RNA editing as a diagnostic test to diagnosis bipolar disorders

VI CONGRESO LATINOAMERICANO DE BIOQUIMICA CLÍNICA

II CONGRESO INTERNACIONAL DEL COLEGIO NACIONAL DE BACTERIOLOGÍA

¡El riesgo es que te quieras quedar!

Cartagena, Colombia 3 al 6 OCTUBRE 2024

Maurizio Ferrari MD

Former IFCC President

Full Professor of Clinical Pathology

Chief Medical Office (CMO), Synlab Italia, Monza, Italy

Today I'll cover:

- Epigenetics/Epitranscriptomics
- A-to-I Editing
- Psychiatric disorders /Bipolar Disorders
- A-to-I editing in different diseases
- A-to I Editing to differentiate Unipolar depression and Bipolar disorders
- Edit B test

Diagnosis: the missing keystone for global health

Biological diagnosis missing in major therapeutic areas, like mental health

Companion diagnostics limited mainly to oncology

70% of medical decision depends on diagnostics

Diagnosis wavering and error aggravate patients' outcome

Prevention and early treatmer made impossible by the lack early diagnosis

The term epigenetics, which he referred to as, “the branch of biology that studies the causal interactions between genes and their products which bring the phenotype into being” (Conrad Waddington 1942)

Chronology of epigenetics

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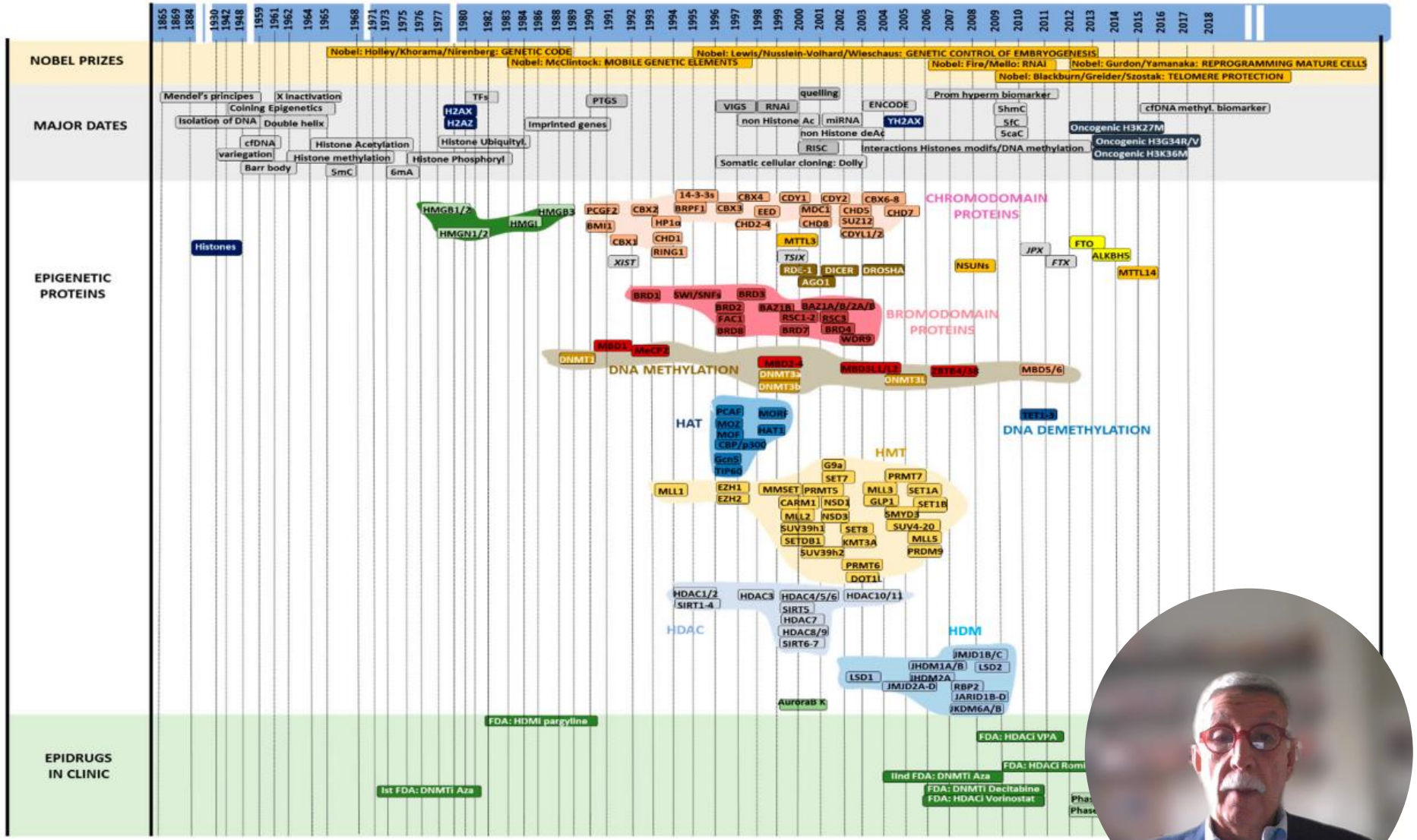
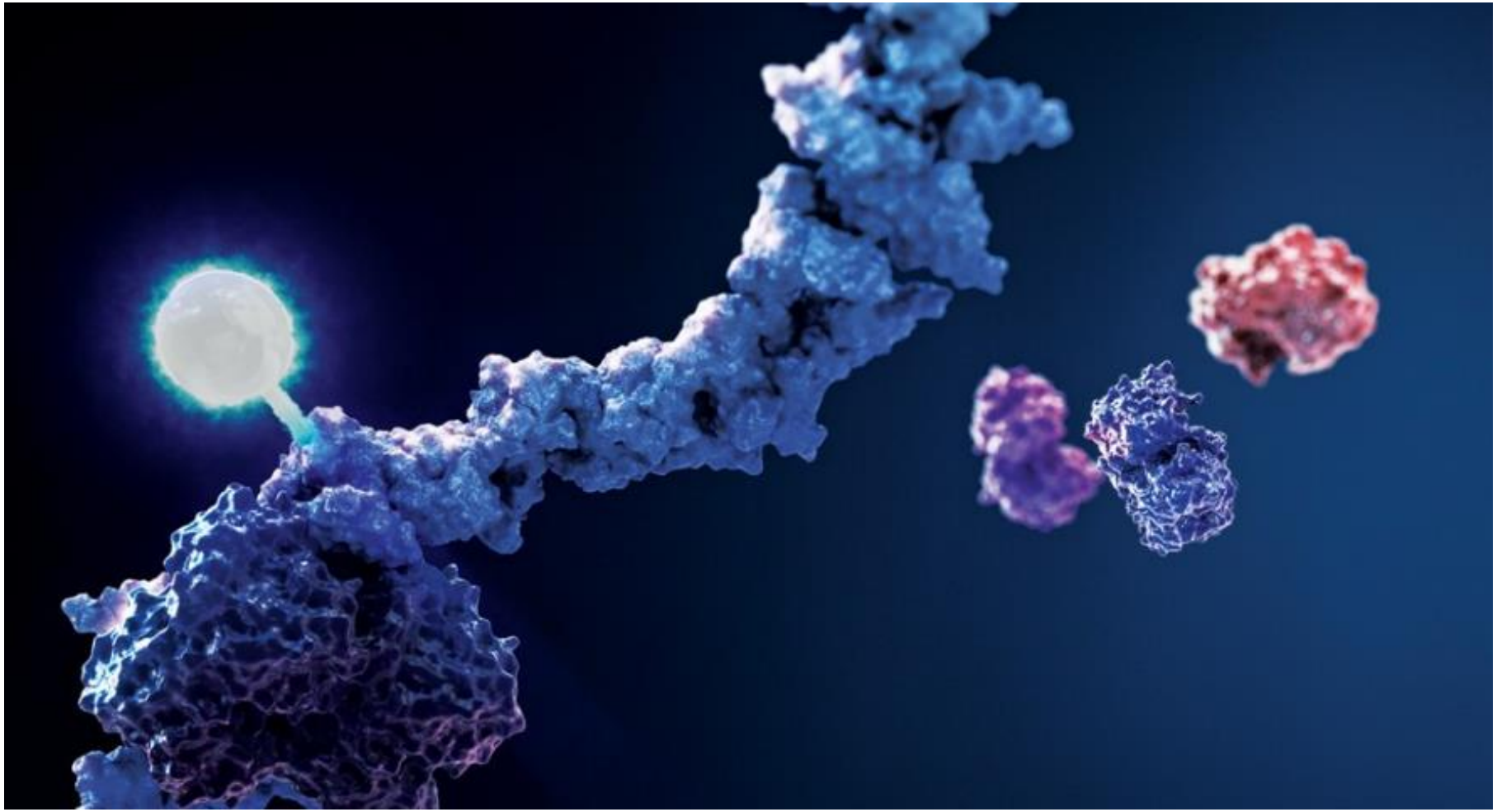
Dates (blue): from 1865 to today
Nobel Prizes (salmon) associated with major genetic or epigenetic discoveries
major dates (gray): important discoveries concerning DNA and chromatin

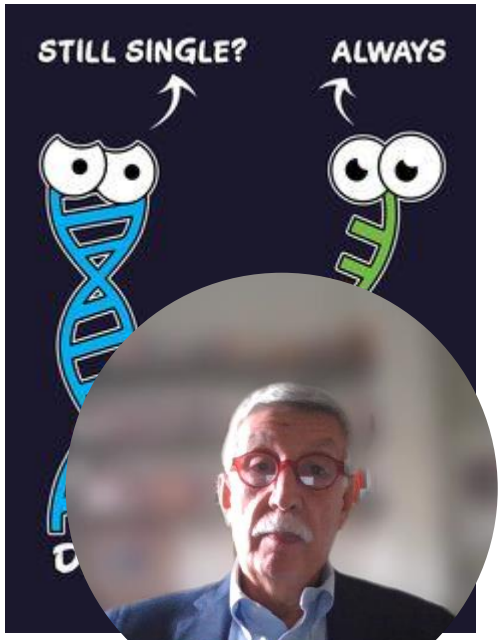
epigenetic proteins (white): year of identification or main writers, readers and erasers of epigenetics (a color code has been associated to proteins involved in a same pathway, e.g., blue for HAT (histone acetyl transferase))

epidrugs in the clinic (green): use of main epidrugs in clinical trials

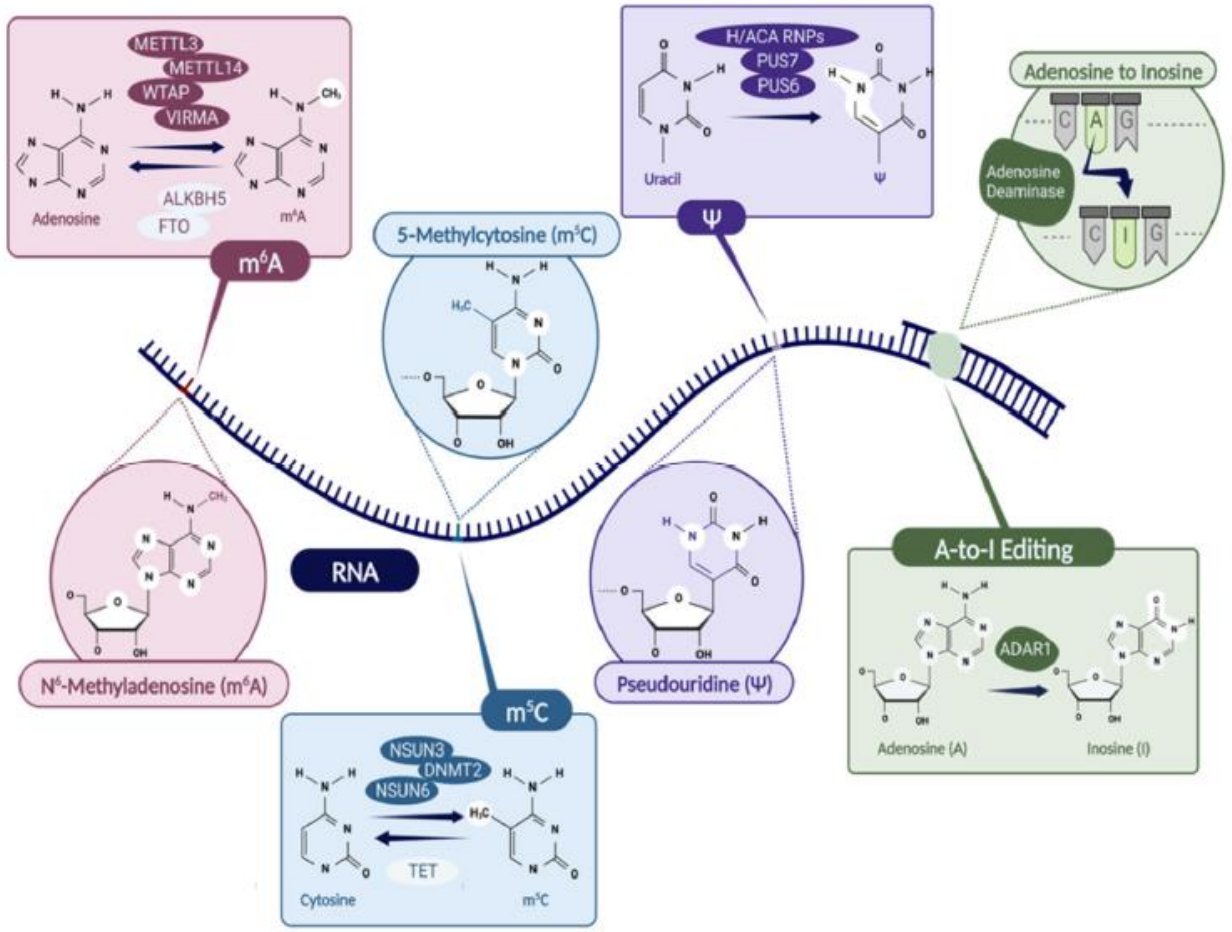


Epitranscriptomics (RNA epigenetics) refers to the posttranscriptional modification of RNA bases (i.e., cytosine and adenosine methylation), which is mediated by specific RNA modification enzymes

