Population level omics data integration to unravel the role of non-coding RNAs in health and disease

X-omics Festival 2023

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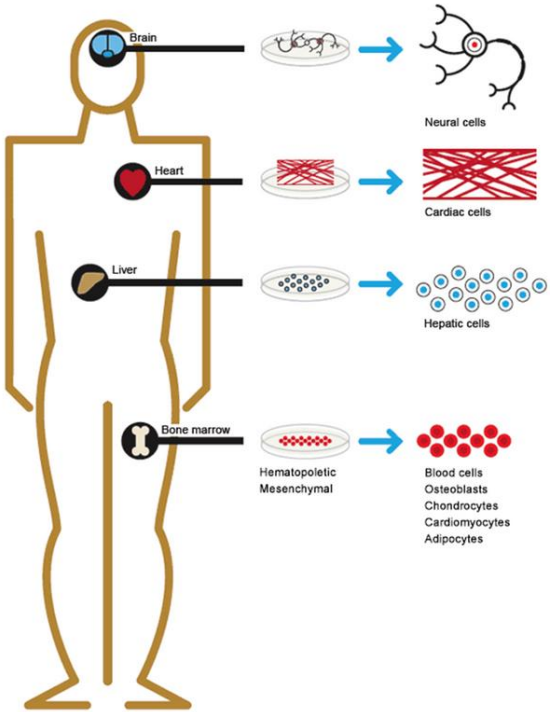
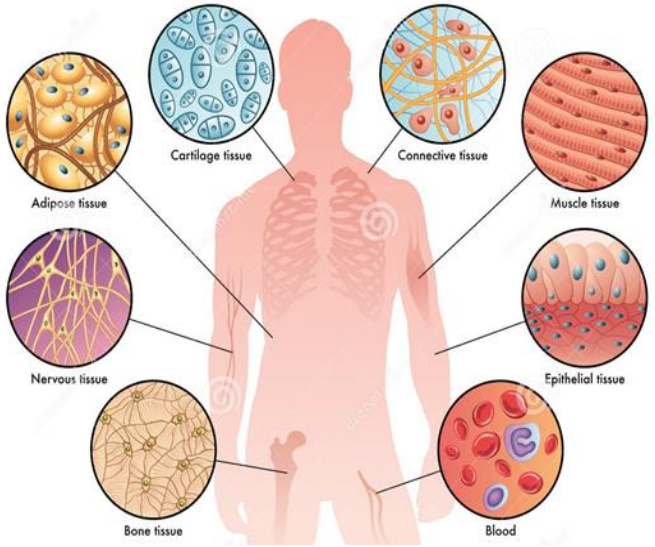
Cell type–specific gene regulation & expression



Adopted from Topper.



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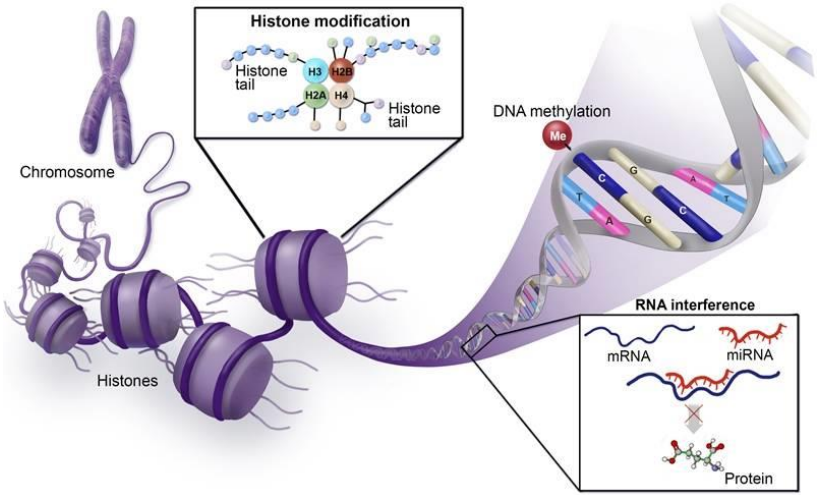
Epigenetic

External modifications to DNA that turn genes on/off. These modifications do not change the DNA sequence, but instead, they affect how cells "read" the genes.

Three main epigenetic marks:

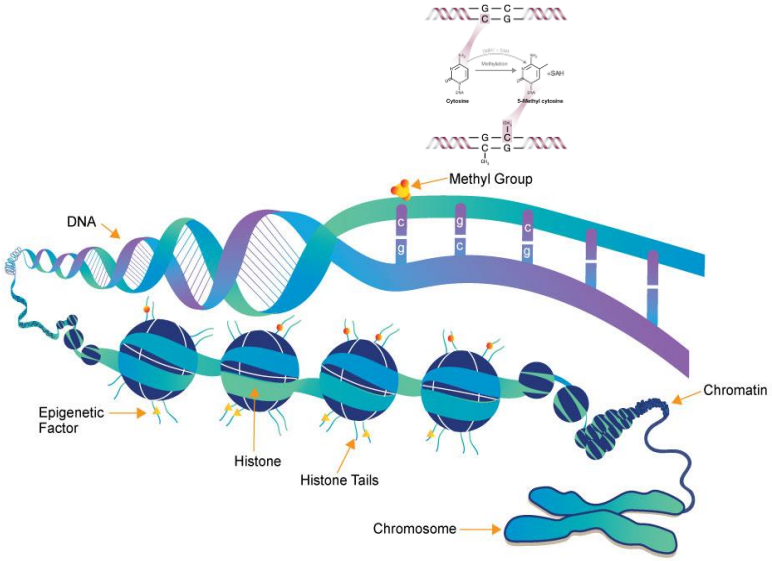
- ❑ DNA methylation
- ❑ Histone modification
- ❑ RNA interference

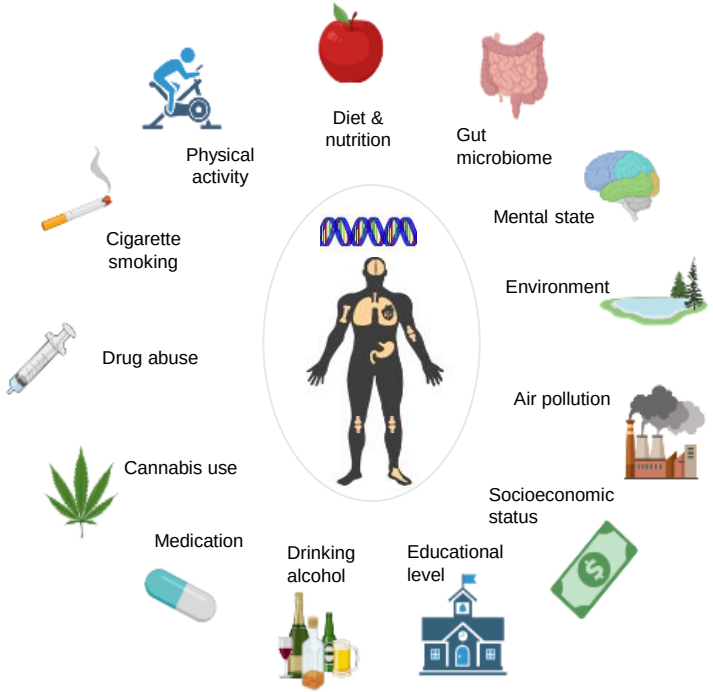


American Society of Hematology.

DNA methylation

A biological process by which a methyl group is added to a cytosine base and change the activity of a DNA segment.





Smoking and alcohol affect DNA methylation

Tobacco smoking leads to extensive changes in DNA methylation.

Zeilinger et al., PLoS One. 2013 May 17;8(5):e63812.

A blood DNA methylation biomarker of alcohol consumption.

Liu et al., Molecular Psychiatry. 2018. 23, 422–433.

European Journal of Epidemiology (2019) 34:1055–1074
https://doi.org/10.1007/s10654-019-00555-w

METHODS

Validated inference of smoking habits from blood with a finite DNA methylation marker set

Maas et al. Clin Epigenet (2021) 13:198
https://doi.org/10.1186/s13148-021-01186-3

RESEARCH Open Access

Validating biomarkers and models for epigenetic inference of alcohol consumption from blood

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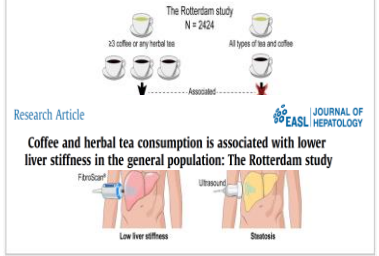
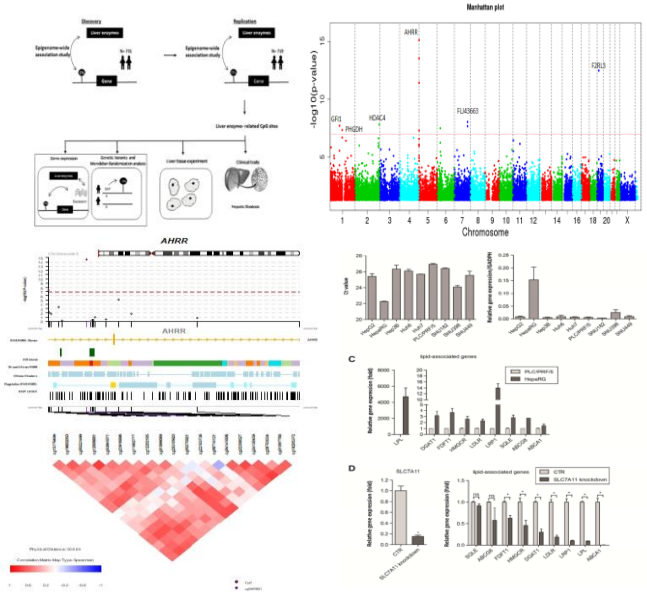
Coffee consumption changes DNA methylation

Drinking coffee & disease risk



MENU nature COMMUNICATIONS Search

Epigenome-wide association meta-analysis of DNA methylation with coffee and tea consumption



Forbes

EDITORS' PICK | 70,192 views | Apr 30, 2020, 12:14am EDT

Here's Some Good News About The Coffee You're Drinking During The Coronavirus Lockdown And Your Health

