

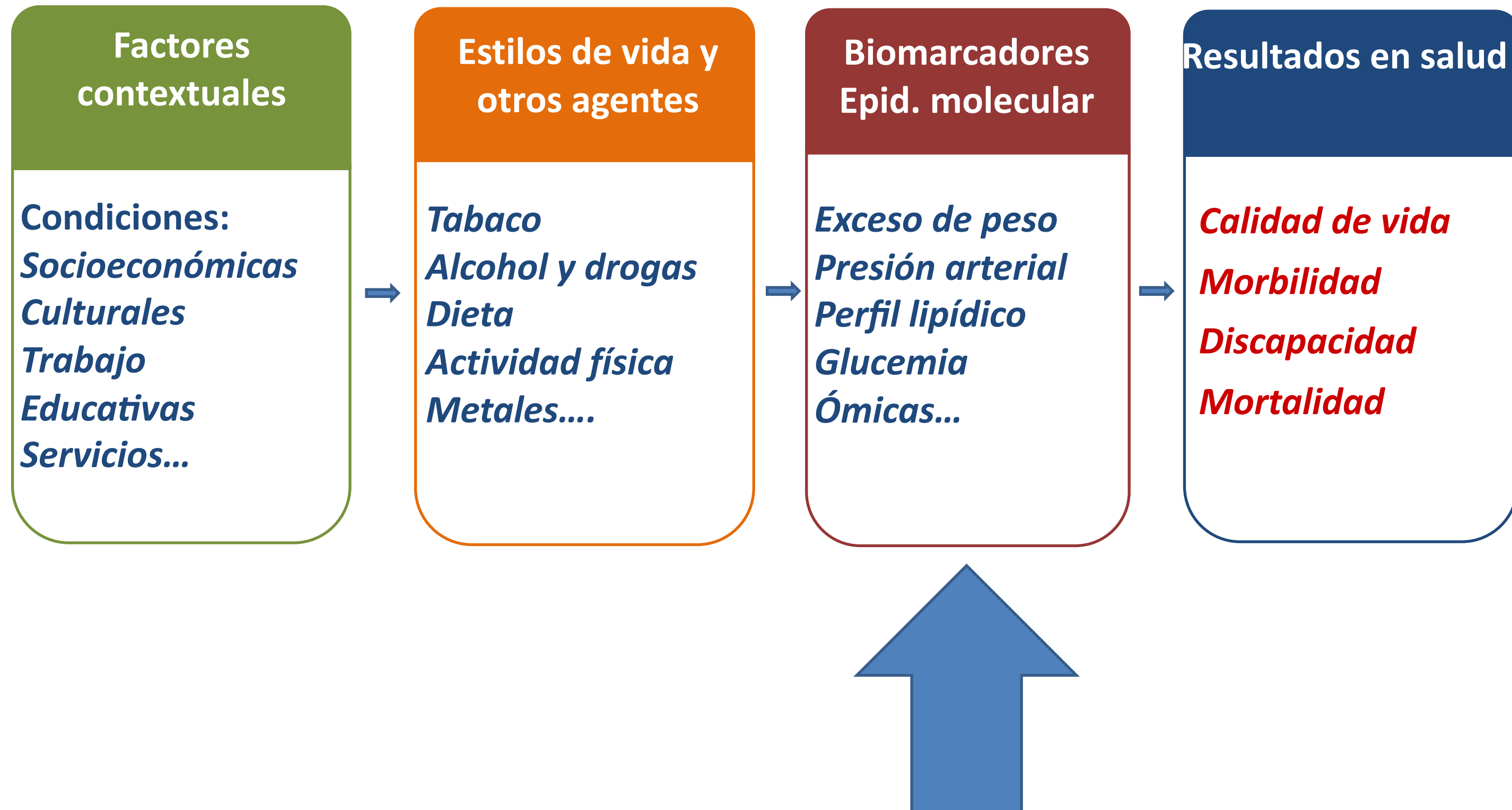
Evaluación de mecanismos para las enfermedades crónicas a partir de datos epidemiológicos.

Intentando desentrañar la “caja negra” desde una perspectiva integradora

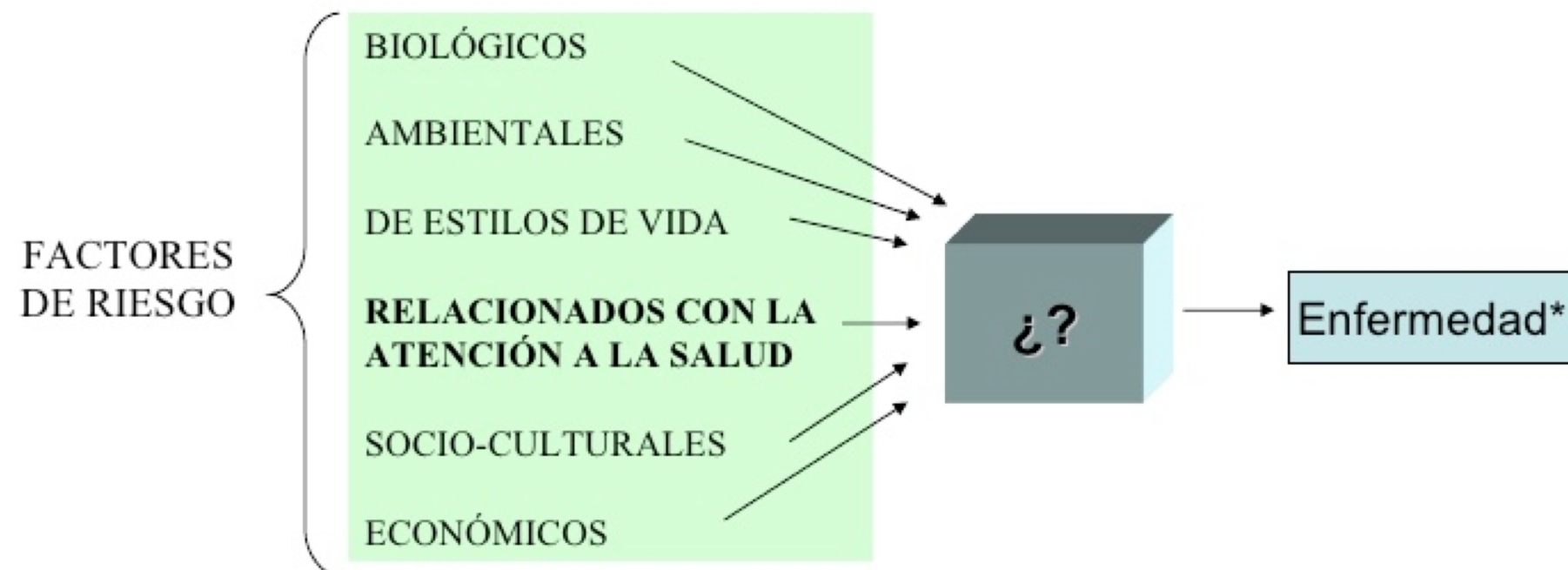
II Jornadas del Centro Nacional de Epidemiología
“Conoce el CNE”

María Téllez Plaza, MD PhD
Científica Titular





MODELO DE LA CAJA NEGRA PARA EL ESTUDIO DE LAS ENFERMEDADES CRÓNICAS



* *Enfermedad como resultado de la acumulación de riesgos en la persona; El riesgo como un atributo personal y susceptible de medición; Se invisibiliza la diversidad de forma en que interactúan estas variables (el contexto) como determinante explicativo de los perfiles epidemiológicos en grupos de poblaciones.*

Fuente:

