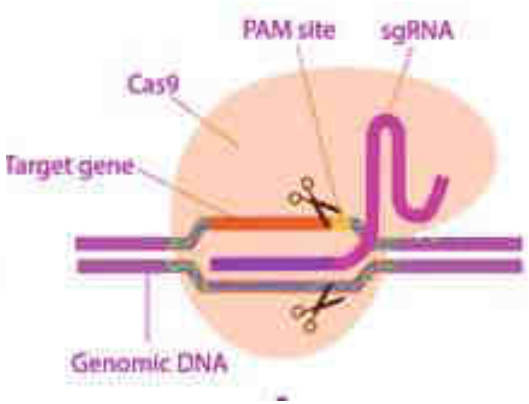
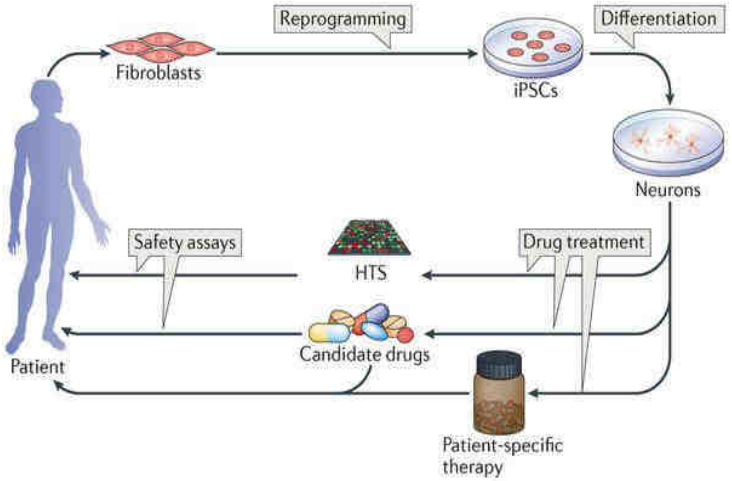
Genetics and drug discovery



CRISPR Tools

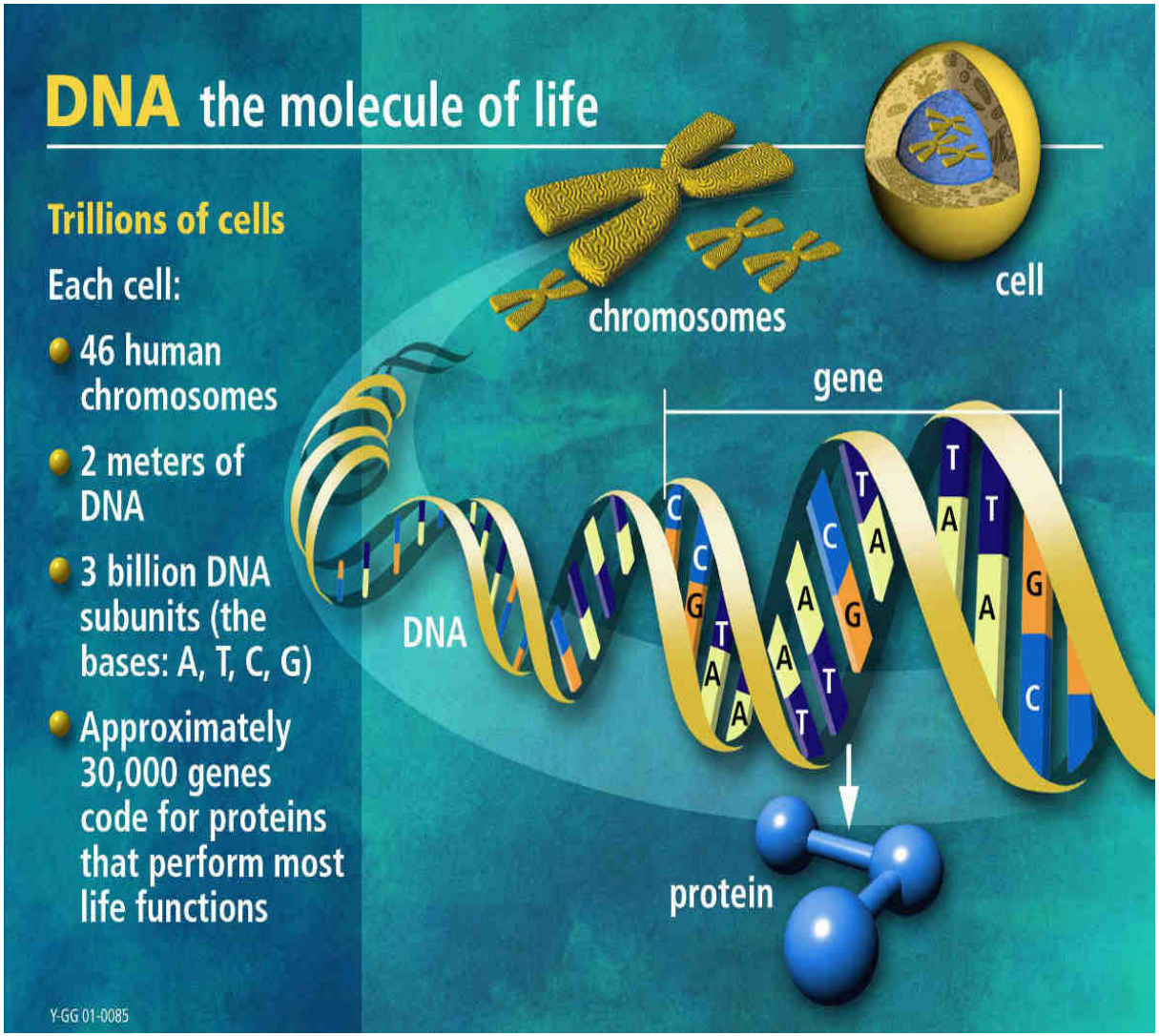


IPSC cells

Human genetics, applications, opportunities and career prospects

Srinivas Reddy Pallerla B.Pharm, PhD
https://srinivaspallerla.weebly.com/

Intro to Human genetics



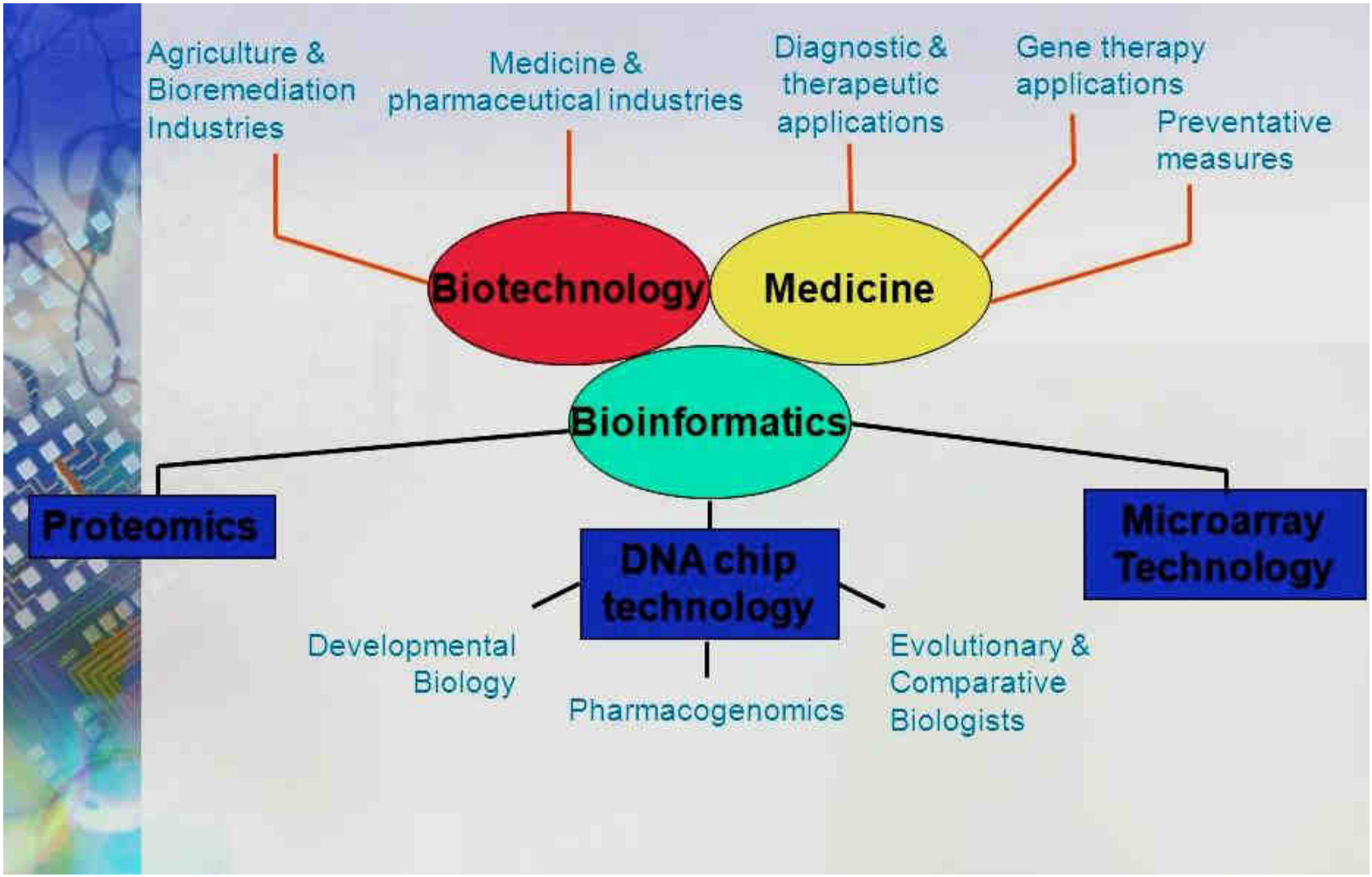
- Only 2% is coding
- we are 99.9% identical

(1 in 1000 bps ~ 3 million bases)

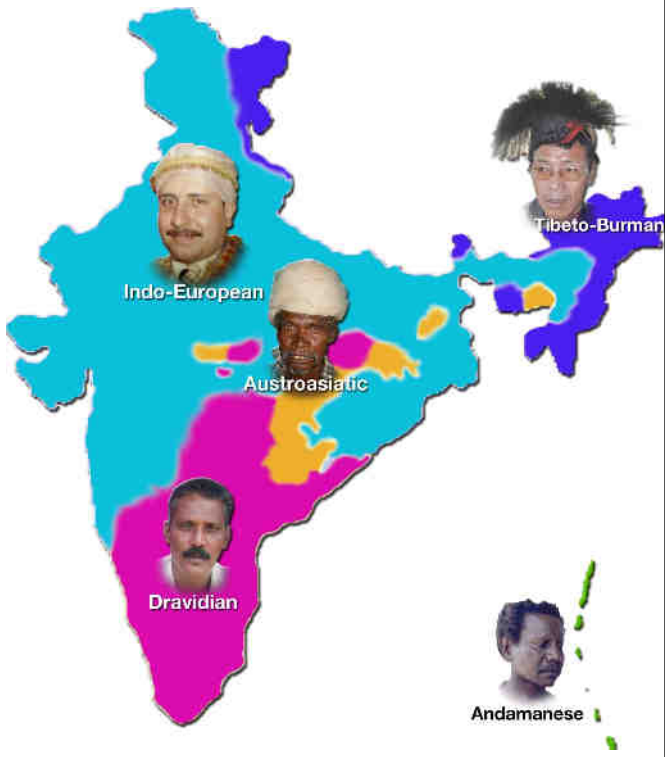
- This is what that makes us Unique
- These differences can be
- 60 new variants

A day might come where whole genome will be sequenced as soon as we born

Why we need to study genetics?



- Whole exome sequencing
- SNP genotyping microarrays



Advances in Sequencing technologies

2001 (Whitehead) (Illumina)

2016

13 Years & 3.8 billion $

12 hours & 1000$

- UK- 100000 , Iceland -100000, Saudi Arabia-100000 samples, US and China - 1 Million & faroe islands - whole population (PLOS Biology | DOI:10.1371/journal.pbio.1002195)
- 1000 genome project (2504 sequenced & 500 are sequenced from India 118 are ITU (**Indian Telugus in UK**)

Sequencing technologies

Sanger

Ion Torrent

Roche 454

Illumina *Seq

Pacific Biosciences

Nanopore

illumina®

Infinium® Global Screening Array-24 v1.0

A powerful, high-quality, economical array for population-scale genetic studies.