David DiSalvo Senior Contributor ©
Healthcare

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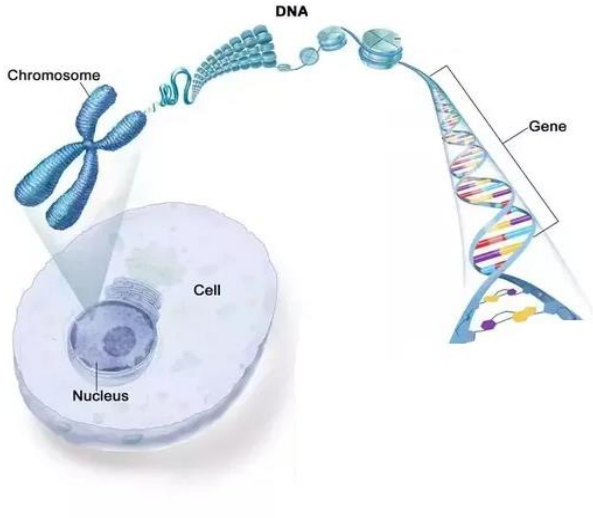
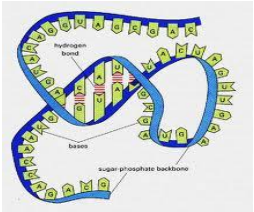
Gene expression is regulated by RNA interference



DNA

Transcription

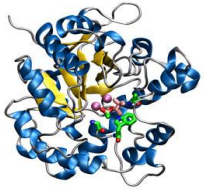
mRNA



non-coding RNAs

Translation

Protein



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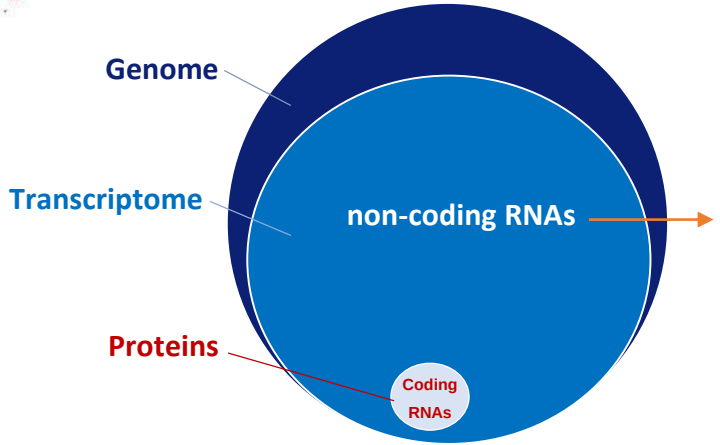
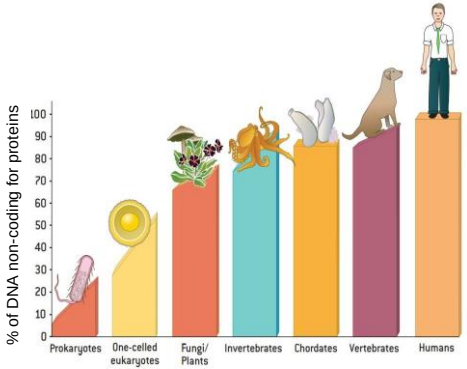
Human genome, coding and non-coding RNAs

> **70**% of the human genome is transcribed into RNAs.

(ENCODE project)

< **5**% of RNAs have the potential to encode proteins.





Mattick, J.S. Sci Am. 291:60-67. 2004

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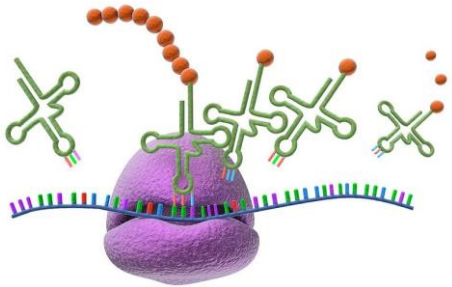
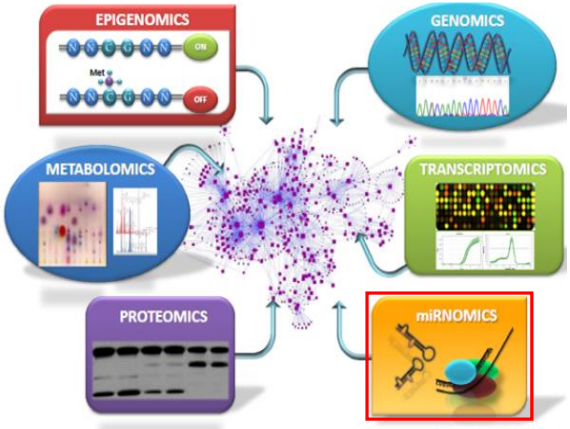
Coding & non-coding, the converging concepts of RNAs

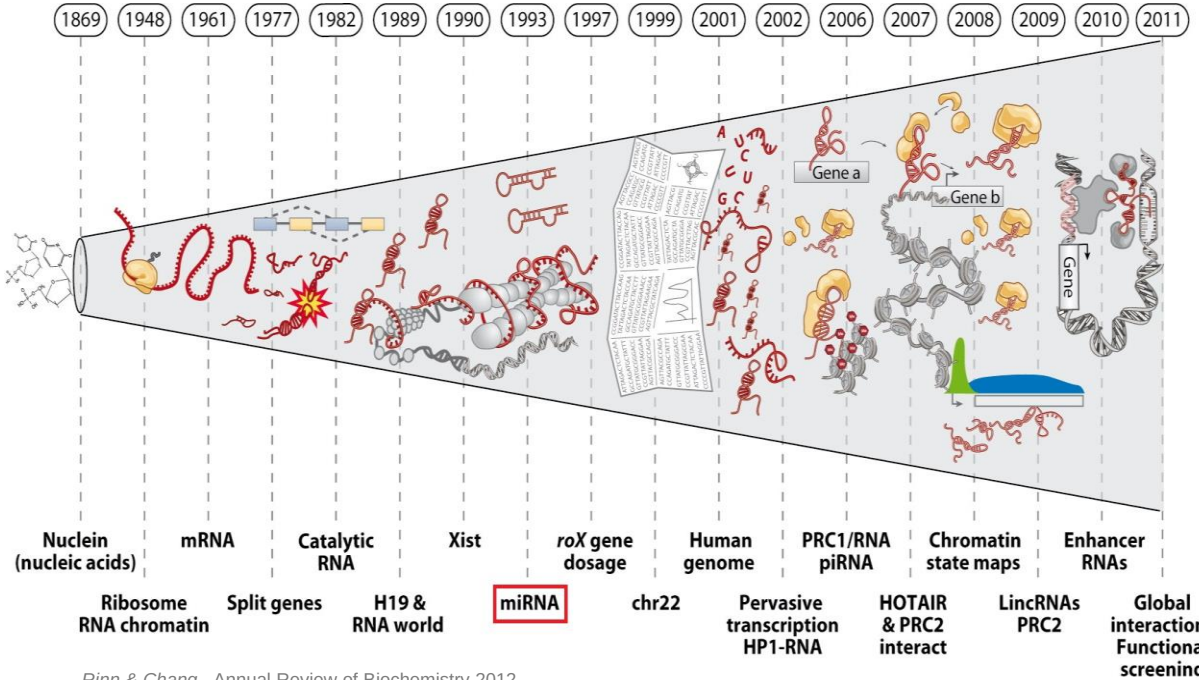


microRNAs (miRNAs)

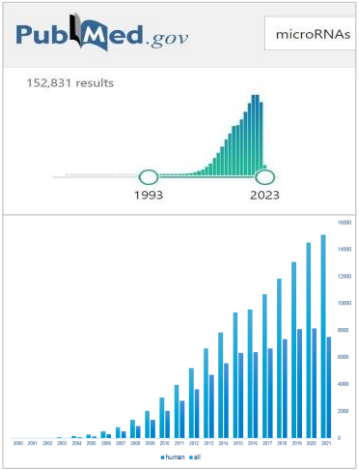
- Small single-stranded forms of RNA
- Highly conserved in different species
- >2000 high-confidence miRNAs in human
- Post-transcriptional regulators of gene expression
- ~2/3 of coding genes are putative targets of miRNAs



The importance of miRNAs in gene regulation has steadily gained appreciation



Rinn & Chang. Annual Review of Biochemistry 2012.



miRNAs are involved in most, if not all, physiological and pathological processes!

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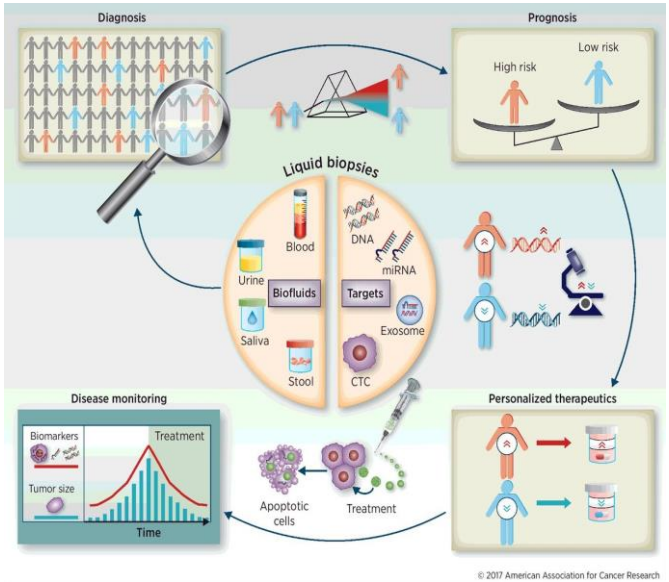
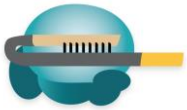
miRNAs in health and disease



- **Providing insights into disease biology**

  (Rayner et al. Science. 2010; Zhu et al. Cell. 2011)

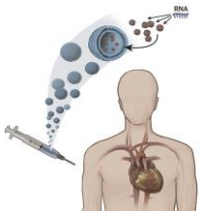


- **Detectable in body fluids as biomarkers**

  (Olson E, Science Tran. Med 2014)

- **Their potential therapeutic targets**

  (Czech MP. N Engl J Med. 2006)



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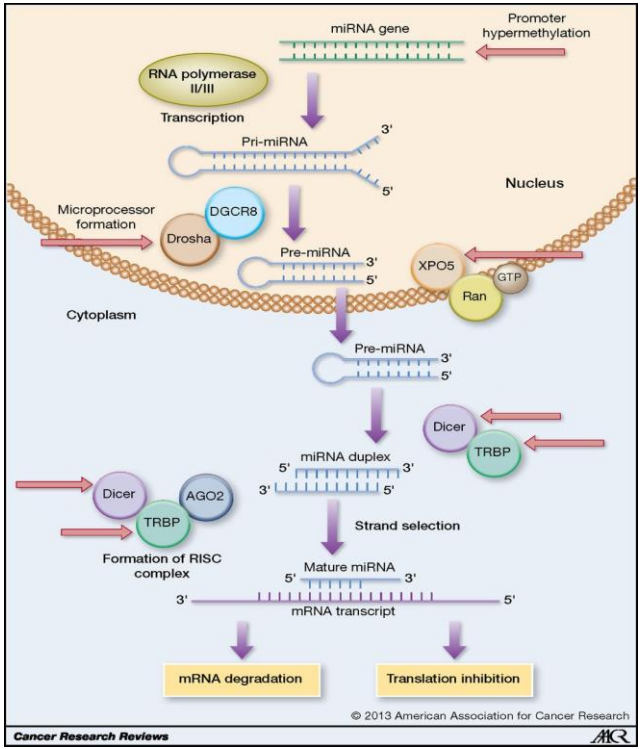
Biogenesis and function of miRNAs