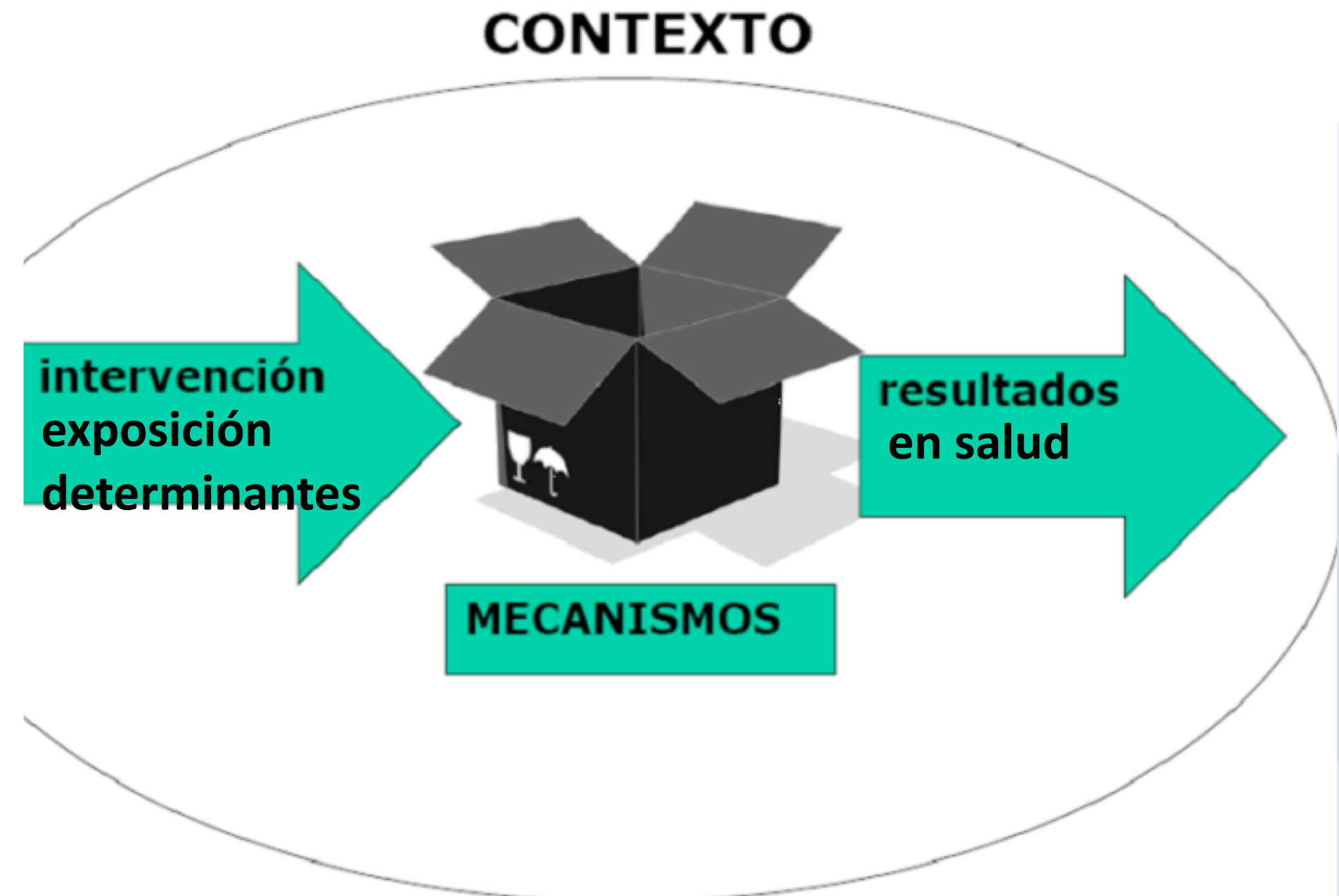
<https://promociondesaludyepidemiologiauacm.wordpress.com/2015/05/28/los-modelos-explicativos-del-proceso-salud-enfermedad-atencion/>

- Una desventaja del modelo, es que implica una deficiente comprensión de los eventos que se investigan, al no ser necesario comprender todo el proceso para adoptar medidas eficaces de control.

El modelo de la caja negra también tiene como limitación la dificultad para distinguir entre los determinantes individuales y poblacionales de la enfermedad.

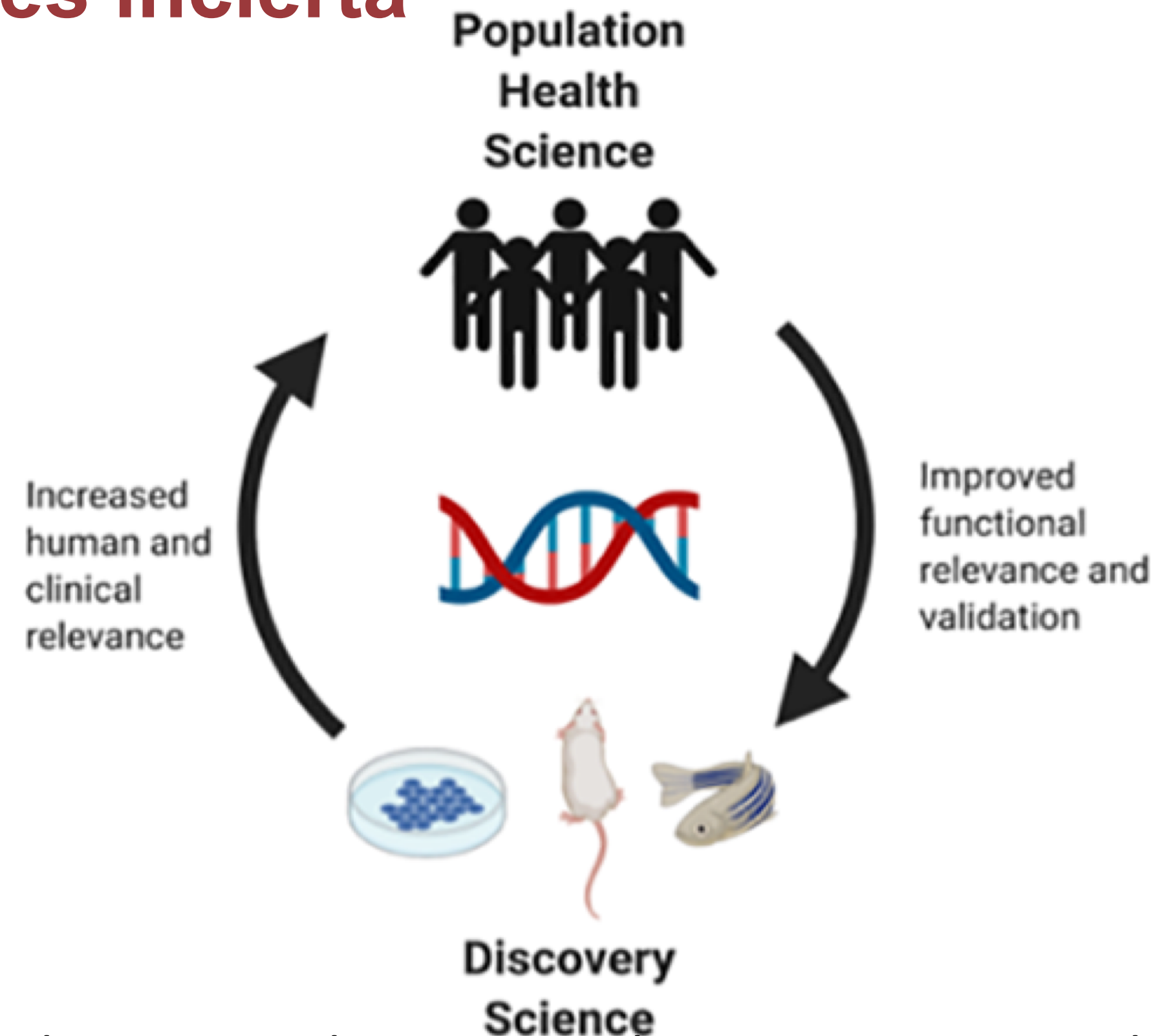
EL ESTUDIO DE MECANISMOS EN EPIDEMIOLOGIA DE LAS ENFERMEDADES CRÓNICAS: INTENTANDO ABRIR LA CAJA

“Epidemiology is the study and analysis of the distribution, patterns and determinants of health and disease conditions in defined populations. It is a cornerstone of public health, and shapes policy decisions and evidence-based practice by identifying risk factors for disease and targets for preventive healthcare” (Fuente: A Dictionary of Epidemiology (6th ed.))

