which may raise risk by 50% or less, that could play a key role in common, complex diseases. (The exception is work by deCODE Genetics in Reykjavik, which has used linkage methods and a proprietary database containing information on much of Iceland's adult population to find some common disease genes.)

As an alternative to traditional linkage studies, researchers have tried searching for "candidate genes" known to play a role in some biologic process, such as insulin production. They looked for associations between mutations in these candidates and common diseases. Hundreds of studies have reported such associations. But few have been reproduced more than once or twice.

The new strategy that's blossomed this spring has fundamentally altered the gene-



Rising to the top. In a genome-wide association study for type 2 diabetes, 386,731 genetic markers, shown here by chromosome, pop up. Those above the higher line appeared to be significantly associated with disease.

Selected Genome-Wide Scan Results

DISEASE	PUBLICATION DATE	SAMPLE SIZE*	GENES OR VARIANTS FOUND	APPROXIMATE INCREASED RISK FOR HOMOZYGOTES†
Macular degeneration	2005	1700	1 new gene	400% to 600%
Inflammatory bowel disease	2006	4500	1 new gene	120%
Prostate cancer	2007	17,500	2 variants in same region (1 new)	123%
Obesity	2007	38,700	1 new gene	67%
Type 2 diabetes	2007	32,500	9 variants (3 new)	80%
Heart disease	2007	41,600	1 new variant	25% to 40%

* Cases and controls including replicates. † For highest risk variant.

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A HapMap harvest of insights into the genetics of common disease

Teri A. Manolio, Lisa D. Brooks, and Francis S. Collins

National Human Genome Research Institute, Bethesda, Maryland, USA.

Table 6
GWA studies in cardiovascular conditions and lipid metabolism

Disease/trait	Sample size Initial	Sample size Replication	Region	Gene	Strongest SNP-risk allele	Risk allele frequency in controls	*P*	OR per copy or for heterozygote (95% CI)	Platform manufacturer and SNPs[A]
QT interval prolongation (115)	100 >445 ms 100 <386 ms	200 >95th pct[B] 200 <5th pct[B]	1q23.3	*NOS1AP*	rs10494366-?	0.36	1×10^{-10}	4.9–7.9 (NR)[C]	Affymetrix: 88,500
Myocardial infarction (116)	1,607 cases 6,723 con	2,080 cases 6,041 con	9p21.3	*CDKN2A/B*	rs1757278-G	0.45	1×10^{-20}	1.28 (1.22–1.35)	Illumina: 305,953
Coronary disease (35)	1,926 cases 2,938 con	See ref. 73	9p21.3	*CDKN2A/B*	rs1333049-C	0.47	1×10^{-13}	1.47 (1.27–1.70)	Affymetrix: 469,557
Coronary disease (75)	1,926 cases 2,938 con	875 cases 1,644 con	9p21.3	*CDKN2A/B*	rs1333049-C	0.47	3×10^{-19}	1.36 (1.27–1.46)	Affymetrix: 377,857
			6q25.1	*MTHFD1L*	rs6922269-A	0.25	3×10^{-6}	1.23 (1.15–1.33)	
			2q36.3	Pseudogene	rs2943634-C	0.65	2×10^{-7}	1.21 (1.13–1.30)	
Atrial fibrillation/ atrial flutter (77)	550 cases 4,476 con	3,363 cases 17,616 con	4q25	Intergenic (near *PITX2*)	rs2200733-T rs10033464-T	0.11[D] 0.08[E]	3×10^{-41} 7×10^{-11}	1.72 (1.59–1.86) 1.39 (1.26–1.53)	Illumina: 315,515
LDL-cholesterol (117)	1,955 hypertensive individ	2,033 individ in 519 families; 1,467 twins[F]	1p13.3	*CELSR2, PSRC1, SORT1*	rs599839-G	0.24	1×10^{-7}	0.95 (0.93–0.97)[G]	Affymetrix: 400,496
LDL-cholesterol (118)	2,758 studied	18,554 studied	1p13.3	*CELSR2, PSRC1, SORT1,*	rs646776-T	0.24	3×10^{-20}	0.16 (0.14–0.18)[H]	Affymetrix: 389,878
			19p13.11	*CILP2, PBX4*	rs10096148-G	0.90	3×10^{-9}	0.10 (0.06–0.14)[H]	
HDL-cholesterol (118)	2,758 studied	18,554 studied	1q42.13	GALNT2	rs4846914-G	0.40	2×10^{-13}	0.07 (0.05–0.09)[I]	Affymetrix: 389,878
Triglycerides (119)	2,758 studied	18,554 studied	7q11.23	*BCL7B, TBL2, MLXIPL*	rs17145738-T	0.87	7×10^{-22}	0.14 (0.10–0.18)[H]	Affymetrix: 389,878
			8q24.13	*TRIB1*	rs17321515-A	0.40	4×10^{-17}	0.08 (0.06–0.10)[H]	
			1q42.13	*GALNT2*	rs4846914-G	0.40	7×10^{-15}	0.08 (0.06–0.10)[H]	
			19p13.11	*CILP2, PBX4*	rs10096140-G	0.90	4×10^{-8}	0.10 (0.06–0.14)[H]	
			1p31.3	*ANGPTL3, DOCK7, ATB4C*	rs12130333-C	0.73	2×10^{-8}	0.11 (0.07–0.15)[H]	
Triglycerides (119)	2,811 studied	10,535 studied	7q11.23	*MLXIPL*	rs3812316-C	0.95	1×10^{-10}	10.5 (5.3–17.7)[J]	Perlegen: 180,410 to 216,774
HDL-cholesterol (120)	8,656 studied	11,399 studied	12q24.11	*MVK/MMAB*	rs2338104-G	0.45	3×10^{-9}	0.48 (NR)[K]	Illumina and Affymetrix[L]
LDL-cholesterol (120)	8,589 studied	7,440–10,783 studied	1p13.3	*CELSR2, PSRC1, SORT1*	rs599839-A	0.77	6×10^{-33}	5.48 (NR)[K]	Illumina and Affymetrix[L]
			19p13.11	*NCAN/CILP2*	rs16996148-G	0.89	3×10^{-9}	3.32 (NR)[K]	
			6p21.32	*B3GALT4*	rs2254287-G	0.38	5×10^{-8}	1.91 (NR)[K]	
Triglycerides (120)	8,684 studied	5,312–9,707 studied	2p23.3	*GCKR*	rs780094-T	0.39	6×10^{-32}	8.59 (NR)[K]	Illumina and Affymetrix[L]
			8q24.13	*TRIB1*	rs17321515-A	0.55	7×10^{-13}	6.42 (NR)[K]	
			7q11.23	*MLXIPL*	rs17145738-C	0.84	2×10^{-12}	8.21 (NR)[K]	
			1p31.3	*ANGPTL3*	rs1748195-C	0.73	2×10^{-10}	7.21 (NR)[K]	
			19p13.3	*NCAN/CILP2*	rs16996148-G	0.92	3×10^{-8}	6.10 (NR)[K]	