Transcription

Processing

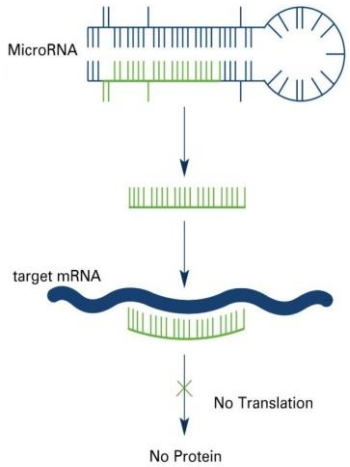
Maturation

Execution



The canonical processing pathway

Gene silencing



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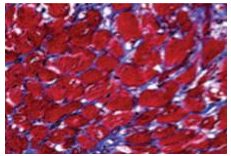
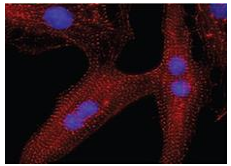
Identification of miRNAs involved in human diseases

Healthy & diseased samples

Cells



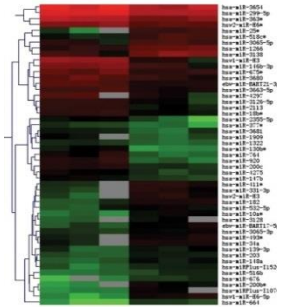
Tissue



RNA extraction

miRNA & gene expression profiling (RNA-seq)

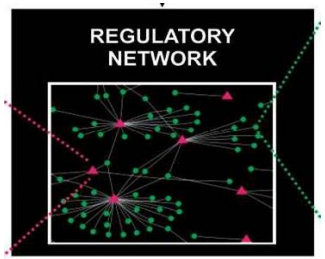
Expression analysis



Differential Exp. analysis (altered miRNAs & genes)

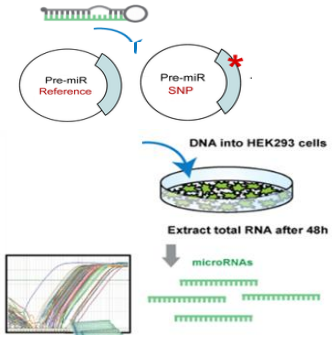
miRNA-gene correlations

miRNA-mediated gene regulation

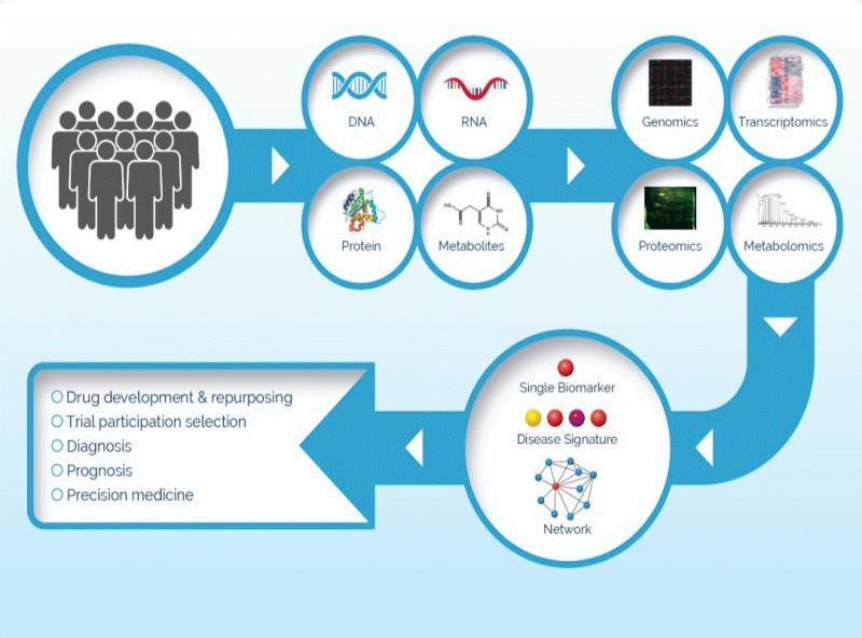


Network analysis

Validation studies



Integrating population level omics data to identify miRNAs implicated in diseases



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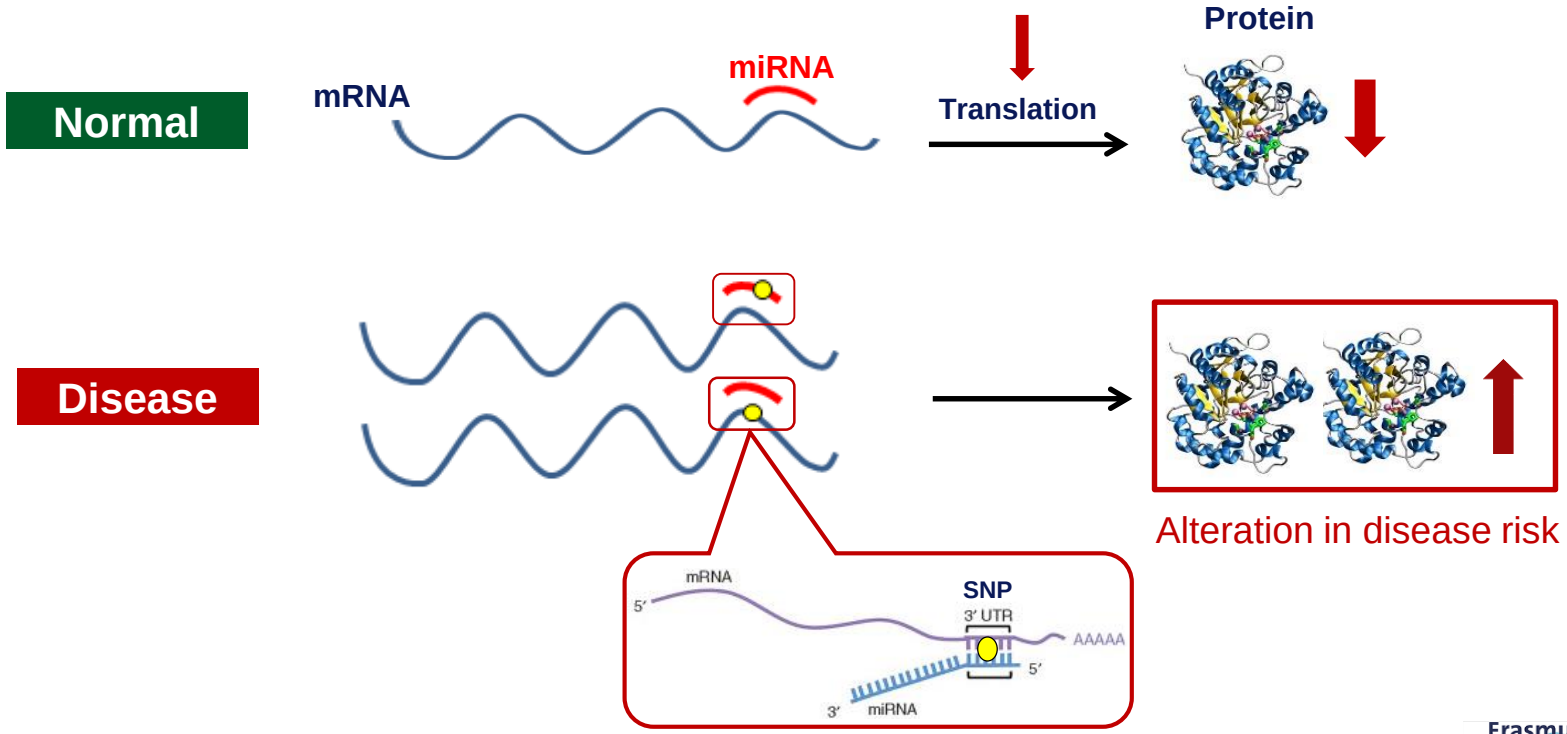
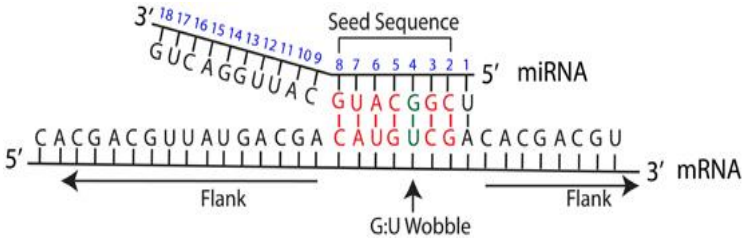
Genetic variants in miRNAs & their binding sites

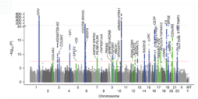


miRNA-related SNPs and their links to diseases



miRNA-related genetic variants	#SNPs
Genetic variants in miRNA-encoding sequences	~250
Genetic variants in miRNA-binding sites within the 3′-UTR of target genes	~50,000

Identification of candidate SNPs



GWAS study on AMD
(Fritsche *et al* 2016)

+

Known variants in miRNAs and binding sites
(PolymiRTS, miRNASNP, PolymiRTS, Patrocles)

=

Identification of miRNAs or miRNA binding sites (BS) associated with AMD

Prove functionality of variants

prioritize candidates by:
- eQTL data
- locus zoom plots
- expression data
- miRNA structure prediction tools