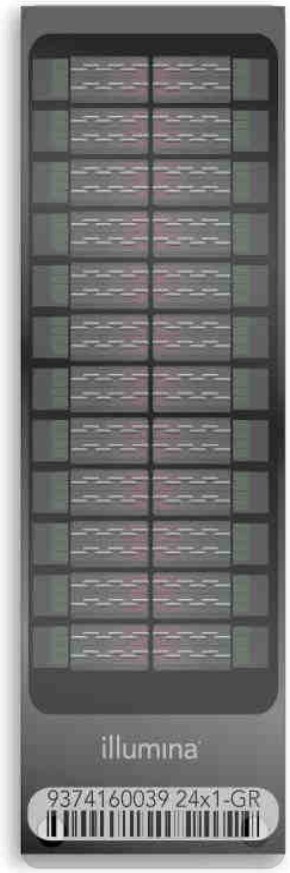


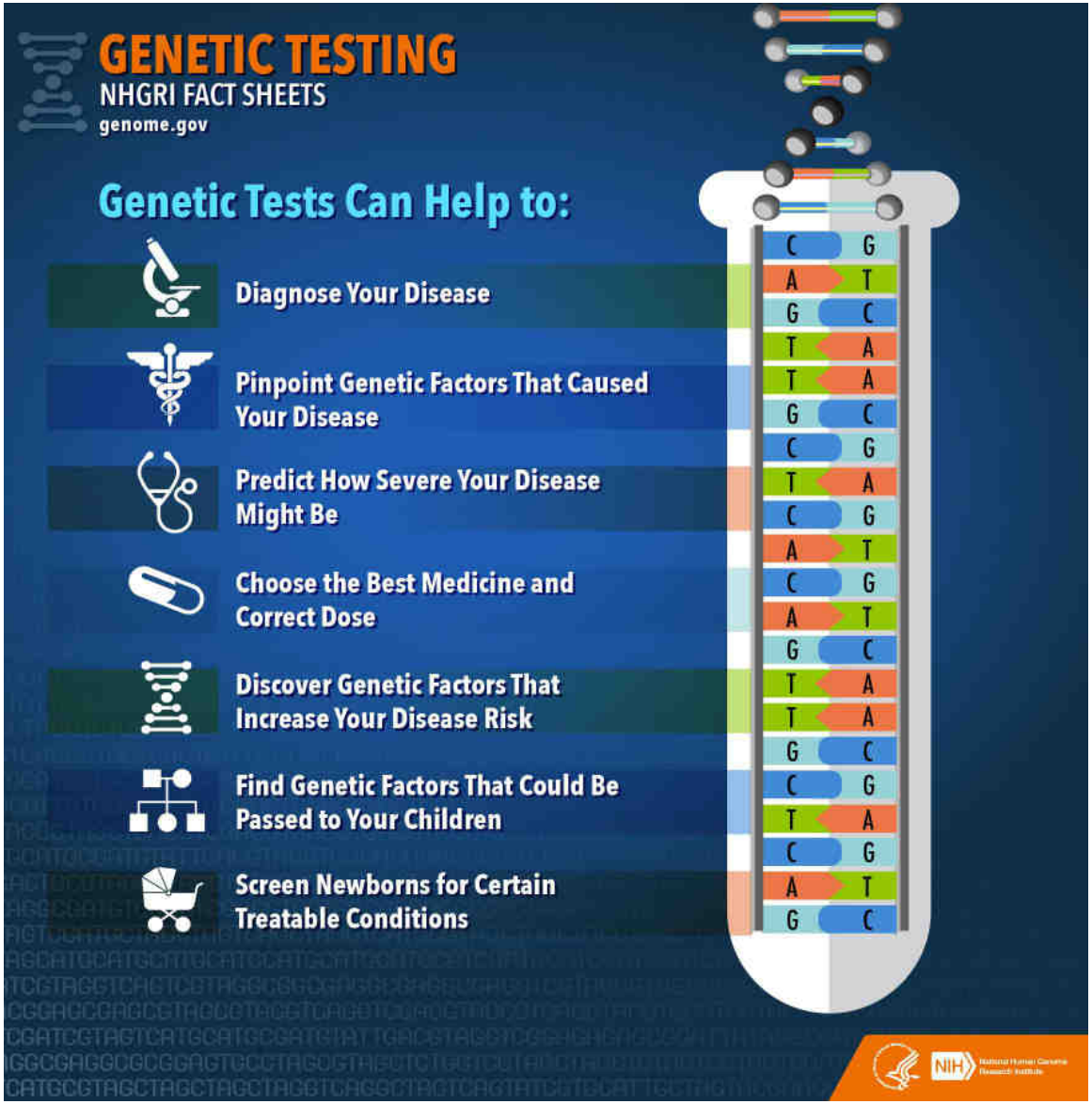
Table 1: Product Information

Feature	Description	
Species	Human	
Total Number of Markers	642,824	
Capacity for Custom Bead Types	50,000	
Number of Samples per BeadChip	24	
DNA Input Requirement	200 ng	
Assay Chemistry	Infinium HTS	
Instrument Support	iScan or HiScan® System	
Sample Throughput[a]	~ 2304 samples/week	
Scan Time per Sample	iScan System 2.5 min	HiScan System 2.0 min

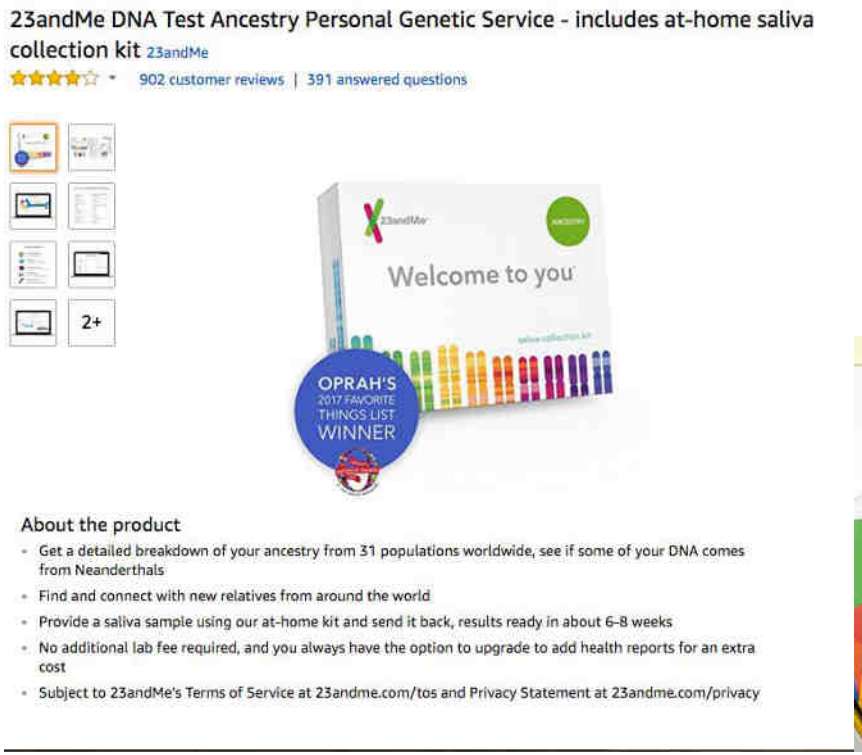
a. Estimate assumes 1 iScan System, 1 AutoLoader, 2 Tecan robots, and a 5-day work week.

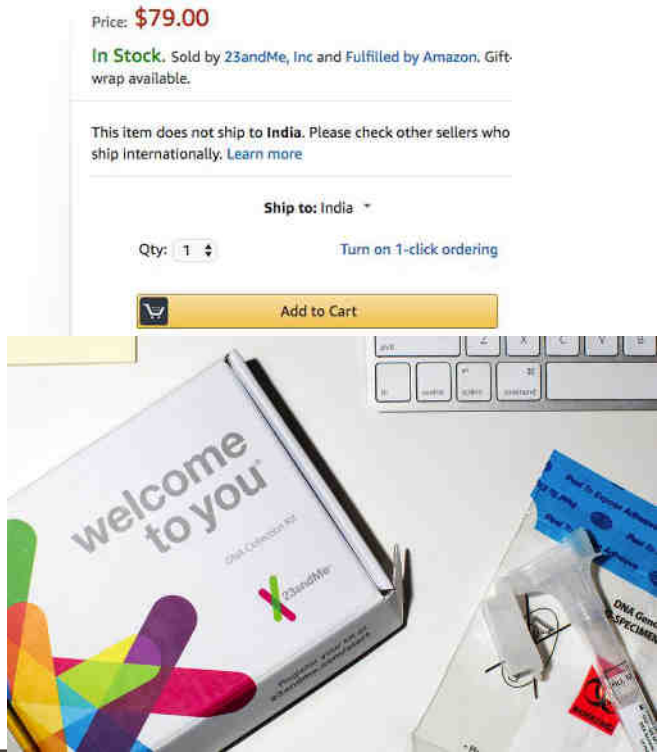
Table 2: High-Value Content

Content	No. of Markers	Research Application/Note
ADME Core and Extended Genes	5816	Drug metabolism and excretion
ADME Core and Extended Genes +/- 10 kb	7246	Drug metabolism and excretion (plus regulatory regions)


GENETIC TESTING
NHGRI FACT SHEETS
genome.gov
Genetic Tests Can Help to:
Diagnose Your Disease
Pinpoint Genetic Factors That Caused Your Disease
Predict How Severe Your Disease Might Be
Choose the Best Medicine and Correct Dose
Discover Genetic Factors That Increase Your Disease Risk
Find Genetic Factors That Could Be Passed to Your Children
Screen Newborns for Certain Treatable Conditions
NIH

You can buy genetic testing on AMAZON.com (e-commerce website)





Pharmacogenetic testing through the direct-to-consumer genetic testing company 23andMe

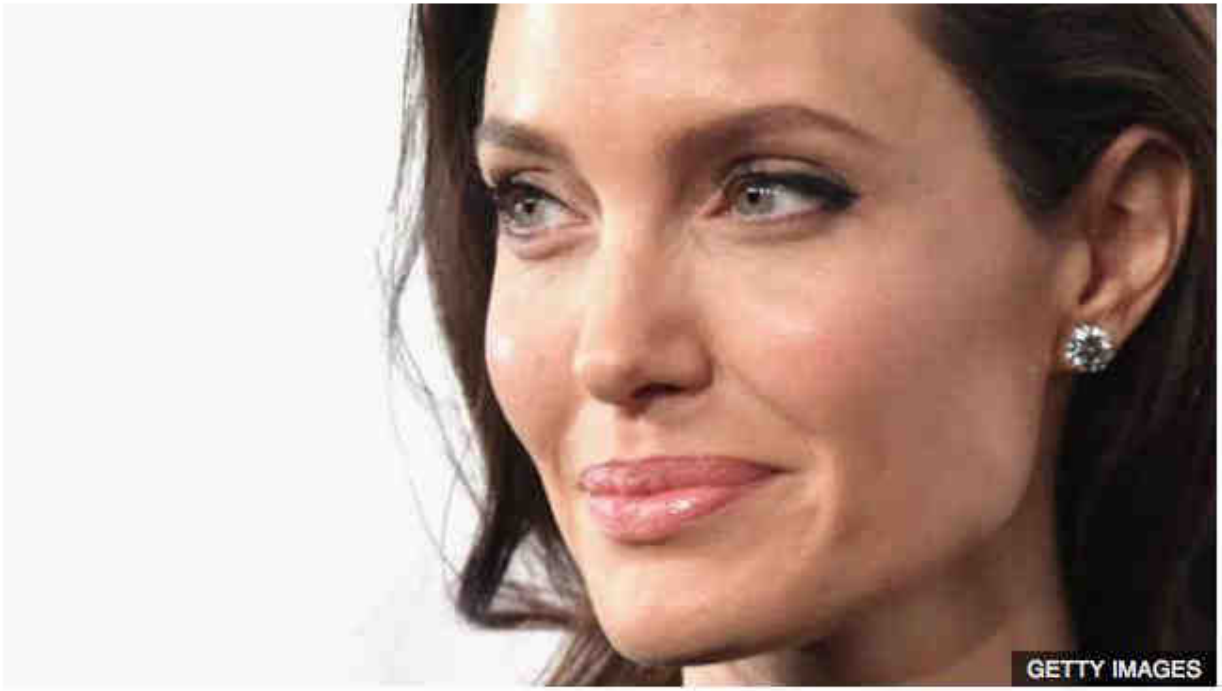
Mengfei Lu[1], Cathryn M. Lewis[1,2] and Matthew Traylor[1*]

Conclusion: 23andMe reports provide some useful pharmacogenetics information, mirroring clinical tests that are in standard use. Other tests are unspecific, providing limited guidance and may not be useful for patients without professional interpretation. Nevertheless, DTC companies like 23andMe act as a powerful intermediate step to integrate pharmacogenetic testing into clinical practice.

Angelina Jolie gene testing for all?

By James Gallagher
Health and science correspondent, BBC News

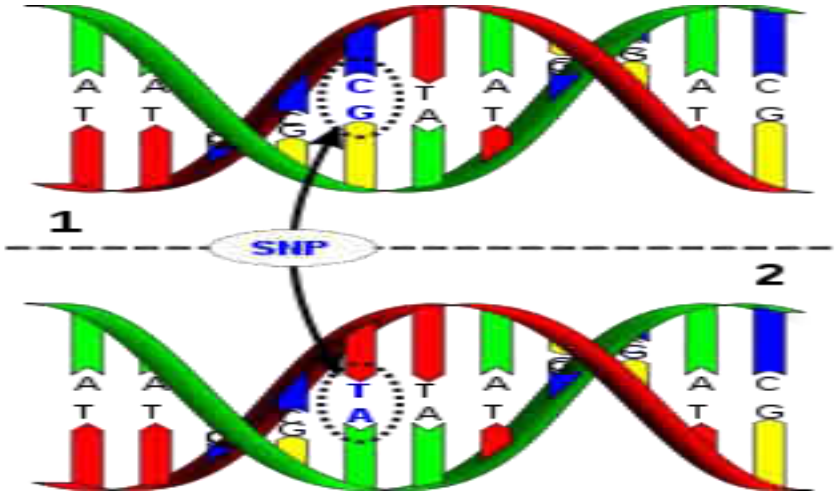
18 January 2018 109 Share



Testing all women for the "Angelina Jolie gene", even if not considered at risk, would prevent cancers, save lives and is cost effective, say doctors.

