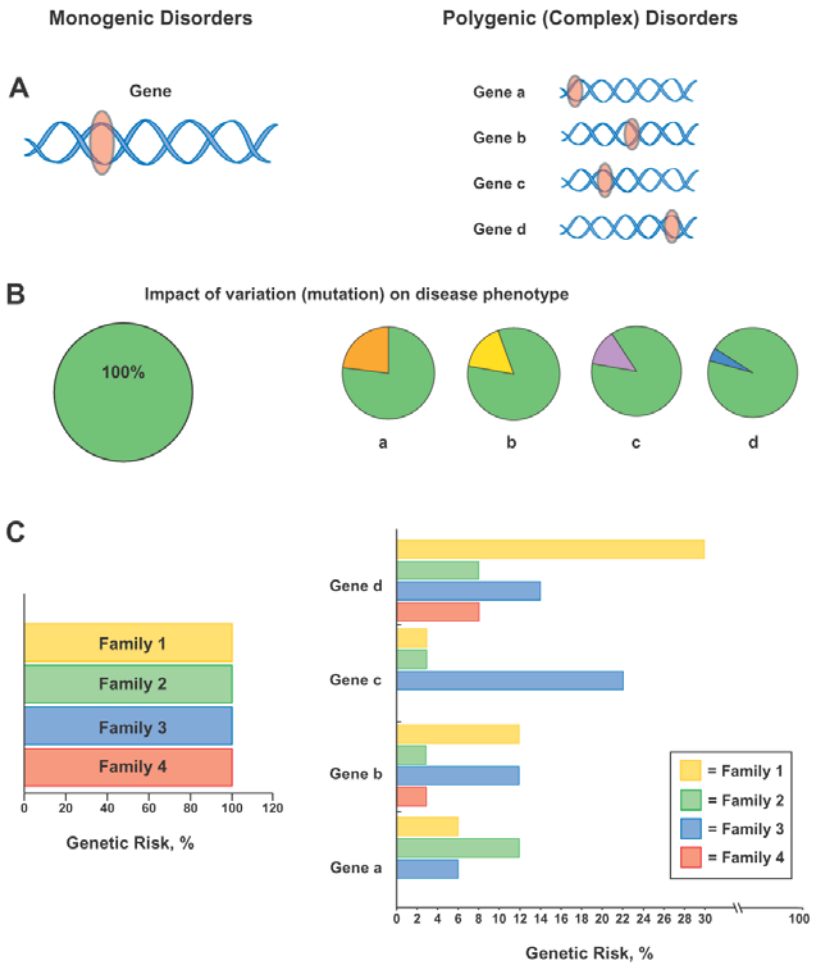
Screening e Counseling genetico: quando e per quale paziente