Studies, statistics, and platforms are reported as described in Table 2. individ, individuals; pct, percentile; ?, risk allele not indicated. [A]Number that passed quality control. [B]Also studied were 7,817 cohort members. [C]Difference (in ms) between homozygotes. [D]In European population; allele frequency was 0.53 in Hong Kong population. [E]In European population; allele frequency was 0.22 in Hong Kong population. [F]One twin selected randomly. [G]Increase in mmol. [H]Increase in SD. [I]Decrease in SD. [J]Increase in percentage. [K]Increase in mg/dl. [L]About 2,261,000 imputed.

Table 7
GWA studies in neuropsychiatric conditions

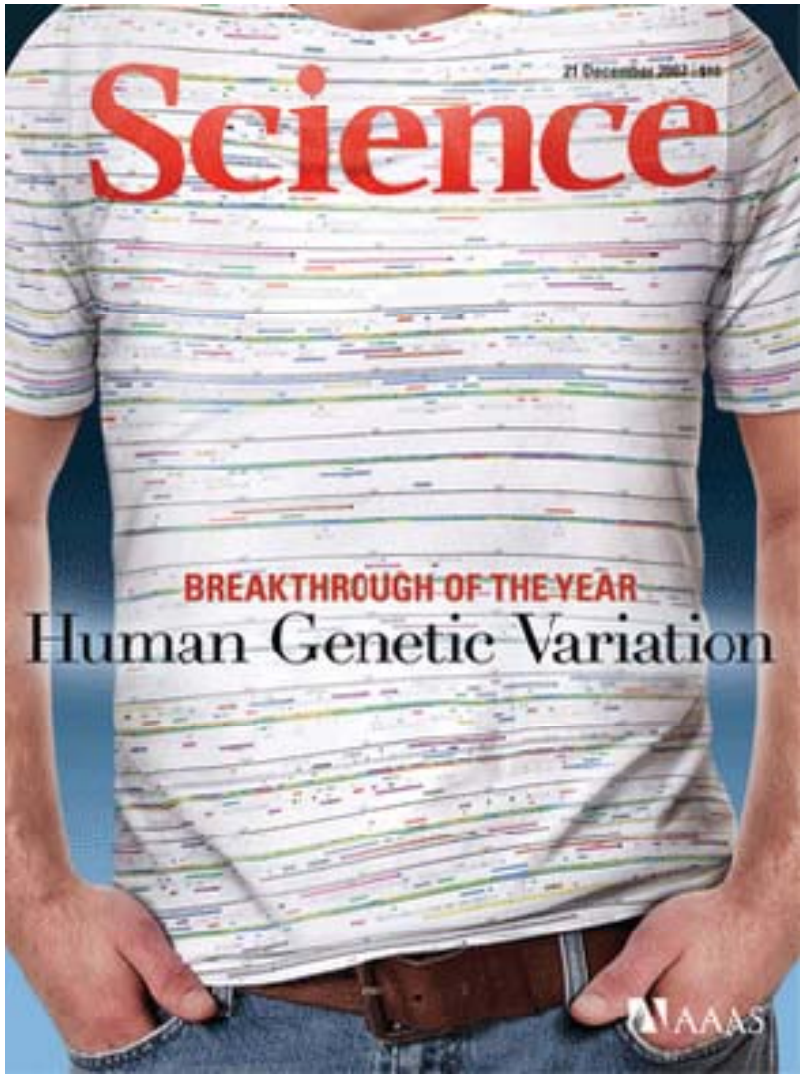
Disease/trait	Sample size Initial	Sample size Replication	Region	Gene	Strongest SNP-risk allele	Risk allele frequency in controls	*P*	OR per copy or for heterozygote (95% CI)	Platform manufacturer and SNPs[A]
Sporadic amyotrophic lateral sclerosis (121)	276 cases 271 con	NR	10q26.13	Intergenic	rs4363506-?	NR	7×10^{-7}	1.90 (1.50–2.40)	Illumina: 549,062
Amyotrophic lateral sclerosis (122)	737 cases 721 con	1,030 cases 1,195 con	7q36.2	*DPP6*	rs10260404-C	0.38	5×10^{-8}	1.30 (1.18–1.43)	Illumina: 311,946
Multiple sclerosis (97)	931 trios 2,431 con	609 trios 2,322 cases 2,987 con	10p15.1 5p13.2	*IL2RA* *IL7RA*	rs12722489-C rs6897932-C	0.85 0.75	3×10^{-8} 3×10^{-7}	1.25 (1.11–1.36) 1.18 (1.11–1.26)	Affymetrix: 334,923
Restless legs syndrome (123)	306 cases 15,664 con	311 cases 1,895 con	6p21.2	*BTBD9*	rs3923809-A	0.66	1×10^{-17}	1.90 (1.50–2.20)	Illumina: 306,937
Restless legs syndrome (124)	401 cases 1,644 con	1,158 cases 1,178 con	2p14 6p21.2 15q23	*MEIS1* *BTBD9* *MAP2K5, LBXCOR1*	rs2300478-G rs9296249-T rs12593813-G	0.24 0.76 0.67	3×10^{-26} 4×10^{-18} 1×10^{-15}	1.74 (1.57–1.92) 1.67 (1.49–1.89) 1.50 (1.36–1.66)	Affymetrix: 236,758
*APOE*e4* carriers with late-onset Alzheimer disease (125)	446 cases 290 con	415 cases 260 con	11q14.1	*GAB2*	rs2373115-G	0.72	1×10^{-10}	4.06 (2.81–14.69)	Affymetrix: 312,316
Schizophrenia (126)	178 cases 144 con	NR	Xp22.33/Yp11.32	*CSF2RA*	rs4129148-C	NR	4×10^{-7}	3.23 (2.04–5.15)[B]	Affymetrix: 439,511
Bipolar disorder (127)	461 cases 563 con	772 cases 876 con	13q14.11	*DGKH*	rs1012053-A	0.83	2×10^{-8}	1.59 (1.35–1.87)	Illumina: 555,235[C]
Bipolar disorder (35)	1,868 cases 2,938 con	NR	16p12.1	*PALB2, NDUFAB1, DCTN5*	rs420259-A	0.72	6×10^{-8}	2.08 (1.60–2.71)	Affymetrix: 469,557

Studies, statistics, and platforms are reported as described in Table 2. ?, risk allele not indicated. [A]Number that passed quality control. [B]Homozygote. [C]Pooled genotyping.