Genetic variation

- Single Nucleotide Polymorphisms
- Most common and well studied form of variation
- Defined by a population frequency > 1%



http://www.gbhealthwatch.com/Trait-Genetics-101.php

Overview:

I. Intro to human genetics, Sequencing and SNP genotyping technologies and applications

II. Pharmacogenetics

III. Application CIRSPR/Cas9 Tech & in basic research and drug discovery,

IV. How genetic studies nailed down a gene that is imp in glucose homeostasis: (My research at CCMB for the past 4 years)
T2D complex disease genetics and functional genomics of HKDC1(Hexokinase domain containing gene 1) gene.

V. Career prospects : After Pharmacy

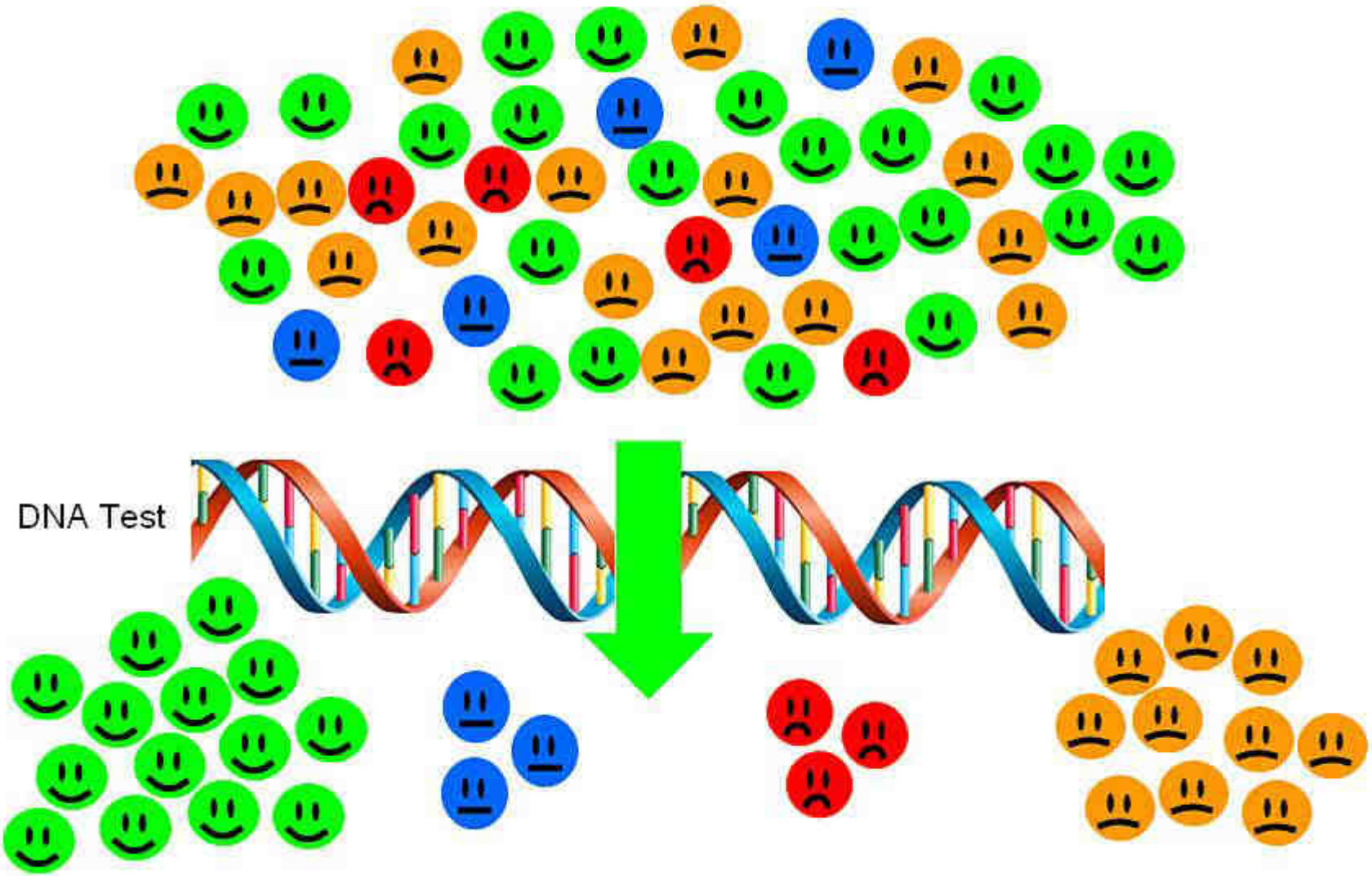
VI. Brief overview on current hot areas in research
Bioinformatics and statistics
Gut microbiota
Metabolomics
induced pluripotent stem cells (iPSC)

Pharmacogenomics & Pharmacogenetics: From DNA to Drug Treatment

"Here's my sequence..."

New Yorker, 2000

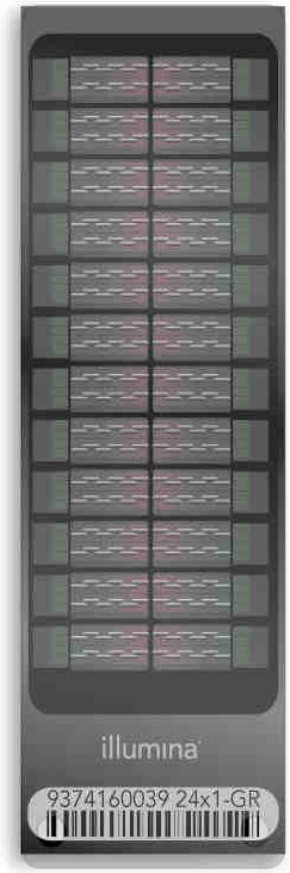
Your DNA Affects Your Response to Drugs



illumina®

Infinium® Global Screening Array-24 v1.0

A powerful, high-quality, economical array for population-scale genetic studies.



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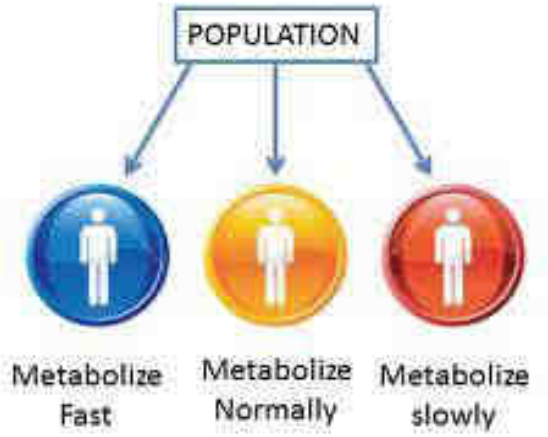
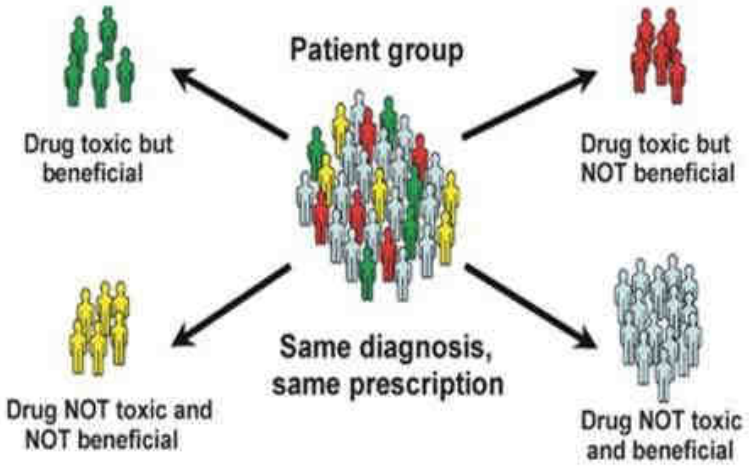
Back to the drugs…

- The utility of pharmacogenetics:
  - Determining appropriate dosing
  - Avoiding unnecessary toxic treatments
  - Ensuring maximal efficacy
  - Reducing adverse side effects
  - Developing or choosing novel treatments
  - Can also explain variable response to illicit drugs





Genetic epidemiology of pharmacogenetic variations in *CYP2C9*, *CYP4F2* and *VKORC1* genes associated with warfarin dosage in the Indian population

Anil K Giri[1,2,†], Nazir M Khan[1,†], Sandeep Grover[1,†], Ismeet Kaur[1], Analabha Basu[1], Nikhil Tandon[4], Vinod Scaria[2,3], IGV Consortium, INDICO[5], Ritushree Kukreti[1], Samir K Brahmachari[1] & Dwaipayan Bharadwaj*[1,2]

[1]CSIR-Institute of Genomics & Integrative Biology, Delhi, 110 020, India
[2]Academy of Scientific & Innovative Research (AcSIR), Anusandhan Bhawan, 2 Rafi Marg Delhi, 110 001, India
[3]National Institute of BioMedical Genomics, Kalyani, 741 251, India
[4]Department of Endocrinology, All India Institute of Medical Sciences, New Delhi, 110 029, India
[5]GN Ramachandran Knowledge Center for Genome Informatics, CSIR-Institute of Genomics & Integrative Biology, Mathura Road, Delhi, 110 020, India
*Author for correspondence:
Tel.: +91 11 29879246
Fax: +91 11 2766 7471
dib@igib.res.in
†Authors contributed equally
‡A complete list of the members of the INDICO consortia can be found in the online Supplementary Material

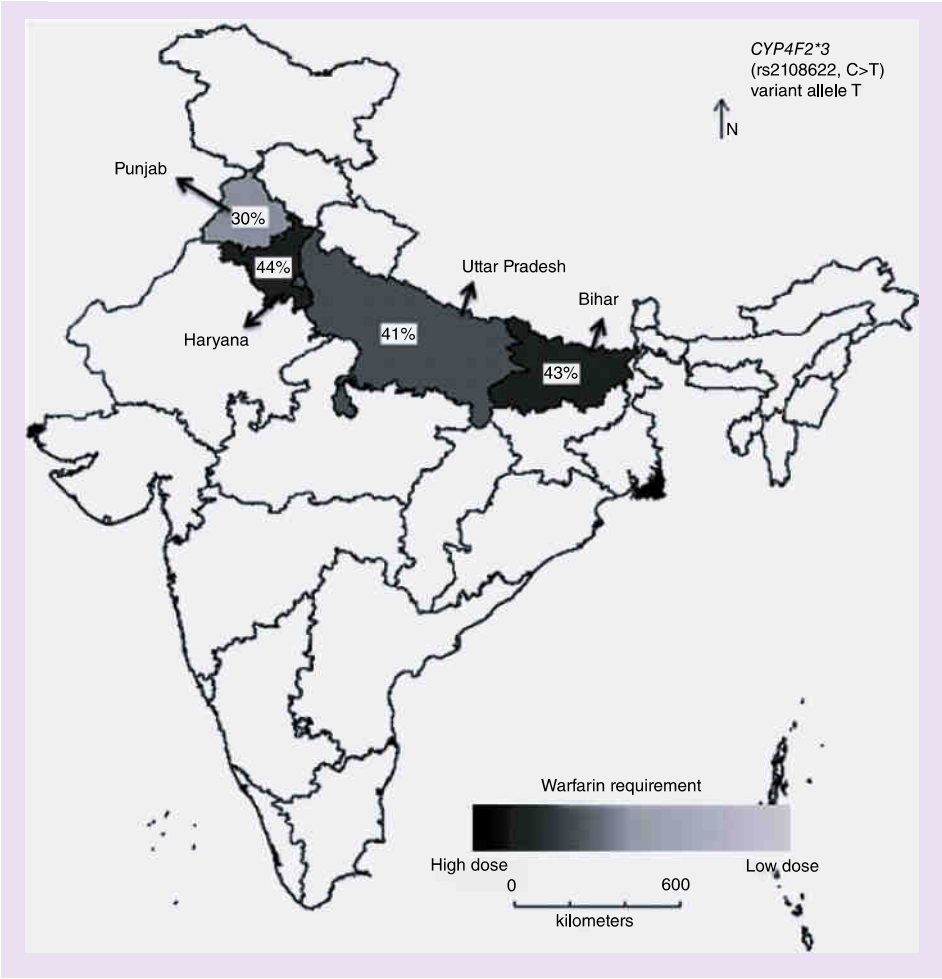


Figure 2. Allele frequency distribution among north Indians. Distribution of *CYP4F2*3* allele frequency (variant allele T) was shown in various states of India. States are shaded according to minor allele frequency.