Anna Maria Di Blasio

Responsabile del Laboratorio di Genetica Medica

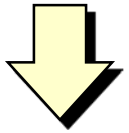
Instituto Auxologico Italiano Milano

Malattie Mendeliane o Monogeniche e Malattie Complesse o Poligeniche



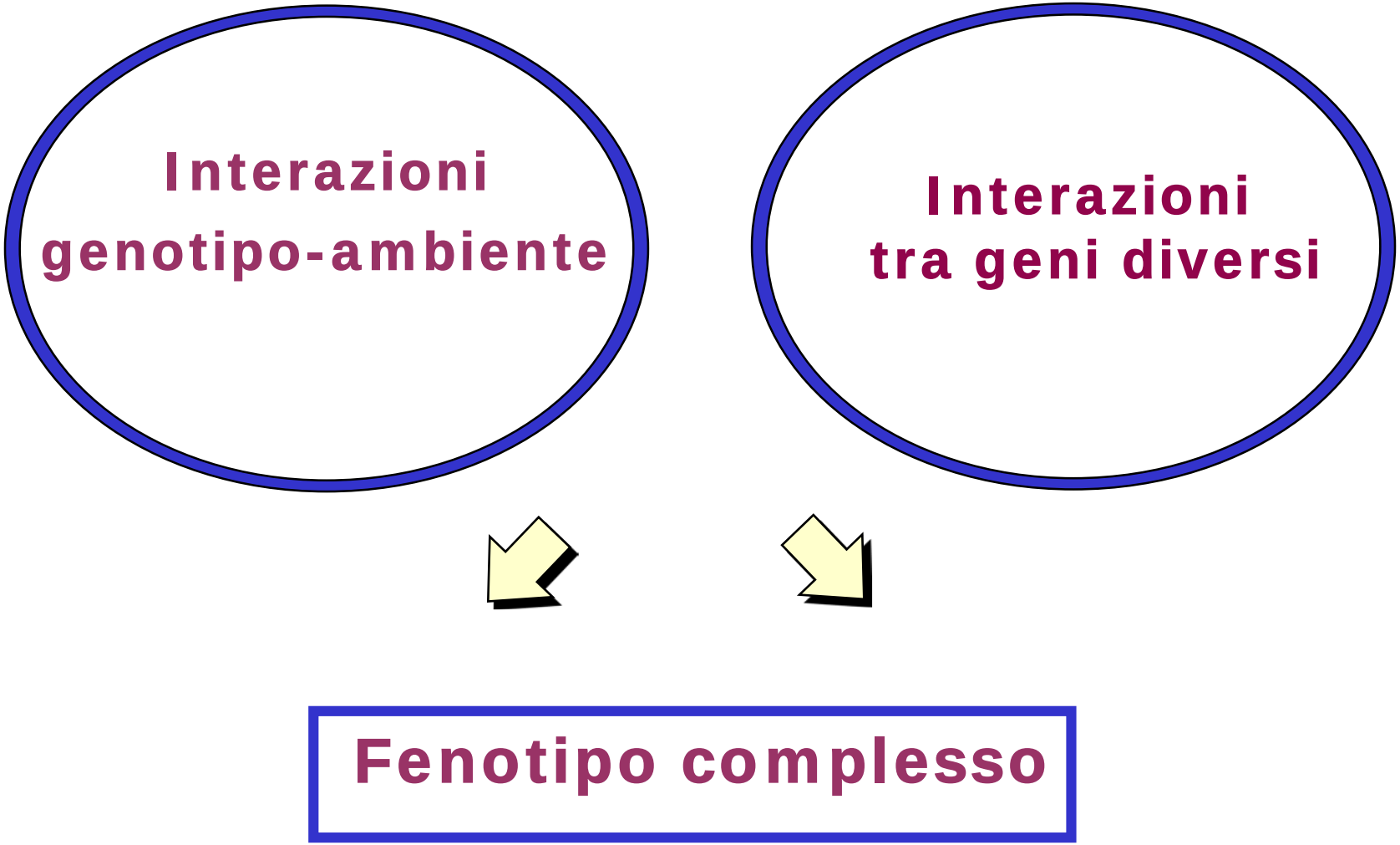
Malattie Mendeliane o Monogeniche

Correlazione diretta genotipo-fenotipo



Estrema Variabilità Fenotipica

Malattie Complesse o Poligeniche



1960's - 1990's

Descriptive epidemiology

Familial aggregation

Heritability studies

Segregation analyses

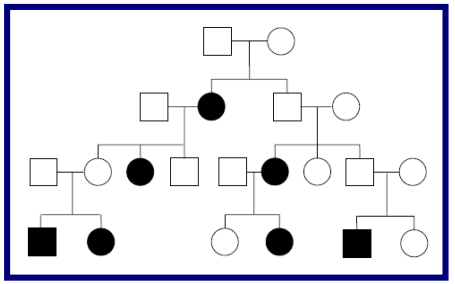
1960's — 1990's

Ethnic variation in disease risk
Risk among immigrants

Estimates the recurrence risk of a disease/trait between family members relative to the risk in the population

Estimate the genetic and environmental contribution to disease/trait

Estimates the genetic model of a disease/trait by looking at multigenerational family data.

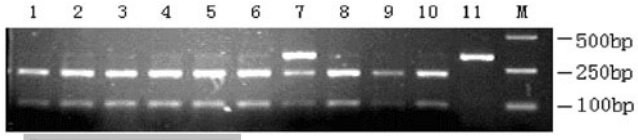


1990’s - 2017

Candidate gene approach

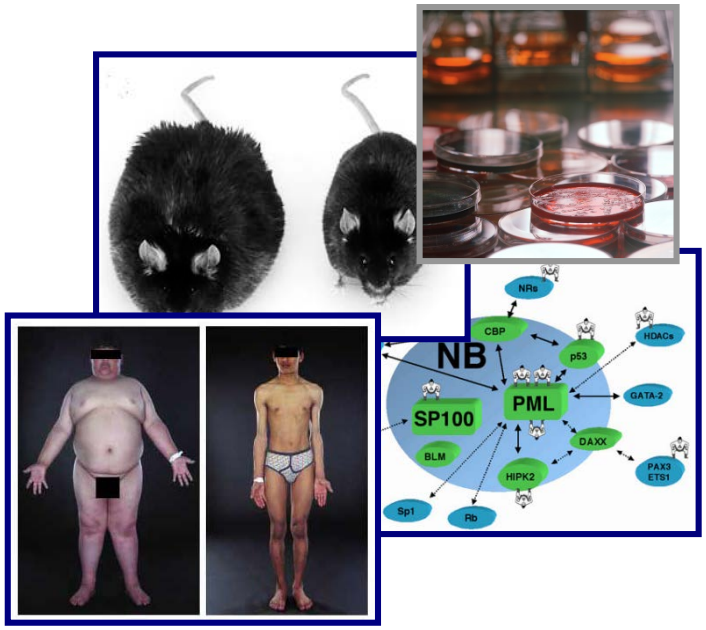
1990’s — 2015

Association analyses between a genetic variant and a trait/disease

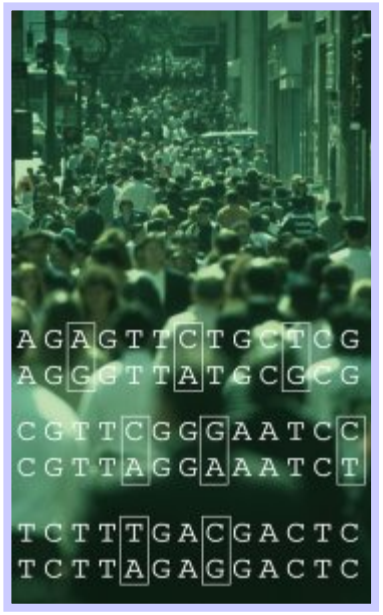


Candidate gene approach

= hypothesis-driven, based on the current understanding of the biology and pathophysiology of the trait/disease