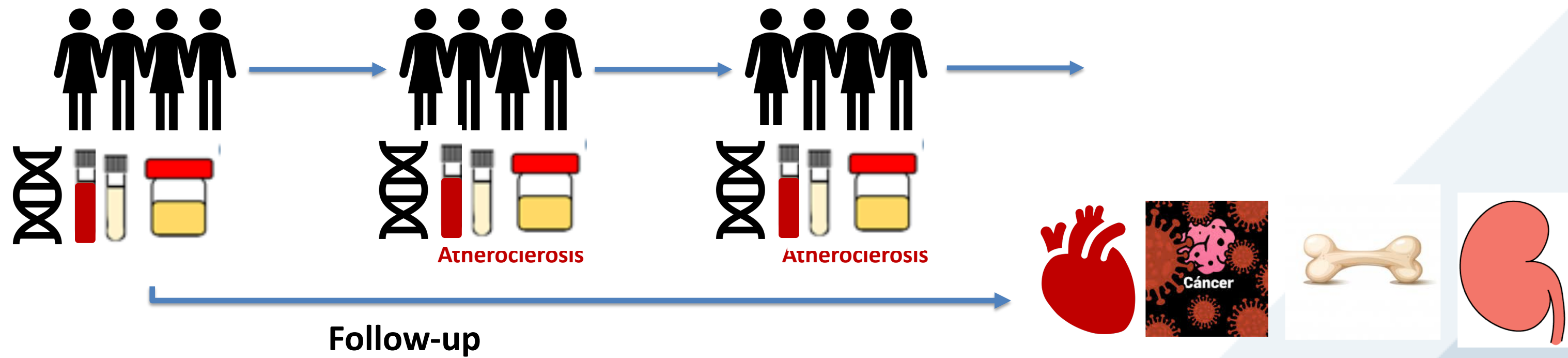


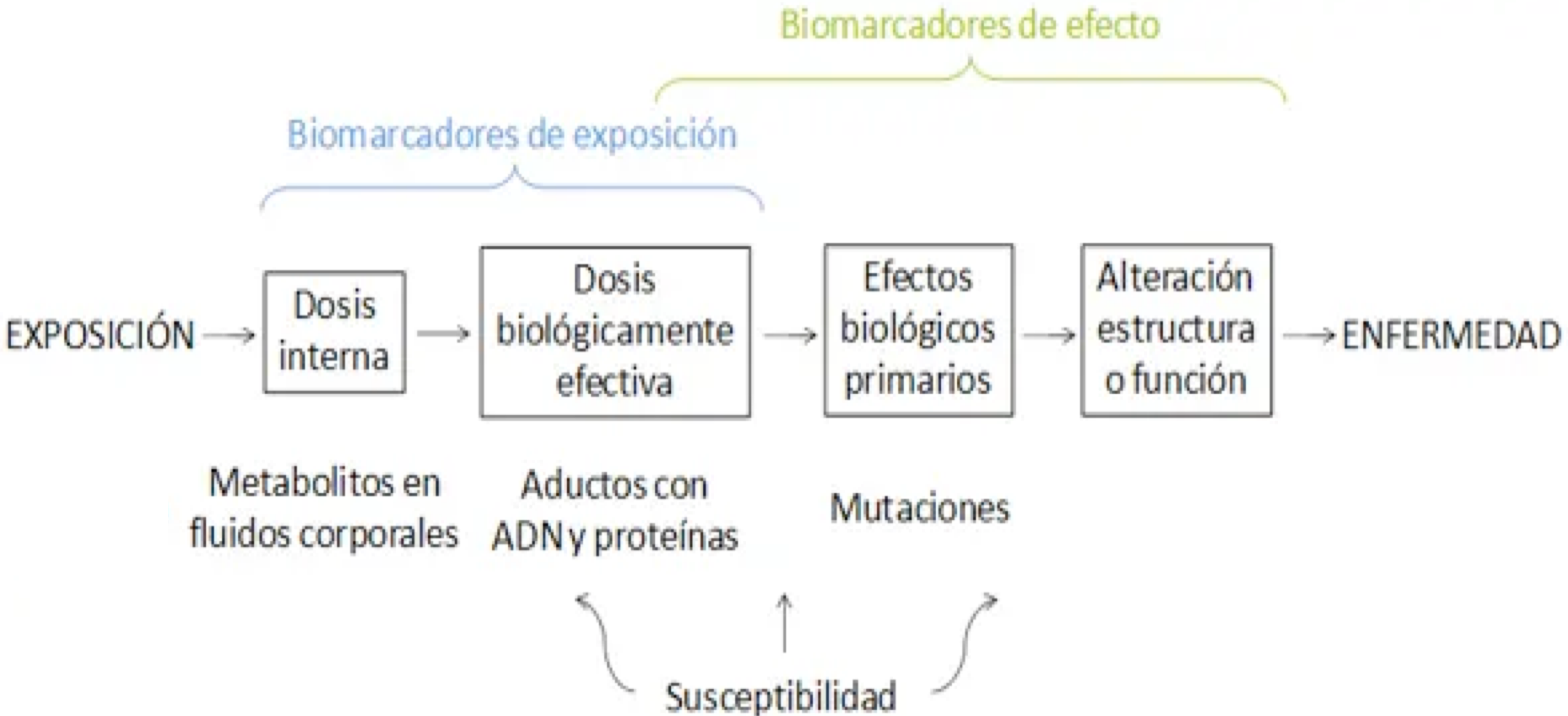
La extrapolación de los resultados experimentales en humanos es incierta

Los estudios mecanísticos a partir de estudios poblacionales pueden indicar qué mecanismos biológicos son más relevantes



ESTUDIOS EPIDEMIOLOGICOS CON TRABAJO DE CAMPO + MUESTRAS BIOLÓGICAS + SEGUIMIENTO EN LOS QUE EL DEPARTAMENTO DE ENFERMEDADES CRÓNICAS PARTICIPA ACTIVAMENTE