Important Issue:

Do the variations identified for any one disease explain much of the disease burden in the population at large?

The Recognition of Genetic Variation as Fundamental to All of Biology and Biomedical Science in Particular



The Limitations of Standard GWA Study Paradigms

- Human GWA studies have resulted in unequivocal statistical associations between DNA sequence variations and diseases/disease traits of all sorts

- Many loci identified are not obvious genes previously implicated in disease pathogenesis (many are not even in genes!)

- Associated genes have, on average, very small effects on disease (Odds Ratios of ~1.2-1.4)

- Collectively, the variations identified for any one disease typically explain a very small fraction of the disease burden in the population (e.g., 4-10%)

- Other genetic factors that might influence a disease that can't be detected via current GWA study paradigms: rare variants, structural variations, subtle epistatic interactions, gene x environment interaction effects, epigenetic factors, etc.

- **Is there a way to identify the effects of, e.g., rare variations, gene and GE interactions, epigenomic factors, etc. contributing to disease? HUGELY IMPORTANT issue**

The case of the missing heritability

When scientists opened up the human genome, they expected to find the genetic components of common traits and diseases. But they were nowhere to be seen. **Brendan Maher** shines a light on six places where the missing loot could be stashed away.

If you want to predict how tall your children might one day be, a good bet would be to look in the mirror, and at your mate. Studies going back almost a century have estimated that height is 80–90% heritable. So if 29 centimetres separate the tallest 5% of a population from the shortest, then genetics would account for as many as 27 of them[1].

This year, three groups of researchers[2–4] scoured the genomes of huge populations (the largest study[4] looked at more than 30,000 people) for genetic variants associated with the height differences. More than 40 turned up.

But there was a problem: the variants had tiny effects. Altogether, they accounted for little more than 5% of height's heritability — just 6 centimetres by the calculations above.

Even though these genome-wide association studies (GWAS) turned up dozens of variants, they did "very little of the prediction that you would do just by asking people how tall their parents are", says Joel Hirschhorn at the Broad Institute in Cambridge, Massachusetts, who led one of the studies[2].

Height isn't the only trait in which genes have gone missing, nor is it the most important. Studies looking at similarities between identical and fraternal twins estimate heritability at more than 90% for autism[5] and more than 80% for schizophrenia[6]. And genetics makes a major contribution to disorders such as obesity, diabetes and heart disease. GWAS, one of the most celebrated techniques of the past five years, promised to deliver many of the genes involved (see 'Where's the reward?', page 20). And to some extent they have, identifying more than 400 genetic variants that contribute to a variety of traits and common diseases. But even when dozens of genes have been linked to a trait, both the individual and cumulative effects are disappointingly small and nowhere near enough to explain earlier estimates of heritability. "It is the big topic in the genetics of common disease right now," says Francis Collins, former head of the National Human Genome Research Institute (NHGRI) in Bethesda, Maryland. The unexpected results left researchers at a point "where we all had to scratch our heads and say, 'Huh?'", he says.

Although flummoxed by this missing heritability, geneticists remain optimistic that they can find more of it. "These are very early days, and there are things that are doable in the next year or two that may well explain another sizeable chunk of heritability," says Hirschhorn. So where might it be hiding?

ILLUSTRATION BY MARK

Genetic Studies Investigating Drug Response Using Retrospective Data from Clinical Trials

The NEW ENGLAND JOUR

LETTERS

nature genetics

SLCO1B1 Va

*HLA-B*5701* genc
drug-induced live

Ann K Daly[1], Peter T Donaldson[1], David B Goldstein[4], Sally John[5], N William Bernal[6], Heather J Cordel for the DILIGEN study[1,2] and Inte

Drug-induced liver injury (DILI) is an im serious liver disease. The antimicrobial a common cause of DILI, but the genetic l remains unclear. We conducted a genon (GWA) study using 866,399 markers in 5 flucloxacillin DILI and 282 controls mat ancestry. The GWA showed an associati histocompatibility complex (MHC) regio association ($P = 8.7 \times 10^{-35}$) seen for r in complete linkage disequilibrium (LD) Further MHC genotyping, which include tolerant controls, confirmed the associat (OR = 80.6, $P = 9.0 \times 10^{-35}$). The asso in a second cohort of 23 cases. In *HLA-I* rs10937275 in *ST6GAL1* on chromosom genome-wide significance (OR = 4.1, *P* These findings provide new insights into of flucloxacillin DILI and have the poten improve diagnosis of this serious disease.

Hepatotoxicity remains the most commo termination of new therapeutic agents (~3 postmarketing withdrawals[1]. The standar symptomatic hepatic adverse drug reaction inhabitants, according to a French study[2]. Id accounts for 13% of acute liver failure cases 75% of affected individuals either die or req

Flucloxacillin is widely used in many Australia for treatment of staphylococcal ir associated with a characteristic cholestati common in females[3] and the elderly and v courses[3,4]. In the UK, the incidence of flucl

[1]Institute of Cellular Medicine and Institute of Hun [2]Harvard Medical School, Boston, Massachusetts, U [3]GlaxoSmithKline, Research Triangle Park, North C Hospital and Medical School, Dundee, UK. [5]Kings C [6]These authors contributed equally to this work. [7]J (a.k.daly@ncl.ac.uk).

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NATURE GENETICS ADVANCE ONLINE PUBLI

BACKGROUND
Lowering low-density lipoprotein rial reductions in cardiovascular produce larger benefits. In rare c therapy, especially when the statin other medications.

METHODS
We carried out a genome-wide asso ers (and additional fine-mapping and 90 controls, all of whom wer trial involving 12,000 participant simvastatin daily involving 20,00

RESULTS
The genome-wide scan yielded a rs4363657 single-nucleotide polyn chromosome 12 ($P = 4 \times 10^{-9}$). SLCO1B1 peptide OATP1B1, which has been The noncoding rs4363657 SNP wa the nonsynonymous rs4149056 SN rabolism. The prevalence of the ris odds ratio for myopathy was 4.5 (of the C allele, and 16.9 (95% CI, genes. More than 60% of these my The association of rs4149056 with simvastatin daily, which also sho cholesterol-lowering effects of sim associated with myopathy.

