Molecular Methods: More than Diagnostics, leading the Way to Disease Management

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Disclosure

- No conflict of interests with the content of this presentation.

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Objectives

- **Describe a brief overview of the history of molecular diagnostics and molecular medicine.**
- **Identify complex disease states that can be attributed to complex molecular interactions.**
- **Explain the innovations in disease management and treatment at the forefront of molecular medicine and the clinical laboratory.**

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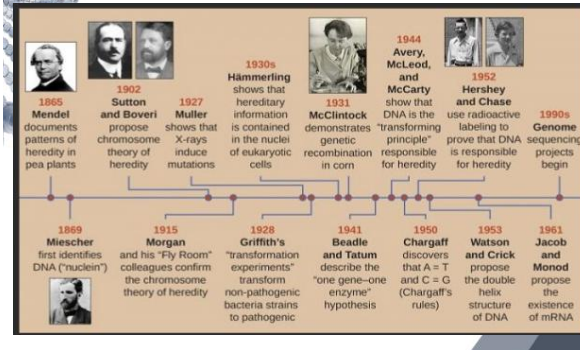
History of Molecular Medicine



History of genetics – Science of Healthy

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Genetics to Molecular Diagnostics

- Molecular Pathology
- Collection of techniques
- Analyze biomarkers in the genome, proteome and beyond!
- Used to diagnose and monitor disease, detect risk and determine therapy.
- Personalized medicine

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Molecular Diagnostics

- 1980, Yuet Wai Kan et al. suggested a prenatal genetic test for Thalassemia.
  - **Restriction endonuclease digestion and genetic variation in the fetus**
- 1995, the Association for Molecular Pathology (AMP) was formed.
- 1999, AMP co-founded The journal of Medical Diagnostics.
- 2012, Genetic hybridization was used to identify a SNP that causes Thalassemia.

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Basics of Molecular Diagnostics

- Microbiology or Human Genetics
- Unique species specific nucleic acid sequences.
- Molecular makes it possible to identify the sequences.

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Molecular Microbiology

- Why is this easier?
  - **Classifications of organisms (genotyping)**
  - **Identification or confirmation of an isolate obtained in culture.**
  - **Early direct detection of pathogens in clinical specimens**
  - **Antibiotic resistance rapid detection**
  - **Detection of mutations**
  - **Identification of toxigenic strains**

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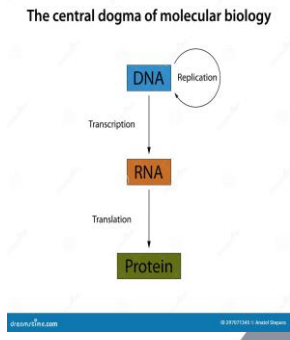
Human Genetics



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Complex Disease States

- A disease that arises from a complex interplay of multiple factors:
- Genetics
- Environmental exposures
- Molecular interactions
- NOT a single genetic mutation

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Impact on Human Health

- Substantial burden to the public health system
- Aging population, increasing
- Requires the development of personalized treatment
  - **New drug discoveries**
  - **Drug repurposing**
  - **Identification of biomarkers**
  - **Improved patient stratification**

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Molecular Networks

- Network of different proteins, genes and signaling molecules that interact.
- Create pathways that can become dysregulated in disease states.
- Different disease outcomes dependent on genetic predisposition and environmental exposure.
- Polygenic Nature

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Interactome

- Biomolecules rarely act individually.
- Cooperate to provide specific functions
- Physical Networks
  - **Stable protein complexes**
  - **High noise in the identification and techniques**
  - **Non-functional protein-protein interactions**
  - **Complemented with functional techniques**

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Functional Networks

- Connect genes with similar or related functions, without physical contact.
- Often demonstrate dependence on expression patterns.
- Gene expression profiling
- DNA methylation
- Chromatin accessibility
- Gene regulatory algorithms