- Pharmaco***genomics***
  - The science of how genes affect the way people people respond to drugs
  - How genes affect…

    …the way our body processes drugs (pharmacokinetics)

    …the interaction of drugs with receptors (pharmacodynamics)

    …the treatment efficacy and adverse side effects

- Pharmaco***genetics***
  - A subset of ‘pharmacogenomics’
  - The study of how ***inherited variation*** affects drug response and metabolism

FDA approved pharmacogenetic tests

Gene	Drug	Consequence
TPMT	6MP	Toxicity
CYP2D6	Tamoxifen	Decreased efficacy
UGT1A1	Irinotecan	Toxicity
CYP2D6	Codeine	Ineffective analgesia

For more info browse following web sites:

PharmGKB

CPIC™
Clinical Pharmacogenetics Implementation Consortium

Warfarin: A dosage story

- Most widely used anticoagulant in the world
  - A "blood thinner"
- Prescribed doses vary widely (1-40mg / daily)
- Therapuetic index is very low
  - High risk of bleeding early in treatment
- Two genes involved in metabolism: *CYP2C9* and *VKORC1*

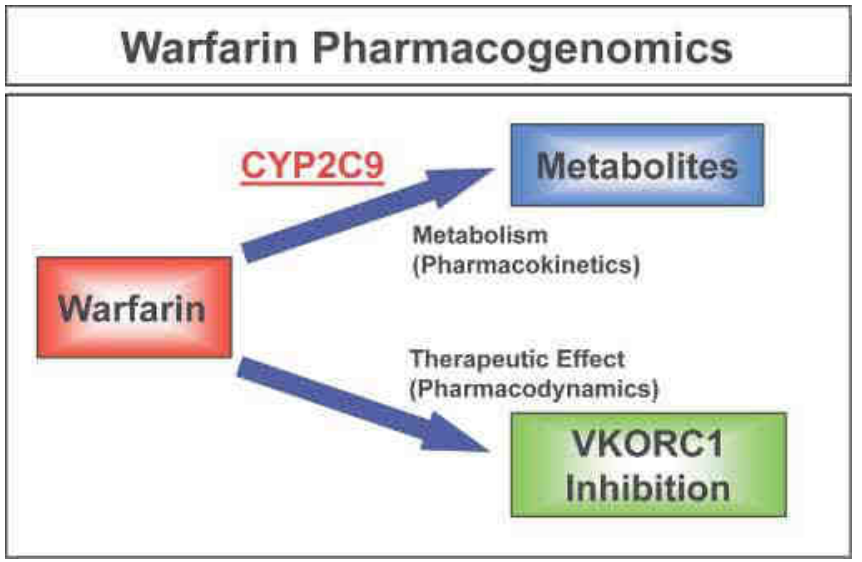


Table 1. Content of commercially available absorption, distribution, metabolism and excretion genotyping products compared to the panel developed in this study.

Company	Assay	Number of genes	Number of markers	Design criteria
	ADME Panel	~180	3000 (plus the ability to spike in an additional 72)	Known and putative functional variation in ADME genes as well as tag SNPs that account for blocks of LD across ADME genes
Affymetrix®	DMET™ Plus Premier Pack	225	1936	Known functional and putative functional variants in ADME genes
Sequenom®	iPLEX® ADME PGx panel	36	192	99% of ADME core list plus additional functional haplotypes
Illumina®	VeraCode ADME Core Panel	34	184	Known functional variants including deletion and duplications in ADME genes as defined by the PharmaADME working group
Roche®	AmpliChip™ CYP450 Test	2	~22	Known functional variants including deletion and duplications in *CYP2D6* and *CYP2C19*
Progenika®	PHARMAchip	36	~90	Known functional variants including deletion and duplications in PK and PD genes.

ADME: Absorption, distribution, metabolism and excretion; LD: Linkage disequilibrium; PK: Pharmacokinetics; PD: Pharmacodynamics.

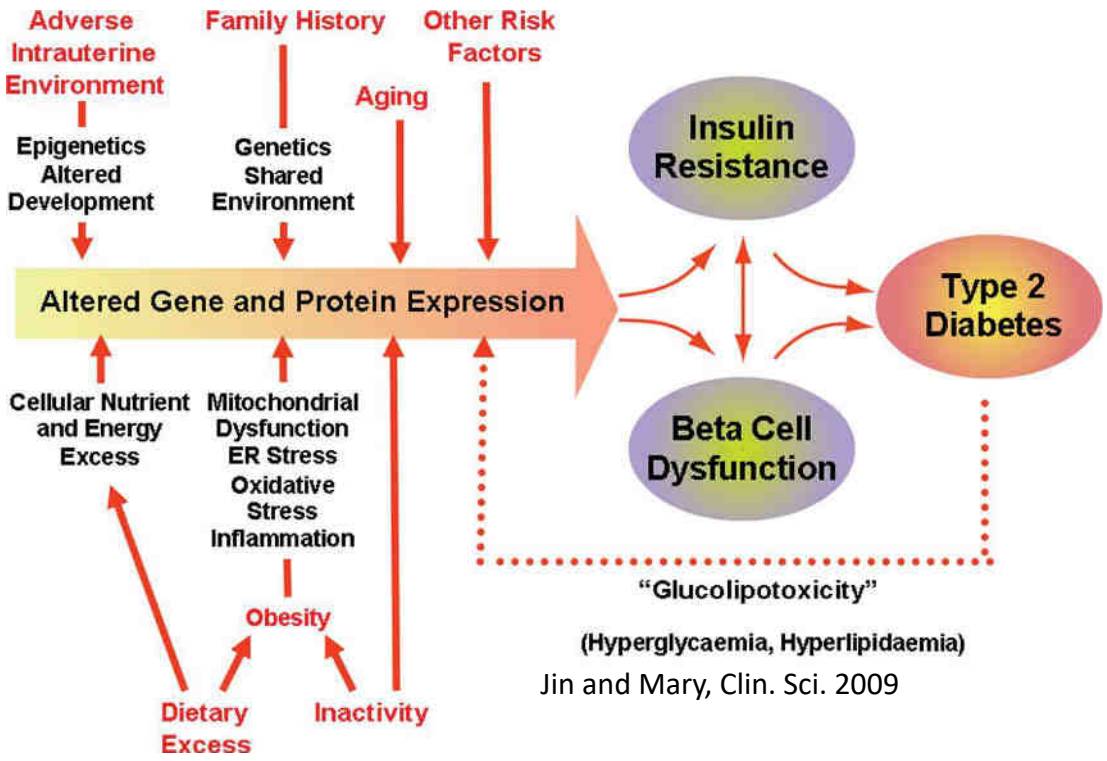
Type 2 diabetes complex disease genetics

Post-GWAS (Genome Wide Association Studies) functional genomics of *HKDC1* (Hexokinase domain containing gene 1) locus in glucose homeostasis and metabolism

Srinivas Reddy Pallerla B.Pharm, PhD
https://srinivaspallerla.weebly.com/

Supervisor: Dr. GR. Chandak, MD, PhD
Center for Cellular and Molecular Biology
Hyderabad

I. Background



Jin and Mary, Clin. Sci. 2009

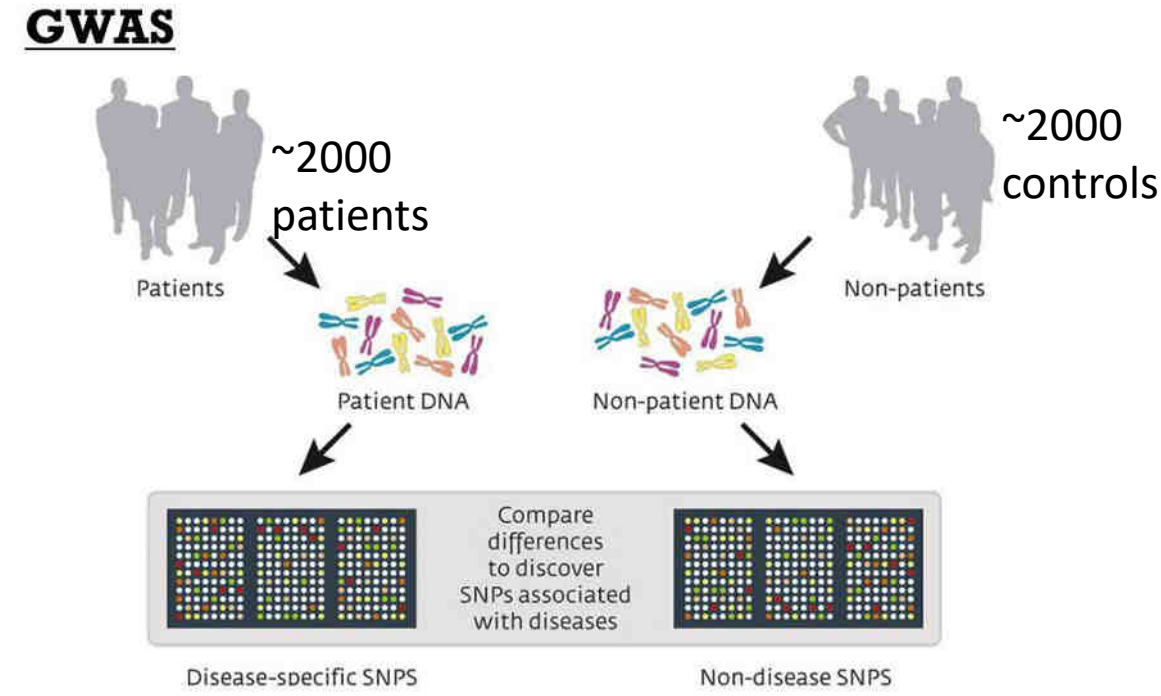
- T2D is a global problem
- India - second highest number of diabetics, 69 million – 2015, reach 123 million by 2040
- Huge economic burden
- T2D can only be treated, but cannot be reversed
- Need to better understand T2D

- Twin and family studies: genetics contribute to the development of T2D
- To understand the genetics of diabetes our lab performed GWAS (Genome wide Association studies)

Genetics of Type 2 diabetes (T2D) in Indians: GWAS

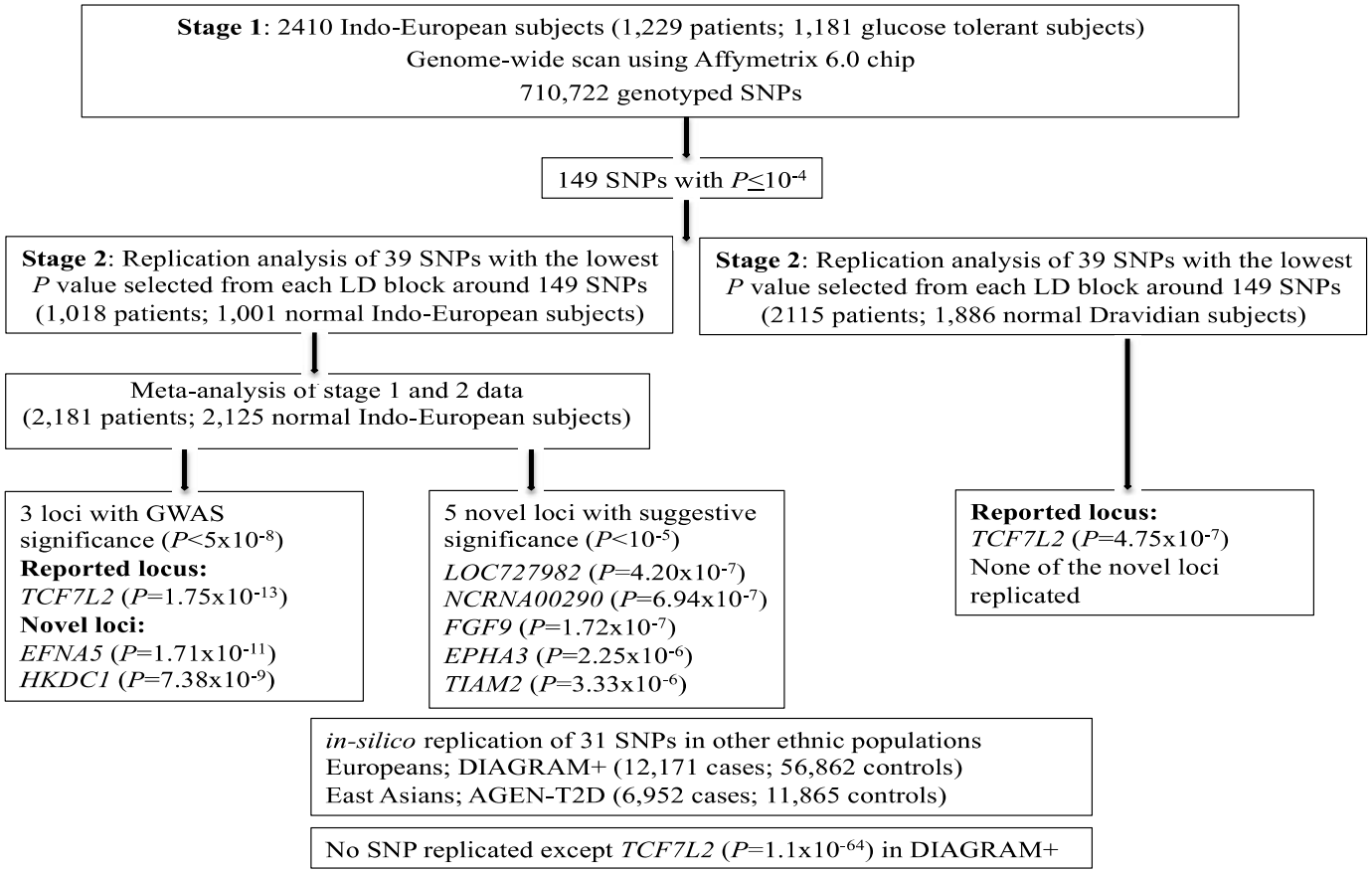
Genetic studies: genotyped 1 million SNPs (Single Nucleotide polymorphisms)
Affymetrix array

Affymetrix
SNP array



http://www.mpg.de/10680/Modern_psychiatry

Type 2 Diabetes Case – Control GWAS Studies - Indian population



- rs7089312 & SNP rs7085830 (OR:1.22 ; $P = 2.03x10^{-5}$ & OR:1.68 ; $P = 7.38x10^{-9}$) variants in HKDC1 (Hexokinase domain containing gene 1) associated for T2D

Association studies to novel therapeutic targets?

Kazuo et al., Expert Opinion on Therapeutic Targets, 2015

Table 1. T2D-associated genes linked to targets of drugs in clinical or preclinical studies.