→

clone variants in vector/order mimic miRNA

→

co-transfect miRNA and target in cultured cells

→

measure effect of SNP on miRNA process

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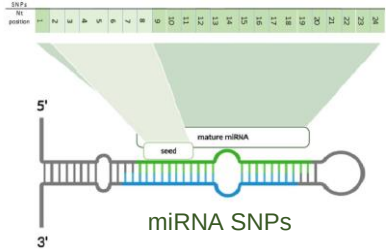
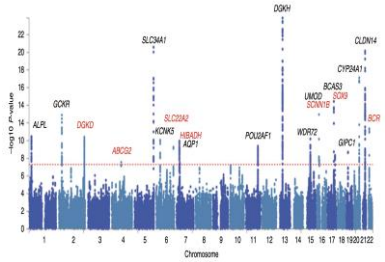
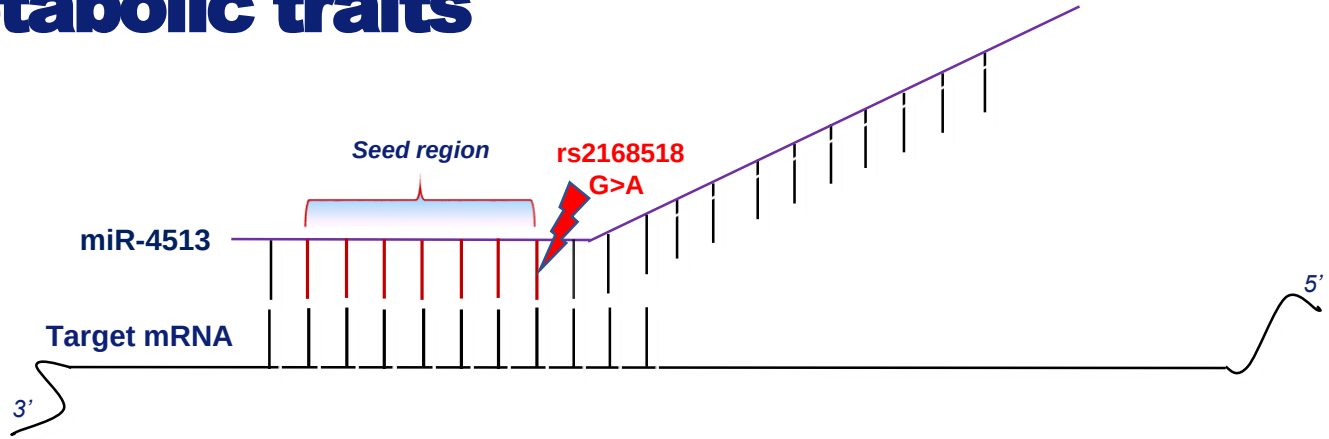
A SNP in miR-4513 associates with several cardio-metabolic traits



miRNA SNPs



GWAS data



↑ Diastolic BP $p=3.5 \times 10^{-12}$	Total Cholesterol $p=5.7 \times 10^{-5}$	Fasting Glucose $p=2.5 \times 10^{-4}$
↑ Systolic BP $p=3.4 \times 10^{-10}$	LDL Cholesterol $p=5.6 \times 10^{-5}$	Cor. Art. Dis. $p=9.2 \times 10^{-4}$

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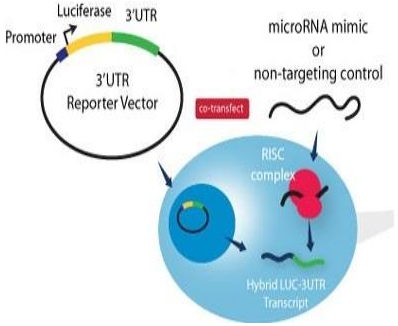
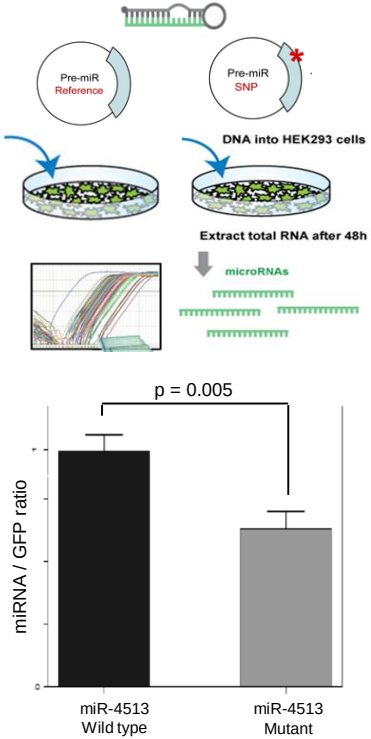
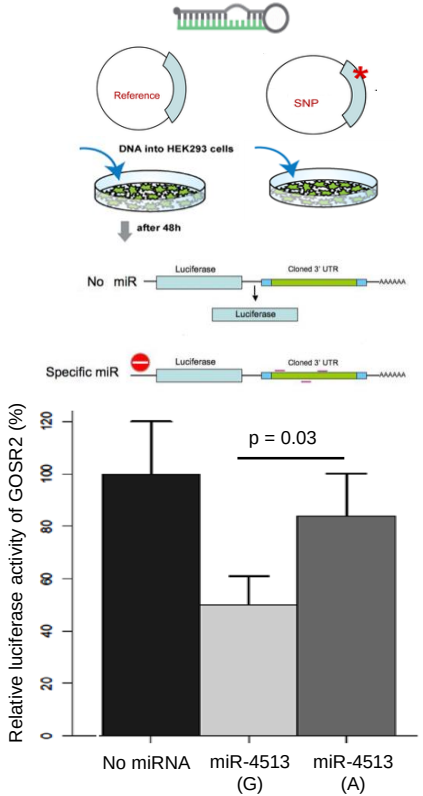
The SNP affects expression and function of miR-4513







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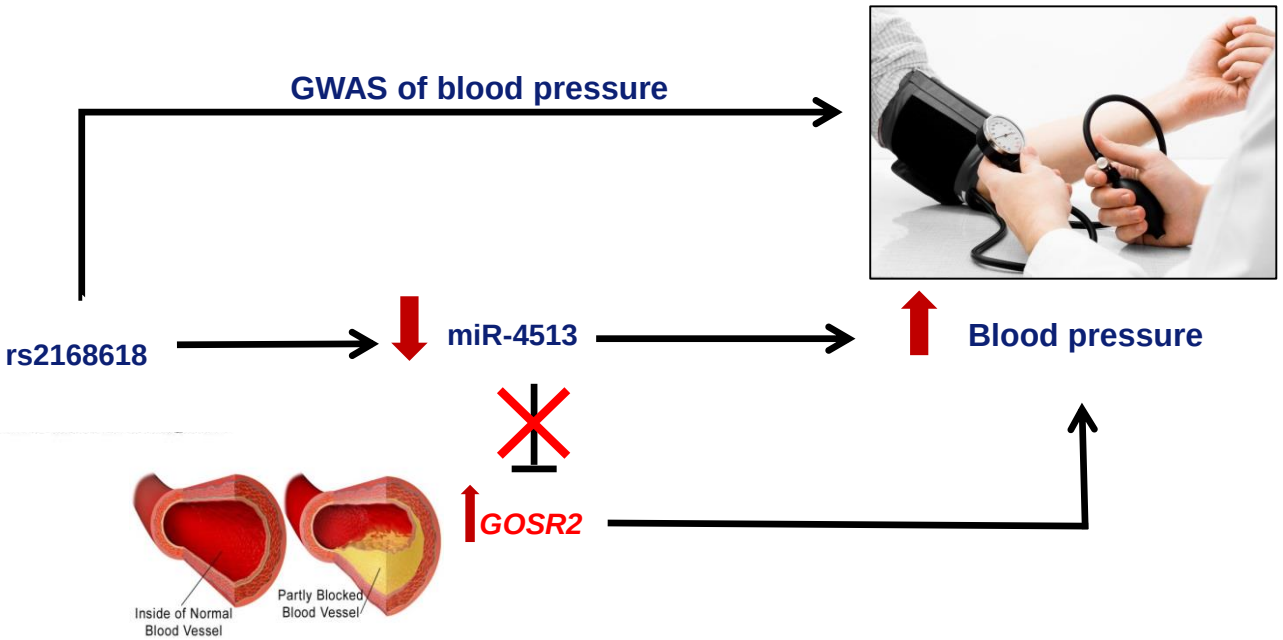
RESEARCH ARTICLE

Human Mutation

OFFICIAL JOURNAL
HGVS
HUMAN GENOME VARIATION SOCIETY
www.hgvs.org

A Genetic Variant in the Seed Region of miR-4513 Shows Pleiotropic Effects on Lipid and Glucose Homeostasis, Blood Pressure, and Coronary Artery Disease



OXFORD

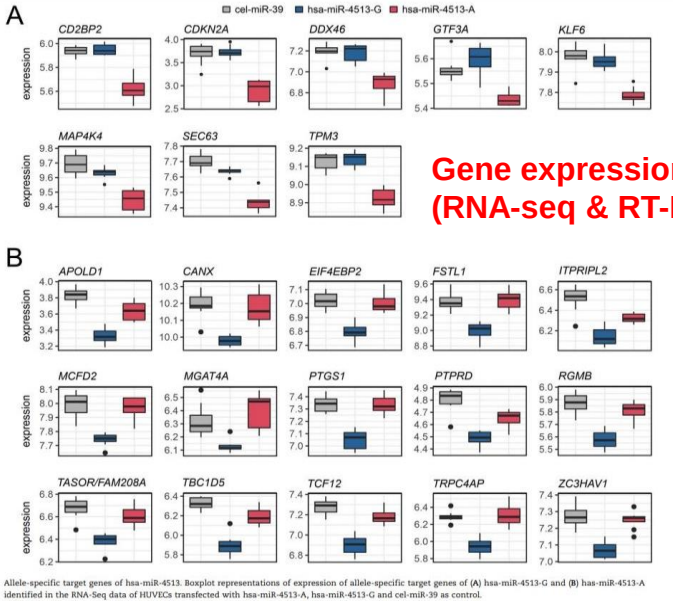
Human Molecular Genetics, 2022, Vol. 31, No. 6 875–887

https://doi.org/10.1093/hmg/ddab292
Advance Access Publication Date: 4 October 2021
General Article

GENERAL ARTICLE

Seed sequence polymorphism rs2168518 and allele-specific target gene regulation of hsa-miR-4513

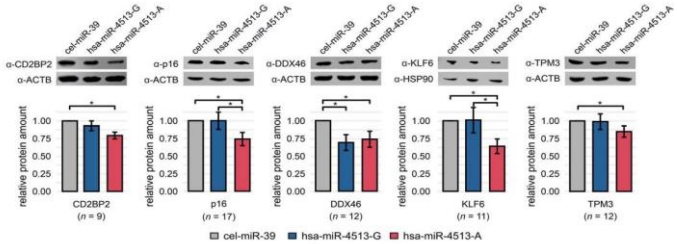
Christina Kiel[1,†], Tobias Strunz[1,‡], Daniele Hasler[2], Gunter Meister[2], Felix Grassmann[1,3,§] and Bernhard H.F. Weber[1,4,*,§,¶]



Allele-specific target genes of hsa-miR-4513. Boxplot representations of expression of allele-specific target genes of (A) hsa-miR-4513-G and (B) has-miR-4513-A identified in the RNA-Seq data of HUVECs transfected with hsa-miR-4513-A, hsa-miR-4513-G and cel-miR-39 as control.

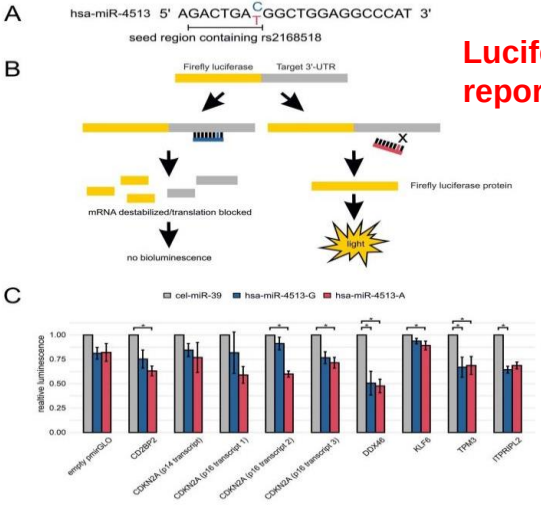
Gene expression (RNA-seq & RT-PCR)

Protein analysis (Western blot)



Allele-specific hsa-miR-4513 target gene protein expression. Representative western blot analyses of five selected target genes with the respective loading controls ACTB or HSP90 (for KLF6). Isoform p16 represents one of the protein products of CDKN2A.