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Modules and Pathways

- Cellular systems are modular
- Functions of cell growth and pathways
- Module-centric approaches
  - **Genotypic data (SNP, copy number alterations)**
  - **Phenotypic data (gene expression profiles)**
- Molecular Pathways (EGFR)

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Molecular Interactions

- Protein-Protein Interactions
- Gene Expression Regulation (Dysregulation)
- MicroRNA Interactions
- Metabolic Dysregulation

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Innovation in Molecular Medicine

- Multi-omics
- GWAS
- Genetic basis and integrative approaches
- High-resolution Methodologies
  - **Type 2 Diabetes**
  - **Osteoarthritis**
  - **Alzheimer's Disease**
  - **Systemic Lupus Erythematosus**

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GWAS

- Identification of risk loci
- Translation to clinical application is difficult
- Strong linkage disequilibrium between variants
- Identification of effector genes of the variants
- Variants in noncoding regions

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What is GWAS?

- Genome-wide set of genetic variants
- Often Single nucleotide polymorphisms
  - **SNP arrays**
- Major human disease traits
- Phenotype of a specific disease or trait
  - **Mendelian randomization**
  - **Colocalization analysis**

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GWAS Techniques

- Chromatin Immunoprecipitation
  - **ChIP-seq**
- Transposase-accessible chromatin sequencing
  - **ATAC-seq**

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What is ChIP-seq?

- Chromatin immunoprecipitation
- Antibody based technology
- Selectively enriches DNA-binding proteins and the DNA targets
  - **Histones, histone modifications, transcription factors, cofactors**
  - **Chromatin state and gene transcription**
- Investigate protein-DNA interactions

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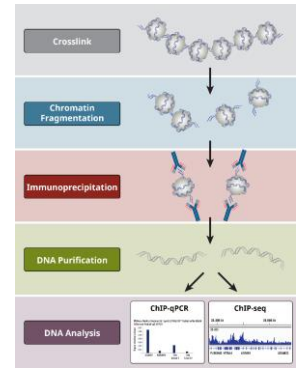
ChIP-seq



Overview of Chromatin Immunoprecipitation (ChIP) | Cell Signaling Technology

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What is ATAC seq

- Technique to evaluate chromatin accessibility
- Identify open chromatin and how it affects gene expression
- Does not require prior knowledge of regulatory elements

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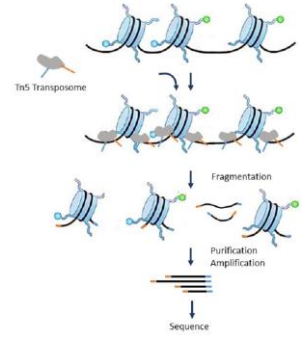
ATAC seq



ATAC-seq.jpg (812×860)

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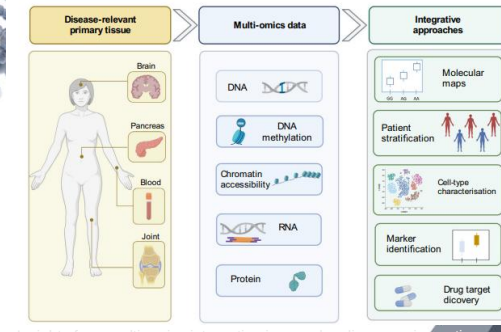
Multi-omics

- Panomics
- Multiple “omes”
  - **Genome**
  - **Proteome**
  - **Transcriptome**
  - **Epigenome**
  - **Metabolome**
  - **Microbiome**

Primary Relevant Tissues



Insights from multi-omics integration in complex disease primary tissues

Disease States

- High prevalence
- Osteoarthritis
  - **40% over the age of 70 years**
- Diabetes
  - **6.28% of the world population**
- Substantial burden on public health
- Underscores the need to develop personalized treatment