Environmental toxic metal contaminants and risk of cardiovascular disease

Systematic review and meta-analysis



37 unique studies

26 cohort studies

11 case-control studies

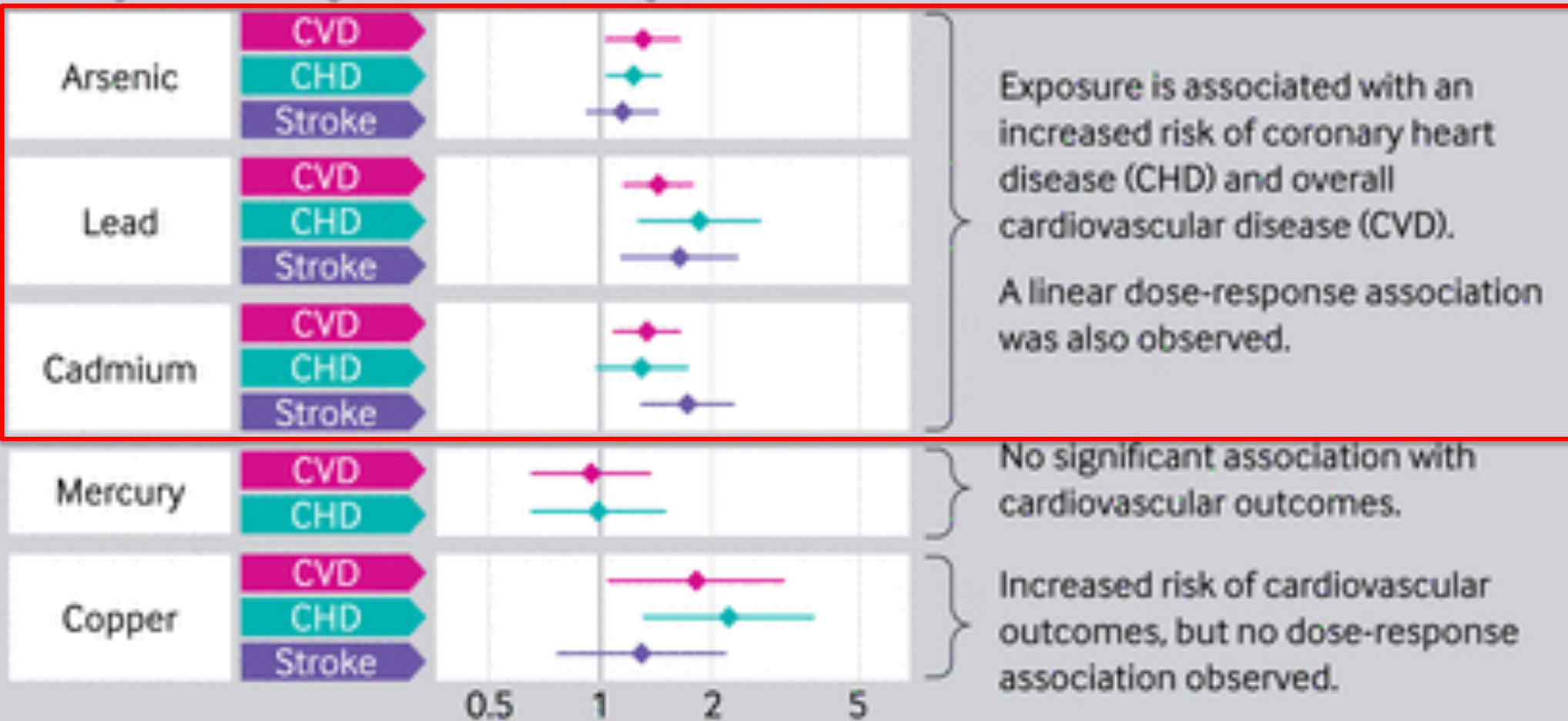
348 259 non-overlapping participants

Study quality

Newcastle-Ottawa score 0–9, high scores better



Exposure Outcome Relative risk (95% CI) Top v bottom third of baseline level



- Revisión sistemática de 37 estudios únicos
- >300,000 participantes
- Exposición al arsénico, el plomo y el cadmio asociada con aumento de riesgo de cardiopatía isquémica y enfermedad cardiovascular con una dosis-respuesta clara

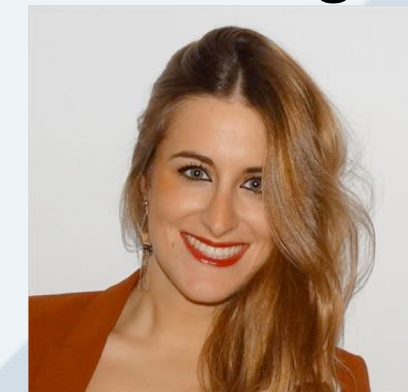
Metales y estrés oxidativo a unas dosis de exposiciones que son propias de la población genera

		GSSG/GSH		MDA		8-Oxo-dG	
		GMR (95%CI)	P-trend	GMR (95%CI)	P-trend	GMR (95%CI)	P-trend
Essential	Co	0.98 (0.89, 1.08)	0.70	0.99 (0.94, 1.03)	0.543	1.03 (0.98, 1.09)	0.195
	Cu	1.06 (0.95, 1.18)	0.30	1.04 (0.98, 1.09)	0.188	1.04 (0.99, 1.10)	0.142
	Mo	1.14 (1.03, 1.27)	0.01	1.01 (0.96, 1.07)	0.611	1.04 (0.94, 1.15)	0.201
	Zn	0.99 (0.88, 1.11)	0.85	1.07 (1.01, 1.14)	0.019	1.07 (1.01, 1.13)	0.02
Non-essential	Sb	0.99 (0.90, 1.09)	0.84	1.00 (0.95, 1.05)	0.883	1.05 (0.96, 1.15)*	0.001*
	Ba	1.17 (1.05, 1.31)	0.006	1.02 (0.96, 1.08)	0.561	1.00 (0.95, 1.06)	0.962
	Cd	1.07 (0.97, 1.19)	0.19	1.12 (1.02, 1.23)	0.02	1.09 (0.99, 1.20)*	0.08
	Cr	1.23 (1.04, 1.46)*	0.002*	0.98 (0.92, 1.03)	0.413	1.04 (0.99, 1.10)	0.131
	V	1.18 (1.00, 1.40)*	<0.001*	0.97 (0.92, 1.03)	0.288	1.03 (0.98, 1.09)	0.256



Fuente: Domingo-Relloso et al. Environment International, 2019

A. Domingo



M. Grau



Models adjusted for sex, education (<high school, >.high school), smoking status (never, former and current smoker), cumulative smoking dose (0, 0–12, >12 pack-years), urine cotinine (<34, 34–500 and >.500 ng/ml), estimated glomerular filtration rate (ml/min per 1.73m²), residence (urban or rural), HDL cholesterol level (mg/dl), total cholesterol level (mg/dl), dyslipidaemia treatment (yes/no), hypertension treatment (yes/no), diabetes mellitus of type 2 (yes/no) and systolic pressure (mmHg).



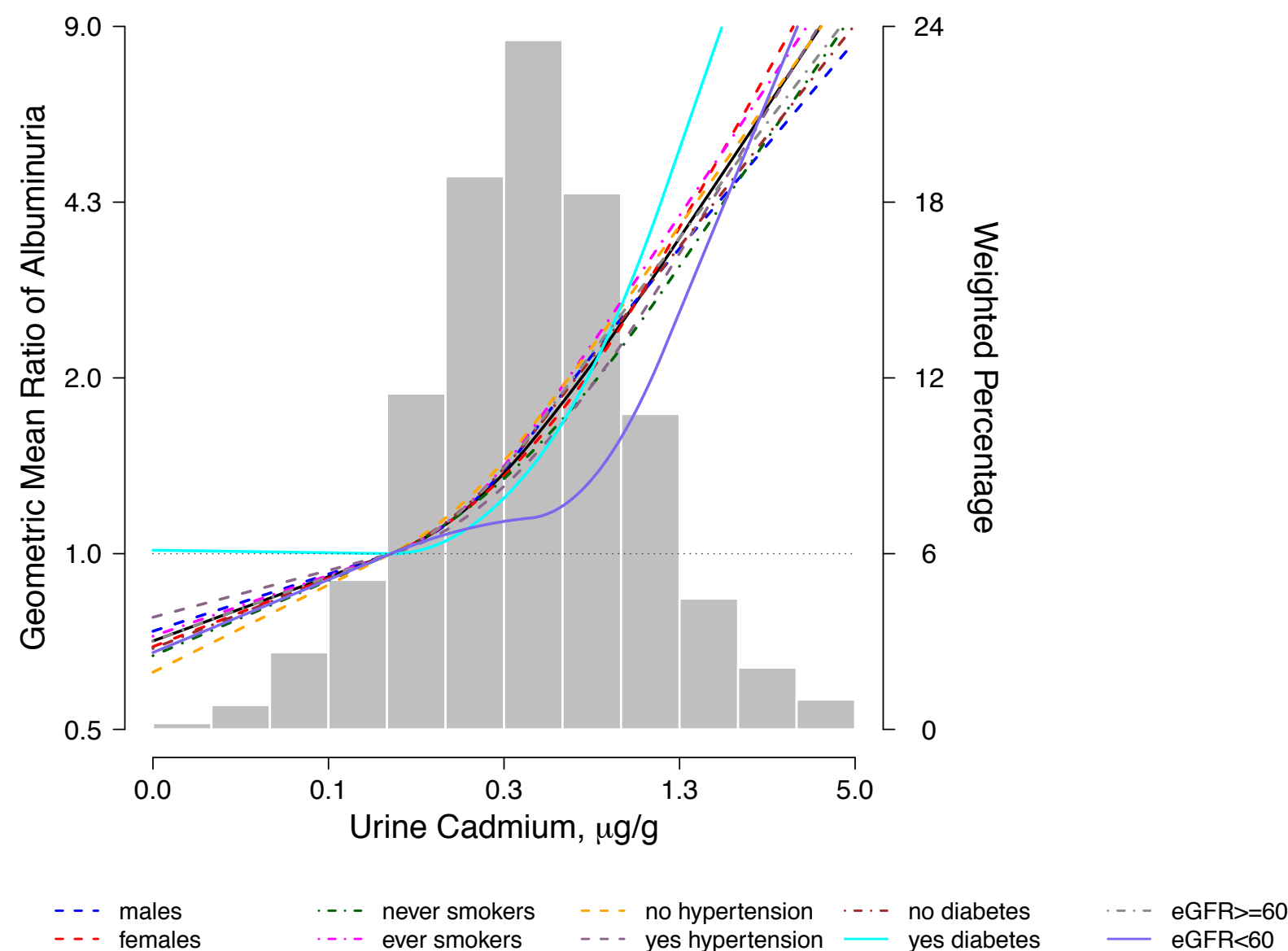
Interacciones gen-ambiente: El cadmio y la albuminuria