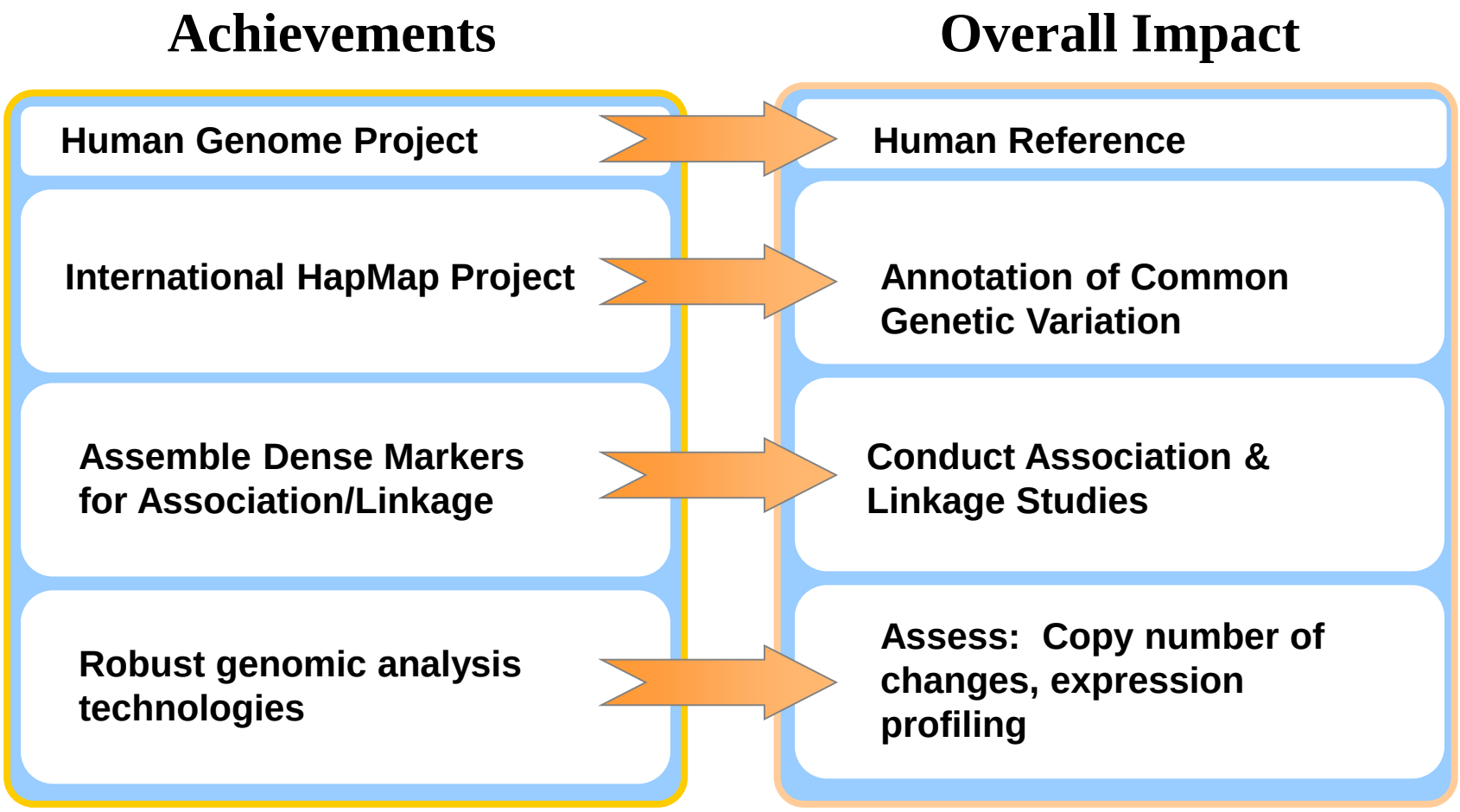


Physiology / Biology



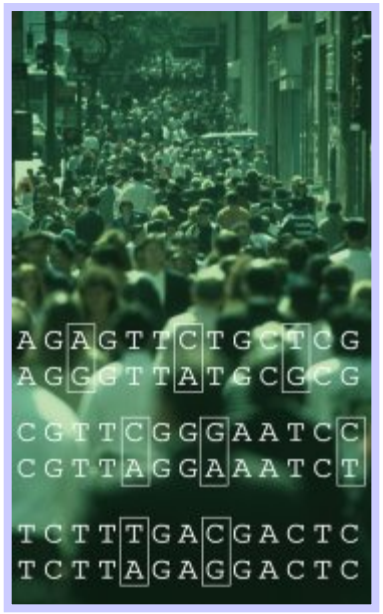
Epidemiology

Milestones in Human Genomics & Disease Susceptibility

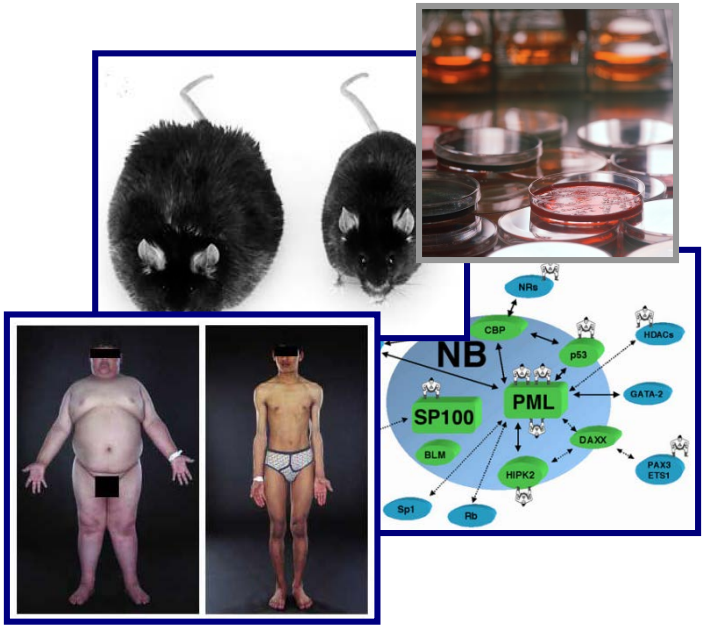


Genome-wide scans

= hypothesis-free, to identify new, unanticipated, genes and thus expand the view of the biology and physiology of disease/trait