Type 2 Diabetes

- Clinically heterogeneous metabolic disease
  - **>450 million individuals worldwide**
- Impairment of insulin secretion, signaling, carbohydrate, lipid and protein metabolism
  - **Defective insulin secretion; pancreatic beta cells**
  - **Lack/reduced response to insulin**
- >700 genetic risk loci have been identified
- >90% mapped to noncoding sequences
- 19% of these variants effect insulin secretion
  - **Pancreatic studies**
  - **Tissue is difficult to access**

Type 2 Diabetes (Continues)

- Islet specific signals are not discernable
  - **1-2% of the pancreas**
- Multi-omics studies are important
  - **7741 cis-expression quantitative loci have been identified**
  - **ChIP seq and ATAC seq to enrich active chromatin states and identified islet specific transcription factors**
  - **47 variants identified with a causal role**

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Type 2 Diabetes (Continues)

- ChIP seq-identified enhancer regions and specific gene promoters
- >1300 enhancer hubs in pancreatic islets containing variants that affect insulin secretion
- All the studies were from postmortem donors, predominantly
- Profiles of living pancreatomized donors revealed mitochondrial and immune response functional differences.

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Type 2 Diabetes Resources

- Translational Human Pancreatic Islet Genotype Tissue-Expression Resource (TIGER)
- TIGER - Database Commons
- Diabetes Epigenome Atlas
- T2D Knowledge Portal: Epigenome Atlas (DGA) | Broad Institute

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Osteoarthritis

- Complex MSD that affects all tissues of diarthrodial joints
  - **Degradation of cartilage**
- A joint disorder, tissues are challenging to access
- Not included in reference databases

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Osteoarthritis Genetics

- GWAS has revealed ~150 genetic risk loci
  - **Unclear of which variants are developmental or progressive**
- Tissues are typically collected during joint replacement
- Recent multi omics studies

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Osteoarthritis Multi omics

- Osteoarthritis cartilage and synovial tissue
- Transcriptomic and proteomic map of genetic variants
  - **Identified 5 putative effector genes**
  - **409 genes linked to cartilage degeneration at the transcriptome and proteome level**
- Correlation has identified 19 compounds that can reverse disease progression at the molecular level

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Osteoarthritis Multi omics (2)

- mRNA and miRNA studies
- 142 miRNAs and 2387 mRNAs linked to cartilage degradation
- Pairing study, functional analysis and generated a miRNA-mRNA network
- Revealed several clusters of interactions

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Alzheimer's Disease

- Neurodegenerative disease
  - **Most common form of dementia**
  - **Accumulation of Beta-amyloid plaques and tau-containing neurofibrillary tangles**
- Complex tissue can only be studies post-mortem
- Brain is heterogeneous
- Consists of different regions and different cell types that work together
  - **Single cell type studies are important**

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AD and Multi omics

- Late-stage AD study
- Identified regulatory elements that influence genes *in cis*
- *APOE* and *CLU* in oligodendrocytes
- Transcription factors in glia cells *(SREBF1)*
  - **Increased representation of binding sites in oligodendrocytes**

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AD Multi omics

- Molecular alterations in AD brain regions
- Gain of histone modifications (H3K27ac and H3K9ac)
- *VGF* and *ATP6V1A* downregulation
  - ***ATP6V1A* promising drug target**
- 173 proteins linked to AD progression
- Amyloid and tau pathways, pointing to different etiological mechanisms

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Systemic Lupus Erythematosus

- Autoimmune disease of multiple organs
  - **Relapse-remitting course**
  - **Autoantibody production and inflammation**
- Peripheral Blood
  - **WBC (leucopenia, lymphopenia)**
  - **Platelets (thrombocytopenia)**
  - **RBC reduction**
- Large patient heterogeneity
  - **Affected organs, disease severity, clinical manifestations**
  - **Active disease, belimumab is effective**

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SLE and Molecular Studies

- GWAS
- >100 SLE risk loci (2023)
- >300 SLE risk loci (2024)
- Easy access to samples (blood)
  - **Transcriptomic data from 1.2 million PBMC's**
- SLE patient stratification into clusters
- 750 differential expressed genes (DEGs)
  - **Upregulated SLE genes and TF binding data using ENCODE has revealed SLE relevant pathways**