CONCLUSIONS
We have identified common varia an increased risk of statin-induced achieve the benefits of statin th trolled Trials number, ISRCTN74

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ORIGINAL CONTRIBUTION

Association of
Genotype With
and Clinical Eff

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William Herzog, MD
Paul A. Gurbel, MD

DUAL ANTIPLATELET THERAPY, including clopidogrel and aspirin, inhibits platelet function, preventing ischemic events and improving outcomes following acute coronary syndromes and percutaneous coronary intervention (PCI).[1,2] To exert an antiplatelet effect, clopidogrel requires conversion to an active thiol metabolite (SR 26334) by hepatic cytochrome P450 (CYP) isoenzymes, which inhibit adenosine diphosphate (ADP)–stimulated platelet

For editorial comment see p 896.

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nature

LETTERS

Genetic variation
treatment-induce

Dongliang Ge[1], Jacques Fellay[1], Alex Erin L. Heinzen[1], Ping Qiu[3], Arthur H. & David B. Goldstein[1]

Chronic infection with hepatitis C virus (HC people worldwide and is the leading cause America[1]. Although the recommended tr infection involves a 48-week course of (PegIFN-α-2b) or -α-2a (PegIFN-α-2a) com (RBV), it is well known that many patients treatment, and that patients of European an cantly higher probability of being cured tha ancestry. In addition to limited efficacy, trea tolerated because of side effects that preven completing therapy. For these reasons, i determinants of response to treatment is a h report that a genetic polymorphism near the interferon-λ-3 (IFN-λ-3), is associated wi twofold change in response to treatment, bo European ancestry ($P = 1.06 \times 10^{-25}$) an ($P = 2.06 \times 10^{-5}$). Because the genotype lead is in substantially greater frequency in Eu populations, this genetic polymorphism al mately half of the difference in response ra Americans and patients of European ancestr

To identify human genetic contributions t response, we have performed a genome-wid more than 1,600 individuals who were part and we included a further 67 patients from ano ment study[9]. Briefly, the IDEAL study compar three treatment regimens involving PegIFN- combined with RBV. It demonstrated simil IFN preparations and a significantly lower ef African-Americans compared with Americans (European-Americans). All patients included Americans who were chronically infected w Patients received 48 weeks of treatment and A total of 1,671 individuals were genotype Human610-quad BeadChip, and we then sear of treatment response as a primary endpoint. treatment response and non-response accordi tions[9], concentrating on sustained virological re the absence of detectable virus at the end of (Supplementary Information I). We include satisfied stringent compliance criteria (Supplen in the analyses of treatment response, and 1,47 analysis of baseline viral load.

We found that a polymorphism on chrom is strongly associated with SVR in all patient gr

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nature medicine

ARTICLES

A GRK5 polymorphism that inhibits β-adrenergic receptor signaling is protective in heart failure

Stephen B Liggett[1,6,7], Sharon Cresci[2,7], Reagan J Kelly[3,7], Faisal M Syed[1], Scot J Matkovich[2], Harvey S Hahn[1], Abhinav Diwan[1], Jeffrey S Martini[4], Li Sparks[1], Rohan R Parekh[1], John A Spertus[5], Walter J Koch[4], Sharon L R Kardia[3] & Gerald W Dorn II[1,2]

β-adrenergic receptor (βAR) blockade is a standard therapy for cardiac failure and ischemia. G protein–coupled receptor kinases (GRKs) desensitize βARs, suggesting that genetic GRK variants might modify outcomes in these syndromes. Re-sequencing of GRK2 and GRK5 revealed a nonsynonymous polymorphism of GRK5, common in African Americans, in which leucine is substituted for glutamine at position 41. GRK5-Leu41 uncoupled isoproterenol-stimulated responses more effectively than did GRK5-Gln41 in transfected cells and transgenic mice, and, like pharmacological βAR blockade, GRK5-Leu41 protected against experimental catecholamine-induced cardiomyopathy. Human association studies showed a pharmacogenomic interaction between GRK5-Leu41 and β-blocker treatment, in which the presence of the GRK5-Leu41 polymorphism was associated with decreased mortality in African Americans with heart failure or cardiac ischemia. In 375 prospectively followed African-American subjects with heart failure, GRK5-Leu41 protected against death or cardiac transplantation. Enhanced βAR desensitization of excessive catecholamine signaling by GRK5-Leu41 provides a 'genetic β-blockade' that improves survival in African Americans with heart failure, suggesting a reason for conflicting results of β-blocker clinical trials in this population.

Heart failure is an incurable syndrome arising from multiple causes that will affect one in five adults, conferring mortality rates of ~25% within a year of diagnosis and ~50% at 5 years after diagnosis[1,2]. The management of heart failure is complicated by disease heterogeneity in both inherited genetic cardiomyopathies[3] and the more common non-familial dilated and ischemic cardiomyopathies[4–7]. We and others have proposed that interindividual differences in genetic polymorphisms involving catecholamine signaling pathways can modify heart failure risk, prognosis or response to treatment. Especially relevant would be pharmacogenomic interactions between genetic variants of catecholamine receptors or their effectors and βAR antagonists (β-blockade), which is a standard therapy for heart failure and myocardial ischemia[8]. This therapy prolongs life and ameliorates symptoms but concomitantly impairs a key mechanism for acutely increasing cardiac output in response to physiological stress. Genetic polymorphisms that influence the balance between beneficial and toxic effects of βAR signaling may therefore be crucial to outcomes of cardiac disease.

An important mechanism for downregulating βAR signaling in heart failure is increased expression of myocardial GRK2, which phosphorylates cardiac βAR, leading to recruitment of β-arrestin and receptor uncoupling from G proteins and downstream signaling effectors[9]. Although several studies have shown that expression of a GRK2-inhibiting peptide can improve cardiac function in experimental models of heart failure[10,11], cardiac-specific ablation of GRK2 in mice actually accelerates catecholamine-induced heart failure[12]. Thus, the effects of GRK2 on heart function seem to depend both on its expression level and on pathophysiological context. The function of the other GRK with high levels of cardiac expression, GRK5 (ref. 13), has not been as well defined. Genetic ablation of GRK5 is not associated with a cardiac phenotype in mice[14], but massive cardiac overexpression of bovine GRK5 depresses cardiac βAR responsiveness[15,16]. Differences between GRK5 and GRK2 in subcellular localization, mechanism of activation and receptor specificity suggest that they may have nonredundant modulatory roles in the heart. Of particular interest is rapid up- and downregulation of GRK2 that correlates with ventricular function[17,18], implying that GRK2 may function predominantly in the acute regulation of βAR signaling. GRK5 expression seems to be less dynamic, suggesting that this GRK may be more important for chronic regulation[19,20]. As such, GRK5-mediated βAR desensitization might provide adaptive, beneficial effects during early ventricular decompensation, before frank cardiac failure.