Epidemiology



Physiology / Biology

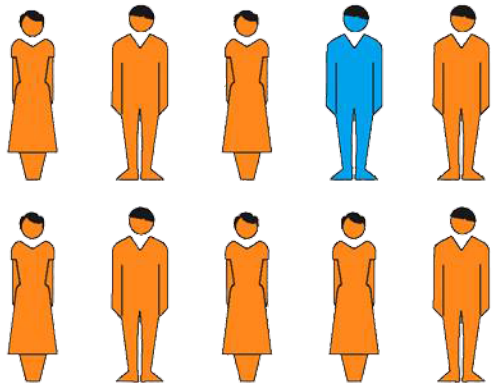
Basic Principle of Genetic Association Studies In Unrelated Individuals

Gene A

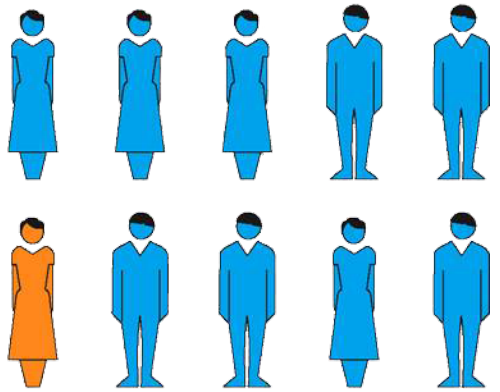
Affected

Unaffected

Gene B



Affected

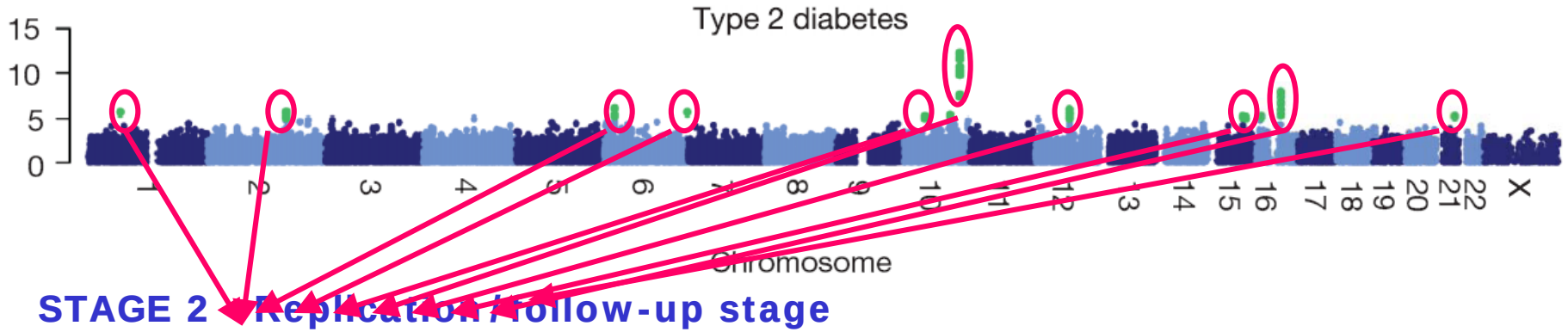


Unaffected

Genome-wide association study

STAGE 1 - Discovery stage

- 300,000 up to 1,000,000 genetic variants (SNPs)
- Case-control or cohort studies
- Large amount of tests → significance threshold $p < 0.0000001$