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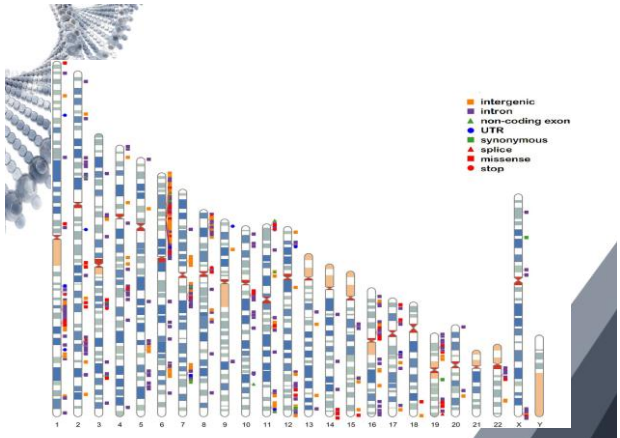

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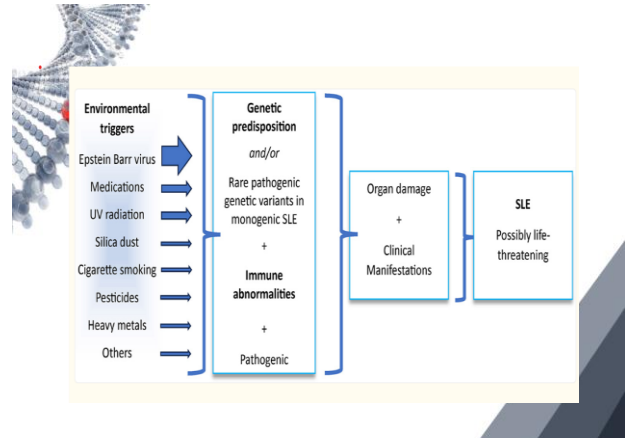

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SLE and Metabolomics Data

- Investigated cardiovascular risk data
- Two robust SLE patient clusters were identified
- Dyslipidemia
- Higher apolipoprotein B and AI rations (Apo2-ApoA1)
- Identified DEGs in isolated T-cells (CD8+ 82 DEGs; CD4+ 417 DEGs)

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Concluding Remarks

- Integration of Multi omics
- Disease etiology in specific tissues
- International collaborations creating databases
- Limitations
  - **Sample size**
  - **Population diversity**
  - **Disease relevant cell types**
    - Many remain undiscovered

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Technology Limitations

- Sequencing technologies are primarily short-read (up to 300 bp)
- Genomic structural variation or highly repetitive regions (telomeres or centromeres) or gene expression limited (long transcripts)
  - **Long read sequencing**

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Sample Limitations

- Joint tissues in osteoarthritis
- Brain tissue in AD
- Pancreatic cells in T2D
- Organoids
  - **Stem cell derived 3D invitro models of human organs**

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Disease Management and Treatment

- Cause versus Consequence
- Complexity
- Some treatments have been identified as noted
- Continued biomarker and molecular studies across disease development stages is still needed
- Many studies have been limited to those with European ancestry

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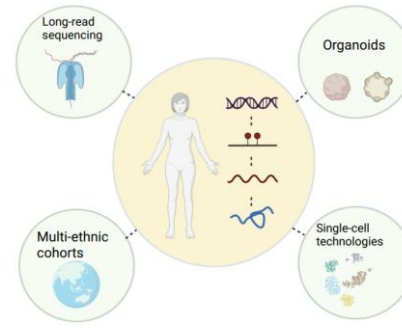

Insights from multi-omics integration in complex disease primary tissues

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Resources

- Human Cell Atlas (http://www.humancellatlas.org)
- ENCODE (www.encodeproject.org)
- ROADMAP (NIH Roadmap Epigenomics - GEO – NCBI)

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