Luciferase reporter assay



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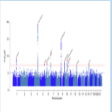
Cross-omics data integration towards understanding genetic regulation & disease association of miRNAs

Step.1

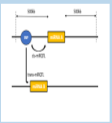
GWAS of miRNAs

Discovery & Internal validation
- Rotterdam study-I (N=1000)
- Rotterdam study-II (N=1000)
- Rotterdam study-IV (N=750)

Replication
- Framingham study (N=5,239)
- Ottawa study Canada (N=710)



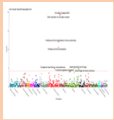
Post-GWAS studies
- Cis and trans miRNA-eQTLs
- Functional annotation



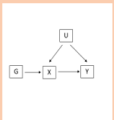
Step.2

Clinical traits & causal inference

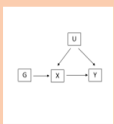
PheWAS
- UK biobank (N= 423,442)
- Hospital Episode Statistics data



MR-PheWAS
- Exposure (miR data Rotterdam)
- Outcome (traits UK biobank)



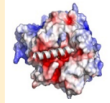
MR (replication)
- Exposure (miR data Rotterdam)
- Outcome (GWAS catalog)



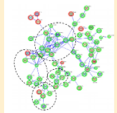
Step.3

Target genes and pathways

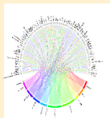
mRNAs and proteins
- mRNA-eQTLs (N= 31,684)
- Protein-QTLs (Up to 30,000)



Target genes analysis
- miRNA-mRNA interactions
- In-silico & in-vitro studies



Metabolites (QTLs)
- ~250 in Nightingale (N~ 120,000)
- >1000 in Metabolon (N~ 10,000)



Step.4

Building ADMIRE web tool

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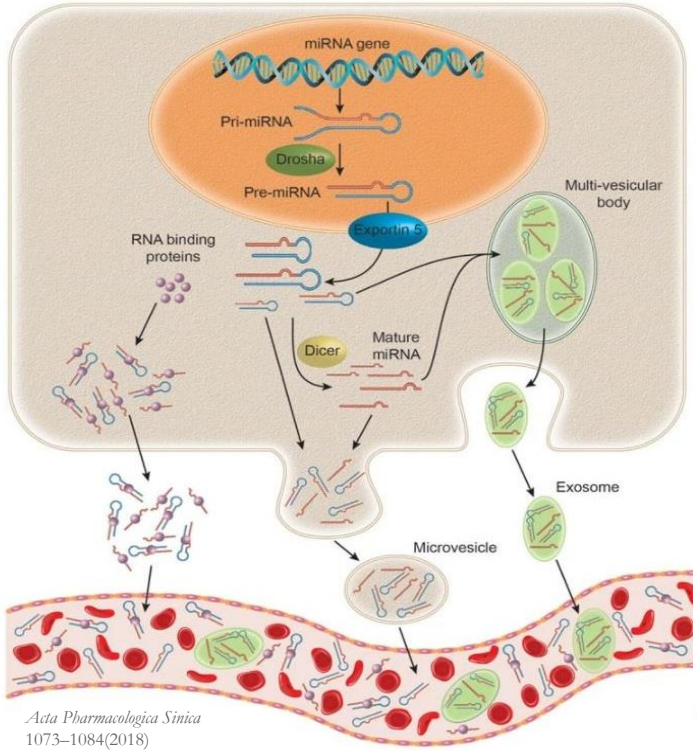
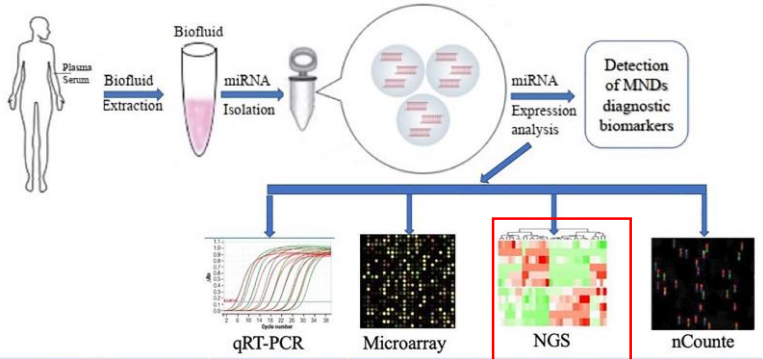
Step.1

Plasma circulating miRNAs in the Rotterdam study



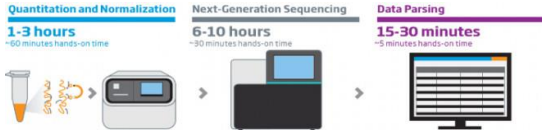
Acta Pharmacologica Sinica
1073–1084(2018)



	qRT-PCR	Microarray	NGS	nCounte
Sensitivity	Very high	Moderate to low	Moderate	Moderate
Specificity	Very high	Moderate to low	Very high	Moderate
Throughput	Moderate to low	Very high	Very high	Moderate
Validation data	No	Yes	Yes	Yes
Normalization	Yes	No	No	Yes
Identification novel miRNAs	No	No	Yes	No

Wang et al. Front Neurosci. 2020 Apr 16;14:354.

HTG
RNA-Seq

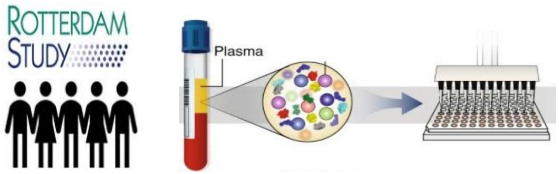


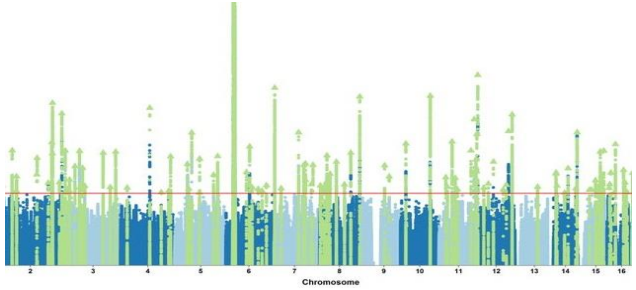
Erasmus MC

Step.1

Genome-wide association study of miRNA levels

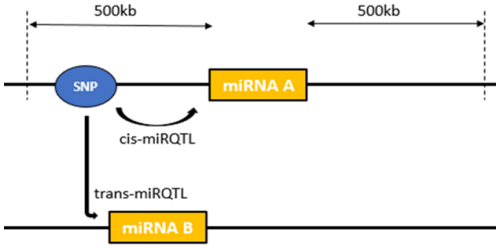
GWAS on plasma levels of 2083 miRNAs





Associating levels of each miRNA to 10M SNPs

Cohort	#participants	#miRNAs
Rotterdam Study	~2,200	2083
Ottawa study	710	2083
Framingham study	5,239	~700



4,310 cis- and trans-miRNA-eQTLs (P<2.4x10e-11) for **64** miRNAs

The miRNA-eQTLs can explain the variance ranged from 2% to 11%

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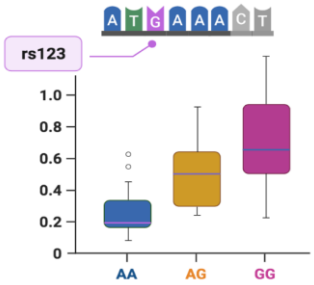
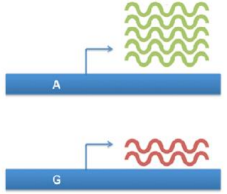
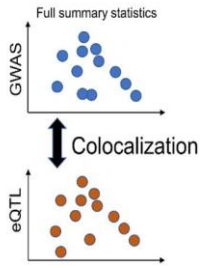
Step.2

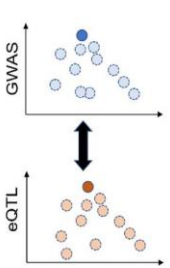
Association of miRNA-SNPs with clinical traits

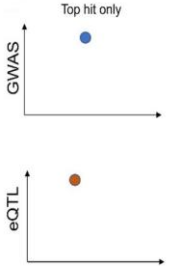
GWAS Catalog











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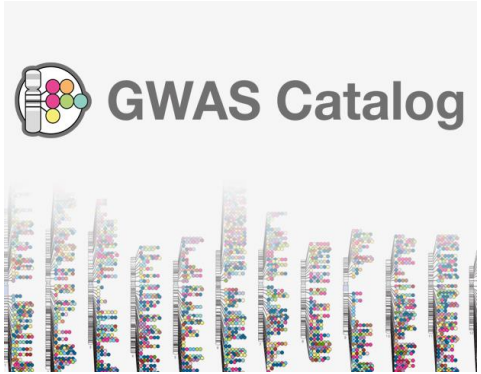
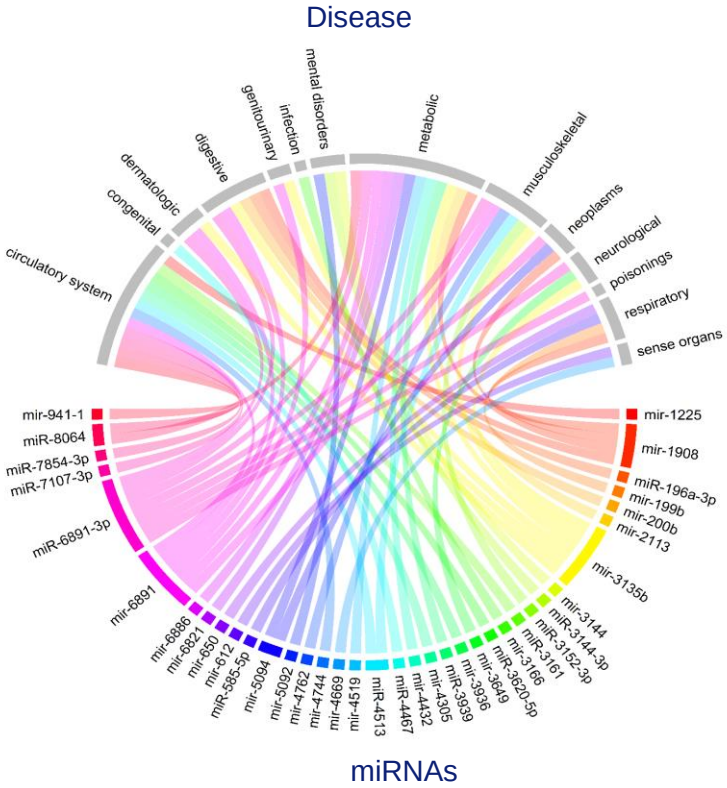
Step.2

Clinical significance of miRNA-related variants



Cis-miRNA-eQTLs were associated with diseases such as blood pressure, cancer, mental health, haematological indices, anthropometric measures, lipid levels.