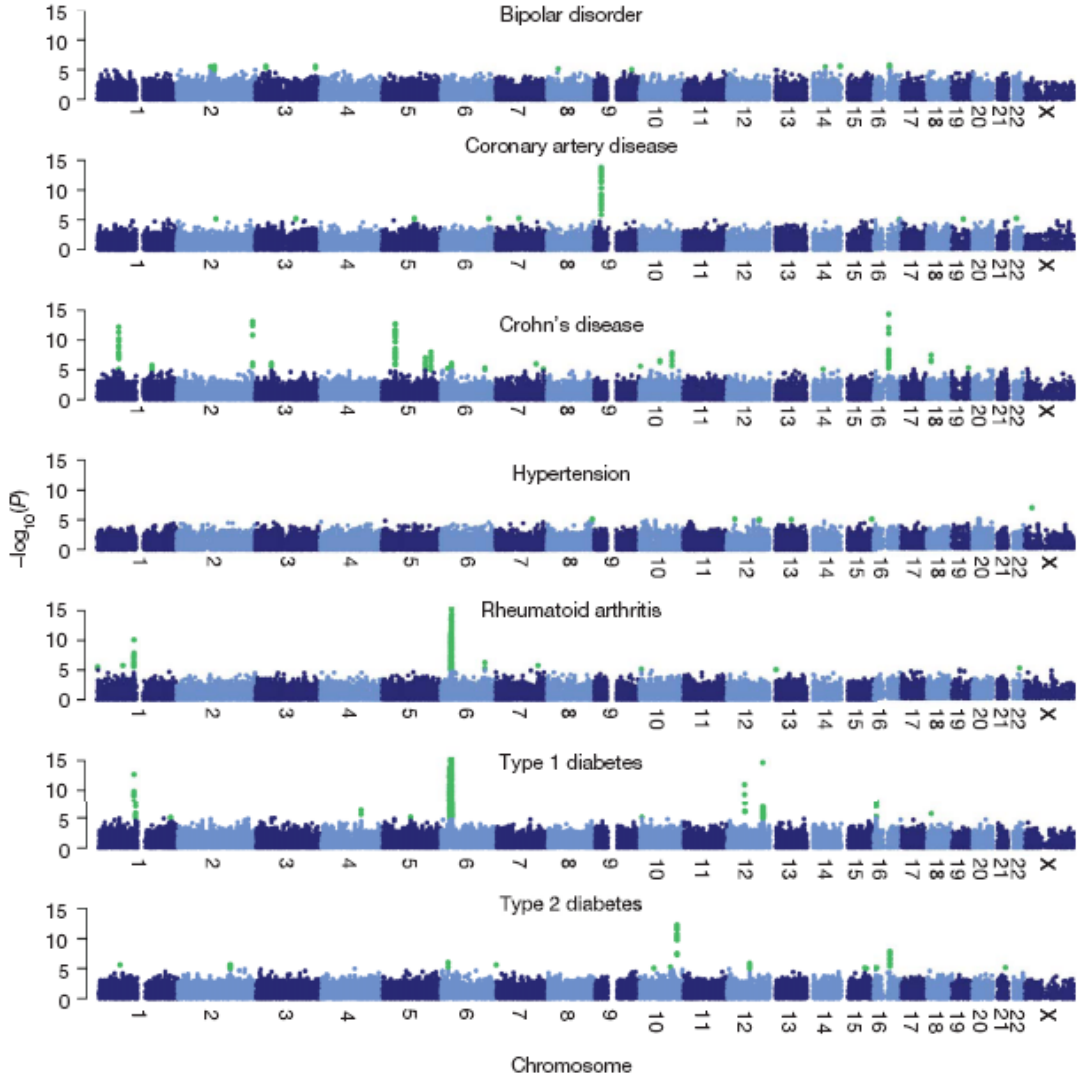
STAGE 2 - Replication / follow-up stage

- 1-100 SNPs that represent top loci
- Case-control or cohort studies – at least equal size of discovery panel
- Meta-analysis of stage 1 & 2 results

Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls

Nature 2007, 447: 661-678

The Wellcome Trust Case Control Consortium*



GWAs in IPF

Noth et al.
Lancet Respir Med 20013

N = 1584
600K SNPs
European-Americans

N = 2257
Europeans-Americans

MUC5B (11p15.5)
TOLLIP (*11p15.5*)
SPPL2C (17q21.31)