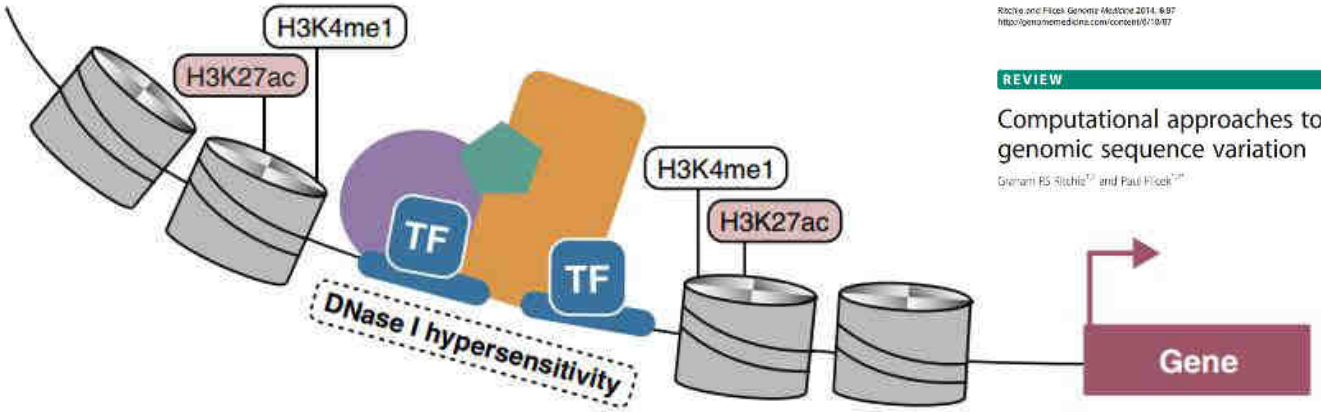
Gene	Index variant	Trait associated with variant	Drug class	Interaction type	Preclinical study in model animals	Clinical trial
KCNJ11/ ABCC8	rs5215	T2D	Sulfonylurea	Inhibitor	Completed	Completed
PPARG	rs4135250	T2D	Thiazolidinedione	Agonist	Completed	Completed
GLP1R	rs10305492	T2D, FG	GLP-1 analogue	Agonist	Completed	Completed
GCK	rs4607517	Fasting glucose, T2D, HOMA-B	Glucokinase activator	Activator	Lowered glucose level	Phase II
GCKR	rs780094	T2D, fasting glucose, triglycerides, fasting insulin	GCKR disrupter	Disrupter	Lowered glucose level	Phase II
ADRA2A	rs10885122	Fasting glucose, T2D, HOMA-B	Alpha2-adrenergic receptor antagonist	Agonist	Improves impaired insulin secretion	Phase I
SLC30A8	rs13266634	Fasting glucose, HOMA B, T2D	Zinc	Substrate	Alleviated the insulin resistance and hyperglycemia	Phase I
MTNR1B	rs10830963	T2D, beta-cell function	Melatonin, melatonin receptor agonists	Agonist	Decreases insulin secretion	Phase III
ADIPOQ	rs1501299	T2D, HOMA-IR, adiponectin level	AdipoRon	Agonist	Pleiotropic effects	NA
ADCY5	rs11708067	T2D, 2 hour glucose, HOMA- B	Forskolin	NA	Lowered fasting glucose	Phase III
FADS1	rs174550	Fasting glucose, T2D, HOMA-B	Alpha-Linolenic acid, icosapent	Ligand	Improved glucose tolerance	Phase I
MC4R	rs12970134	T2D, BMI, waist circumference, insulin resistance	MC4-agonist	Agonist	Decreased food intake and decreased body weight	Phase II
PAM	rs35658696	T2D	*N*-alpha-acetyl-3,5-diiodotyrosylglycine	NA	NA	NA

Notes: BMI, body mass index; HOMA-B, homeostasis model assessment of beta-cell; HOMA-IR, homeostasis model assessment of insulin resistance; NA, not applicable; T2D, type 2 diabetes..

II. Post-GWAS functional characterization of *HKDC1* locus

HKDC1 locus on Chromosome 10q22

***HKDC1* SNPs associated for T2D**



Genome Medicine

REVIEW

Computational approaches to interpreting genomic sequence variation

Figure 1 Model of enhancer function. Transcriptional enhancer elements are noncoding stretches of DNA that regulate gene expression levels, most often in *cis*. Active enhancer elements are located in open chromatin sensitive to DNase I digestion and flanked by histones marked with H3K4me1 and H3K27ac. Enhancers are often bound by a number of transcription factors (TF), such as p300 (blue). Mediator and cohesin are part of a complex (orange, green and purple) that mediates physical contacts between enhancers and their target promoters.

HKDC1 in glucose homeostasis and metabolism?

Molecular evolution of the vertebrate hexokinase gene family: Identification of a conserved fifth vertebrate hexokinase gene. Irwin & Tan Genomics Proteomics. 2008. – Probable enzyme and highly conserved from early vertebrates to humans

- Hexokinases (HKI, HKII, HKIII & HKIV) : Glucose to Glucose-6-P
- Play important role in development and glucose homeostasis and metabolism
- What is the role of HKDC1 in glucose homeostasis and metabolism?

Key questions:

1. What is the phenotype of *Hkdc1* knockout in mice?

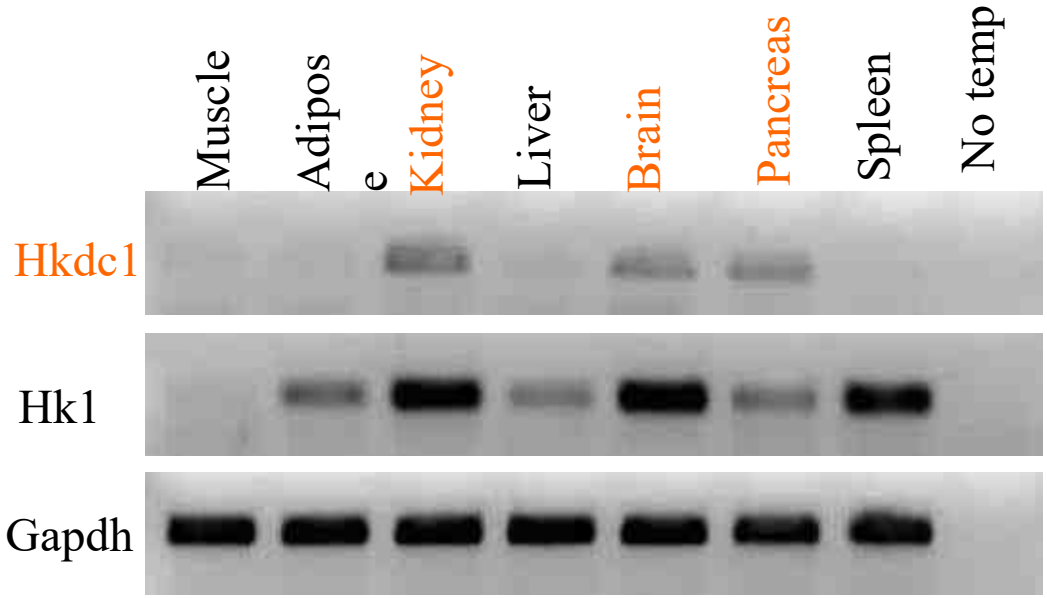
2. Does human *HKDC1* gene code for a protein with the biochemical properties similar to other hexokinases?

- **Gestational diabetes GWAS study found variant (rs4746822) in *HKDC1* associated with diabetes during pregnancy (Hayes et al., 2013)**

1: *Hkdc1* KO in mouse: diabetic?

- Before generating KO mice – gene expression in various tissues
- Adult mouse – metabolically active tissues isolated and expression was checked

Expression in adult metabolically active tissues



- **GTEx – human tissue expression database**

Ubiquitous with highest in intestine, thymus & kidney

Hkdc1 is expressed in kidney, brain and pancreas

Hkdc1 KO in mouse: (Work in progress @ CCMB)

Pipeline – Generating Hkdc1 KO mice :

Purchased From KOMP	336 ES clones : 6 clones +ve	Blastocyst injections	Hkdc1 KO mice is generated & currently phenotypic characterization underway

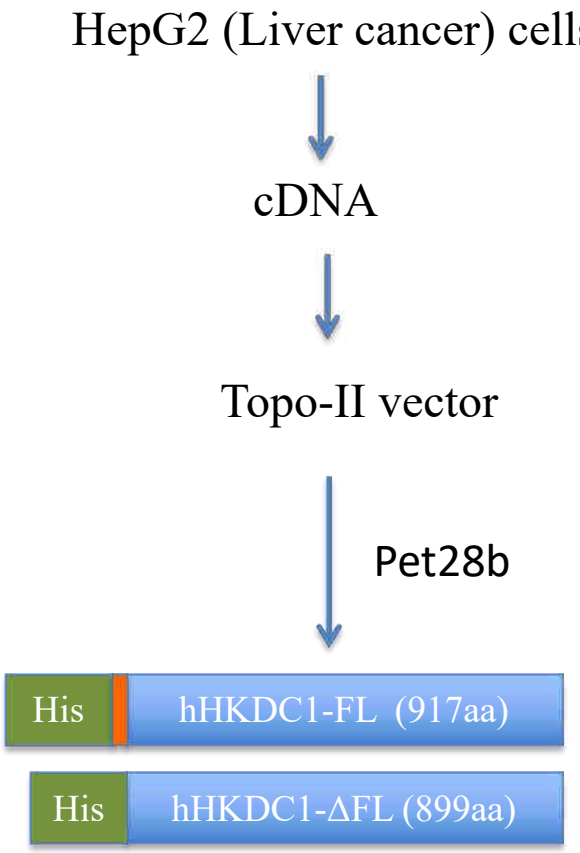
HKDC1 Is a Novel Hexokinase Involved in Whole-Body Glucose Use, Ludvik et al. Endocrinology, 2016.

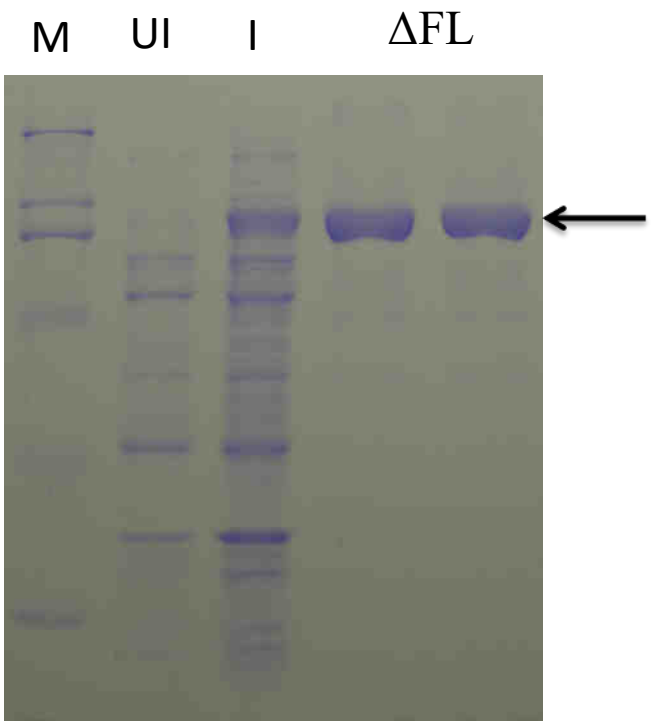
GWAS resulted in identification of novel gene HKDC1: glucose homeostasis & metabolism

Phenotype ? Causal tissue ? Causal mechanism ?

CCMB Transgenic facility team : Dr. Chandrashekar P (In-charge), Purnima, Jyothi & Sarathi

2. Cloning, expression & biochemical characterization of Human HKDC1



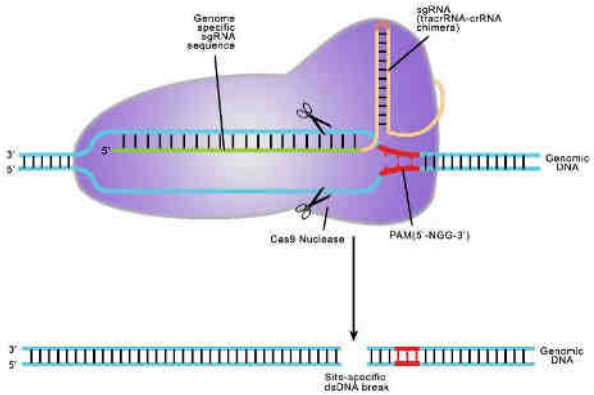


Specific activity - U/mg	Km Glucose- (mM/L)	Km ATP- (mM/L)
0.42 +/-0.1	~0.3	~ 0.2

Amruthanjali & Gunjan (Junior research fellows)

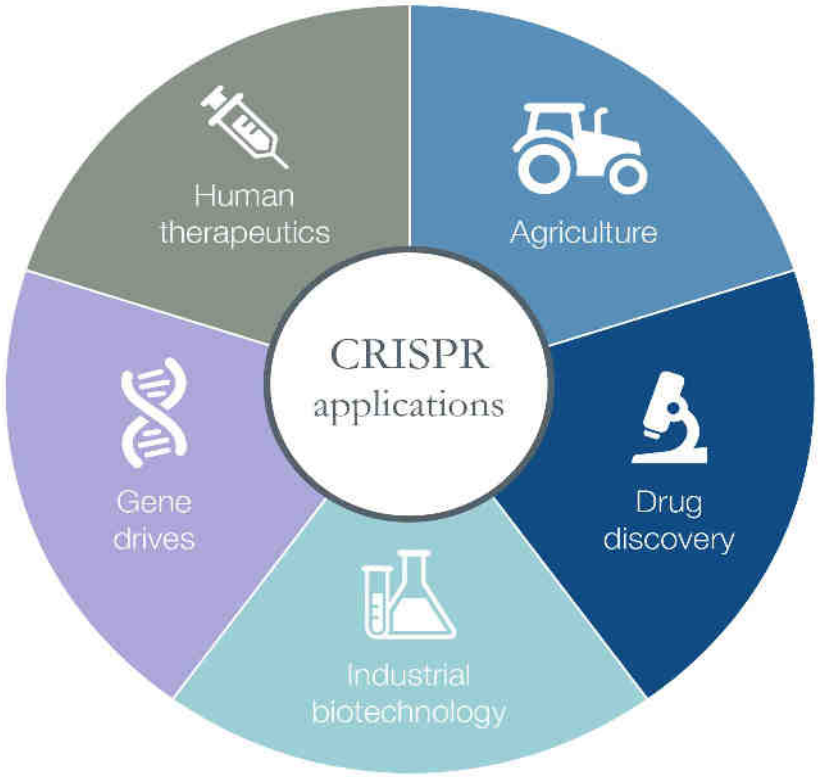
Application of CRISPR gene editing in drug discovery and therapeutics