To examine this notion, we searched for human genetic variants of GRK2 and GRK5 that might modify the risk or outcome of heart failure, or alter the response to heart failure therapy. We identified a

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NATURE MEDICINE ADVANCE ONLINE PUBLICATION 1

The Efficiency of Targeted or Restricted-Entry Trials

Vol. 10, 6759–6763, October 15, 2004 Clinical Cancer Research 6759

Perspective

Evaluating the Efficiency of Targeted Designs for Randomized Clinical T

STATISTICS IN MEDICINE
Statist. Med. 2005; 24:329–339
Published online 18 November 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/sim.1975

Richard Simon
Biometric Research
Maryland

ABSTRACT

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On the efficiency of targeted clinical trials

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ORIGINAL ARTICLE

Statistical Methods for Targeted Clinical Trials under Enrichment Design

Jen-Pei Liu,[1,2] Jr-Rung Lin[1]*

Background/Purpose: After completion of the Human Genome Project, disease targets at the molecular level can be identified. Treatment for these specific targets can be developed with the individualized treatment of patients becoming a reality. However, the accuracy of diagnostic devices for molecular targets is not perfect and statistical inference for treatment effects of the targeted therapy is biased. We developed statistical methods for an unbiased inference for the targeted therapy in patients who truly have the molecular targets. Methods: Under the enrichment design, for binary data, we propose using the expectation maximization (EM) algorithm with the bootstrap method, to incorporate the inaccuracy of the diagnostic device for detection of the molecular targets for inference of the treatment effects. A simulation study was conducted to empirically investigate the performance of the proposed estimation and testing procedures. A numerical example illustrates the application of the proposed method.
Results: Simulation results demonstrated that the proposed estimation method was unbiased, with adequate precision, and the confidence interval provided satisfactory coverage probability. The proposed testing procedure adequately controlled the size with sufficient power. The numerical example showed that a statistically significant treatment effect could be obtained when the inaccuracy of the diagnostic device was taken into account.
Conclusion: Our proposed estimation and testing procedures are adequate statistical methods for the inference of the treatment effect for patients who truly have the molecular targets. [*J Formos Med Assoc* 2008;107(12 Suppl):S35–S42]

Key Words: diagnostic accuracy, enrichment design, targeted treatment

As a result of recent insights into genomics and pharmacogenomics, molecular disease targets can be identified and utilized for treatment.[1–5] At the same time, diagnostic devices for detection of disease using state of the art biotechnology such as microarray, polymerase chain reaction (PCR), mRNA transcript profiling, and single nucleotide polymorphisms, have also become possible. As a result, treatments specific for the patients with the identified molecular targets can be developed, and patients benefit from the treatment without suffering serious or even fatal toxicity. Consequently, personalized medicine may finally become a reality.

Targeted therapy is a type of treatment that uses drugs or other means, such as monoclonal antibodies, against the identified molecular targets that are involved in disease pathogenesis. Targeted clinical trials are those that evaluate the efficacy and safety of targeted therapies.[6] The current

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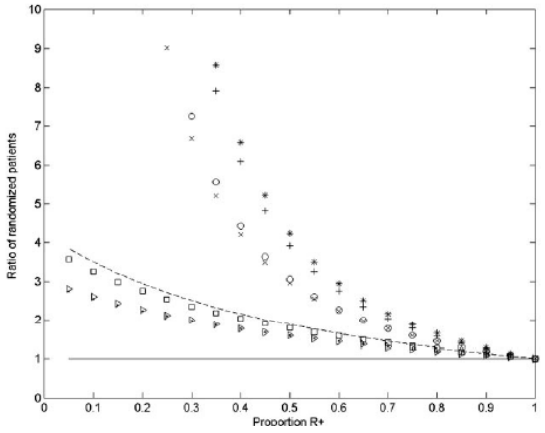

Fig. 1 Number of randomized patients required for untargeted design relative to that for targeted design. The *horizontal axis* represents the proportion of patients who express the target and are expected to be responsive to the new treatment. Case 0: No treatment effect for R− patients ($\delta_0 = 0$): ○: $\delta_1 = 0.2$, $p_c = 0.1$; ×: $\delta_1 = 0.4$, $p_c = 0.1$; +: $\delta_1 = 0.2$, $p_c = 0.5$; ∗: $\delta_1 = 0.4$, $p_c = 0.5$. Case 1: Treatment effect for R− patients is half as large as that for R+ patients ($\delta_0 = \delta_1/2$): ▷: $\delta_1 = 0.2$, $p_c = 0.1$; ·: $\delta_1 = 0.4$, $p_c = 0.1$; □: $\delta_1 = 0.2$, $p_c = 0.5$; –: $\delta_1 = 0.4$, $p_c = 0.5$.

http://linus.nci.nih.gov/brb/samplesize/td.html

- Do not consider sampling burden associated with recruiting subjects with genetic profiles
- Do not consider specific genetic variations and their utility for design of a clinical trial
- Do not consider relevance to current, historical, or future clinical trials of new therapies

The Efficiency of Targeted or Restricted-Entry Trials

Chapter 11
Predictive Biomarker Classifiers in the Design of Pivotal Clinical Trials

Richard Simon

Contents

Abstract In this chapter we distinguish the use of predictive biomarkers from surrogate endpoint biomarkers. We also distinguish the use of predictive biomarkers for selecting patients for pivotal clinical trials of a new drug from the use of predictive biomarkers for optimizing the utilization of an existing drug. We summarize the key steps in the development of predictive biomarker classifiers for use in new drug development. We discuss the design of targeted clinical trials in which a predictive biomarker classifier is used to restrict entry, and present results comparing the efficiency of targeted trials relative to standard randomized pivotal trials. We also discuss alternative designs in which the predictive biomarker classifier is not used to restrict entry of patients but is used to prospectively define an analysis plan for evaluating the new drug in classifier negative and positive patients. The development of predictive biomarker classifiers can be subjective, but pivotal trials should test hypotheses about the effectiveness of a new drug in subsets defined in a completely prespecified manner by a predictive classifier, and should not contain any subjective components. The data used to develop the predictive classifier should be distinct from the data used to evaluate a new drug in subsets determined by the classifier. The purpose of the pivotal trial is to evaluate the new drug in patient groups defined prospectively by the predictive classifier, not to refine or reevaluate the classifier or its components. New drug development should move from a correlative science mode to a predictive medicine mode.