M. Grau



Objetivo: Evaluar la hipótesis de que portadores de genotipos específicos son más susceptibles al cadmio. **Las interacciones gen-ambiente también pueden apuntar a mecanismos relevantes**



SLC30A4: Transportador de zinc en el retículo endoplásmico. No tiene papel conocido en la albuminuria

RAC1: Rho-GTPasa, con un papel en el mantenimiento de los podocitos y la integridad de los túbulos proximales. Asociado con disfunción endotelial

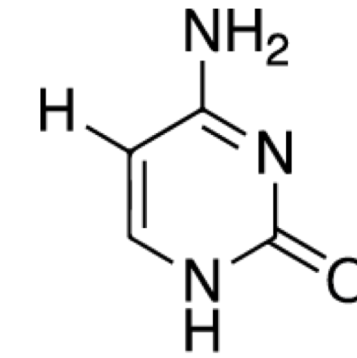
GMR of albuminuria levels comparing 80th to 20th percentile of cadmium distribution, by genotypes of SNPs with significant interactions at the Bonferroni level

SLC30A4	RAC1	N	GMR (95% CI)	P - int
rs3087816	rs4720672			<0.001
T/T + T/C (ref)	T/T (ref)	892	1.82 (1.65, 2.01)	
T/T + T/C (ref)	T/C + C/C	344	3.02 (1.85, 4.94)	
C/C	T/T (ref)	34	2.43 (2.07, 2.85)	
C/C	T/C + C/C	14	19.1 (8.02, 45.49)	

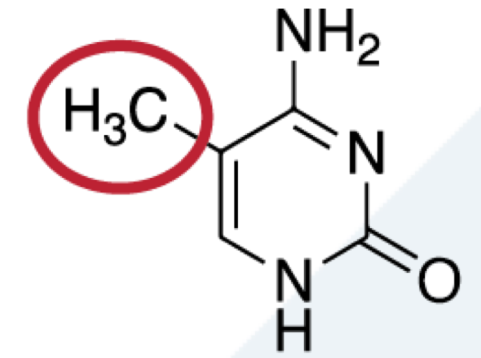
Fuente: Grau-Perez et al. Environment International 2017

La epigenética y la metilación del DNA

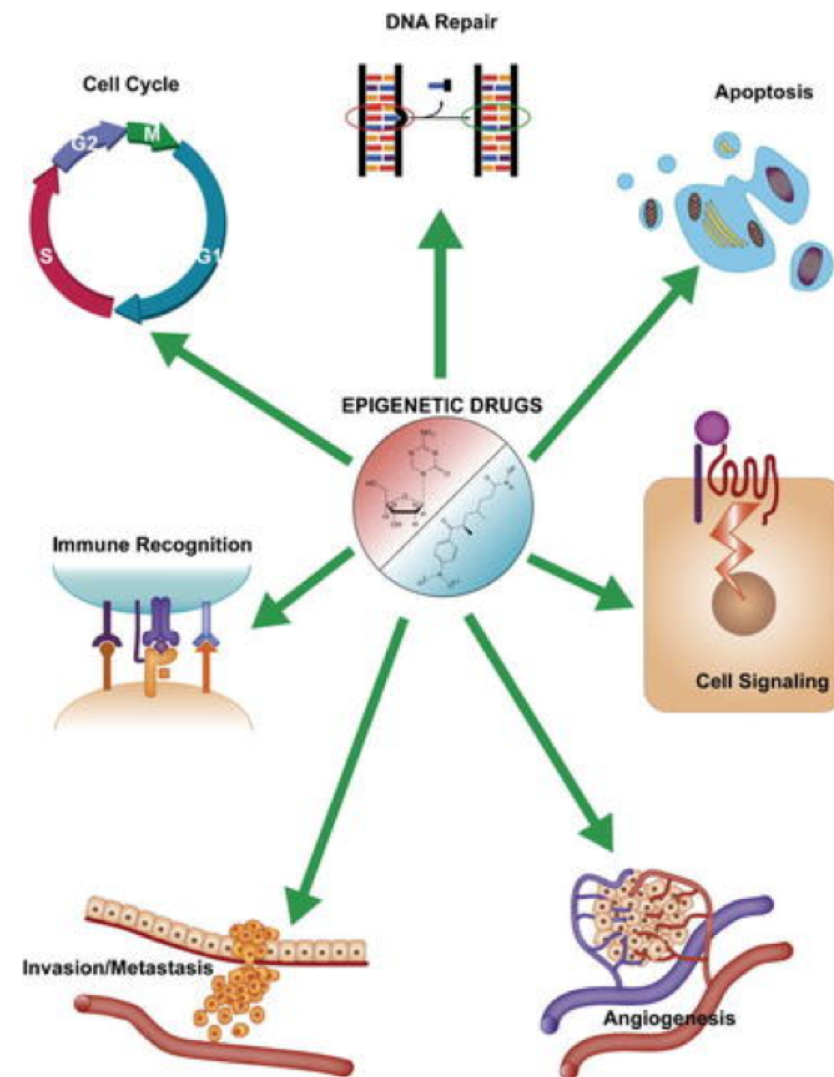
❖ **Metilación del DNA:** cambia la expresión de los genes sin alterar la secuencia genética



Cytosine



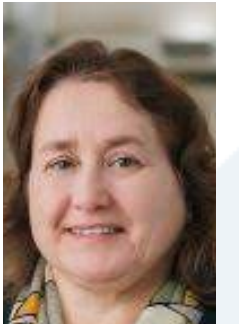
methyated Cytosin



Sigalotti et al. 2007

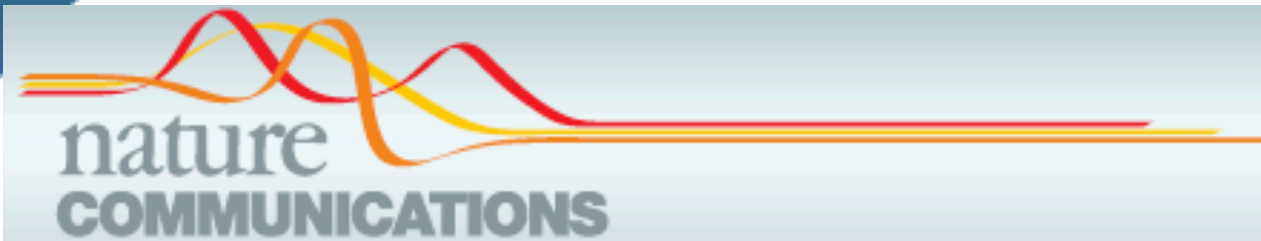
- ❖ **Sensible al ambiente**
- ❖ **Se puede heredar**
- ❖ **Reversible y modificable farmacológicamente**