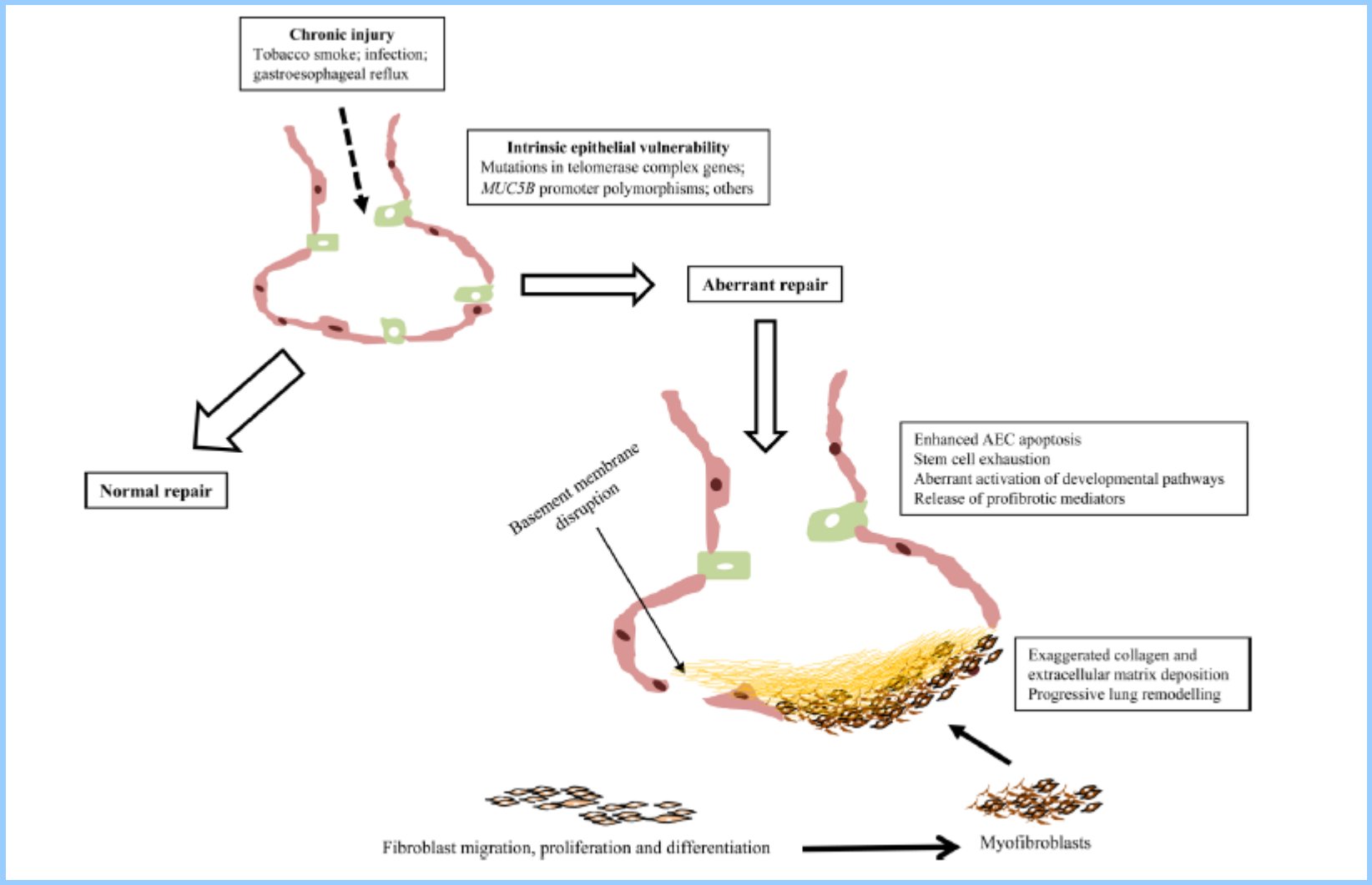
Fingerlin et al.
Nature Genetics 2013

N = 6299
600K SNPs
White Europeans

N = 2766
White Europeans

TERT (5p15.33)
MUC5B (11p15.5)
TERC (3q26.2)

Dall'Associazione Genetica alle Ipotesi Patogenetiche

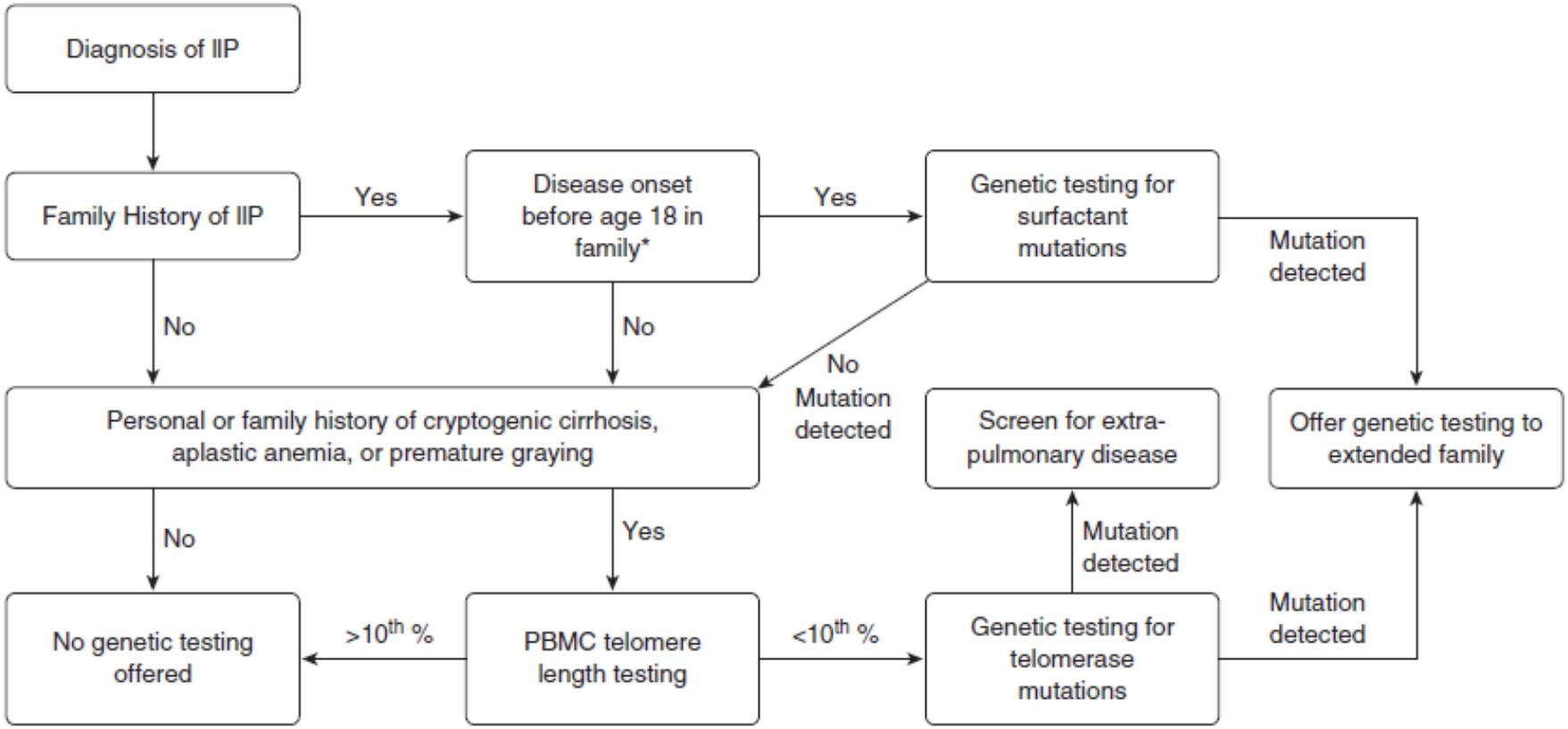


PULMONARY PERSPECTIVE

Genetic Evaluation and Testing of Patients and Families with Idiopathic Pulmonary Fibrosis

Jonathan A. Kropski[1], Lisa R. Young[1,2], Joy D. Cogan[3], Daphne B. Mitchell[1], Lisa H. Lancaster[1], John A. Worrell[4], Cheryl Markin[1], Na Liu[1], Wendi R. Mason[1], Tasha E. Fingerlin[5], David A. Schwartz[6], William E. Lawson[1,7], Timothy S. Blackwell[7,8,9], John A. Phillips III[3,10], and James E. Loyd[1]