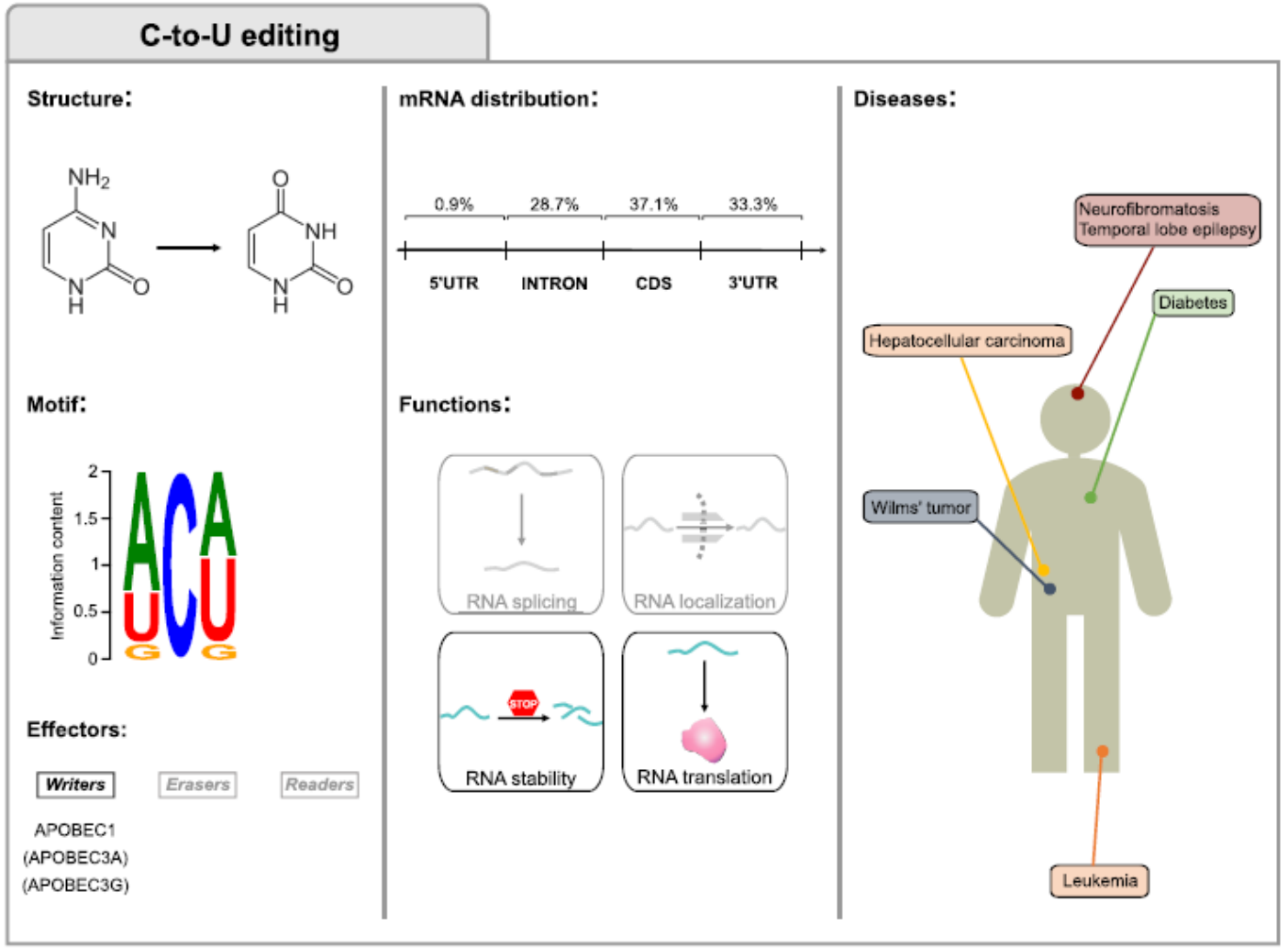




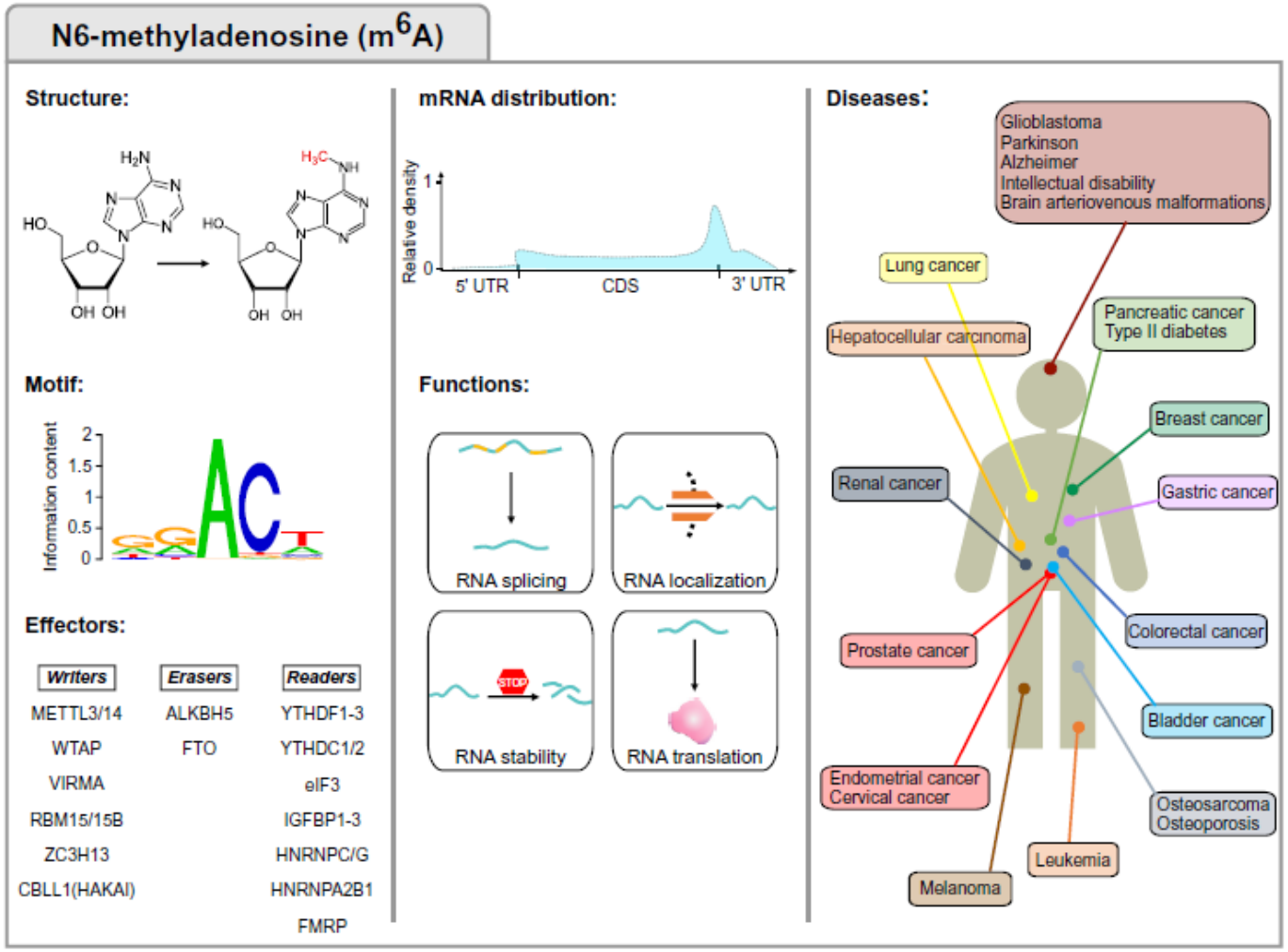
Epitranscriptomic modifications

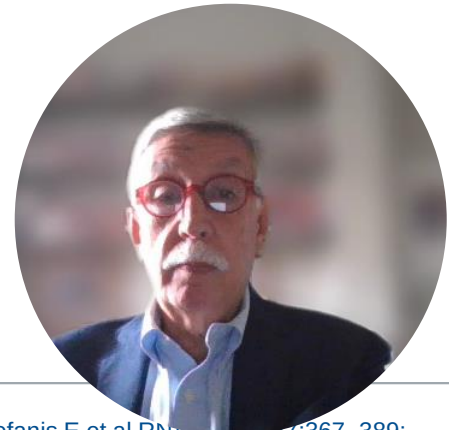


C-to-U editing



N6-methyladenosine (m6A) modification

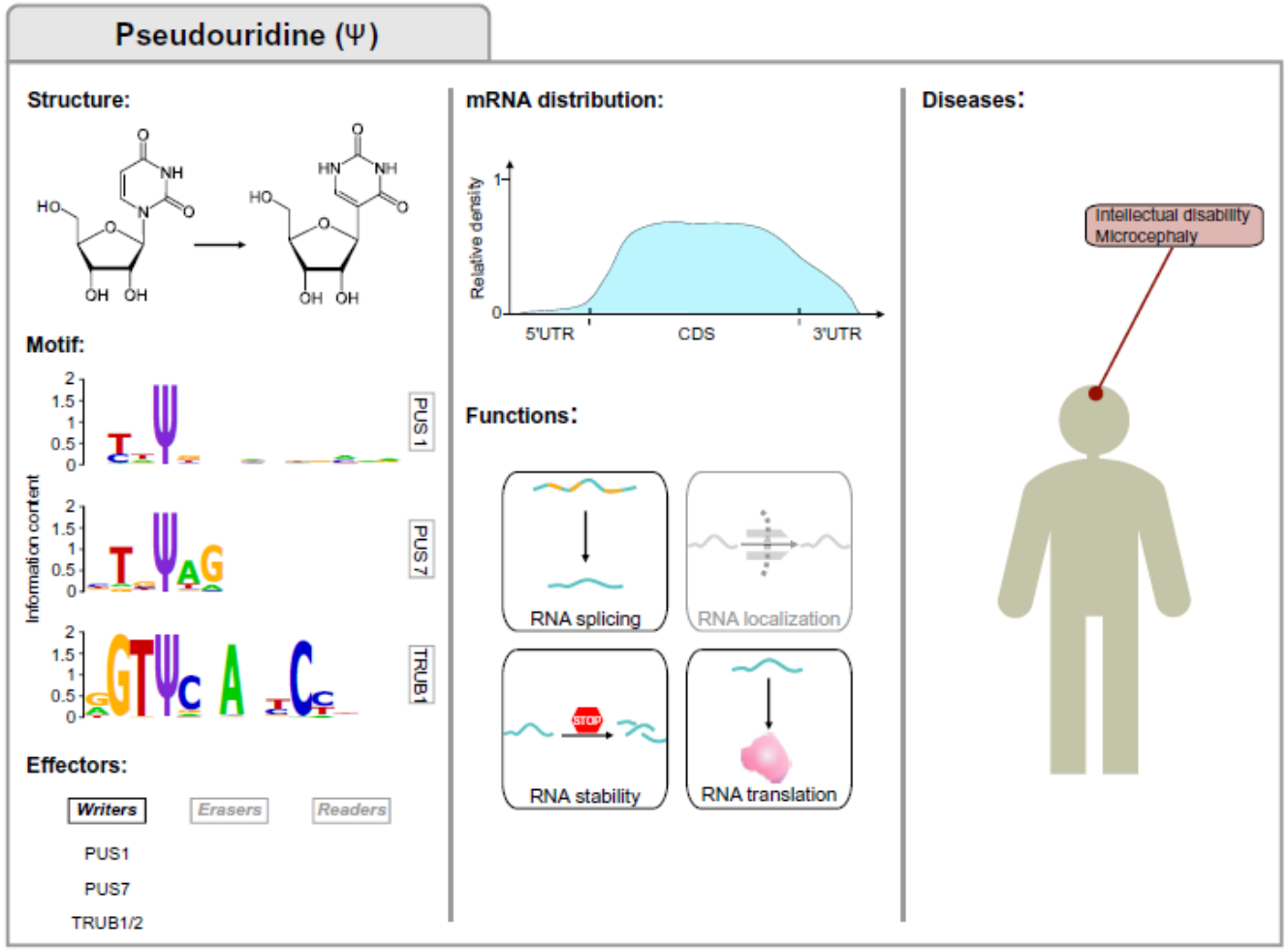




Destefanis E et al RNA (2021) 27:367–389;