Los metales y el tabaco como determinantes del metiloma y el riesgo cardiovascular



- 1: Schlosser P, et al. Meta-analyses identify DNA methylation associated with kidney function and damage. *Nat Commun.* 2021 Dec 9;12(1):7174.
- 2: Domingo-Relloso A, et al. Arsenic exposure and human blood DNA methylation and hydroxymethylation profiles in two diverse populations from Bangladesh and Spain. *Environ Res.* 2021 Sep 10;204(Pt B):112021.
- 3: Navas-Acien A, et al. Blood DNA Methylation and Incident Coronary Heart Disease: Evidence From the Strong Heart Study. *JAMA Cardiol.* 2021 Nov 1;6(11):1237-1246.
- 4: Domingo-Relloso A, et al. DNA methylation and cancer incidence: lymphatic-hematopoietic versus solid cancers in the Strong Heart Study. *Clin Epigenetics.* 2021 Feb 25;13(1):43.
- 5: Christiansen C, et al. Novel DNA methylation signatures of tobacco smoking with trans-ethnic effects. *Clin Epigenetics.* 2021 Feb 16;13(1):36.
- 6: Crocker KC, et al. DNA methylation and adiposity phenotypes: an epigenome-wide association study among adults in the Strong Heart Study. *Int J Obes (Lond).* 2020 Nov;44(11):2313-2322.
- 7: Domingo-Relloso A, et al. Cadmium, Smoking, and Human Blood DNA Methylation Profiles in Adults from the Strong Heart Study. *Environ Health Perspect.* 2020 Jun;128(6):67005.
- 8: Bozack AK, et al. Locus-Specific Differential DNA Methylation and Urinary Arsenic: An Epigenome-Wide Association Study in Blood among Adults with Low-to-Moderate Arsenic Exposure. *Environ Health Perspect.* 2020 Jun;128(6):67015.
- 9: Grau-Perez M, et al. Mendelian Randomization and the Environmental Epigenetics of Health: a Systematic Review. *Curr Environ Health Rep.* 2019 Mar;6(1):38-51.
- 10: Kihl SF, et al. MLML2R: an R package for maximum likelihood estimation of DNA methylation and hydroxymethylation proportions. *Stat Appl Genet Mol Biol.* 2019 Jan 17;18(1):/j/sagmb.2019.18.issue-1/sagmb-2018-0031/sagmb-2018-0031.xml.
- 11: Riffo-Campos AL, et al. In silico epigenetics of metal exposure and subclinical atherosclerosis in middle aged men: pilot results from the Aragon Workers Health Study. *Philos Trans R Soc Lond B Biol Sci.* 2018 Jun 5;373(1748):20170084.
- 12: Istas G, et al. Identification of differentially methylated BRCA1 and CRISP2 DNA regions as blood surrogate markers for cardiovascular disease. *Sci Rep.* 2017 Jul 11;7(1):5120.
- 13: Ruiz-Hernandez A, et al. Environmental chemicals and DNA methylation in adults: a systematic review of the epidemiologic evidence. *Clin Epigenetics.* 2015 Apr 29;7(1):55.
- 14: Tellez-Plaza M, et al. Association of global DNA methylation and global DNA hydroxymethylation with metals and other exposures in human blood DNA samples. *Environ Health Perspect.* 2014 Sep;122(9):946-54. doi: 10.1289/ehp.1306674.

Los metales y el tabaco como determinantes del metiloma y factores relacionados con el riesgo cardiovascular



ARTICLE

<https://doi.org/10.1038/s41467-021-27234-3>

OPEN

Meta-analyses identify DNA methylation associated with kidney function and damage

Chronic kidney disease is a major public health burden. Elevated urinary albumin-to-creatinine ratio is a measure of kidney damage, and used to diagnose and stage chronic kidney disease. To extend the knowledge on regulatory mechanisms related to kidney function and disease, we conducted a blood-based epigenome-wide association study for estimated glomerular filtration rate ($n = 33,605$) and urinary albumin-to-creatinine ratio ($n = 15,068$) and detected 69 and seven CpG sites where DNA methylation was associated with the respective trait. The majority of these findings showed directionally consistent associations with the respective clinical outcomes chronic kidney disease and moderately

kidney function and damage. *Nat Commun.* 2021 Dec

India, Bangladesh and Spain. *Environ Res.* 2021 Sep 10;204(Pt

2): Evidence From the Strong Heart Study. *JAMA*

Cardiovascular: atropoietic versus solid cancers in the Strong Heart

Study: A multi-ethnic trans-ethnic effects. *Clin Epigenetics.* 2021 Feb

2): A genome-wide association study among adults in the Strong

Heart Study: Profiles in Adults from the Strong Heart Study.

Epigenetics: An Epigenome-Wide Association Study in Blood 2021 Jun;128(6):67015.

Journal of Health: a Systematic Review. *Curr Environ*

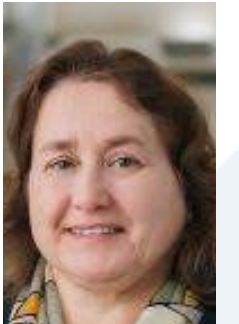
Health: A genome-wide association study in Blood 2021 Jun;128(6):67015.

Journal of Health: a Systematic Review. *Curr Environ*

Health: A genome-wide association study in Blood 2021 Jun;128(6):67015.

Journal of Health: a Systematic Review. *Curr Environ*

Health: A genome-wide association study in Blood 2021 Jun;128(6):67015.



Los metales y el tabaco como determinantes del metiloma y factores relacionados con el riesgo cardiovascular



JAMA Cardiology | Original Investigation

Blood DNA Methylation and Incident Coronary Heart Disease Evidence From the Strong Heart Study

Ana Navas-Acien, MD, PhD; Arce Domingo-Relloso, MS; Pooja Subedi, MPH; Angela L. Riffo-Campos, PhD; Rui Xia, PhD; Lizbeth Gomez, MPI; Karin Haack, PhD; Jeff Goldsmith, PhD; Barbara V. Howard, PhD; Lyle G. Best, MD; Richard Devereux, MD; Ali Tauqeer, MD, PhD; Ying Zhang; Amanda M. Fretts, PhD; Gernot Pichler, MD, PhD, MSc; Daniel Levy, MD; Ramachandran S. Vasan, MD; Andrea A. Baccarelli, MD, PhD; Miguel Herreros-Martinez, MS; Wan-yee Tang, PhD; Jan Bressler, PhD; Myriam Fornage, PhD; Jason G. Umans, MD, PhD; Maria Tellez-Plaza, MD, PhD; M. Daniele Fallin, PhD; Jinying Zhao, MD, PhD; Shelley A. Cole, PhD



ARTICLE

<https://doi.org/10.1038/s41467-021-27234-3>

OF

Meta-analyses identify factors associated with kidney disease

Chronic kidney disease is a major public health problem. Estimated glomerular filtration rate (eGFR) is a measure of kidney function, and a low eGFR is associated with kidney disease. To extend the knowledge on renal function and disease, we conducted a blood-based meta-analysis of 10 studies (n = 33,605) (n = 15,068) and detected 69 and seven CpG sites with the respective trait. The majority of these 10 associations with the respective clinical outcomes

IMPORTANCE American Indian communities experience a high burden of coronary heart disease (CHD). Strategies are needed to identify individuals at risk and implement preventive interventions.