Indicazioni al test genetico nei pazienti con IPF



Box 1 Pulmonary and extrapulmonary features of telomere syndromes

Pulmonary manifestations
Idiopathic pulmonary fibrosis
Non-specific interstitial pneumonia
Hypersensitivity pneumonitis
Bronchiolitis obliterans organising pneumonia
Unclassifiable pulmonary fibrosis
Premature-onset emphysema
Combined pulmonary fibrosis and emphysema
Haematological manifestations
Macrocytosis
Cytopenias (mostly thrombocytopenia)
Aplastic anaemia
B, T and natural killer cell immunodeficiency
Malignancies (myelodysplastic syndromes, acute myeloid leukaemia)
Gastrointestinal manifestations
Cryptogenic liver fibrosis/cirrhosis
Nodular atrophic liver
Liver transaminase elevation
Splenomegaly
Cutaneous manifestations
Premature hair greying/loss
Nail ridging
Bone manifestations
Osteoporosis
Avascular necrosis
Increased cancer risk (mainly epithelial cancers)
Chemotherapy/radiotherapy intolerance

Il Test ed il Counseling genetico nei pazienti con IPF: prospettive

- **Consentire la diagnosi negli stadi iniziali o subclinici**
- **Predire l'andamento clinico della malattia**
- **Predire la risposta al trattamento (PANTHER-IPF ; GERM'O'P)**

Il Test ed il Counseling genetico nei pazienti con IPF: limiti

- **Incompleta penetranza e variabilità fenotipica**
- **Rilevazione di varianti geniche rare di significato biologico incerto (VUS)**
- **Necessità di eseguire comunque un attento follow up indipendentemente dal risultato del test genetico**

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 20 centimetres separate the tallest 5% of a population from the shortest, then genetics would account for as many as 17 of them[1].

This year, three groups of researchers[2–4] scoured the genomes of huge populations (the largest study[3] 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?

Right under everyone's noses

The inability to find some genes could be explained by the limitations of GWAS. These studies have identified numerous one-letter variations in DNA called single nucleotide polymorphisms (SNPs) that co-occur with a disease or other trait in thousands of people. But a given SNP represents a much bigger block of genetic material. So, for example, if two people share one of these variants at a key location, both may be scored as having the same version of any height-related gene in that area, even though one person actually has a relatively rare mutation that has a huge effect on height. The association study might identify a variant responsible for the height difference, says Teri Manolio, director of the Office of Population Genomics at the NHGRI, but averaging across hundreds of people could give the appearance that its effects are pretty wimpy. "It's going to be diluted," she says.

Finding this type of missing heritability is conceptually easy, because it involves closer scrutiny of the genes already in hand. "Just exploring, in a very dense way, genetic variation at the loci that have been discovered is probably going to [explain] another increment of missing heritability," Hirschhorn says. Researchers will need to sequence candidate genes and their surrounding regions in thousands of people if they are to unearth more associations with the disease.

Helen Hobbs and Jonathan Cohen of the University of Texas Southwestern Medical Center in Dallas did this in an attempt to capture all the variation in *ANGPTL4*, a gene their studies had linked to cholesterol and triglyceride concentrations. They sequenced the gene in around 3,500 individuals from the Dallas Heart Study and found that some previously unknown variants had dramatic effects on the concentration of these lipids in the blood[7]. Mark McCarthy of Britain's Oxford Centre for Diabetes, Endocrinology and Metabolism says that such studies could reveal much of the missing heritability, but not a lot of people have had the enthusiasm to do them. This could change as the cost of sequencing falls.

Out of sight

Other variants, for which GWAS haven't even begun to provide clues, will prove even harder to find. In the past, conventional genetic studies for inherited diseases such as cystic fibrosis identified rare, mutated genes that have a high penetrance, meaning that the gene has an effect in almost everyone who carries it. But it quickly became apparent that high-penetrance variants would not underlie most common diseases because evolution largely keeps them in check.

What powered the push into genome-wide association was a hypothesis that common diseases would be caused by common, low-penetrance variants when enough of them showed up in the same unlucky person. Now that hypothesis is being questioned. "A lot of people are recognizing that screening for common variation has delivered less than we had hoped," says David Goldstein, professor of genetics at Duke University in Durham, North Carolina.