Trans-miRNA-eQTLs were associated with diseases/traits eg. cancer, cardiometabolic, haematological indices, allergy.



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Step.2

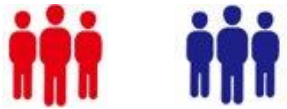
miRNAs implicated in multiple traits (UK Biobank)

UK Biobank with N~425K participants

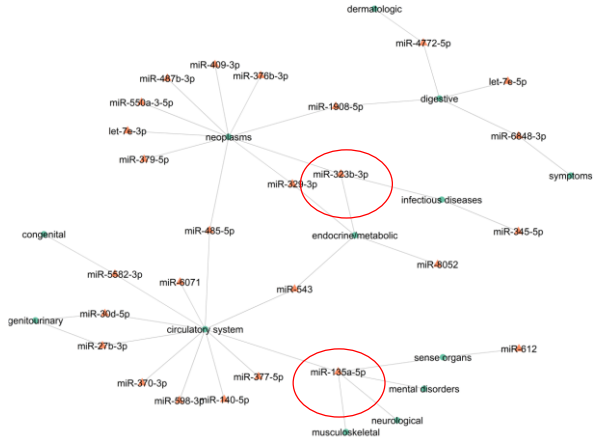
905 clinical traits (>200 cases) in 16 disease categories

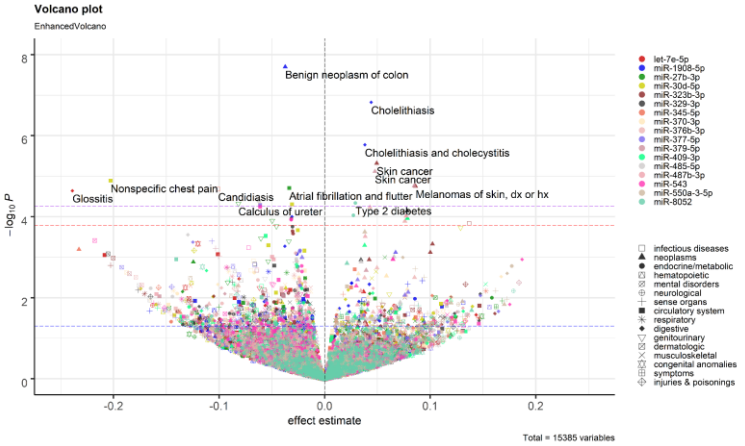
Phenome-wide association studies (PheWAS):

- Single cis-instrument analysis for 85 miRNAs
  ***9** miRNAs* ⟹ ***23** clinical outcomes*
- GRS cis-instrument analysis for 119 miRNAs
  ***17** miRNAs* ⟹ ***24** clinical outcomes*



Association analysis across phenome
Phenotype 1 ~ SNP+ sex + age
Phenotype 2 ~ SNP+ sex + age
…

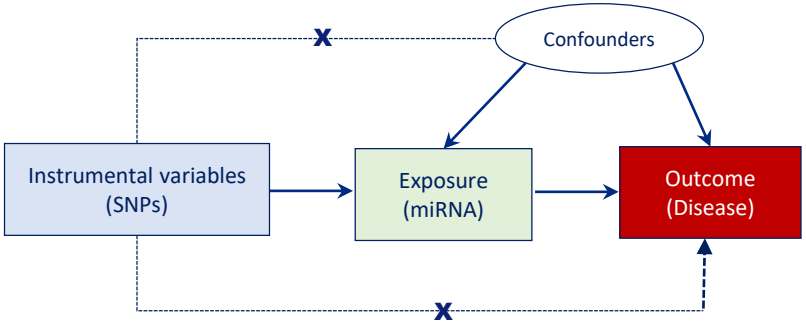


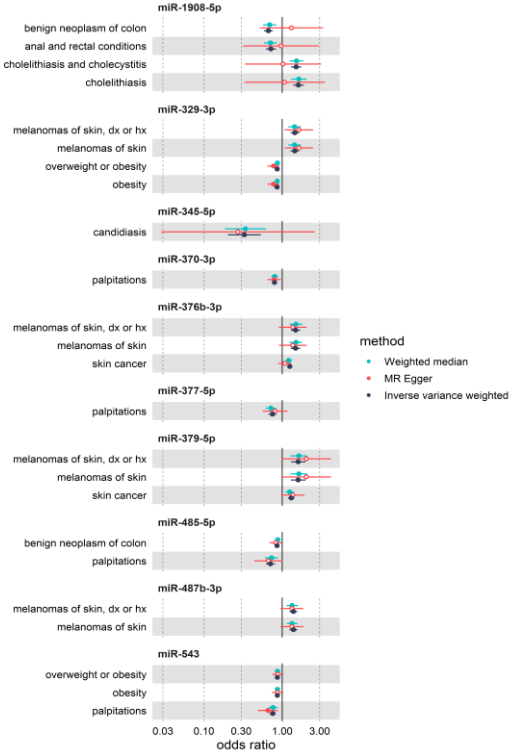


Step.2

Causal link between miRNAs and diseases (MR)

Mendelian randomization

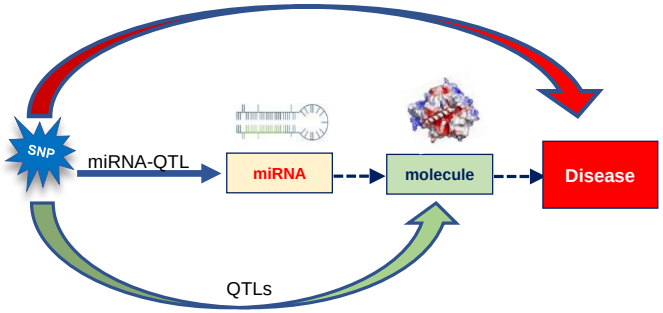


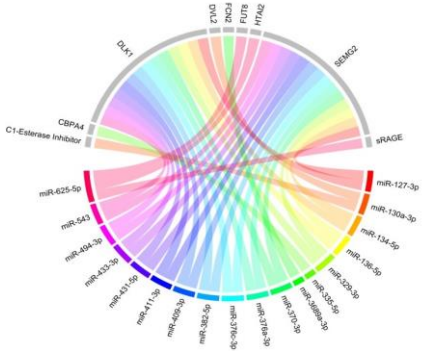


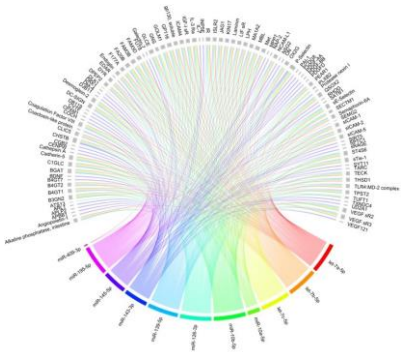
Multivariable MR confirmed a causal role for some miRNAs in complex diseases like cancer and obesity.

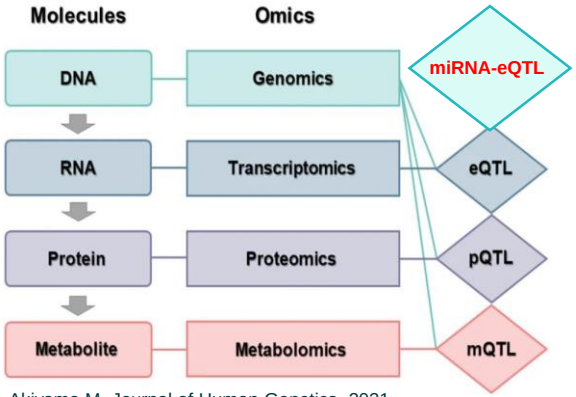
Step.3

Multi-omics study for miRNA-regulated pathways









Akiyama M. Journal of Human Genetics, 2021.

- **miRNA-QTLs** (n~8,000) ⟶ **64** miRNAs
- **Gene-eQTLs** (n~32,000) ⟶ **39** miRNAs and **146** genes
- **Protein-QTLs** (n~30,000) ⟶ **29** miRNAs and **112** proteins
- **Metabolite-QTLs** (n~10,000) ⟶ **13** miRNAs and >**200** metabolites

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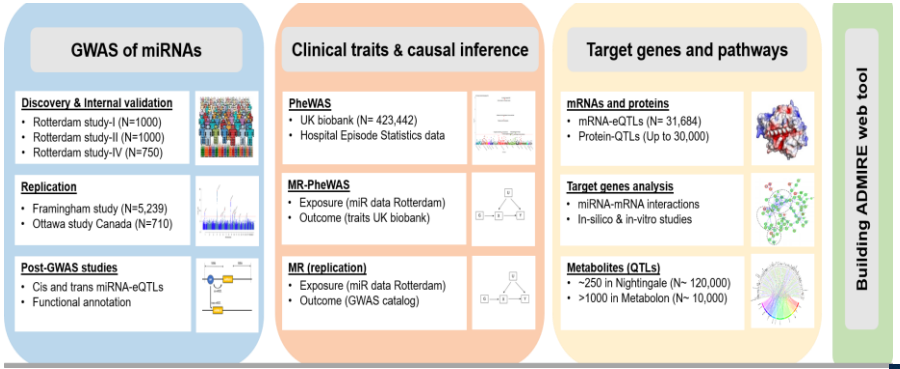
Step.4

Atlas of genetic regulation & Disease association of miRNAs Expression (ADMIRE)



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An atlas of genetic regulation and disease associations of microRNAs

Rima Mustafa, Michelle M.J. Mens, Arno van Hilten, Jian Huang, Gennady Roshchupkin, Tianxiao Huan, Linda Broer, Paul Elliott, Daniel Levy, M. Arfan Ikram, Marina Evangelou, Abbas Dehghan, Mohsen Ghanbari

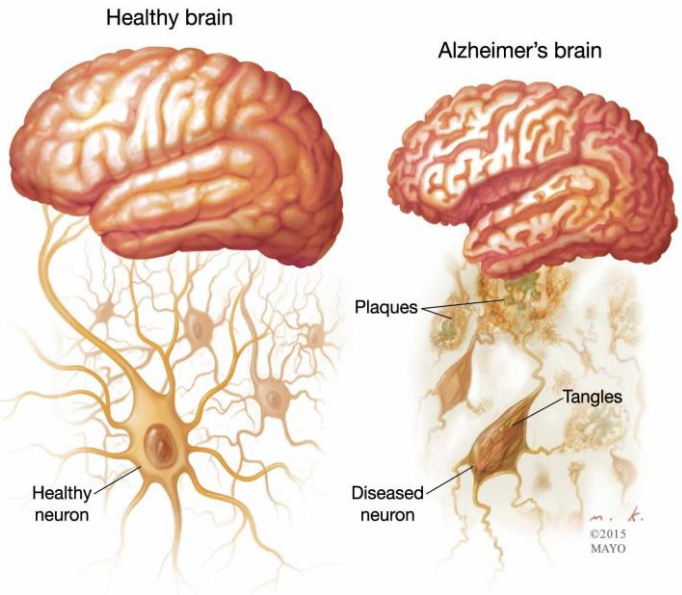
doi: https://doi.org/10.1101/2022.11.10.22282180

Alzheimer's disease (AD)

manifested by the progressive loss of memory and cognitive decline.