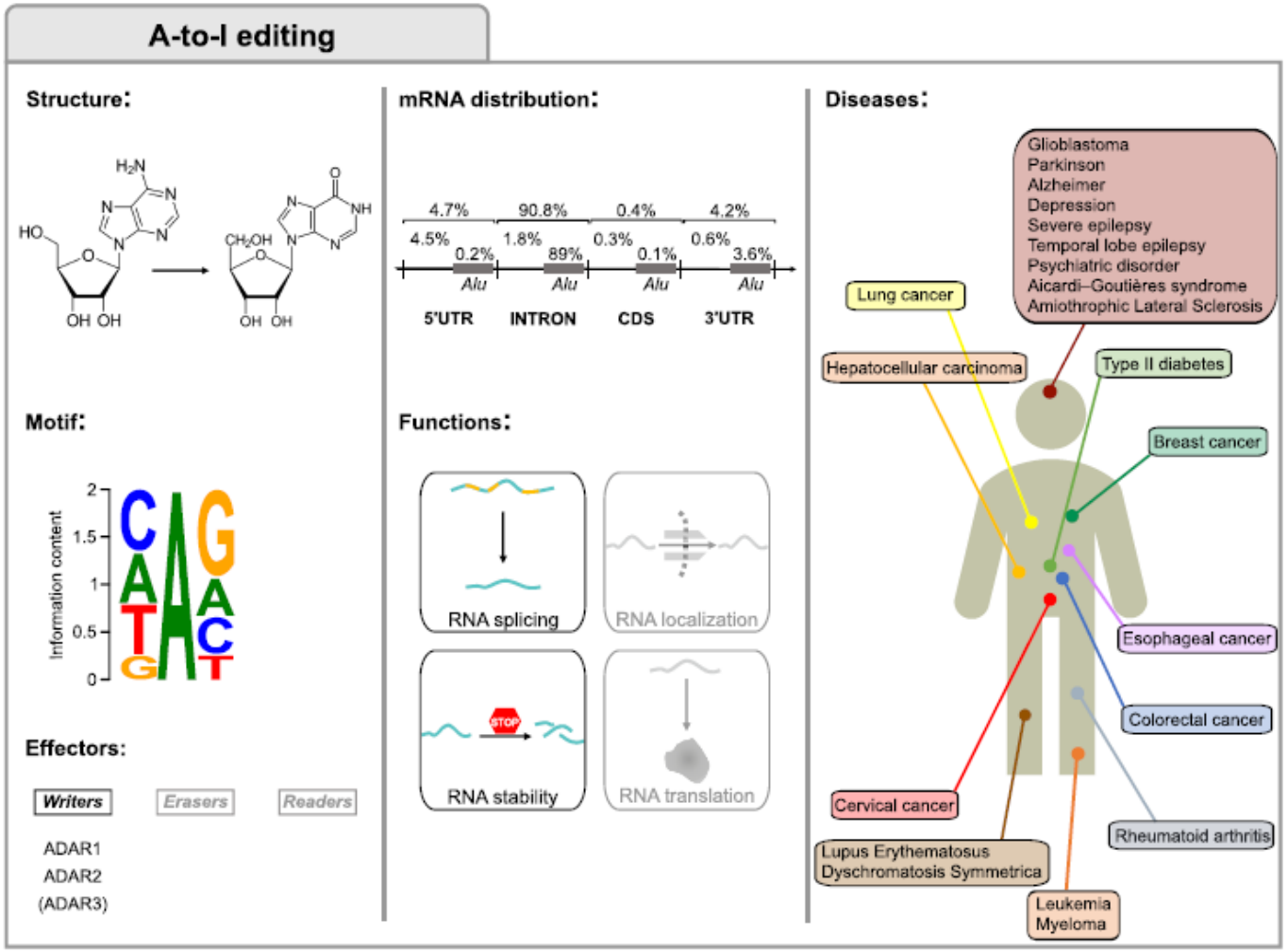
Pseudouridine (Ψ) modification

b



Destefanis E et al RNA ( ) :367–389;

A-to-I editing



Destefanis E et al [illegible] 367–389;

RNA editing, a key mechanism with critical cascading consequences

A-to-I Editing is :

An epitranscriptomic modification

A deamination of adenosine into an inosine

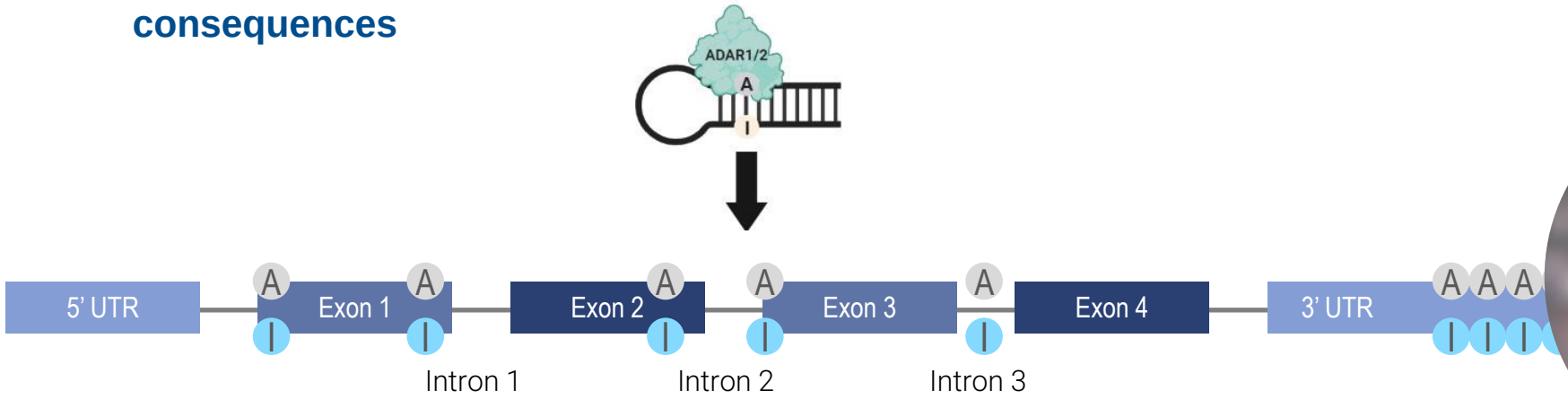
Site/structure specific

Regulated by ADARs (adenosine deaminase acting on dsRNA) enzymes

Which dysregulation implies severe consequences

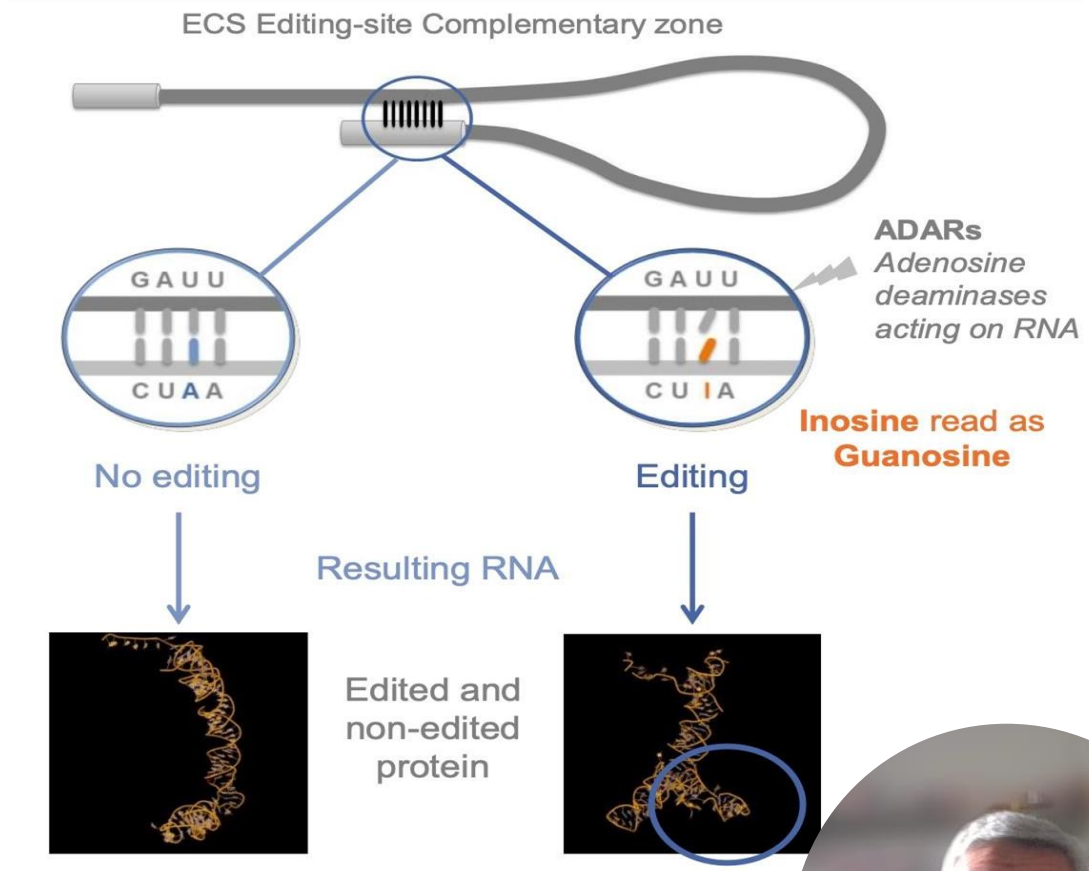
RNA editing impacts key mechanisms…

- RNA structure and stability
- Splicing
- Gene expression
- Protein synthesis
- Signal transmission



RNA EDITING – How it works?