II. CRISPR/Cas9 genome editing technology



- CRISPR technology is a simple yet powerful tool for editing genomes.
- Faster, cheaper, easier, precise, multiplex gene editing

- Applications of CRISPR technology



AstraZeneca
IMED Biotech Unit

Transforming and Translating Drug Discovery – Use of CRISPR/Cas9 in Target Discovery, Hit Finding, Translational Studies & Gene Therapy

Lorenz Mayr, VP & Global Head
IMED Discovery Sciences – Biological Reagents & Assay Development

February 21, 2017
CRISPR 2017, Boston/MA

CRISPR/Cas9 in Drug Discovery

Summary

Precise-Genome Editing (PGE) with **CRISPR/Cas9** is a breakthrough technology for drug discovery at Pharma & Biotech with applications in:

- Personalised Medicine ('precision medicine')
- Target Finding
- Target Validation
- Hit/Lead Finding
- Safety & DMPK
- Possibly also **Therapeutic Genome Editing** (TGE) – we are currently building disease models and refine technologies for NHEJ, HDR, delivery technologies

AstraZeneca and our **collaboration partners (academia/industry)** will continue to drive the future development of **CRISPR/Cas9 technologies, and** we are very open to **additional partnerships ('open innovation')**

Jayamuki college of Pharmacy

Careers after pharmacy : **INTERDISCIPLINARY KNOWLEDGE imp**

Research : Basic or Translational: PhD
Diagnostics technologies
Marketing
Biotechnology: product development
Forensics
Food technology & analytics
Teaching
Drug discovery
Data scientist:
Genetic counselor
Managing Clinical trials
Managing cohorts (prospective/retrospective)

SKY is the limit

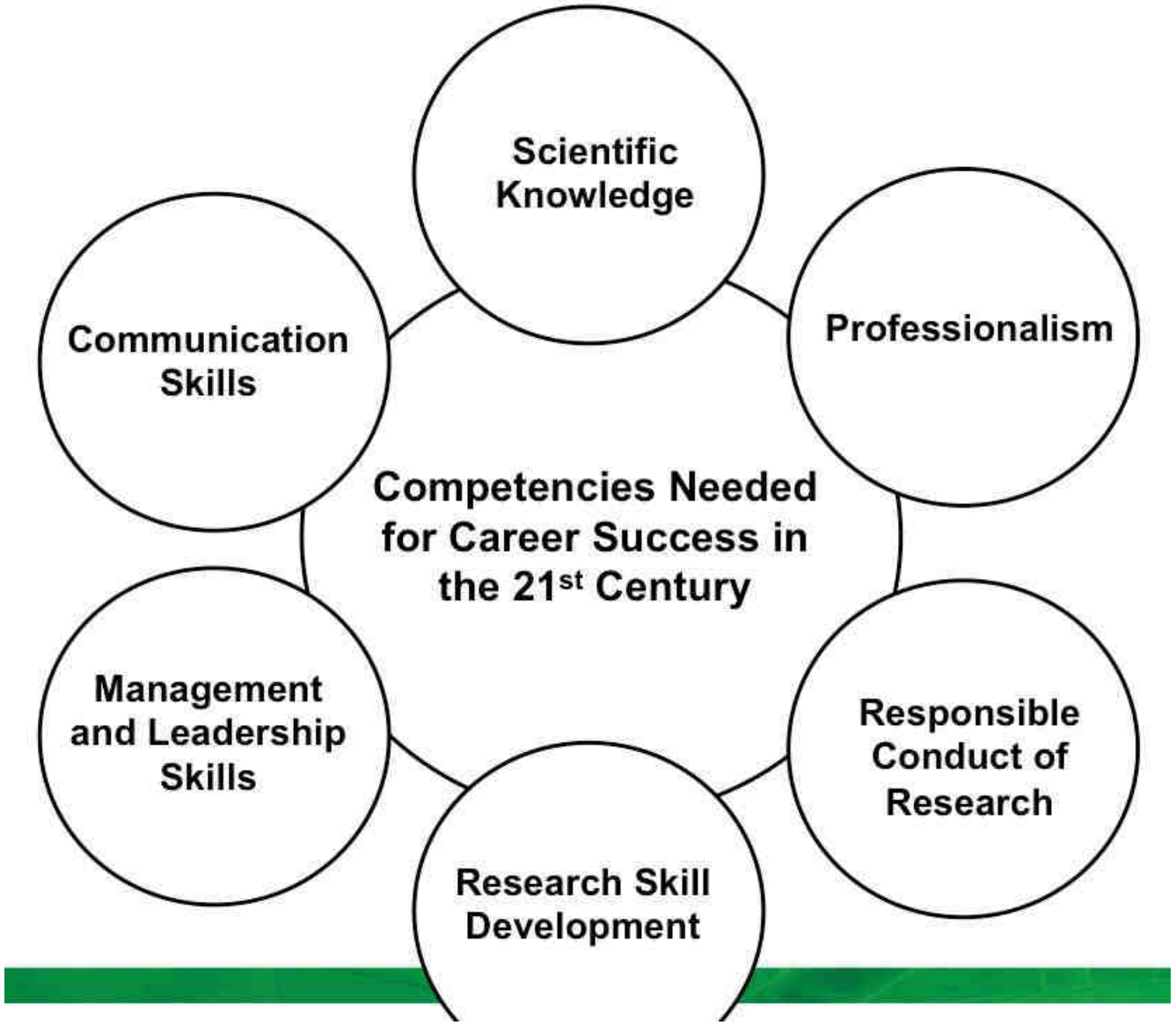
www.bioinformatics.ca

Canadian Bioinformatics Workshops

Careers in Research

- Interdisciplinary Knowledge : Pharmacy + additional knowledge
- IF you aim for something you need to build skills that are necessary in that area (Read books), watch motivational videos, networking, public relations
- Soft skills : communication, writing, presenting,
- Collaborative skills
- Explore information in the area of your interest in public databases
- Imagination is the key for success
- Upgrade your skills in the area of your interest
- Take risks and work hard (Noting is going to be easy _ work hard and smart)

(The me I see the me I will be) : You will be what you think


Scientific Knowledge
Professionalism
Communication Skills
Competencies Needed for Career Success in the 21st Century
Management and Leadership Skills
Responsible Conduct of Research
Research Skill Development

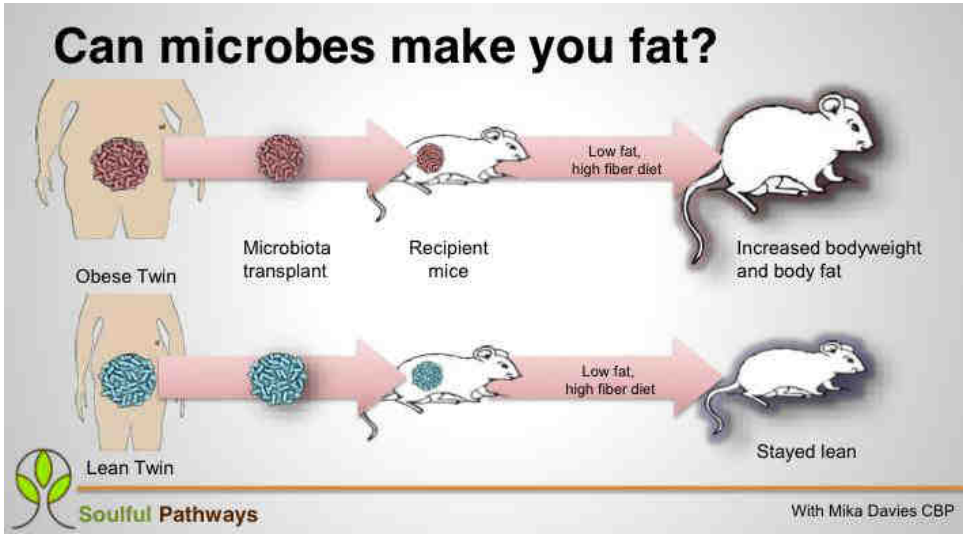
Gut Microbiota

Gut Microbiota

100 Trillion Microbes!

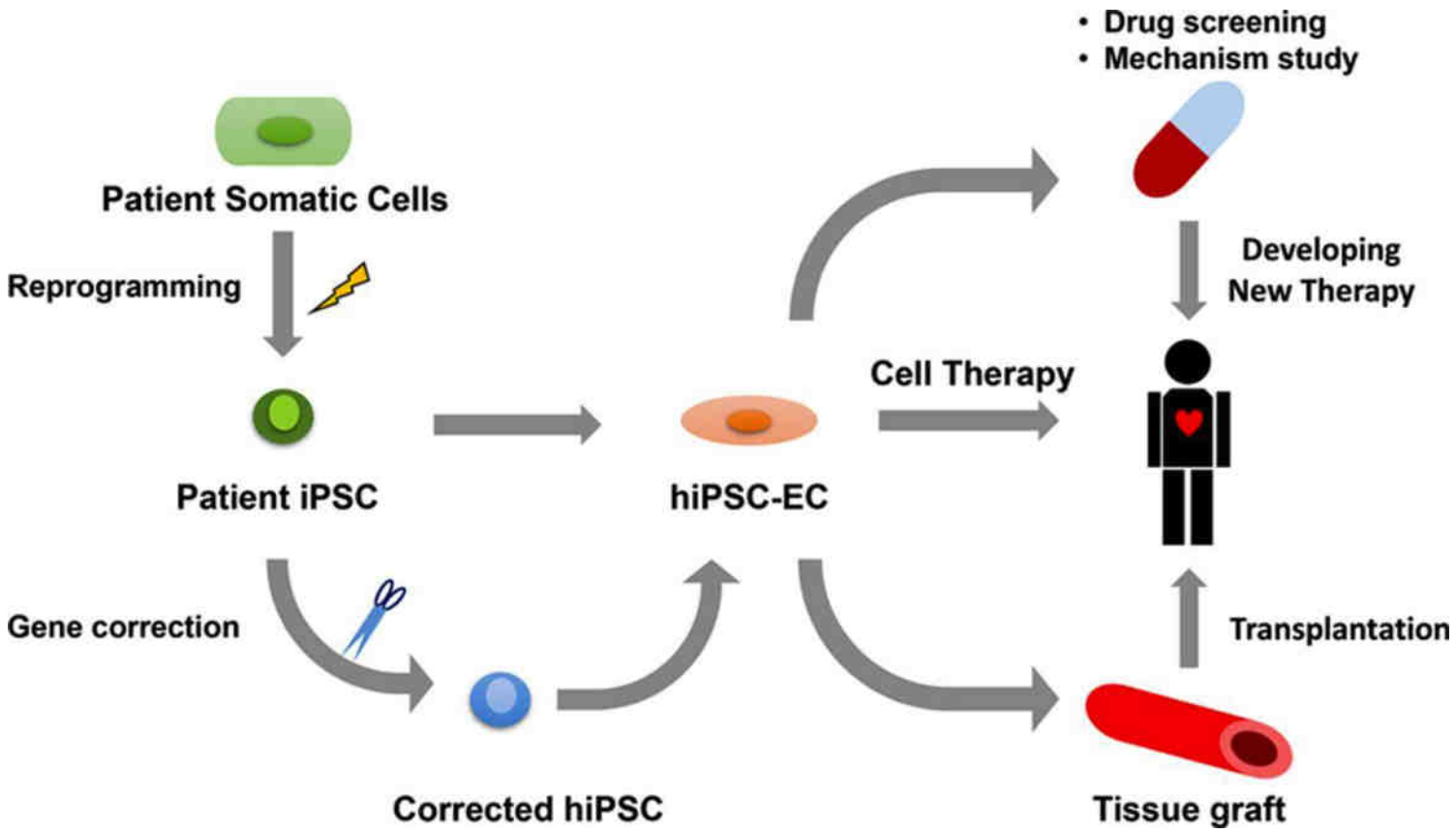
A healthy diverse microbiome:

- Protects us against pathogens
- Trains our immune system
- Essential for healthy digestion
- Determines whether we are fat or lean
- Enhances brain function and mood
- Reduces the tendency to form kidney stones
- Keeps your skin radiant



https://www.slideshare.net/soulfulpathways/feel-better-already-microbiome-health

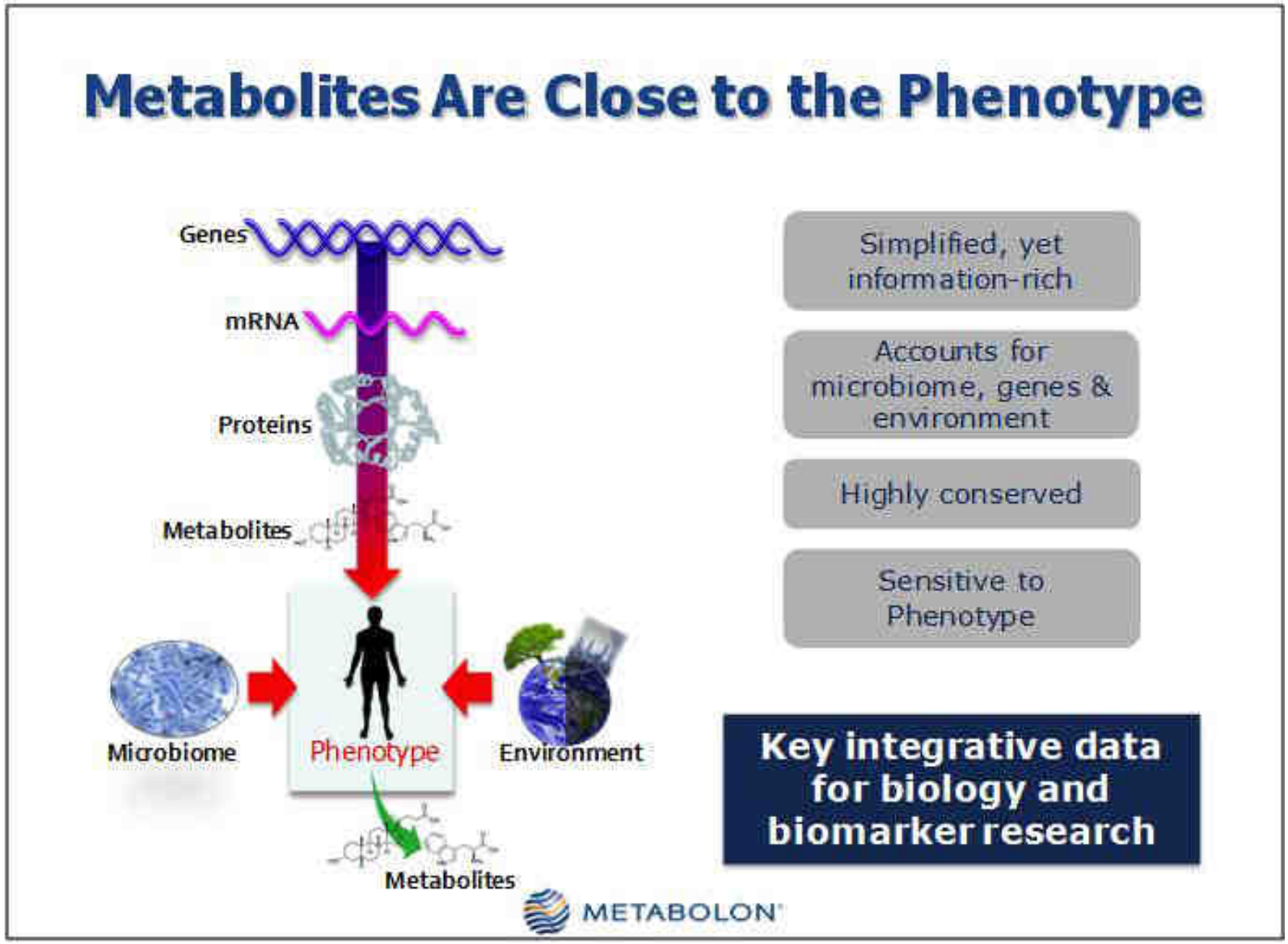
Application of Patient derived iPSC in Therapeutics and drug discovery



Differentiation, Evaluation, and Application of Human Induced Pluripotent Stem Cell–Derived Endothelial Cells
Yang Lin, Chang-Hyun Gil, Mervin C. Yoder

Metabolomics

Metabolomics profiling to identify the biomarkers that pe rdispose



General approach to metabolomics

Biological Samples

Human & Animal
- fluids
- tissues

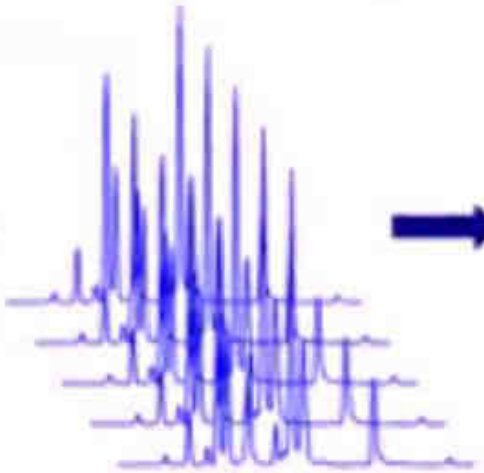
Yeast

Bacteria

Cultured cells

Growth media

Analytical Profiling



Key features:
- multiple metabolites
- signal proportional to [metabolite]
- precision & robustness

Statistics & Data Analyses

- univariate and multivariate statistics
- correlations
- pathway analyses

Scientific Discovery

- Signatures of disease/biomarkers
- Metabolic phenotypes
- Drug activity and efficacy
- Discovery of new biological mediators

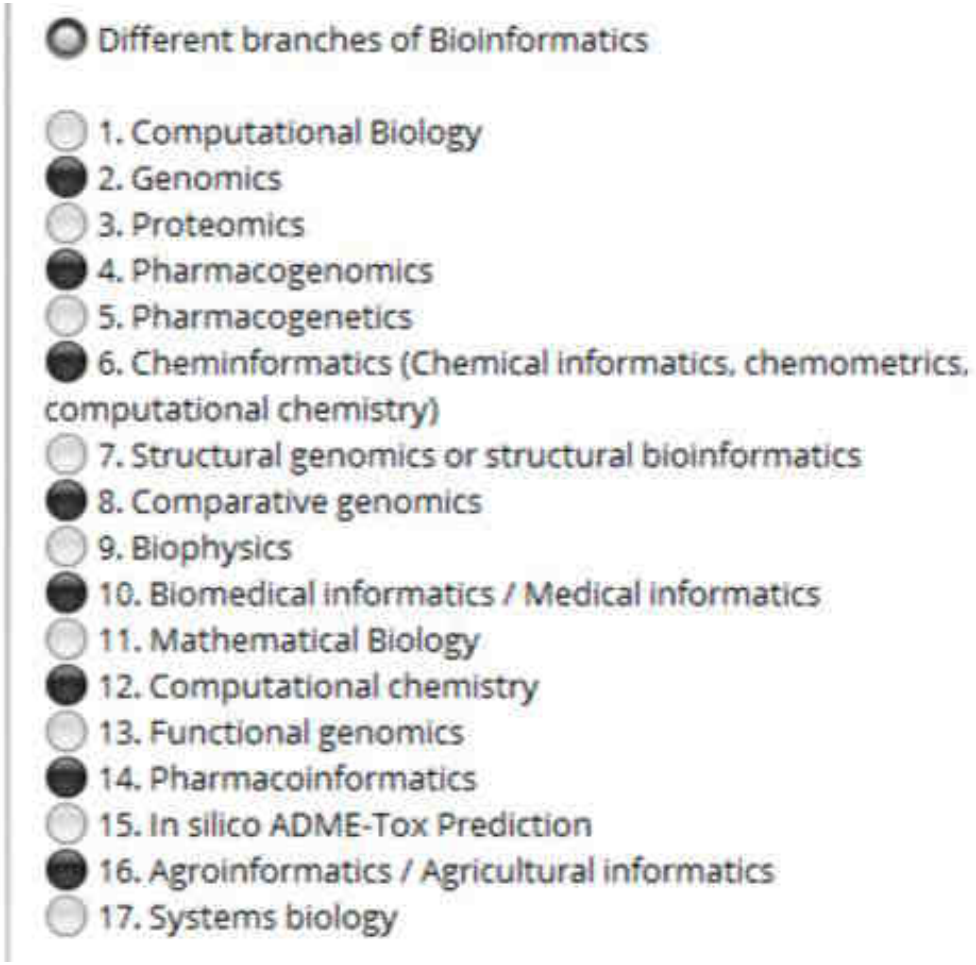
Metabolomics Applications

- Genetic Disease Tests
- Nutritional Analysis
- Clinical Blood Analysis
- Clinical Urinalysis
- Drug Compliance
- Transplant Monitoring

Toxicology Testing
Clinical Trial Testing
Fermentation Monitoring
Food & Beverage Tests
Nutraceutical Analysis
Drug Phenotyping
Water Quality Testing
Petrochemical Analysis

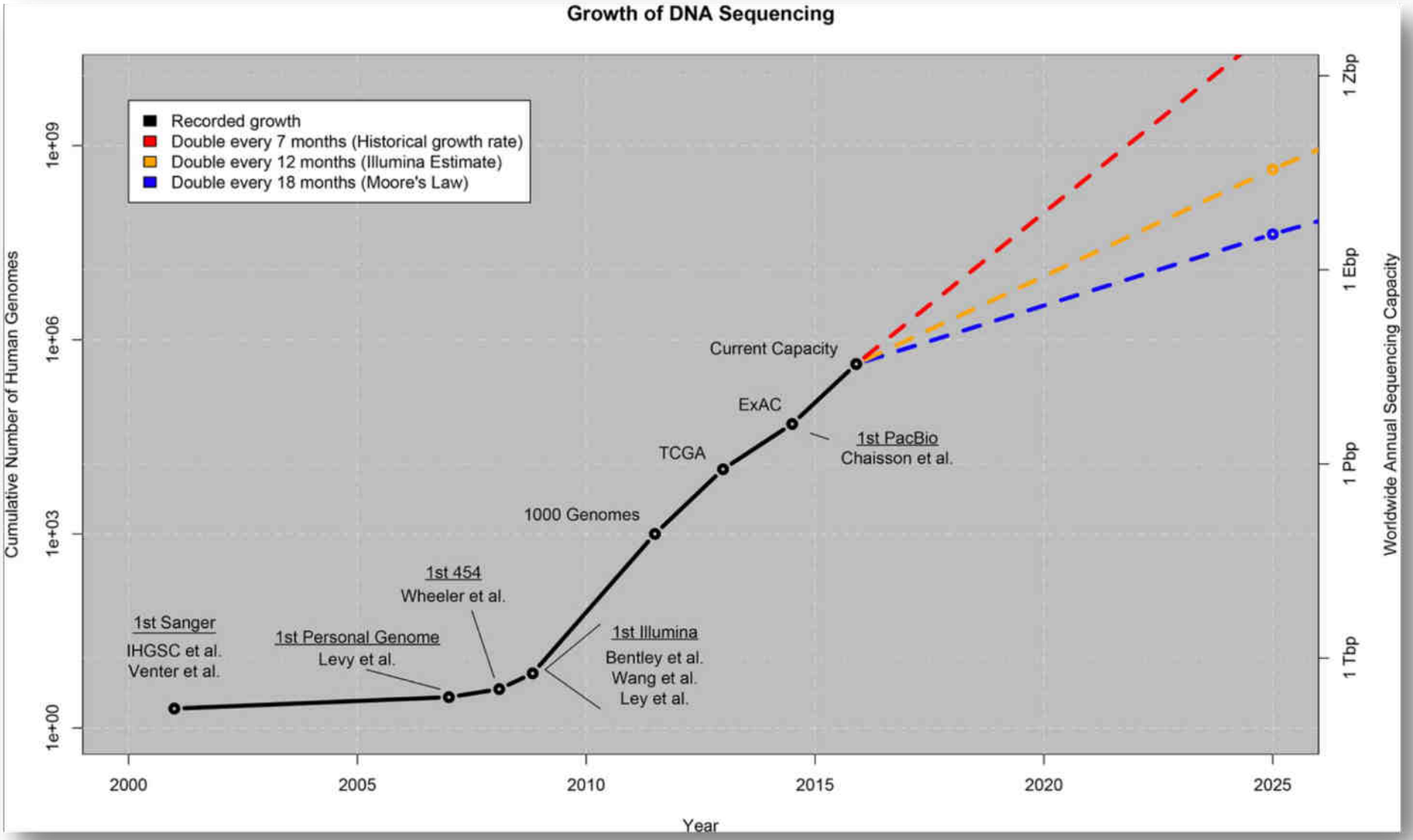
Bioinformatics and statistics

Bioinformatics is an interdisciplinary field that develops and improves on methods for storing, retrieving, organizing and analyzing biological data. A major activity in bioinformatics is to develop software tools to generate useful biological knowledge.