Amyloid plaques & neurofibrillary tangles in the brain are two neuropathological hallmarks of AD.

>50 risk loci identified by GWAS that explain ~30% of the disease heritability.



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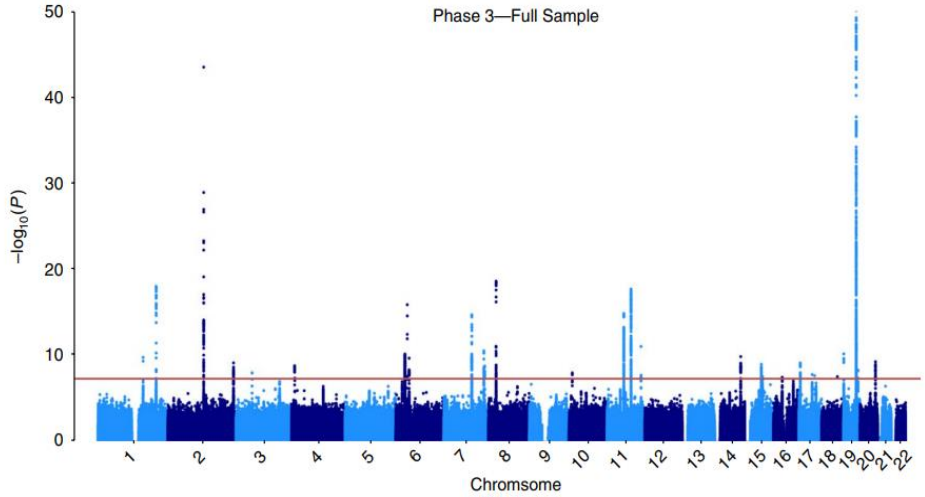
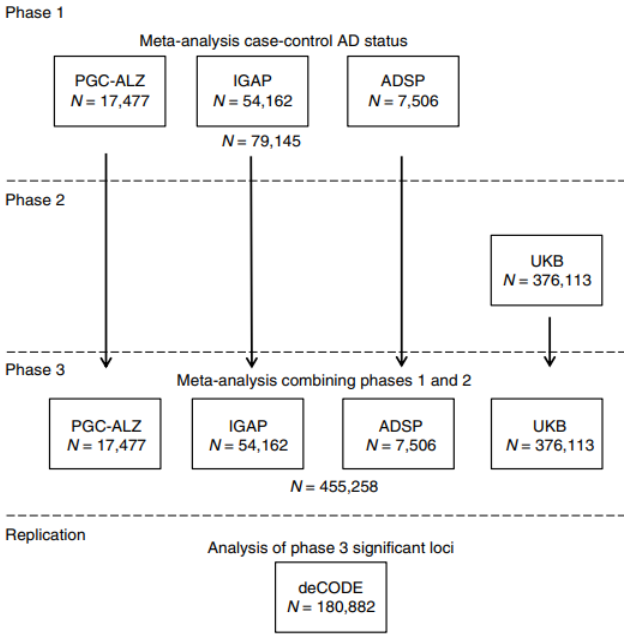
ARTICLES
https://doi.org/10.1038/s41588-018-0311-9
nature genetics

Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk



GWAS meta-analysis for AD risk ($N = 455,258$). Manhattan plot displays all associations per variant ordered according to their genomic position on the *x* axis and showing the strength of the association with the $-\log_{10}$-transformed *P* values on the *y* axis.



Jansen et al., *Nature Genetics* 51: 404–413 (2019).

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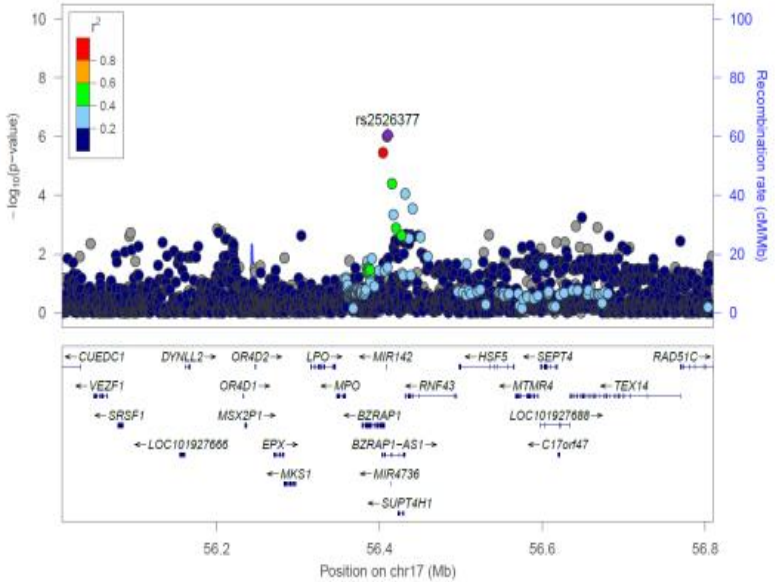
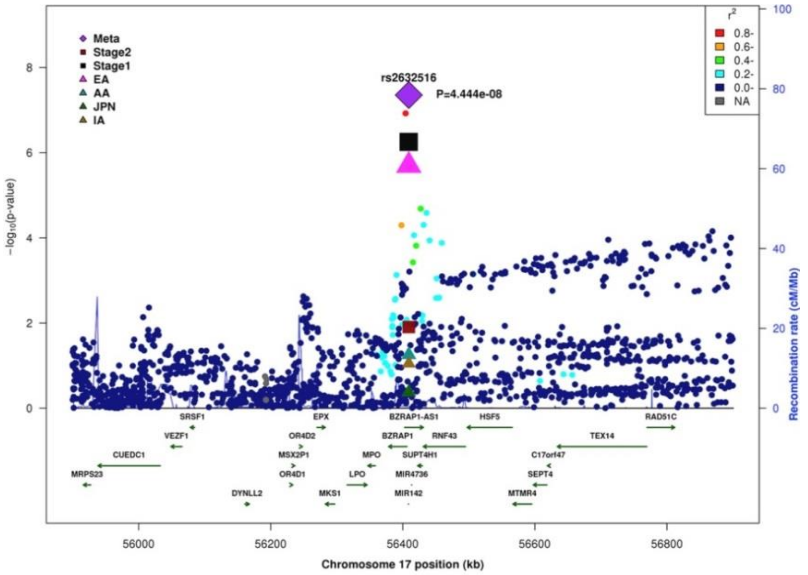
A non-coding SNP at 17q22 associated with AD



Jansen et al., *Nature Genetics* 51: 404–413 (2019).



Jun et al., *Alzheimers Dementia* 13(7):727-738 (2017).

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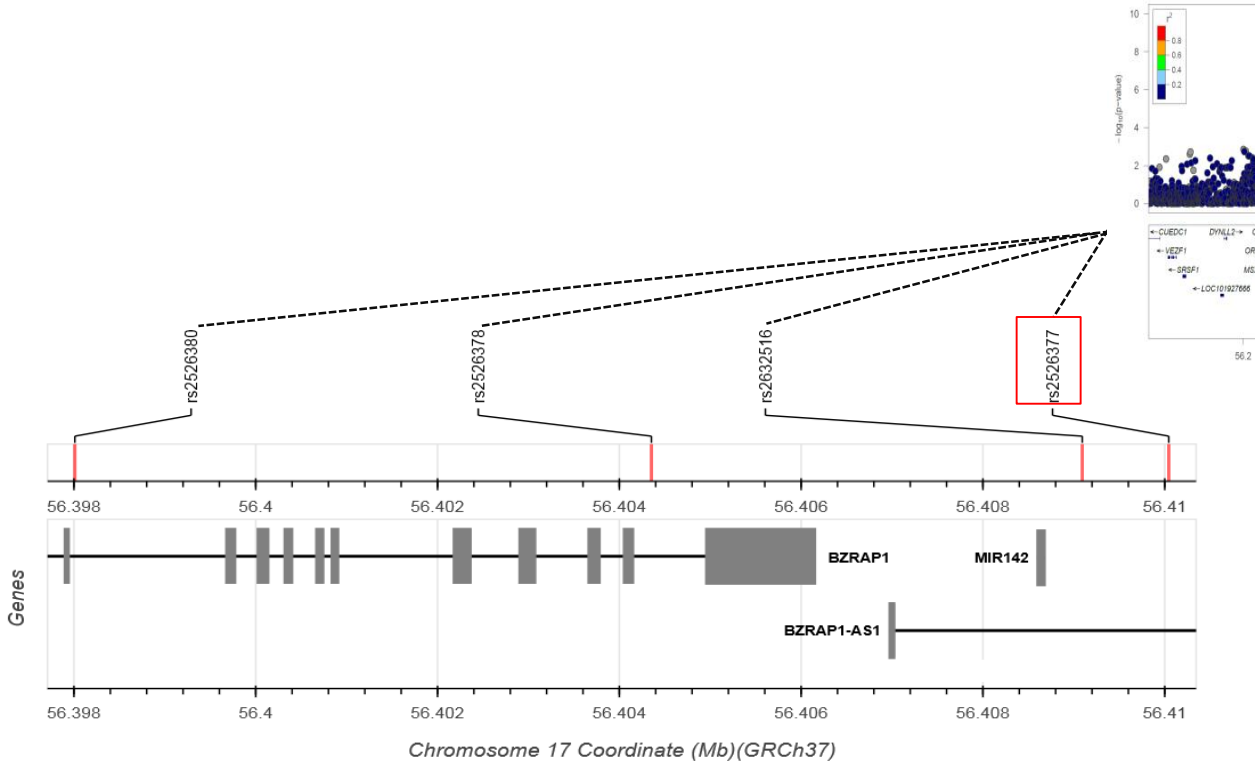
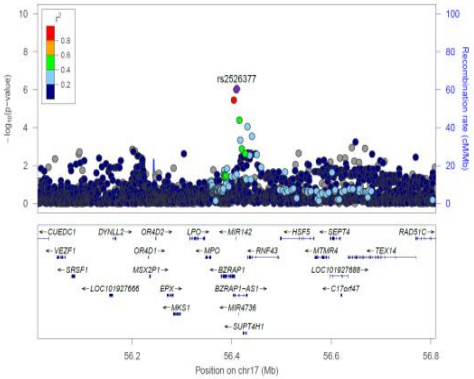
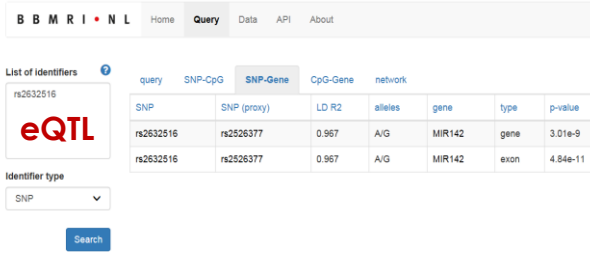
SNPs in high LD at 17q22 associated with AD