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N. Cohen (ed.) *Pharmacogenomics and Personalized Medicine*,

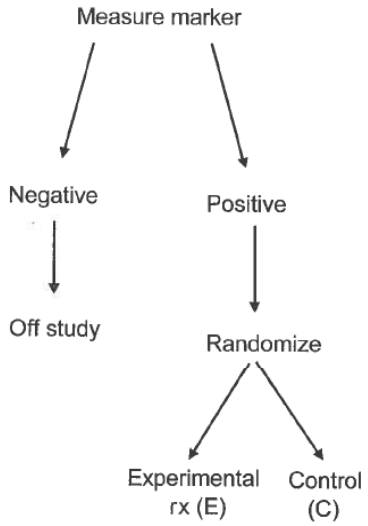

Table 11.1 Efficiency of targeted design

Proportion classifier positive	$\delta / \delta_+ = 0$		$\delta / \delta_+ = .5$	
	Randomized for standard design / Randomized for targeted design	Randomized for standard design / screened for targeted design	Randomized for standard design / Randomized for targeted design	Randomized for standard design / screened for targeted design
.5	4	2	1.8	0.89
.4	6.25	2.5	2.0	0.82
.3	11.1	3	2.4	0.71
.2	25	5	2.8	0.56
.1	100	10	3.3	0.33

screen than are required for randomization with the standard design. Hence, this targeted design is most appropriate when the treatment benefit is expected to be quite specific for classifier positive patients. When the proportion of patients who are classifier positive exceeds 50%, the efficiency advantages of the targeted trial are reduced.

Hypothetical Restricted-Entry Preventive Trials

- Leverage results of existing trials; constrain event rates to observed rates
- Assume genotype frequency and effect sizes from actual GWA studies
- Compute sample size for a trial restricted to individuals with a specific genotype

Table 2. Example Clinical Trials Investigating the Ability of Certain Compounds for Disease Prevention

Outcome	Intervention	Ref	I Rate	C Rate	Red	N I	N C	Pow N	R Pow/Act
Myocardial Infarction (MI)	Atorvastatin	1	0.049	0.062	20.968	4995	5006	3830	0.766
Prostate Cancer	Finasteride	2	0.184	0.244	24.590	4368	4692	577	0.127
Obesity*	Behavioral	3	0.055	0.080	31.250	237	249	1243	5.110
Type II Diabetes (T2D)	Metformin	4	0.210	0.290	27.568	1556	1563	361	0.231
Alzheimers*	Antioxidants	5	0.234	0.244	4.098	10241	10228	22462	2.195

Key: Rate = incidence rate of the outcome among individuals on the active compound or the control compound for the study referenced; N= sample size for the group receiving the drug ("I") and not receiving the drug ("C"). "Red" is the percentage reduction in disease due to the intervention. "Pow N" gives the sample sizes necessary for both the Intervention and Control groups in order to detect the observed effect assuming a power level of 0.8 and a type I error rate of 0.05. "R Pow/Act" provides the ratio of the computed sample size necessary to detect the observed effect ("Pow N") to the actual total sample sized used in the studies. The asterisk denotes studies that did not observe a significant difference in outcome rates between the intervention and control groups. References used in the Table are: 1: LaRosa et al. (2005); 2: Thompson et al. (2003); 3: Gortmaker et al. (1999); 4: Salpeter et al. (2008); 5: Heart Protection Study Collaborative Group (2002).

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Hypothetical Restricted-Entry Preventive Trials

Table 3. Projected Sample Sizes for Genotype-based Trials that Test Compounds Listed in Table 1.

Outcome	Intervention	SNP	Ref	Locus	Freq	OR	Gen N	Screen	R Act	R Pow
MI	Atorvastatin	rs10757278	1	9p21	0.22r	1.72	2693	24485 ± 294	0.539	0.703
Prostate	Finasteride	rs16901979	2	8q24	0.07d	1.53	471	13463 ± 422	0.104	0.817
Obesity	Behavioral	rs1421085	3	FTO	0.41a	1.56	1006	4910 ± 84	4.142	0.809
T2D	Metformin	rs10811661	4	CDKN2A	0.83a	1.20	355	857 ± 3	0.228	0.984
Alzheimers	Antioxidants	rs4420638	5	APOE4	0.17a	4.01	14238	167508 ± 904	1.391	0.634

Key: SNP = Single Nucleotide Polymorphism or locus identified in the study listed under the Ref column associated with the Outcome; Freq is the frequency of the risk genotype(s) with r=recessive, d=dominant, and a=additive allelic effects; "OR" is the effect of the allele on the outcome; "Gen N" denotes the Intervention and Control group sample sizes necessary for detecting a one sided test effect of the intervention at a power level 0.8 and type I error rate of 0.05 assuming that the intervention works just as well among the targeted subjects as the original study group referenced in Table 1. "Screen" gives the total number of individuals (and standard error) that must be screened (i.e., genotyped) in order to identify the requisite number with the appropriate genotype based on the mean negative binomial distribution. "R Act" and "R Pow" denote the ratio of the actual trial sample size and the projected sample size for the actual effect observed to the genotype-based sampling sample size, respectively relative to the original study listed in Table 2. Mode indicates the assumed genotypic or allelic effect: Rec=recessive; Dom=dominant; Add= additive (allelic); References in the Table are 1: Helgodottir et al. (2007); 2. Zheng et al. (2008); 3: Dina et al. (2007); 4: Diabetes Genetics Initiative (2007); 5: Coon et al. (2007).

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Note: It has been shown that genetic variations associated with a disease are largely independent of traditional risk factors, so genetic sampling adds to risk-based sampling

Hypothetical Restricted-Entry Preventive Trials

Table 5. Projected Sample Sizes for Multilocus Genotype-based Trials that Test Some Compounds Listed in Table 1.

Outcome	Drug	# of Fctrs	FH	Freq	OR	Gen N	Screen	R Act	R Pow
Prostate Cancer	Finasteride	≥1	-	0.899	1.87	533	1234 ± 11	0.123	0.962
Prostate Cancer	Finasteride	≥2	-	0.531	2.13	468	1766 ± 39	0.104	0.813
Prostate Cancer	Finasteride	≥3	-	0.171	2.50	398	4661 ± 150	0.088	0.691
Prostate Cancer	Finasteride	≥4	-	0.022	4.47	377	34314 ± 1235	0.083	0.654
Prostate Cancer	Finasteride	≥1	+	0.850	2.25	535	1260 ± 14	0.118	0.929
Prostate Cancer	Finasteride	≥2	+	0.514	2.66	436	1700 ± 40	0.096	0.757
Prostate Cancer	Finasteride	≥3	+	0.214	2.91	380	3552 ± 114	0.084	0.659
Prostate Cancer	Finasteride	≥4	+	0.038	4.52	369	19448 ± 701	0.082	0.640
Prostate Cancer	Finasteride	≥5	+	0.003	9.46	471	314099 ± 10216	0.104	0.816

Key: Column titles are the same as in Table 3. The "# of Fctrs" column refers to the number of genetic factors (SNPs) with frequency and a cumulative effect given in the "Freq" and "Odds Ratio" columns as reported in the relevant reference. "FH" denotes that whether or not a positive Family History is included as one of the risk factors along with the SNPs. Data were taken from Zheng et al. (2008).