OBJECTIVE To investigate the association of blood DNA methylation (DNAm) with incident CHD using a large number of methylation sites (cytosine-phosphate-guanine [CpG]) in a single model.

DESIGN, SETTING, AND PARTICIPANTS This prospective study, including a discovery cohort (the Strong Heart Study [SHS]) and 4 additional cohorts (the Women's Health Initiative [WHI], the Framingham Heart Study [FHS], the Atherosclerosis Risk in Communities Study ([ARIC]-Black, and ARIC-White), evaluated 12 American Indian communities in 4 US states;

+ Supplemental content

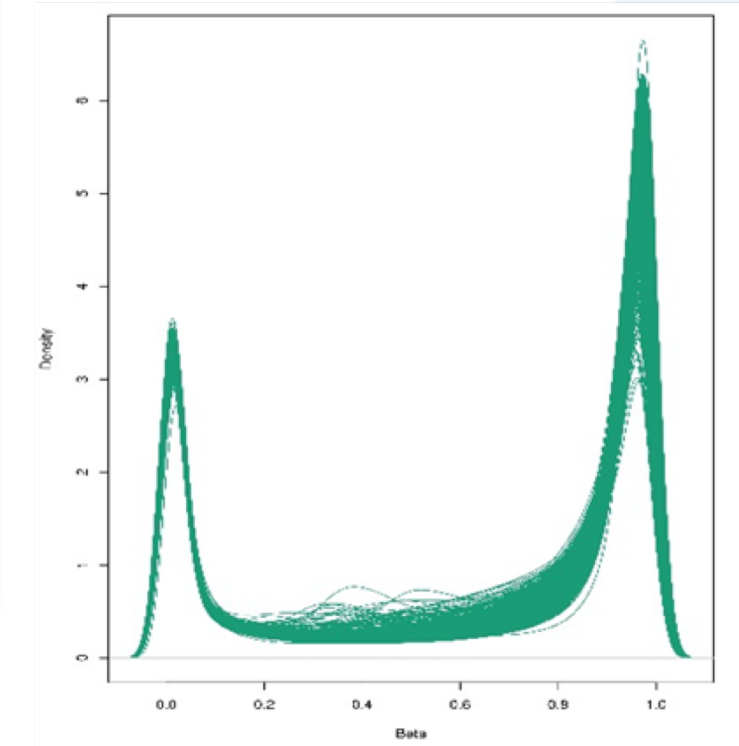


Desarrollo metodológicos/aplicaciones para abordar el papel de marcadores “ómicos” en las enfermedades crónicas

1. Selección de variables de marcadores multi-dimensionales correlacionados
2. Análisis de mediación para evaluar mediadores correlacionados en el context de datos de supervivencia
3. Integración de resultados en mecanismos con evidencia disponible en bases de datos bioinformáticas



Illumina MethylationEPIC BeadChip (850K): over 850K CpG sites per sample at single nucleotide resolution



1. Selección de variables en el contexto de datos ómicos correlacionados: extensión del paquete “SIS”

❖ Versión extendida del paquete de R *SIS* (próximamente en CRAN) acopla :

- ❖ **Iterative Sure Screening (ISIS):**
seleccionar las variables relevantes de un grupo de variables correlacionadas
- ❖ **Adaptive Elastic-Net (AENET):**
estimación de parámetros menos sesgada, que puede favorecer el descubrimiento biológico, así como una mejor precisión predictiva



Arce Domingo



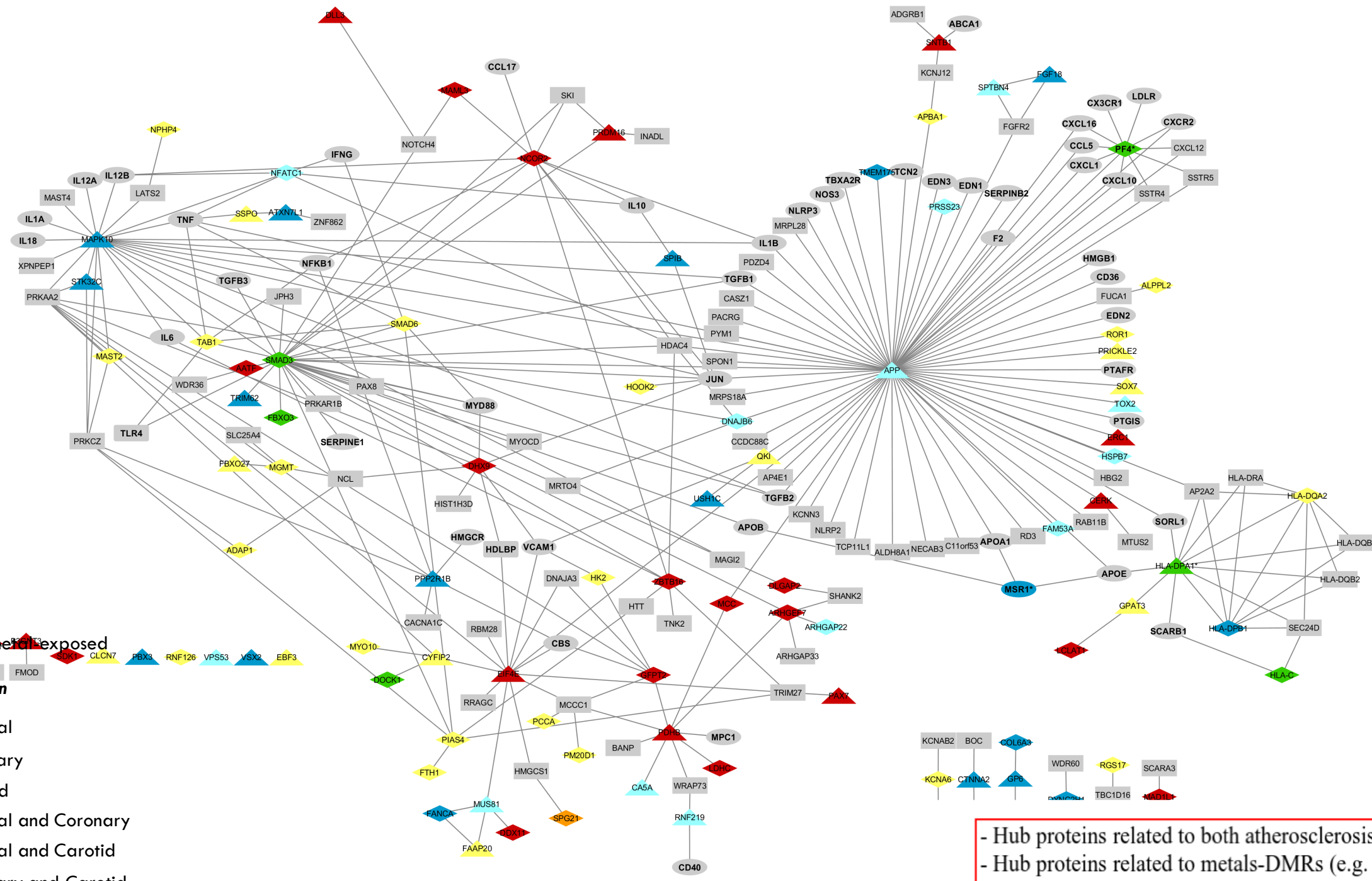
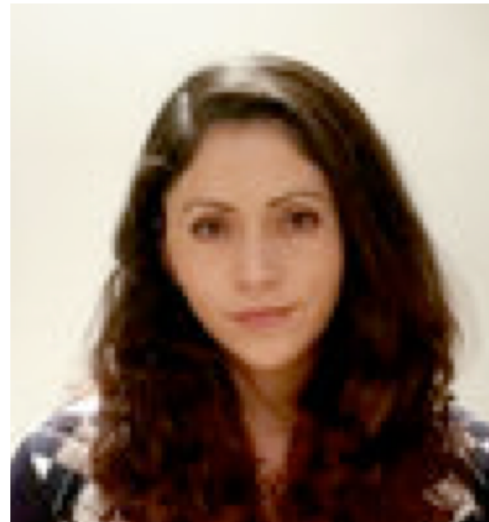
Dr. Yang Feng