- Change structure and stability of RNA
- Aminoacid changes
- Modifies the protein synthesis
- Modulates the synaptic function
- Involved in psychiatric disorders and inflammatory processes



RNA modifying adenosine-to-inosine (A-to-I) is th
of post-transcriptional modification, catalyzed by

The global burden of psychiatric disorders

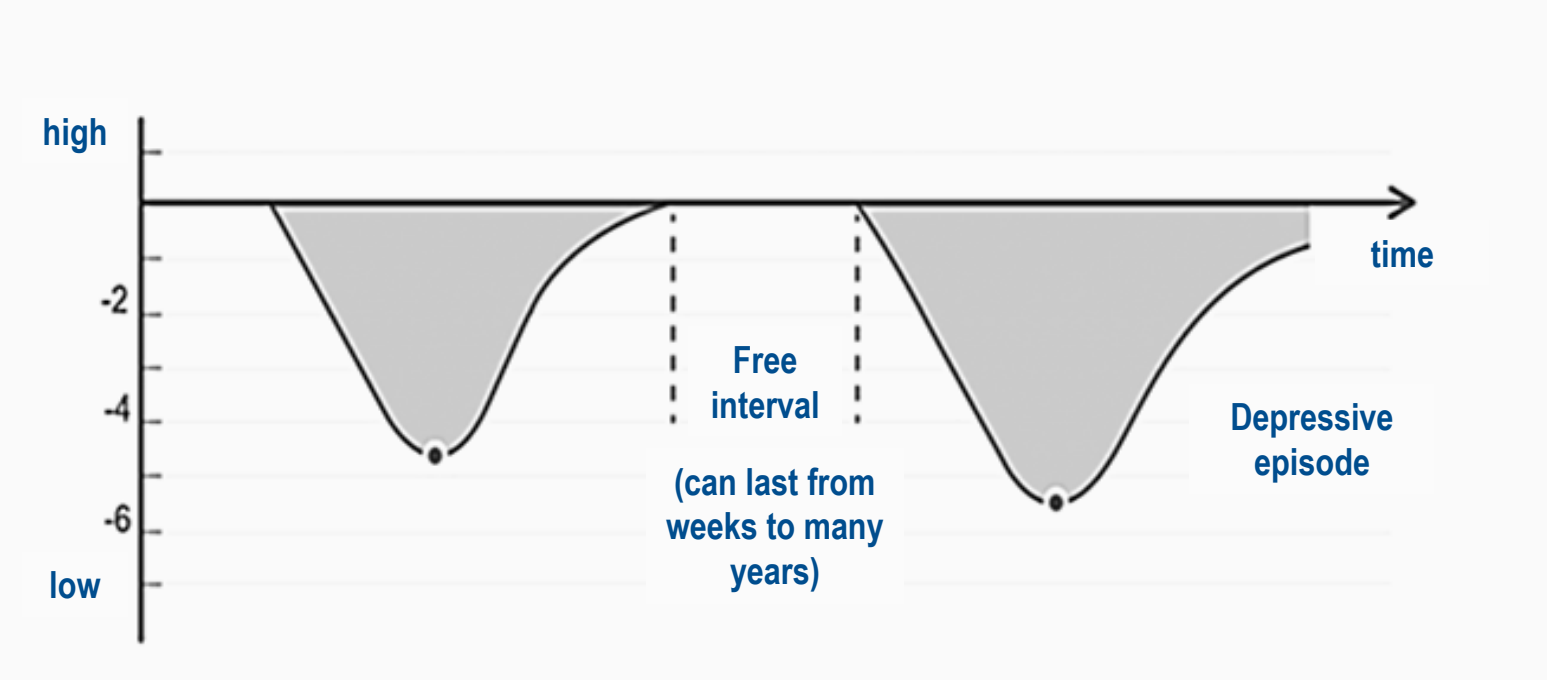
- Psychiatric disorders comprise different types of mental disorder, including anxiety disorder, major depressive disorder (MDD), bipolar disorder (BP), post-traumatic stress disorder, schizophrenia (SZ), eating disorders, neurodevelopmental disorders, disruptive behavior, and dissocial disorders. All disorders have in common a clinically significant disturbance in an individual's cognition, emotional regulation, or behavior and are normally associated with distress or impairment in important areas of functioning.
- In 2019, there were 970 million people around the world living with a psychiatric disorder.
- More than half of those people struggle with anxiety disorder (301 million) or depression (280 million), both of which are also common in children and adolescents.
- BP was experienced by 40 million people.
- Obsessive-compulsive disorder (OCD) affects 1%-3% of the worldwide populati
- Suicide is strongly connected with psychiatric disorders (in particular, depres

The global burden of depression

- Depression is one of the most common mental health disorder affecting near 10% of men and 20% of women worldwide , and is associated with a significant increased mortality, mostly due to suicidal behavior.
- Within mood disorders, bipolar disorder (BD) is one of the most frequent and disabling ones, affecting 1% of the world's population, characterized by episodes of mania, hypomania, and alternating or intertwining episodes of depression.
- As a consequence, the average interval between onset of BD symptoms and proper diagnosis is estimated to be around 7 years.
- Various clinical interview-based instruments are available and routinely used in practice by psychiatrists to diagnose BD, including evaluation of manic symptoms by Young Mania Rating Scale (YMRS), the Altman self-rating scale (ASRM) or the Mood Disorder Questionnaire (MDQ).
- Biological markers to set the boundaries between the different subtypes of depre... are lacking and a major research goal is to identify reliable and clinically usef... biomarkers to differentiate BD from unipolar depression.
- Recent studies have shown an association between depression and RNA alt... epitranscriptomic mechanisms, including RNA methylation , microRNAs , an... editing.

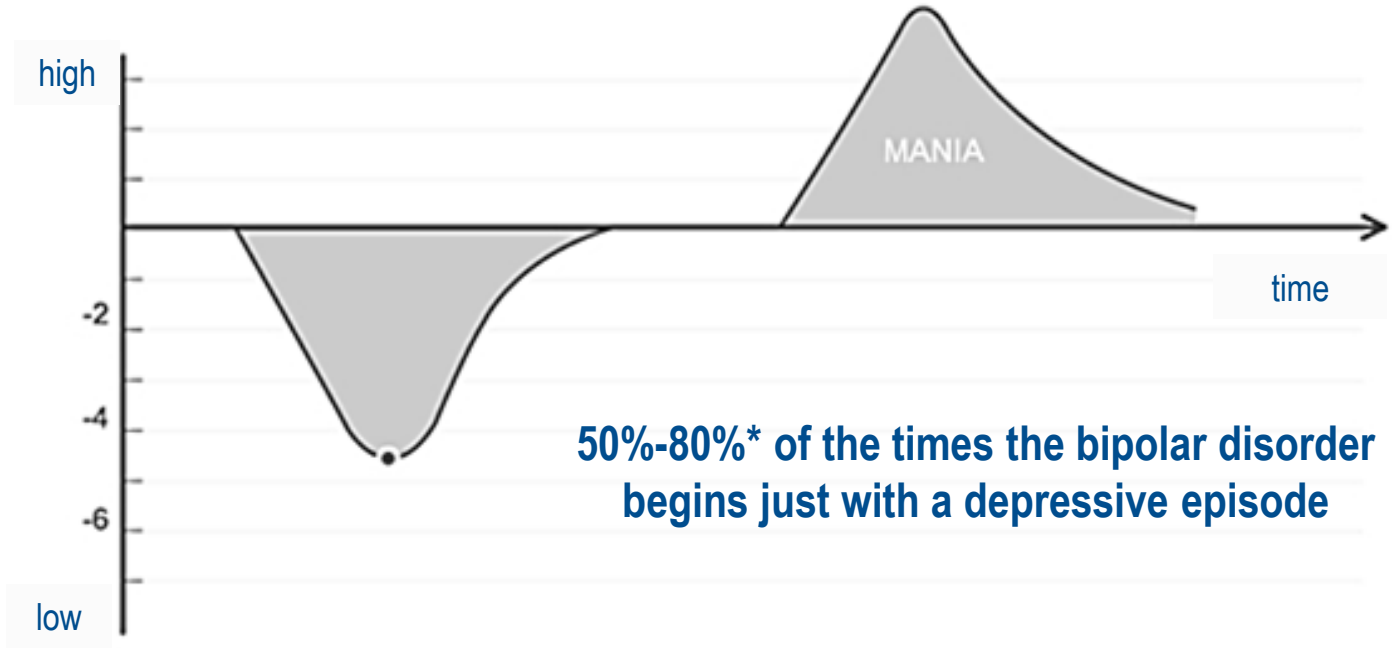
SYMPTOMS

MAJOR DEPRESSIVE DISORDER (UNIPOLAR)



SYMPTOMS

BIPOLAR DISORDER



50%-80%* of the times the bipolar disorder begins just with a depressive episode

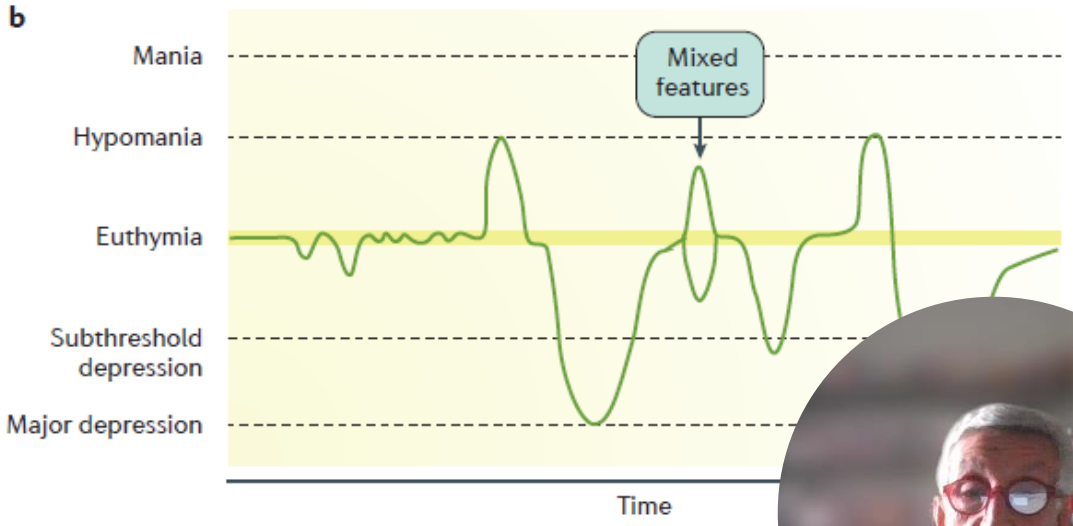
https://ifightdepression.com/it/per-tutti/sottotipi-di-depressione

*Duffy et al., 2007, Mesman et al., [illegible] et al., 2015

Main subtypes of bipolar disorder

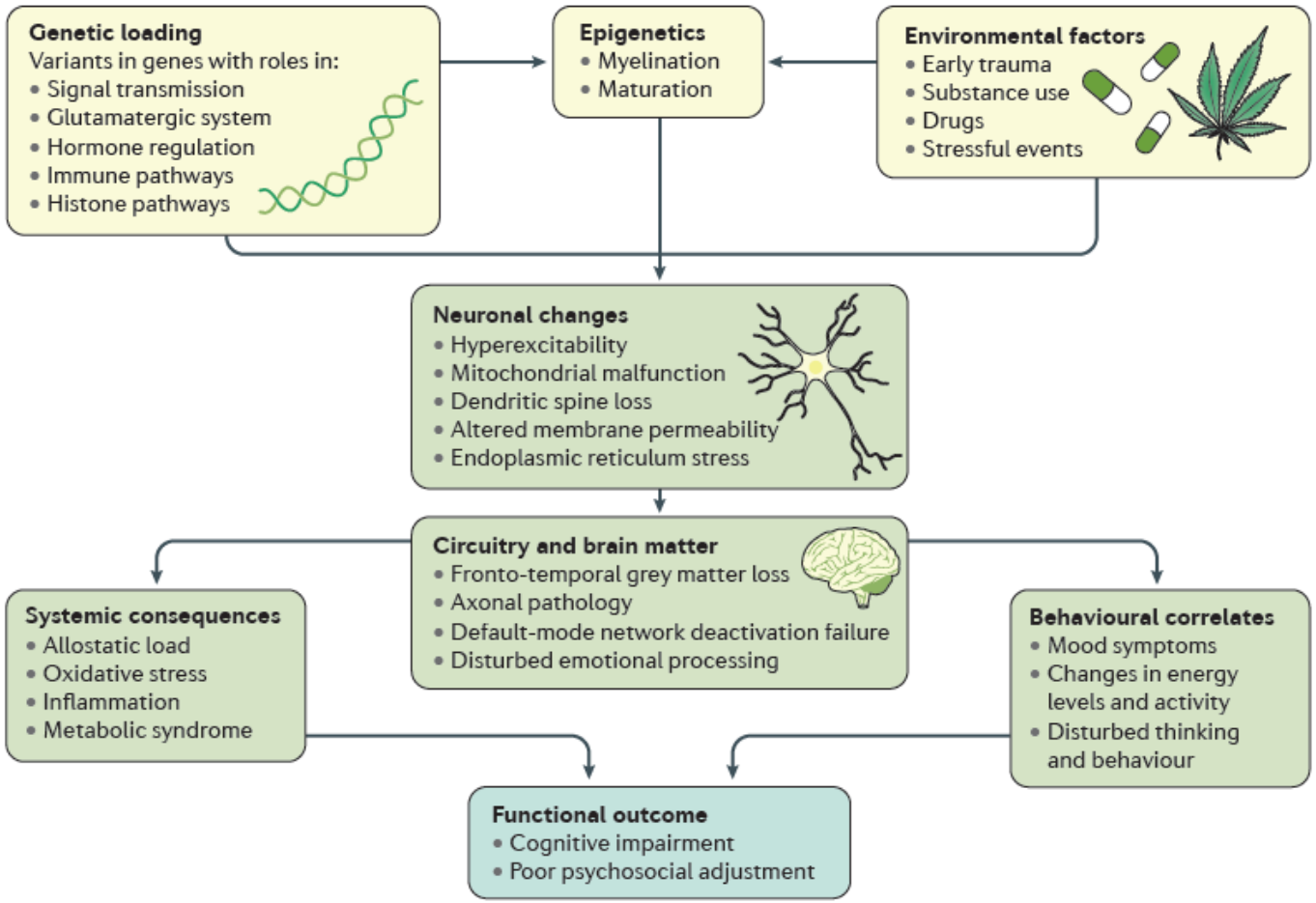
Bipolar I disorder is characterized by at least one episode of mania