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A Genotype-Restricted Pharmacogenetic Trial

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Safety and Efficacy Study of Fx-1006A in Patients With Familial Amyloidosis

This study has been completed.
Information provided by FoldRx Pharmaceuticals

This Tabular View shows the required WHO registration data elements as marked by †

Descriptive Information Fields

Brief Title †	Safety and Efficacy Study of Fx-1006A in Patients With Familial Amyloidosis
Official Title †	Safety and Efficacy of Orally Administered Fx-1006A in Patients With Familial Amyloid Polyneuropathy (FAP): A Randomized, Double-Blind, Placebo-Controlled Study
Brief Summary	This study will examine whether Fx-1006A is effective in halting the progression of Familial Amyloid Polyneuropathy (FAP).
Detailed Description	Deposition of TTR amyloid is associated with a variety of human diseases. Deposition of amyloid fibrils of variant TTR (primarily V30M) in peripheral nerve tissue produces the condition called FAP. The prevention of the formation of amyloid by stabilization of the TTR native state should constitute an effective therapy for amyloid diseases. Therapeutic intervention with a TTR stabilizer drug, such as Fx-1006A, is hypothesized to stop progression of the disease in FAP patients. FAP is a uniformly fatal disease and Fx-1006A is intended to halt the relentless neurological deterioration FAP patients experience. This Phase 2/3 study will enroll early to mid-stage FAP patients in order to interrupt and stabilize the disease at a point in time where progression of motor and autonomic dysfunction can be maximally effected. Male and female patients with FAP with documented V30M TTR mutation will receive Fx-1006A or placebo once daily for a period of eighteen (18) months.

Recruitment Information Fields

Recruitment Status †	Completed
Enrollment †	127
Start Date †	December 2006
Completion Date	December 2007
Eligibility Criteria †	Inclusion Criteria: 1. Amyloid documented by biopsy. 2. Documented V30M TTR mutation. 3. Peripheral and/or autonomic neuropathy with a Karnofsky Performance Status ≥50. 4. Patient is 18-75 years old. 5. If female, patient is post-menopausal, surgically sterilized, or willing to use an acceptable method of birth control. If male with a female partner of childbearing potential, willing to use an acceptable method of birth control for the duration of the study. For both females and males, birth control must be used for at least 3 months after the last dose of study medication. 6. Patient is, in the opinion of the investigator, willing and able to comply with the study medication regimen and all other study requirements. Exclusion Criteria: 1. Chronic use of non-steroidal anti-inflammatory drugs (NSAIDs). 2. Primary amyloidosis. 3. If female, patient is pregnant or breast feeding. 4. Prior liver transplantation. 5. No recordable sensory threshold for vibration perception in both feet, as measured by CASE IV. 6. Positive results for hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (HCV), and/or human immunodeficiency virus (HIV). 7. Renal insufficiency or liver function test abnormalities. 8. New York Heart Association (NYHA) Functional Classification ≥III. 9. Other causes of sensorimotor neuropathy (B12 deficiency, Diabetes Mellitus, HIV treated with retroviral medications, thyroid disorders, alcohol abuse, and chronic inflammatory diseases). 10. Co-morbidity anticipated to limit survival to less than 18 months. 11. Patient received an investigational drug/device and/or participated in another clinical investigational study within 60 days before Baseline.

Only V30M TTR Mutation Carriers are Eligible

The Ultimate Study Design for Personalizing Medicine: The 'N-of-1' Clinical Trial

- A single patient is the object of the trial (e.g., natural in clinical practice; anesthesia)
- Treat the patient with different drugs in an objective, designed manner
- Consider randomizing the treatments, blinding, washout periods, etc.
- Patient monitoring via continuous time data collection (e.g., wireless devices)
- Statistical issues: number of data collections, correlations between observations
- Not appropriate for all diseases/conditions (e.g., acute, life-threatening)

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Combining Single Patient (N-of-1) Trials to Estimate Population Treatment Effects and to Evaluate Individual Patient Responses to Treatment

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ABSTRACT. When treating individual patients, physicians may face difficulties using the evidence from center-based randomized control trials (RCTs) due to limitations in these studies' generalizability. Therefore, they often perform their own "informal" tests of treatment effectiveness. Single patient ("N-of-1") trials provide a structured design for more rigorous assessment of medical treatments of chronic diseases, but are applied only to the index patient. We present a hierarchical Bayesian random effects model to combine N-of-1 studies to obtain an estimate of treatment effectiveness for the population and to use this population information to aid in the evaluation of an individual patient's trial results. The model's treatment effect estimates are adjustments between the population estimate and the individual's observed results. This adjustment is based upon the within-patient and between-patient heterogeneity. We demonstrate this patient-focused method using published data from 23 N-of-1 trial results comparing amitriptyline and placebo for the treatment of fibromyalgia. J CLIN EPIDEMIOL 50;4:401–410, 1997. © 1997 Elsevier Science Inc.