Genomics, Big Data, and Medicine Seminar Series -- Eric D. Green

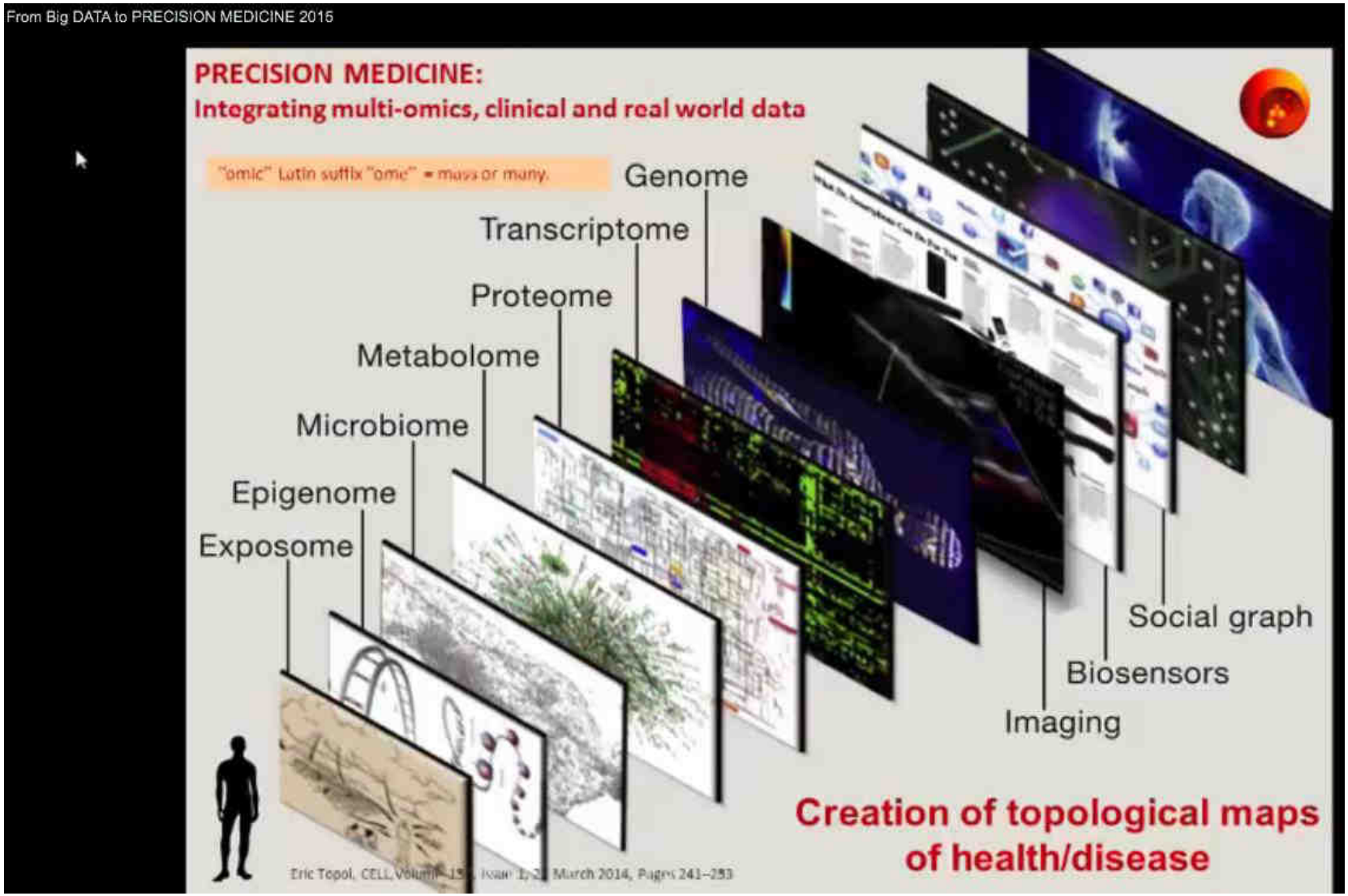
The Largest Current Bottleneck in Genomics...



https://goo.gl/3RhFkH

Thank you and happy to receive questions

Future is here…Integromics .. Big Data and Precision Medicine …



Application of Human genetics and genomics

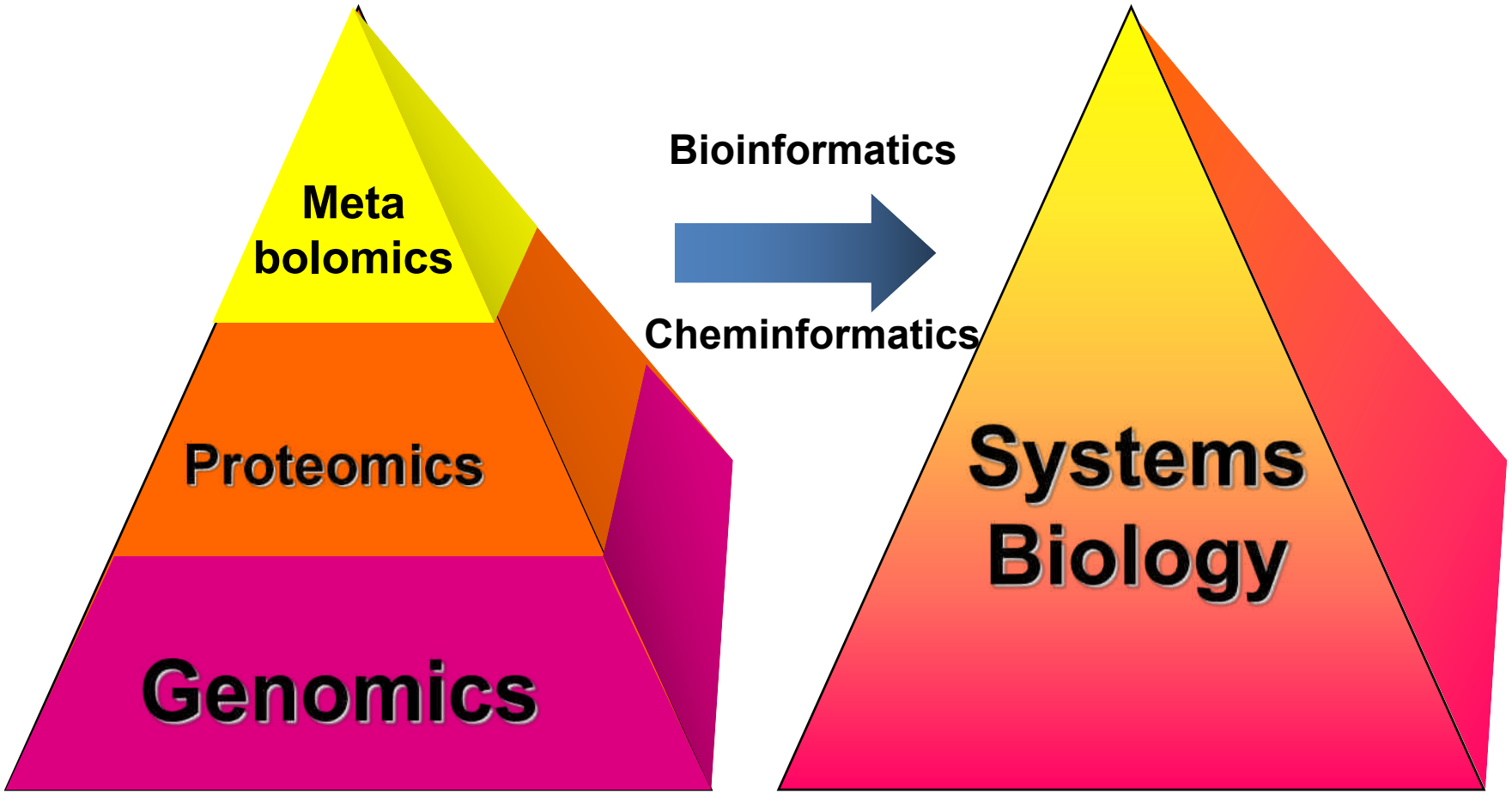
- Career prospects in research:

1. What opportunities you have in research:

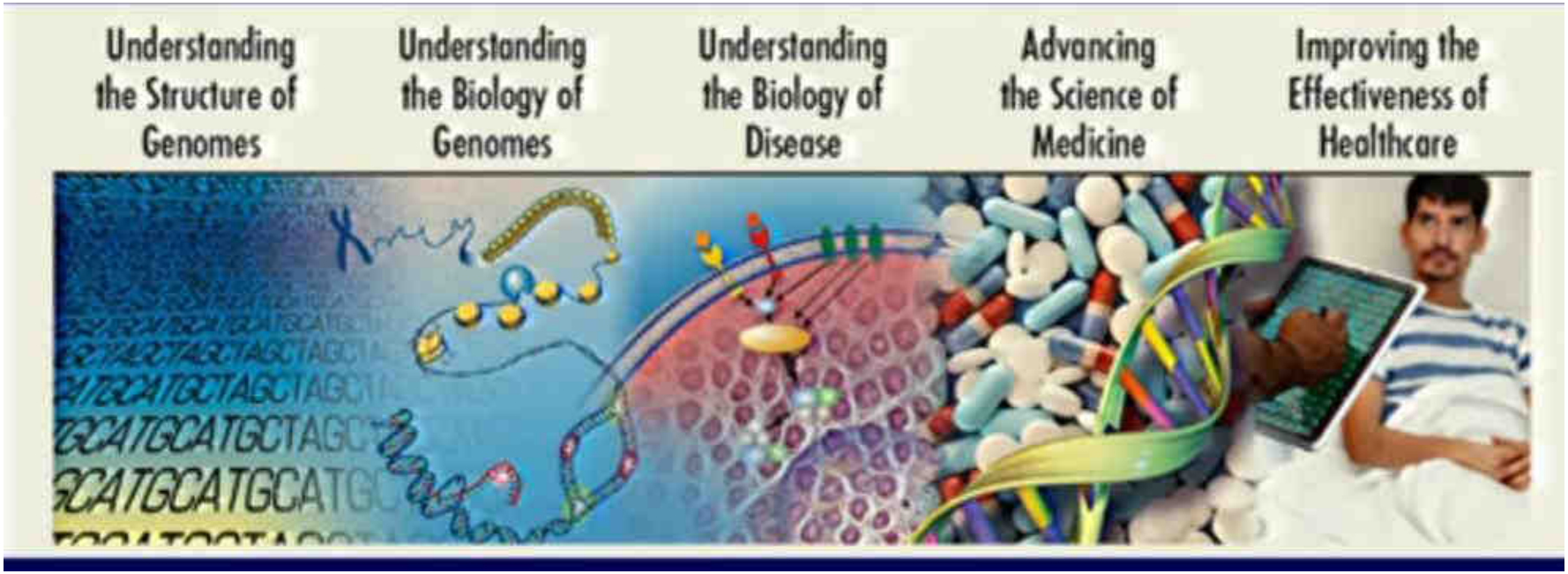
Career prospects for pharmacy students:

- Genetics: pharmacogenetics, molecular mechanisms, diagnostics, preventive strategies.
- Next Generation Sequencing technologies
- Big data: Statistics and bioinformatics
- Personalized medicine
- Gene editing technology: CRISPR/Cas9 tech
- iPSC derived invitro models: Drug testing
- Publicly available information : lectures and info
- Interdisciplinary skills
- Gut microbiota
- Metabolomics

Metabolomics Enables Systems Biology

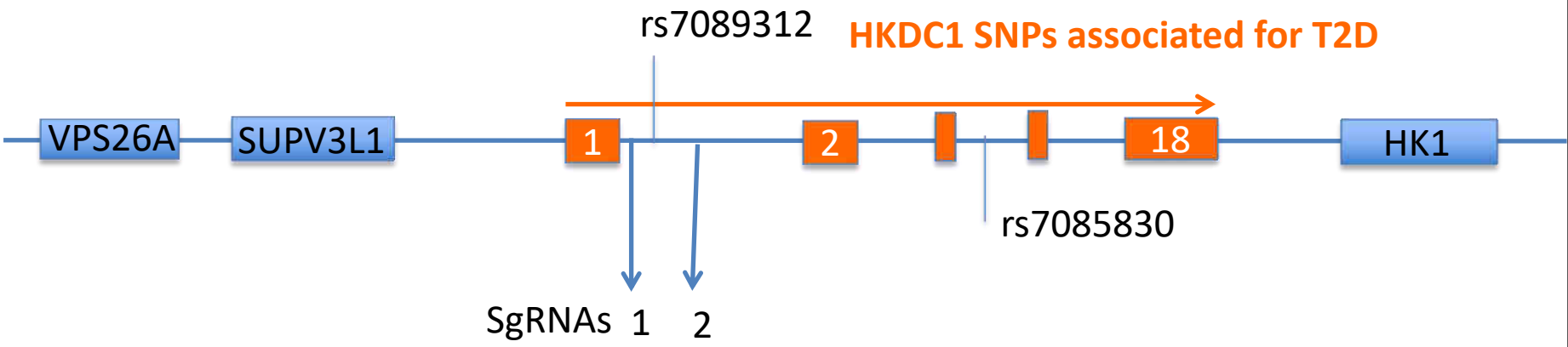


Ultimate goal of these genetic studies



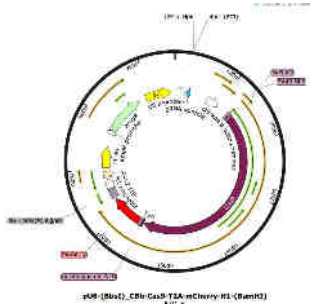
Adapted from Dr. ERIC GREEN, Director, genetic division, NIH, USA

Preliminary study done using CRISPR/Cas9 technology
rs7089312 enhancer locus

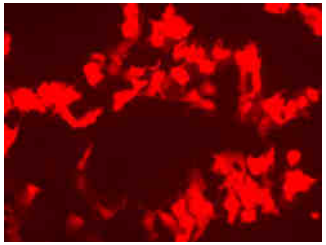


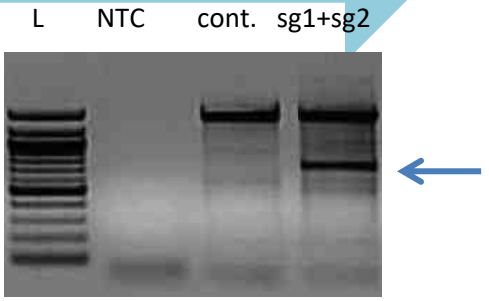
Designed guide RNAs → Guide RNAs cloned → Transfected → Confirmed: PCR & Seq

crispr.mit.edu



pU6-hHkdc1- (Sg1 + Sg2)





CRISPR/Cas9 results show deletion of enhancer locus