Bipolar II disorder is characterized by at least one hypomanic and one depressive episode



Vieta E et al, NATURE REVIEWS, DISEASE PR… 18008, 2018

Multifactorial model of bipolar disorders



Vieta E et al, NATURE REVIEWS, DISEASE PR… 18008, 2018

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Carvalho et al. *Translational Psychiatry* (2020)10:152
https://doi.org/10.1038/s41398-020-0835-5

Translational Psychiatry

REVIEW ARTICLE Open Access

Evidence-based umbrella review of 162 peripheral biomarkers for major mental disorders

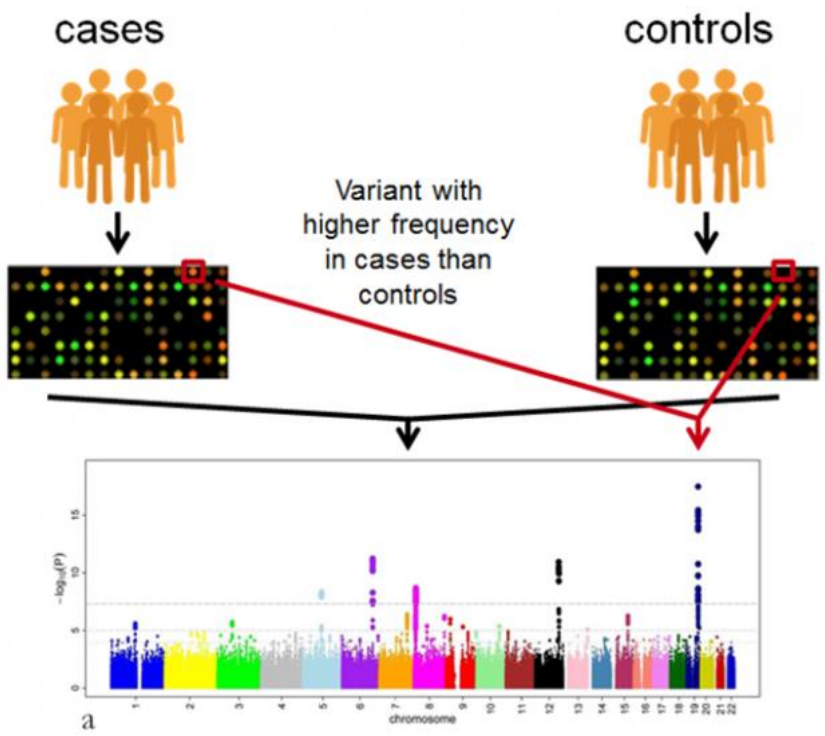
André F. Carvalho[1,2], Marco Solmi[3,4,5], Marcos Sanches[6,7], Myrela O. Machado[8], Brendon Stubbs[9,10], Olesya Ajnakina[11], Chelsea Sherman[12], Yue Ran Sun[12], Celina S. Liu[12], Andre R. Brunoni[13,14], Giorgio Pigato[15,16], Brisa S. Fernandes[17], Beatrice Bortolato[18], Muhammad I. Husain[19,20], Elena Dragioti[21], Joseph Firth[22,23], Theodore D. Cosco[24,25], Michael Maes[26,27], Michael Berk[27,28,29,30], Krista L. Lanctôt[31,32,33,34,35], Eduard Vieta[36], Diego A. Pizzagalli[37], Lee Smith[38], Paolo Fusar-Poli[39,40,41], Paul A. Kurdyak[42,43,44], Michele Fornaro[45], Jürgen Rehm[46,47,48,49,50,51,52] and Nathan Herrmann[53,54,55]

Table 1 Peripheral biomarkers supported by convincing and highly suggestive evidence across major mental disorders.

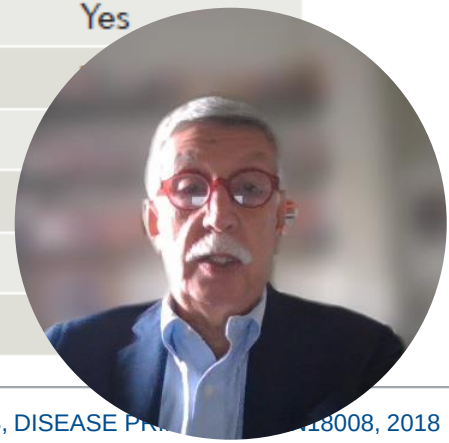
Biomarker (ref. no.)	Alzheimer's disease	Autism spectrum disorder	Bipolar disorder	Major depressive disorder	First-episode psychosis	Schizophrenia
Between-group meta-analyses						
Adiponectin[166]						↓
Anti-Gliadin IgA[118]						↑
Apolipoprotein E[167]	↓					
Arachidonic acid[a] [101]						↑
BDNF[44,110]	↓			↓		
Cortisol[168]						↑
Cortisol awakening response[119]					↓	
Basal cortisol awakening[b] [87]			↑			
CRP[80,102]			↑[c]	↑		
Fibroblast growth factor-2[111]				↑		
Glutamate[91]				↑		
IGF-1[84]			↑[d]	↑		
IL-6[8]				↑		
TGF-Beta 1[11]		↑				
sIL-2 receptor[7,8]				↑		↑
TNF-Alpha[8]				↑		
Folate[105]						↓
Folic acid[59]	↓					
Malondialdehyde[109]						↑
Nerve growth factor[122]						↓
NMDAR[85]			↑			↑
Total cholesterol[94]				↓		
Copper[46]	↑					
Vitamin E[36]	↓					
Vitamin B6[b] [123]						↓
KYNA/3HK[75]				↓		
KYNA/QUIN[75]				↓		
KYN-ACID[75]				↓		
Neurotrophin-3[82]			↑			
Uric acid[81]			↑			
5-hydroxytryptamine[64]		↑				
Glutathione (fasting)[62]		↓				
GSSG[69]		↑				
GSSG (fasting)[62]		↑				
Homocysteine[59]	↑					
Within-group Meta-analyses						
Adiponectin[166]						
IL-6[9]				↓		
Lipid peroxidation Markers[130]				↑		

BDNF brain-derived neurotrophic factor, *IGF* insulin-like growth factor, *IL* interleukin, *INF* interferon, *GSH* glutathione, *GSS*
acid, *Quin* quinolinic acid, *MDA* malondialdehyde, *NMDAR* N-methyl-o-aspartate receptor antibody seropositivity, *NGF* n
quinolinic acid, *sIL-2 Receptor* soluble interleukin 2 receptor, *TGF* transforming growth factor, *TNF* tumor necrosis factor,
[a]Source: Red blood cells.
[b]Convincing evidence criteria. Others biomarkers are supported by highly suggestive evidence.
[c]Euthymia and Mania.
[d]Mania.

GWAS findings in bipolar disorder

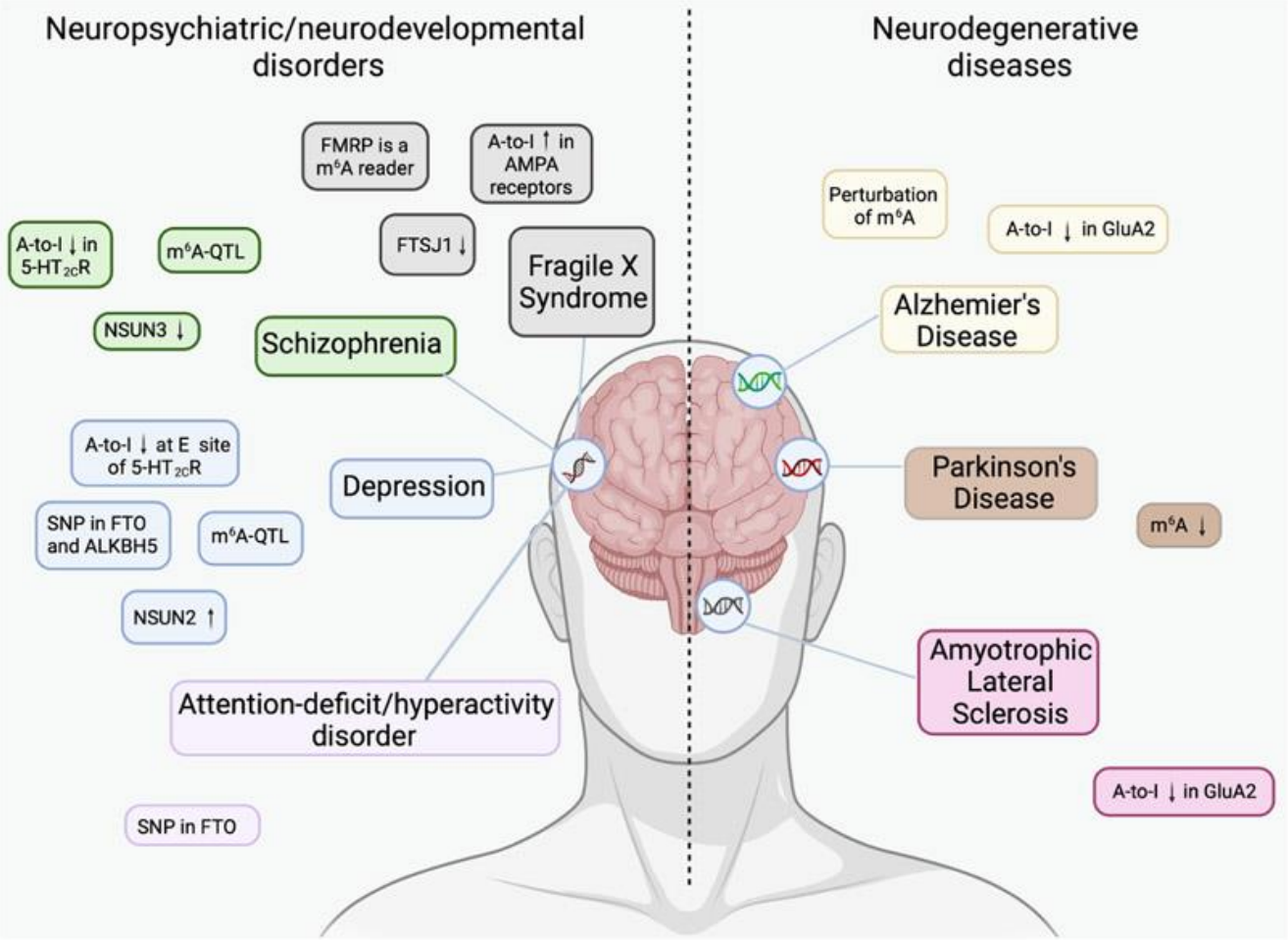


Gene[a]	Locus	Reproduced
PTGFR	1p31.1	No
LMAN2L	2q11.2	Yes
Several genes	3p21	Yes
TRANK1	3p22.2	Yes
ADCY2	5p15.31	No
MIR2113 and *POU3F2*	6q16.1	Yes
SYNE1	6q25.2	Yes
MAD1L1	7p22.3	No
ELAVL2	9p21.3	No
ADD3	10q25.1	No
ANK3	10q21.2	Yes
TENM4	11q14.1	Yes
CACNA1C	12p13.33	Yes
RHEBL1 and *DHH*	12q13.12	
DGKH	13q14.11	
ERBB2	17q12	
NCAN	19p13.11	
TRPC4AP	20q11.2	



Vieta E et al, NATURE REVIEWS, DISEASE PR… …18008, 2018

Perturbation of the epitranscriptome is associated with neuropsychiatric disorders and neurodegenerative diseases



RNA editing is widely involved in human health

Neuro Psychiatric disorders

Depression/ Bipolar disorder *Berg, 2008; Chimienti, 2019, Salvetat 2022*
Schizophrenia *Breen, 2019; Ansell, 2021*
Alzheimer *Khermesh, 2016; Patel, 2021*
Suicide *Weissmann, 2016; Salvetat, 2021*
Epilepsy *Krestel ,2013*

Cancer

Hepatocellular carcinoma *Chen. 2013 Yu 2019*
Breast *Fumagalli. 2015, Li, 2021*
Colorectal cancer *Wei 2022*
Metastatic Melanoma *Nemlich 2018*
Gastric *An, 2021*