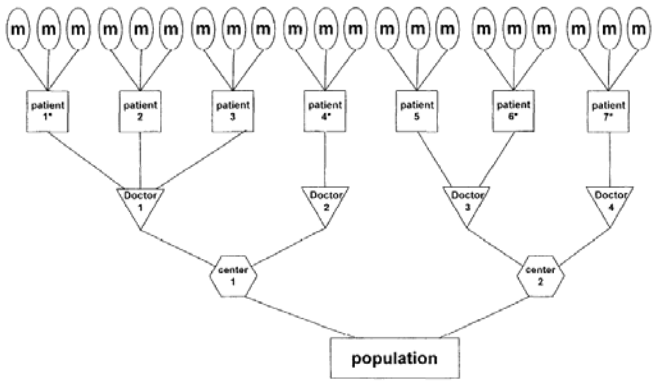
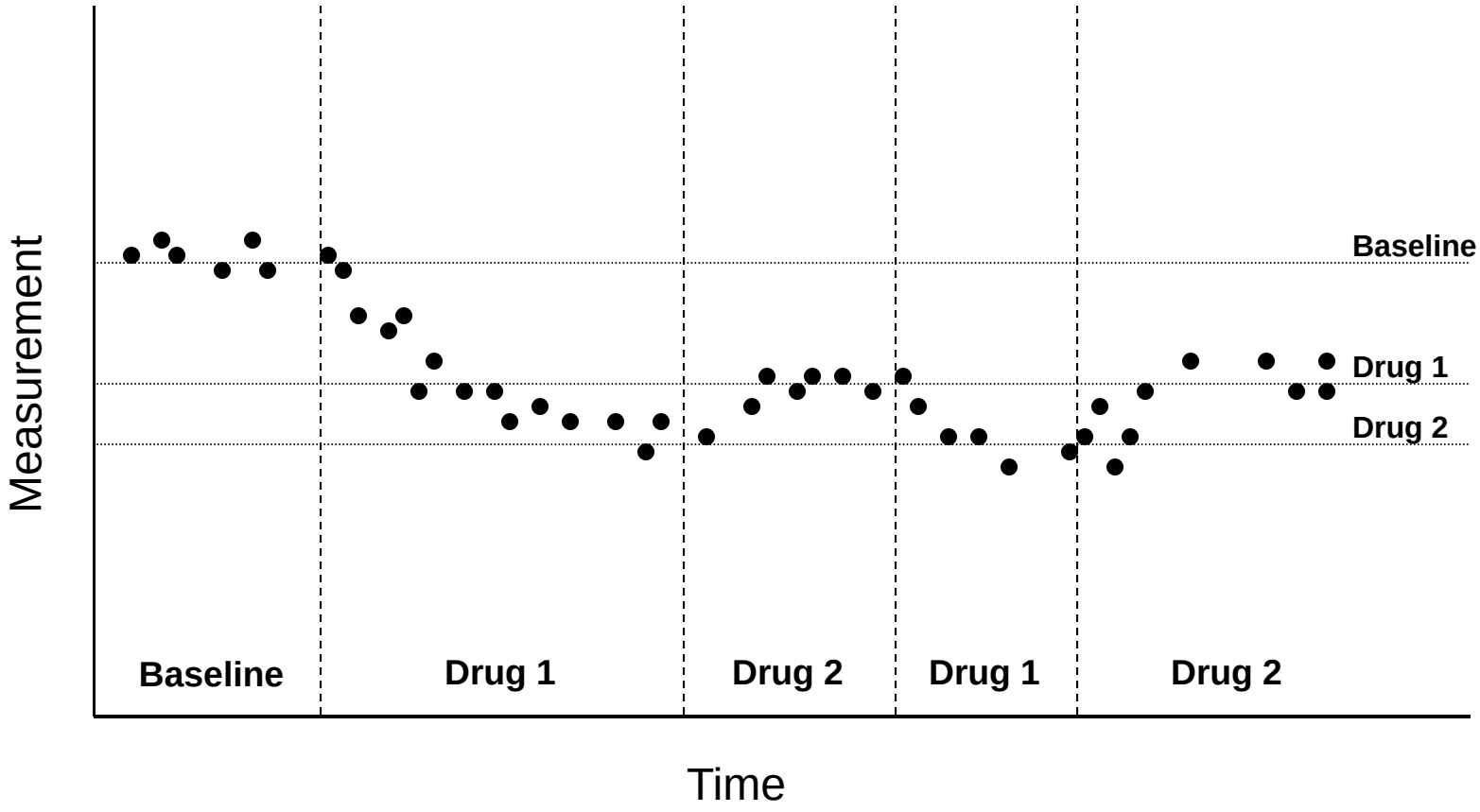


FIGURE 1. Schematic representation of population groupings for analysis of individual patient studies. Each measurement (*m*) represents the difference in disease status score determinations during a paired treatment period from the N-of-1 trial of a given patient. One or several of the patients might be under the care of a given physician. Each physician might work alone or with others to form a center, which might be described by some geographical structure ultimately defining the population. In addition, individual patients may have a specific characteristic (*) that may affect response to treatment.

Design of ‘N-of-1’ Clinical Trials



Example ‘N-of-1’ Clinical Trials

Table 1. Individual and Combined N-of-1 Studies Investigating the Utility of an Intervention

Disease	Reference	# Trials	Intervention (Dx)	Results
COPD	Nonoyama et al. (2008)	26	Ambulatory Oxygen	Reported use of oxygen is biased
OCTD	Hackett et al. (2008)	1	L-arginine diet	L-arginine improved health
Brain injury	Martin & Whyte (2007)	NR	Methylphenidate	No benefit of Methylphenidate
Childhood arthritic pain	Huber et al. (2007)	6	Amitriptyline	No benefit of Amitriptyline
Oral mucositis	Sung et al. (2007)	16	Topical vitamin E	No benefit of Topic vitamin E
Chronic fatigue	Baicus & Baicus (2007)	4	Spirulina	No effect of spirulina
Osteoarthitis	Yelland et al. (2007)	56	Paracetamol/Celecoxib	Paracetamol more effective
Nausea from Chemo	Nathan et al. (2006)	12	Metopimazine	Metopimazine use is beneficial
Anticoagulation	Pereira et al. (2005)	7	Generic/Brand Warfarin	No difference between Generic/Brand
Skeletal cramping	Woodfield e al. (2005)	13	Qunine	Heterogeneity in Qunine response
Sleep disturbances	Wegman et al. (2005)	15	Temazepam	Temazepam is beneficial
Cystic fibrosis	Suri et al. (2003)	48	Recombinant DNase	Marginal improvements with Dx
Migraine	Haas & Sheehe (2004)	32	Dextroamphetamine	Improvements with Dextroamphetamine
Sleep disturbances	Coxeter et al. (2004)	42	Valerian	Valerian did not improve sleep
COPD	Smith et al. (2004)	27	Eformoteral	No effect of Eformoterol
Osteoarthritis	Wegman et al. (2003)	13	NSAIDs	Heterogeneity in response to NSAIDs
Depression	Janssen et al. (2001)	5	Methylphenidate	Two patients improvement with Dx
Cystic Fibrosis	Bollert et al. (1999)	52	Recombinant DNase	Marked Improvements after Dx
ADHD	Kent et al. (1999)	43	Methylphenidate	Improvement with Methylphenidate
Chronic pain	Haines & Gaines (1999)	21	Ketamine	Small subgroup responded to Dx

Conclusions

- Markers of disease risk and treatment response are, in fact, being identified
- Drugs tested in individuals genetically susceptible leads to greater efficiency
- Drugs can be designed to target specific genomic ‘lesions’ (i.e., targeted therapies)
- ‘N-of-1’ trials can be conducted on each individual to determine optimal therapy

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