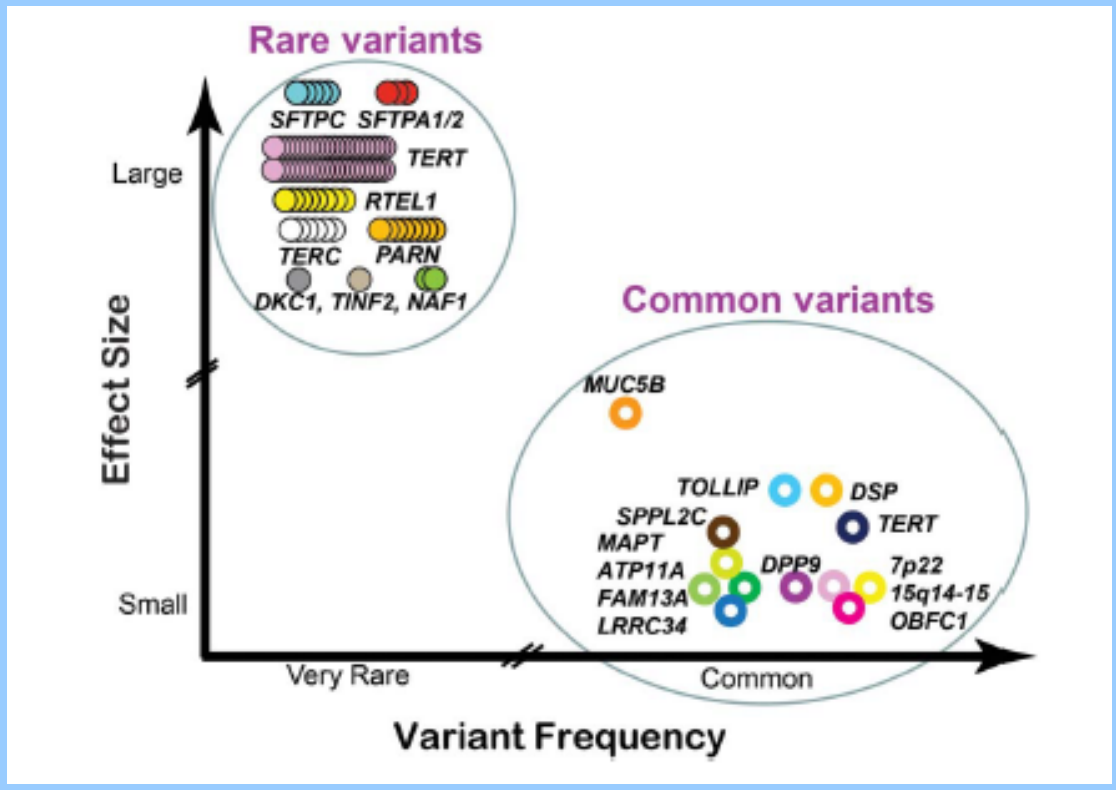
But between those variants that stick out like a sore thumb, and those common enough to be dredged up by the wide net of GWAS, there is a potential middle ground of variants that are moderately penetrant but are rare enough that they are missed by the net. There's also the possibility that there are many more-frequent variants that have such a low penetrance that GWAS can't statistically link them to a disease.

These very-low-penetrance variants pose some problems, says Leonid Kruglyak professor of ecology and evolutionary biology at Princeton University in New Jersey. "You're talking about thousands of variants that you would have to invoke to get near 80% or 90% heritability." Taken to the extreme, practically every gene in the genome could have a variant that affects height, for example. "You don't like to think about models like that," Kruglyak says.

If rare, moderately penetrant or common, weakly penetrant variants are the culprits, then bumping up the number of people in existing association studies could help find previously missed genetic associations. Peter Visscher of the Queensland Institute of Medical Research in Brisbane, Australia, says that a meta-analysis of height studies covering roughly 100,000 people is in the works. Lowering the stringency with which an association is made could drag up more, but confidence in the hits would drop.

At some point it might make sense to stop using SNPs, and start sequencing whole genomes. Collins suggests that the NHGRI's 1,000 genomes project, which aims to sequence the genomes of at least 1,000 people from all over the world, could go a long way towards finding hidden heritability, and many more genomes may become possible at the price of sequencing falls.

Not everyone supports an all-out sequencing onslaught. Goldstein warns against


Rare variants
SFTPC
SFTPA1/2
TERT
RTEL1
TERC
PARN
DKC1, TINF2, NAF1
Common variants
MUC5B
TOLLIP
DSP
SPPL2C
TERT
MAPT
ATP11A
DPP9
7p22
15q14-15
FAM13A
LRRC34
OBFC1
Large
Small
Effect Size
Very Rare
Common
Variant Frequency

Next Generation Sequencing

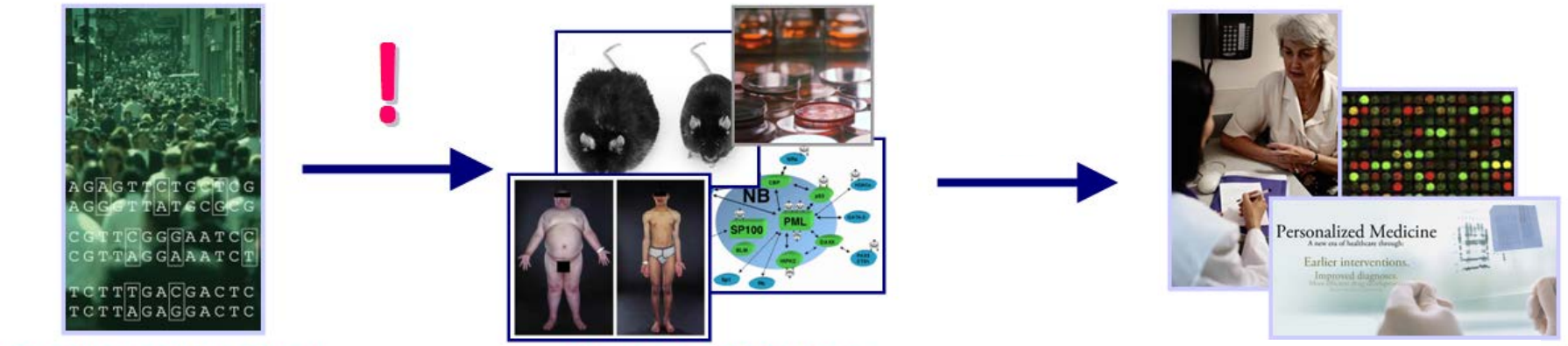
15Gb / 3.5 days

750Gb / 3.5 days

Conclusioni

- Negli ultimi → numerosi nuovi loci di suscettibilità per malattie complesse, incluso l' IPF, sono stati identificati

- La maggior parte sono loci 'inattesi' → che promettono di evidenziare nuovi meccanismi biologici



Genetic epidemiology

Physiology/Biology

GWAS Studies: Just the Start……

"This is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning."

Sir Winston Churchill @ Lord Mayor's Luncheon,
Mansion House following the victory at El Alamein in North Africa
London, 10 November 1942.