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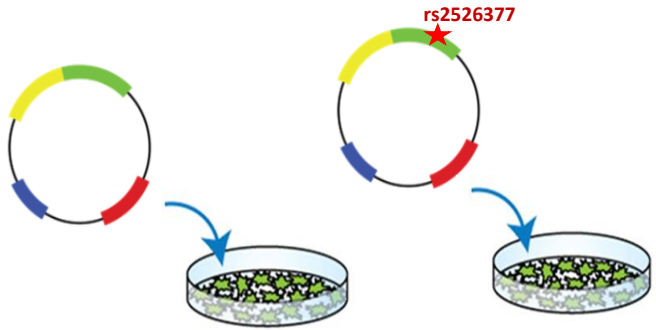
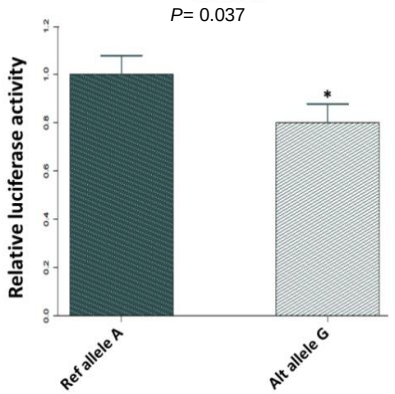
rs2526377 influences on promoter activity of miR-142





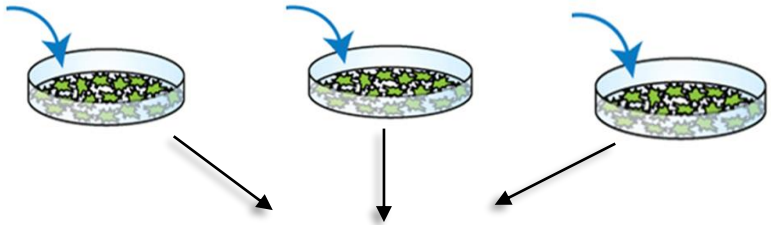
Overexpression and inhibition studies of miR-142

Human iPSC-derived neural progenitor cells

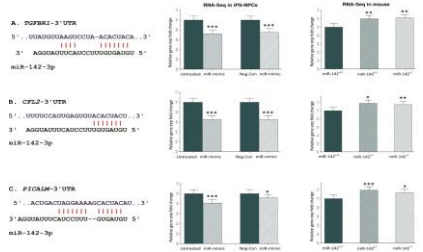
A. Untreated cells

B. miR negative control

C. miR-142 mimic



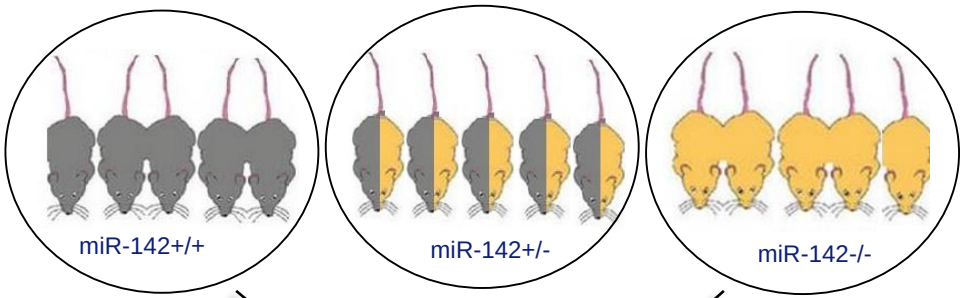
RNA-Seq and differential gene expression (A, B & C)



Down-regulated target genes of miR-142-3p (n=58)

Down-regulated target genes of miR-142-5p (n=28)

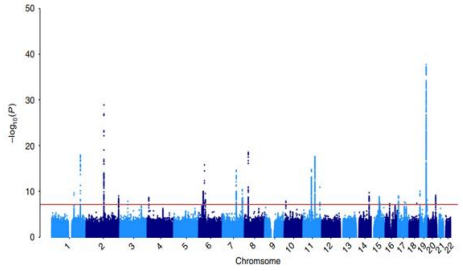
miR-142 Knock-out mice

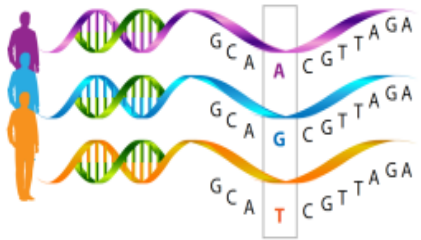


RNA-Seq and differential gene expression of the brain (3 groups)

Up-regulated target genes of miR-142 in the hippocampus of KO vs WT mice

The SNP confers AD risk by deregulation of miR-142





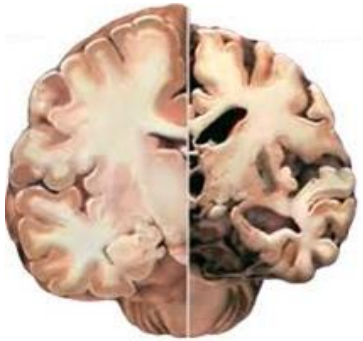
A SNP in 17q22 negatively associated with AD in GWAS data

rs2526377 (G allele)

miR-142

Target Genes (e.g. *TGFBR1*, *PICALM*)

Alzheimer's disease



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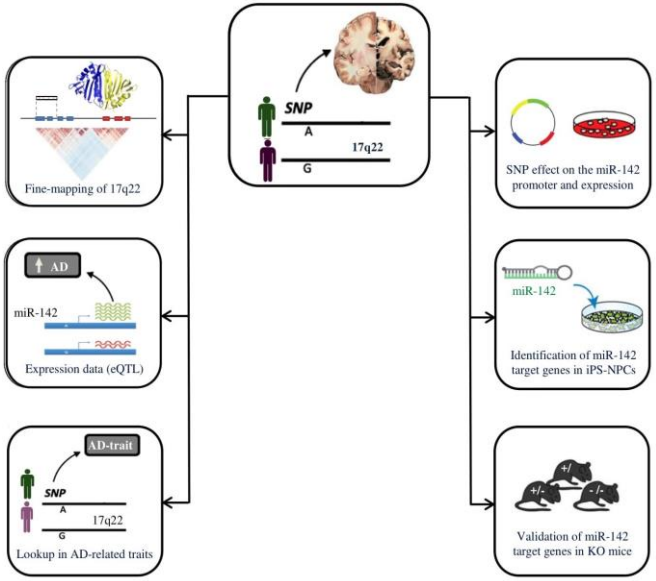
miR-142 regulates the AD hallmark pathways



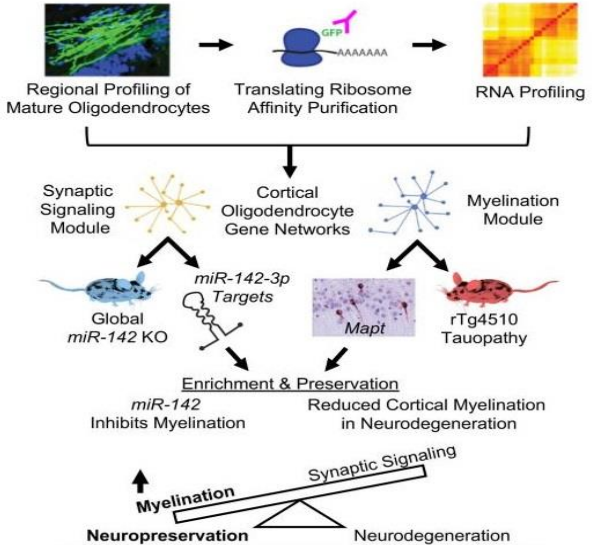
Received: 25 January 2019 | Revised: 13 June 2019 | Accepted: 11 July 2019

DOI: 10.1002/humu.23872

RESEARCH ARTICLE

Human Mutation HGVS HUMAN GENOME VARIATION SOCIETY WILEY

A functional variant in the miR-142 promoter modulating its expression and conferring risk of Alzheimer disease



Human Molecular Genetics, 2021, Vol. 30, No. 1 103–118

doi: 10.1093/hmg/ddaa252
Advance Access Publication Date: 8 February 2021
General Article

OXFORD

GENERAL ARTICLE

miR-142-3p regulates cortical oligodendrocyte gene co-expression networks associated with tauopathy

The potential of miR-142 as biomarker for AD

Research Article
Profiling of microRNAs in Alzheimer's disease

TRANSPARENT PROCESS OPEN ACCESS EMBO Molecular Medicine

Alteration of the microRNA network during the progression of Alzheimer's disease

Sørensen *et al. Translational Neurodegeneration* (2016) 5:6
DOI 10.1186/s40035-016-0053-5

Translational Neurodegeneration

miR-142 is among 15 upregulated miRNAs in the hippocampus of AD patients

RESEARCH Open Access

miRNA expression profiles in cerebrospinal fluid and blood of patients with Alzheimer's disease and other types of dementia – an exploratory study

Sofie Sølvsten Sørensen[1], Ann-Britt Nygaard[2] and Thomas Christensen[1*]

miR-142 is among the upregulated miRNAs in the CSF and plasma of AD patients

Challenges in multi-omics research

Sample size: Obtaining a sufficiently large sample size to provide a good statistical power.

Data quality: Several factors may influence the quality of omics data (eg. sample preparation, measurement accuracy, and analytical variability). Standardization of data collection and QC are required to minimize the impact of these factors and help the reproducibility and comparability of results.

Data integration: Integrating omics data from different platforms is complex and require sophisticated bioinformatics approaches.

Data interpretation: The interpretation of results can be challenging due to the complexity and heterogeneity of biological systems. Appropriate statistical and computational methods are required to identify relevant biological pathways and biomarkers.

Ethics and privacy: Multi-omics research in population-based studies raises several ethical and privacy concerns regarding the storage, sharing & use of sensitive personal information.

Thank you for your attention!

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