RNA editing

Inflammation Infectious diseases

Systemic Lupus erythematosus *Roth, 2018*
Aicardi Gouttiere *Rice,2012*
Systemic Inflammation *Salvetat, 2019*
Hepatitis C Virus *Pujantelli, 2018*
Ebola *Khadka, 2021*
SARS-CoV2 *Liu, 2021*

Metabolic diseases

Obesity *Yu, 2022*
Cardiac dysfunction *El Azzouzi, 2020*
Atherosclerosis *Vlachogiannis, 2021*
Ischemia *Van der Kwast, 2018, 2020*
Diabetes *Xie, 2021*

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nature neuroscience

ARTICLES
https://doi.org/10.1038/s41593-018-0287-x

Widespread RNA editing dysregulation in brains from autistic individuals

Stephen S. Tran[1,2], Hyun-Ik Jun[2], Jae Hoon Bahn[2], Adel Azghadi[2], Gokul Ramaswami[3], Eric L. Van Nostrand[4,5,6], Thai B. Nguyen[4,5,6], Yun-Hua E. Hsiao[7], Changhoon Lee[3], Gabriel A. Pratt[4,5,6,8], Verónica Martínez-Cerdeño[9], Randi J. Hagerman[10], Gene W. Yeo[4,5,6,8], Daniel H. Geschwind[3,11,12*] and Xinshu Xiao[1,2,13,14*]

ARTICLES
https://doi.org/10.1038/s41593-019-0463-7

nature neuroscience

Global landscape and genetic regulatio… RNA editing in cortical samples fro… with schizophrenia

Michael S. Breen[1,2,3*], Amanda Dobbyn[2,4,5], Qin Li[6], Panos Rousso…
Eli Stahl[1,2,5,9,10], Andrew Chess[2,8,9,11], Pamela Sklar[8,9,31], Jin Billy Li…
Joseph D. Buxbaum[1,2,3,8,13*] and CommonMind Consortium[32]

OXFORD

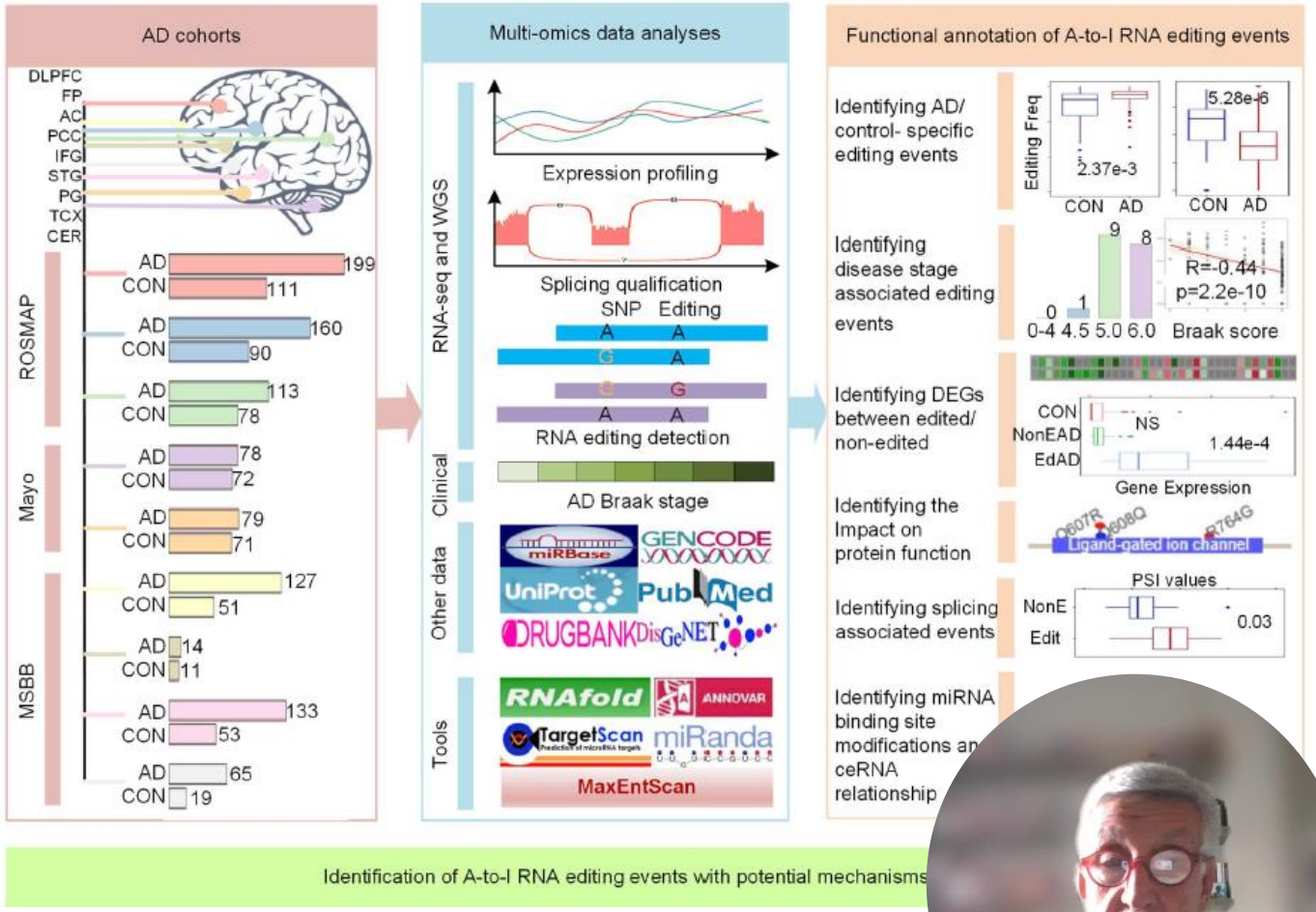
Briefings in Bioinformatics, 22(5), 2021, 1–12

https://doi.org/10.1093/bib/bbaa384
Problem Solving Protocol

ADeditome provides the genomic landscape of A-to-I RNA editing in Alzheimer's disease

Sijia Wu, Mengyuan Yang, Pora Kim and Xiaobo Zhou

The flowchart describes the used AD cohorts,multi-omics data analyses, functional annotation of A-to-I RNA editing and the potential AD mechanism related to A-to-I RNA editing events.
The AD cohorts panel shows the number of AD samples and controls from nine brain regions. For the multi-region and multi-cohort-based AD samples, we performed multiple functional annotations using diverse bioinformatics approaches.
Through these analyses, we identified multiple potential mechanisms related to the A-to-I RNA editing events in AD.



RNA editing biomarkers selection

Discovery cohort

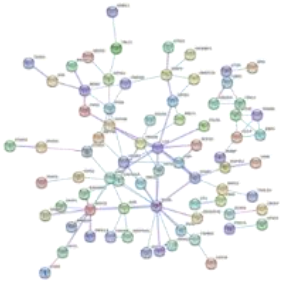
Biological sample preparation

Next Generation Sequencing (NGS)

Quality check

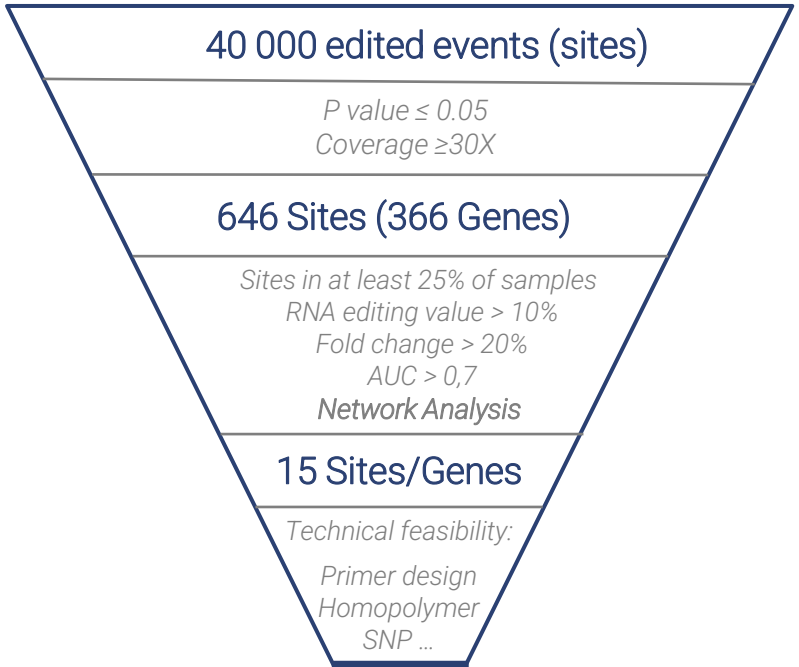
Editome analysis

Biostatistical analysis



Network analysis

Biomarkers identificati



Salvetat et al, [illegible] (2022)

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Artificial Intelligence procedure

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For model development & internal validation

Single center
(Montpellier Univ. Hospital)

255 subjects

RNA editing analysis*

Train Data (~70% / 60%)

Test Data (~30% / 40%)

Preprocessing model

Preprocessing

Train

Validation

Test

Training model

Tuning hyperparameters

k x Cross Validation

Internal testing model

Number of evaluated models

N=5000

RandomForest ExtraTree Algorithm

Selection model

Final Model

Internal Performance Evaluation

Performance prediction

For external validation

Multicentric
(Switzerland 3 centers)

143 subjects

Replicative Data (External Test)

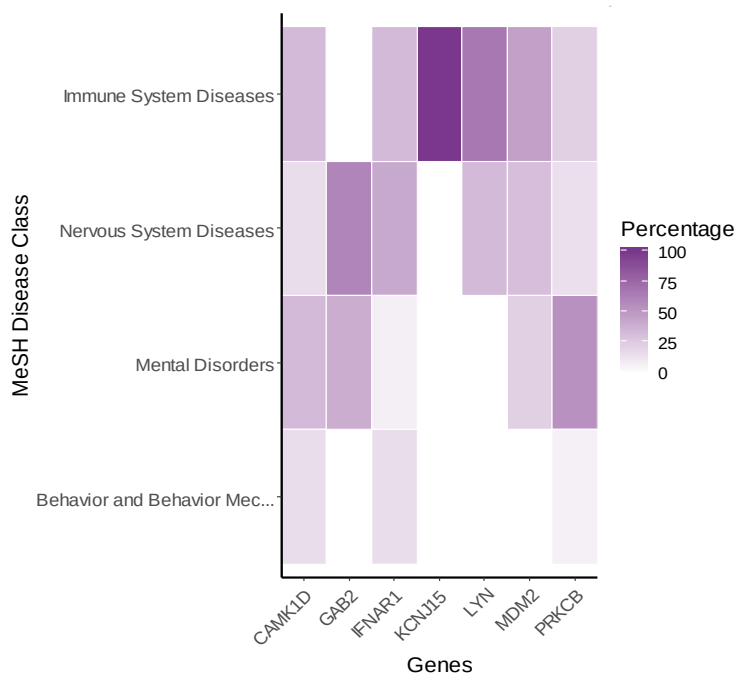
Preprocessing

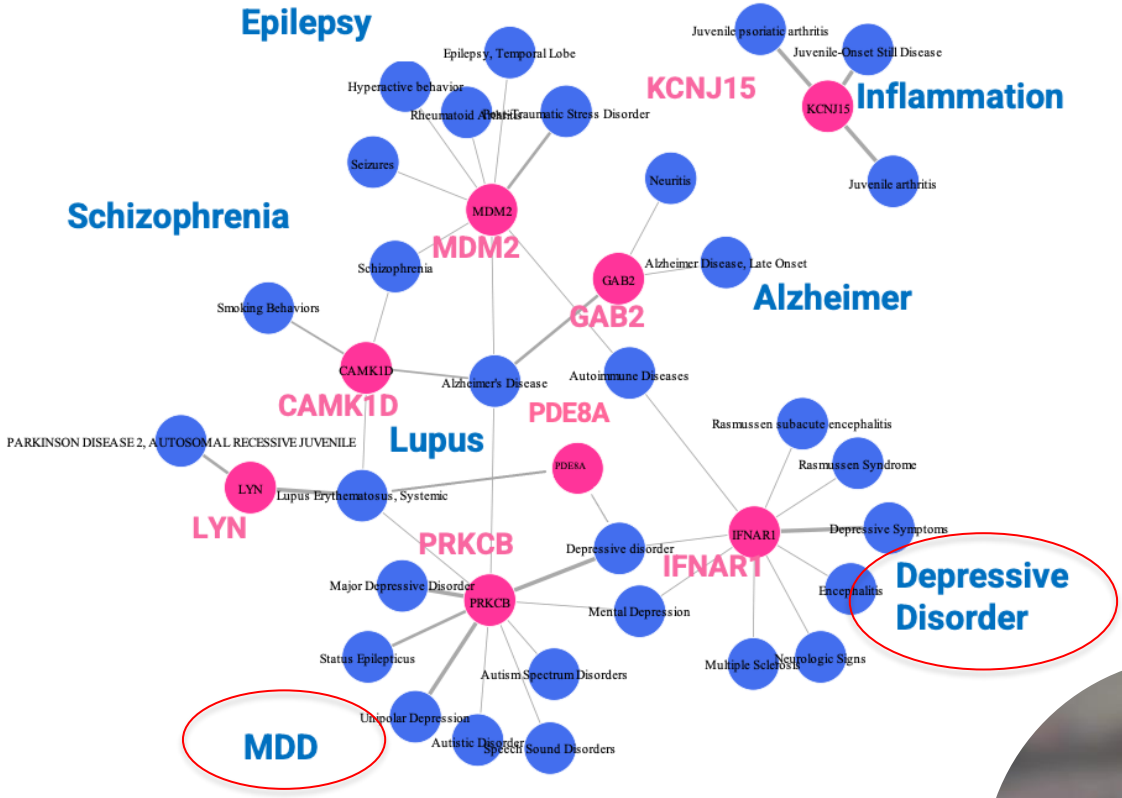
External testing model

External Performance Evaluation

Performance prediction

Panel of biomarkers selected for the study





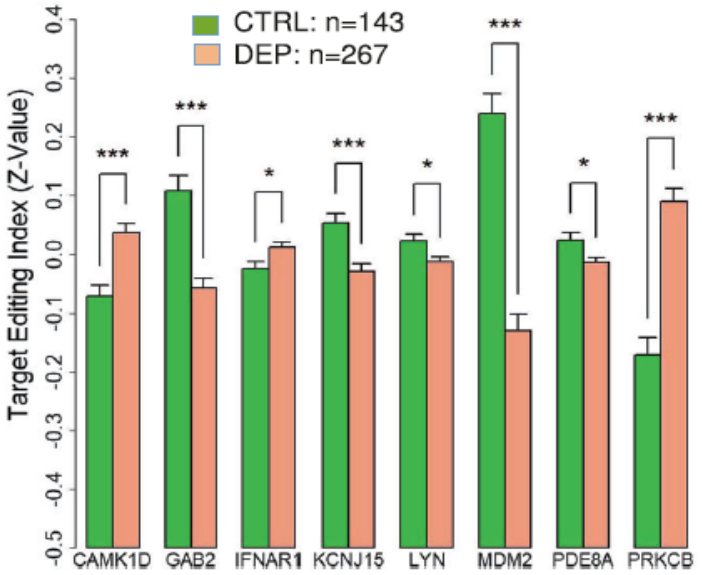
Selected targets belong to different networks: Reactome pathway enrichment, GO Onto DisGeNET network analysis on the 8 targets shows that mainly immune system and nervo diseases, were involved.

Salvetat et al, Transl (2022)

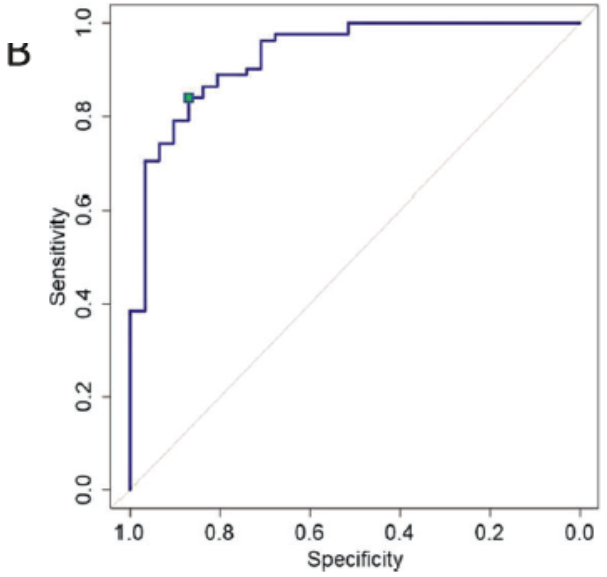
A game changer for bipolar disorder diagnosis using RNA editing-based biomarkers

Nicolas Salvetat[1], Francisco Jesus Checa-Robles[1], Vipul Patel[1], Christopher Cayzac[1], Benjamin Dubuc[1], Fabrice Chimienti[1], Jean-Daniel Abraham[1], Pierrick Dupré[1], Diana Vetter[1], Sandie Méreuze[1], Jean-Philippe Lang[1,2], David J. Kupfer[3], Philippe Courtet[4] and Dinah Weissmann[1]✉

Translational Psychiatry (2022)12:182 ; https://doi.org/10.1038/s41398-022-01938-6

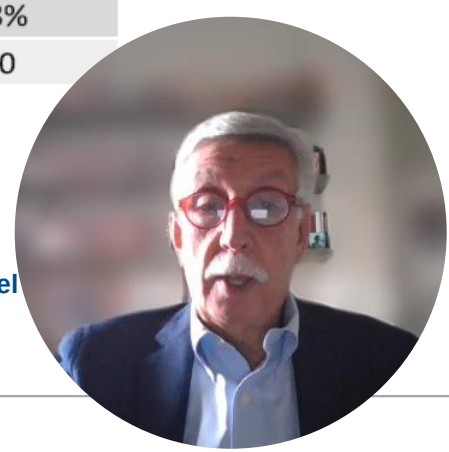


TEI for depressed patients (DEP; n = 267) and healthy controls (CTRL; n = 143);



ROC curve and diagnostic performance of Random Forest model for DEP vs CTRL classification

	Ctrl vs DEP
AUC ROC	**0.930**
CI 95%	[0.879 ; 0.982]
Se	**84.0%**
Sp	**87.1%**
PPV	94.4%
NPV	67.5%
Acc	84.8%
Threshold	0.50



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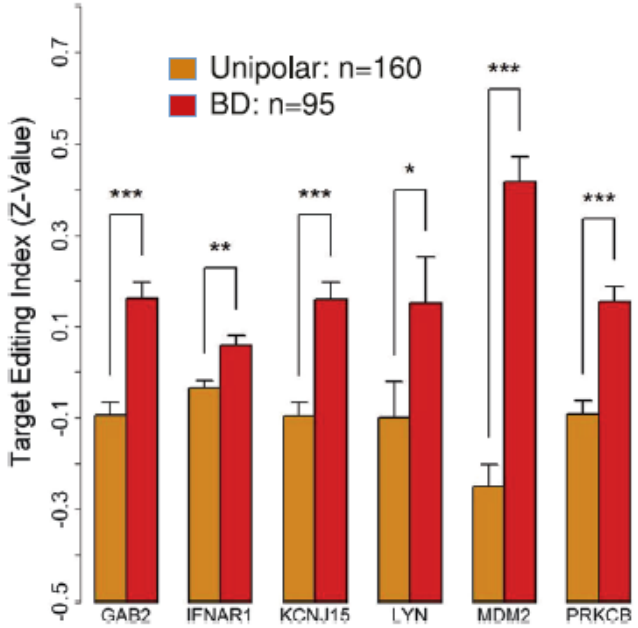
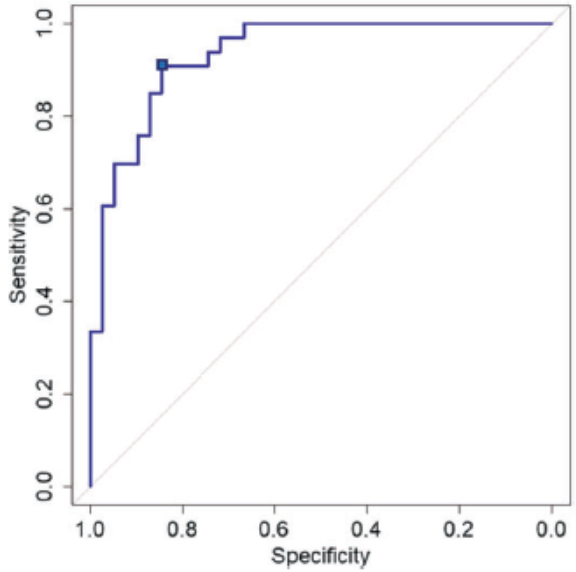
Nicolas Salvetat[1], Francisco Jesus Checa-Robles[1], Vipul Patel[1], Christopher Cayzac[1], Benjamin Dubuc[1], Fabrice Chimienti[1], Jean-Daniel Abraham[1], Pierrick Dupré[1], Diana Vetter[1], Sandie Méreuze[1], Jean-Philippe Lang[1,2], David J. Kupfer[3], Philippe Courtet[4] and Dinah Weissmann[1]✉

Translational Psychiatry (2022)12:182 ; https://doi.org/10.1038/s41398-022-01938-6



TEI for unipolar (n = 160) and bipolar disorder (BD; n = 95)



ROC curve an diagnostic performance of Random Forest model for unipolar vs

	Unipolar VS BD
AUC ROC	**0.935**
CI 95%	[0.882 ; 0.988]
Se	**90.9%**
Sp	**84.6%**
PPV	83.3%
NPV	91.7%
Acc	87.5%
Threshold	0.50

Comparison between subclasses. Cohort 2, N = 87

Comparison between Controls, Bipolar euthymic, Bipolar Depressed, Bipolar Hypomaniac/Maniac and Bipolar Mixed patients

Multiplex analysis of RNA editing

Psychiatry Research 328 (2023) 115422

Contents lists available at ScienceDirect

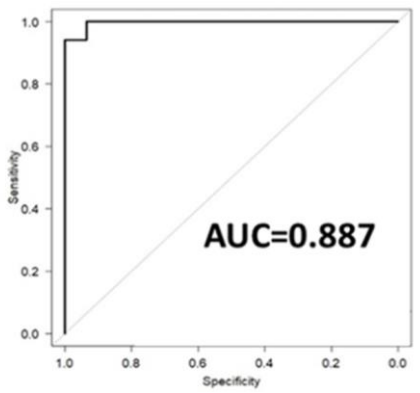
Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

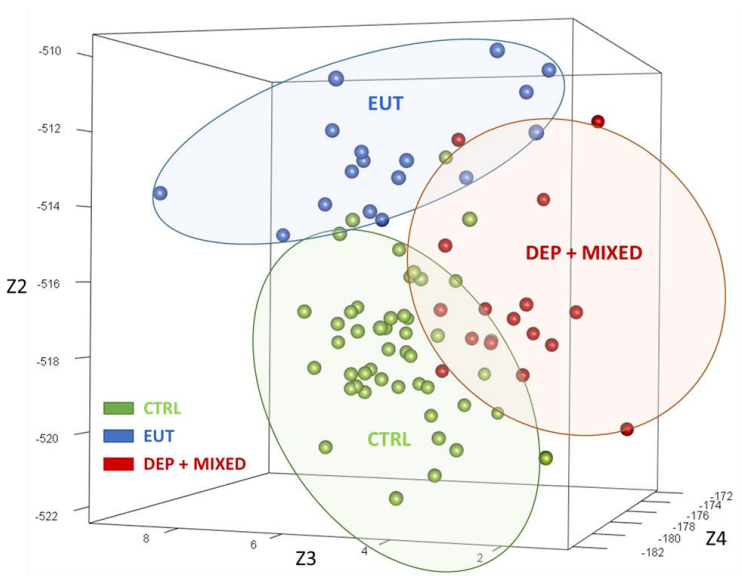
Euthymic and depressed bipolar patients are characterized by different RNA editing patterns in blood

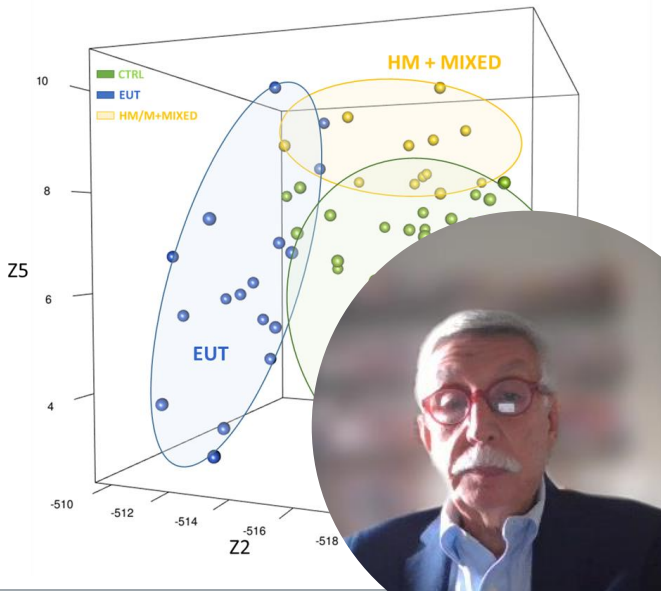
Mirian A.F. Hayashi [a,b,l,*], Nicolas Salvetat [c,l], Christopher Cayzac [c], Francisco Jesus Checa-Robles [c], Benjamin Dubuc [c], Sandie Mereuze [c], João V. Nani [a,b], Franck Molina [c], Elisa Brietzke [d], Dinah Weissmann [c,*]

	Ctrl vs BP	Ctrl vs EUT	Ctrl vs DEP+MIXED	EUT vs DEP+MIXED	Ctrl vs HM/M+MIXED	EUT vs HM/M+MIXED
AUC	0.887	0.996	0.938	0.993	0.993	0.971
Sp	89.4	100	91.5	94.1	100	94.1
Se	85	94.1	87.5	100	91.7	91.7



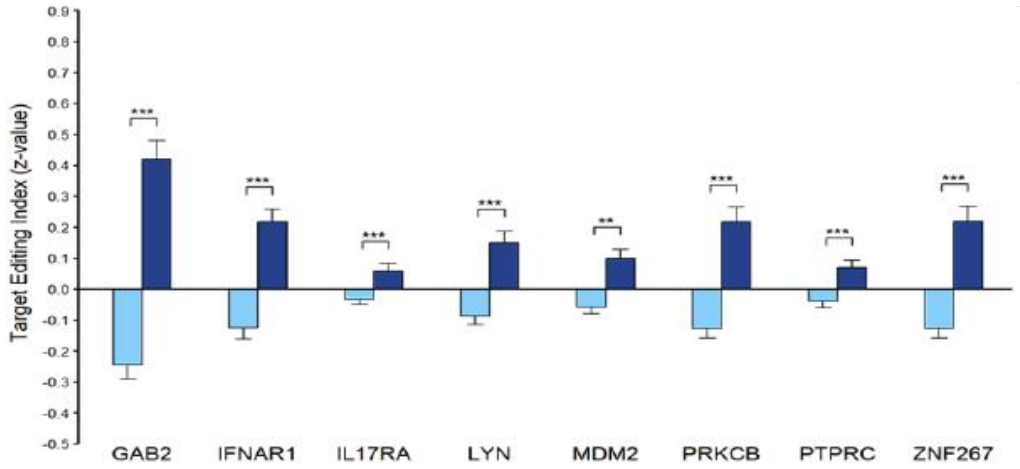
ROC curves of the RNA editing biomarkers combination for CTRL *vs* BD.

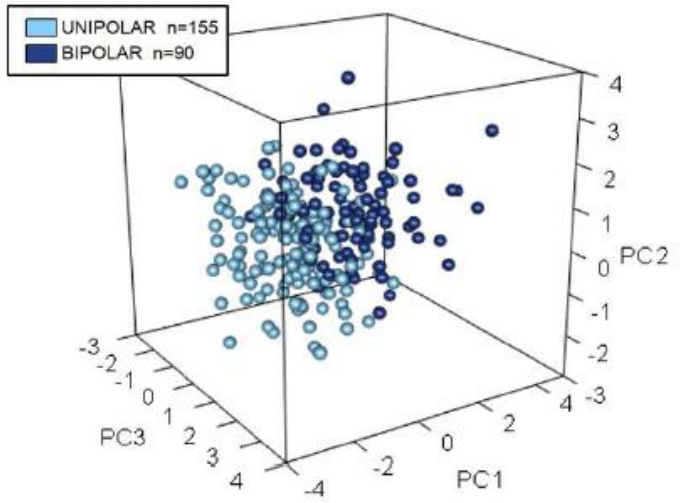




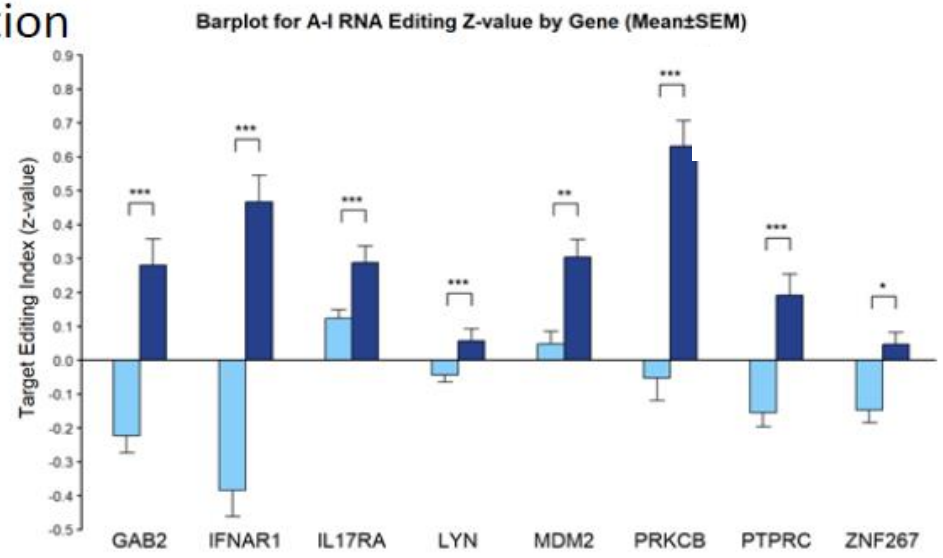
RNA EDITING - biomarkers

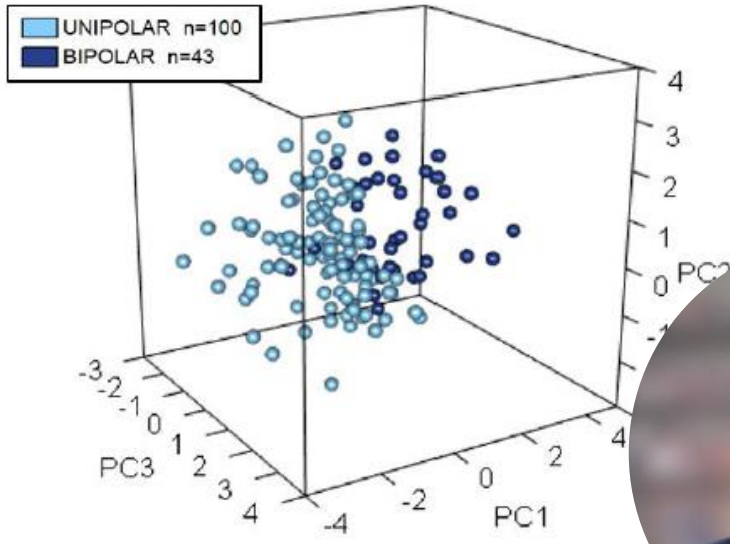
Model





Validation





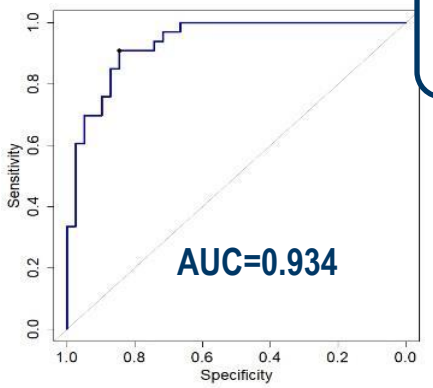
myEDIT-B – CLINICAL VALIDATION

Clinical study

Single center
(Montpellier Univ. Hospital)

255 patients
160 Unipolar, 95 Bipolar
in depressive mood

RNA editing analysis
& AI*



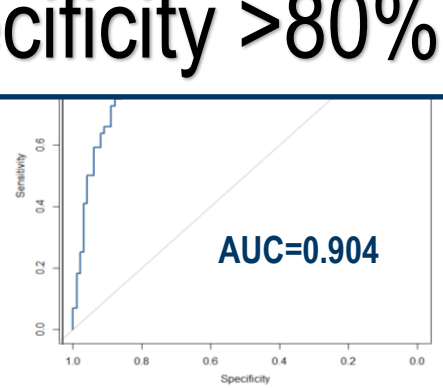
Specificity	84.6 %
Accuracy	87.7 %

Replication Study on an Independent Cohort

Multicentric
(Switzerland 4 center)

143 patients
99 Unipolar, 44 Bipolar in
depressive mood

RNA editing analysis
& AI*



	Performance
Sensitivity	86.4 %
Specificity	80.8 %
Accuracy	82.5 %

Sensitivity and specificity >80%

- Multiplex analysis of RNA editing - Random Forest EUROPEAN population

Diagnosing bipolar disorder is a major pain point for mental health professionals

The gold standard -clinical evaluations & scales- does not differentiate depression from BD

Depression or BD?
- Clinically similar
- 12 M new patients / year in EU, US

Mis-diagnosis of BD
- Up to 40% of supposed depressions could be BD

Diagnostic wandering
- Over 7 years in average

Under-diagnosis of BD

Prevalence of BD: up to 4 %
>145M patients undiagnosed

* ALCEDIAG analysis, Angst J et al, 2011, Ghaemi, J.Aff.Dis 1999, M[illegible]011

Change the game in diagnostics

HOW:

Harness the unexplored power of RNA & AI in the diagnostics

VISION:

Give a new life to patients with enhanced diagnostics and precision medicine

myEDIT-B – WORKFLOW

Blood sample → RNA sequencing in central lab → Data analysis on EDIT-B web platform → Report to lab / clinician → Diagnosis

RNA

Qiasymphony ® Estraction

Library Preparation

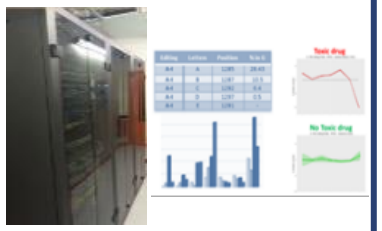
Specific regions in the 8 target biomarkers

NGS

NGS analysis performed on Illumina® platform

Data sequencing

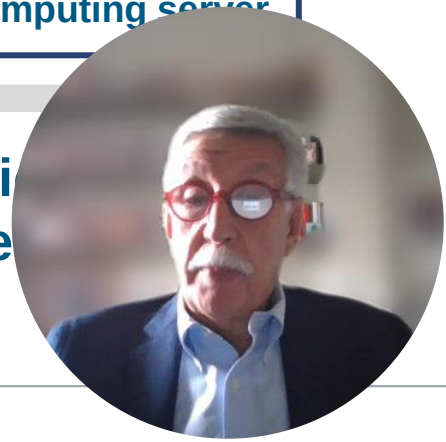
Data analysis



Computing server

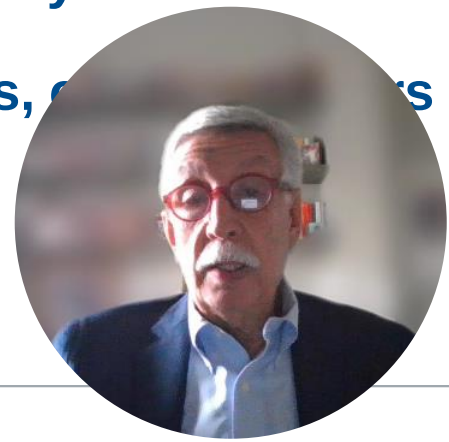
Wet lab

Bioinformatic & Bi
pipeline



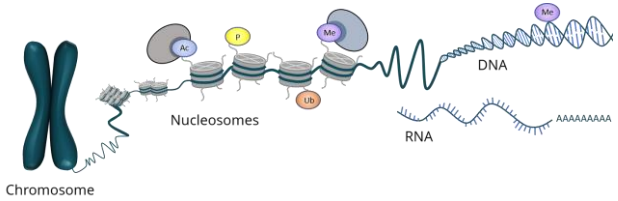
Take Home Messages

- Epigenetics for diagnostics
- RNA and AI for personalized medicine
- Breakthrough innovation for diagnostics and mental health
- EDIT-B is the first blood test to differentiate bipolar from unipolar depressed patients with high performance (> 80%)
- EDIT-B is the ideal decision-making support tool for helping to reduce the diagnostic wandering from years to days
- An international supportive network of clinicians, [illegible]rs & partners



SYNLAB

EUROPE'S NUMBER ONE
MEDICAL DIAGNOSTICS PROVIDER



VI CONGRESO LATINOAMERICANO DE BIOQUIMICA CLÍNICA

II CONGRESO INTERNACIONAL DEL COLEGIO NACIONAL DE BACTERIOLOGÍA

¡El riesgo es que te quieras quedar!

Cartagena, Colombia 3 al 6 OCTUBRE 2024

Thank you for your attention

COLABIOCLI
Confederación Latinoamericana de Bioquímica Clínica

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