Selección de posiciones diferencialmente metiladas en relación al Índice de Masa Corporal (IMC) según métodos de regularización en el Strong Heart Study

	Aenet	LASSO	Elastic-net ($\alpha=0.05$)	MSAenet	SCAD	MCP	Regresión lineal
N variables selected	222	221	222	113	93	42	-
Medidas de rendimiento							
Error c. medio entrenamiento	22.89	14.50	15.73	24.25	14.71	20.18	15.97
Error c. medio validación	31.78	38.29	35.38	42.0	41.65	33.77	35.84
Time de computación (días)	9.9	9.8	8.7	7.3	1.7	0.9	Immediate

3. Integración de resultados de investigación con información relevante de bases de datos bioinformáticas

A. Riffo



Redes de interacción de proteínas asociadas regiones diferencialmente metiladas en relación a la exposición a metals y a la aterosclerosis

Gene identification

- Differentially methylated in atherosclerosis
- Differentially methylated in metal-exposed
- Differentially methylated in atherosclerosis and metal-exposed

Type of variable (Outline)

- Binary
- Continuous
- Both

* Significantly atherosclerosis related protein

Bold Effectors

Arterial origin

- Femoral
- Coronary
- Carotid
- Femoral and Coronary
- Femoral and Carotid
- Coronary and Carotid
- Femoral, Coronary and Carotid

- Hub proteins related to both atherosclerosis and metals-DMRs (e.g. SMAD3, NOP56...)
 - Hub proteins related to metals-DMRs (e.g. SSTR5, HDAC4, AP2A2, CXCL12, SSTR4 ...)

Retos en el estudio de mecanismos a partir de datos epidemiológicos y trabajo en marcha

RETOS

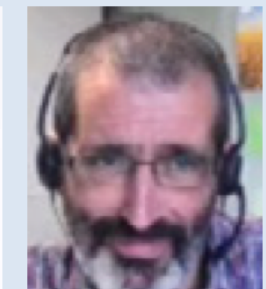
- Integración adicional (multinivel):
 - Factores Contextuales
 - Trayectorias vitales
- ¿Factores causales o correlacionales?
- Confusión residual y otros sesgos...

TRABAJO EN MARCHA

- Proyecto PID2019-108973RB-C21: “**El papel causal del selenio en la diabetes: un estudio omico para informar a la medicina de precisión (SelenOMICs)**”
- **Subproyecto 1 (ISCIII): Mendelian randomization study of Se and diabetes-related endpoints: an integrative approach**



M. Téllez



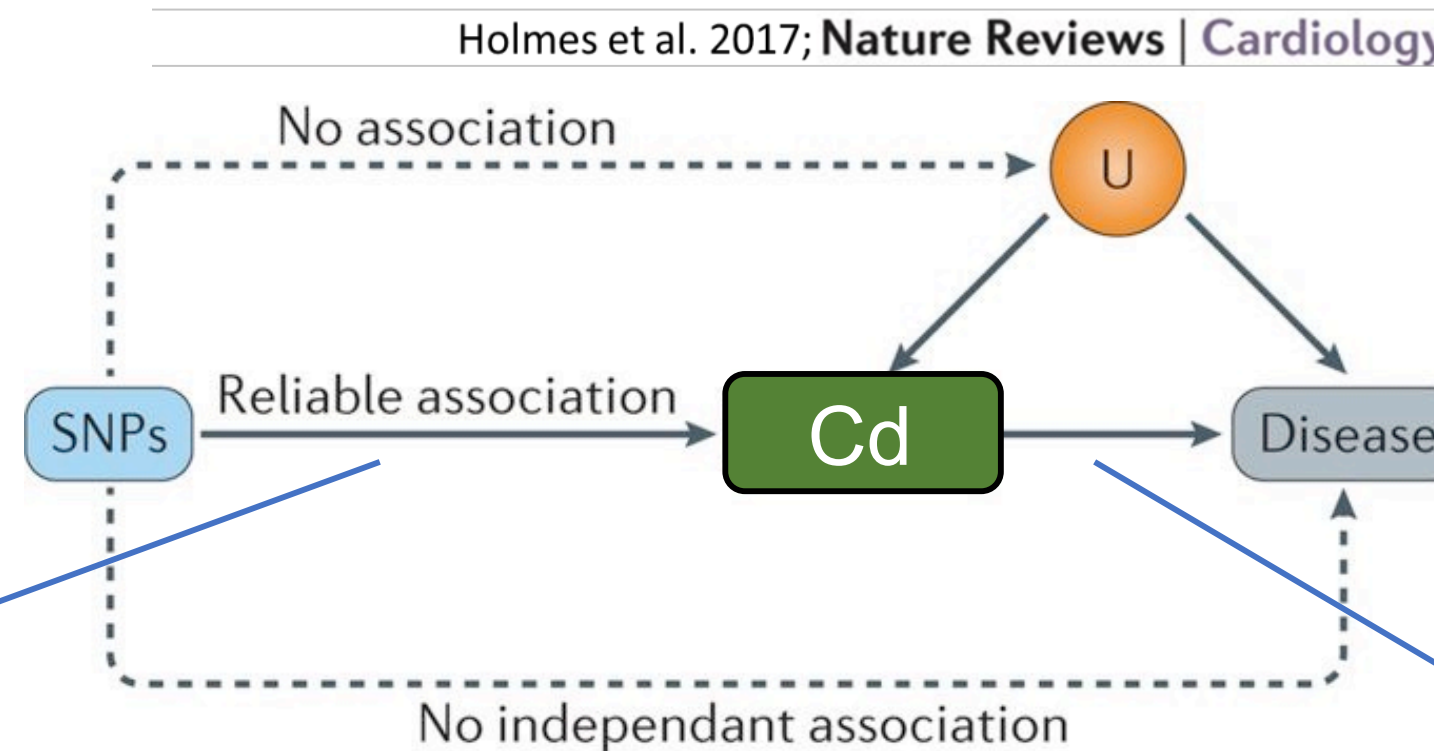
R. Pastor



D. Monleon

ALEATORIZACIÓN MENDELIANA DEL SELENIO Y LA DIABETES: UNA ESTRATEGIA DE ANÁLISIS CUASI-EXPERIMENTAL

GENES AS PROXY OF EXPOSURE



Metals associated with cardiovascular disease, do metals **cause** cardiovascular disease?

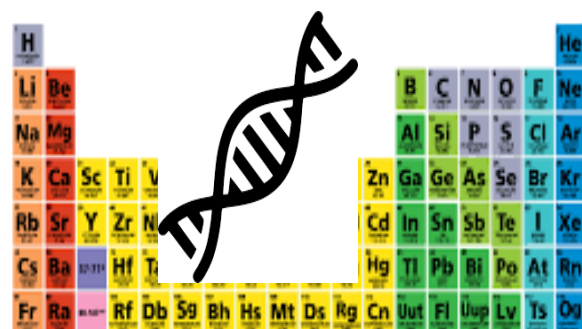
Check whether people with genetically elevated cadmium biomarker levels have more cardiovascular disease



GWAS (SNPs) -> Selenio

SNPs ->

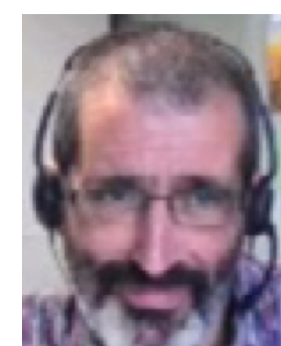
Resistencia a la insulina
Diabetes
Complicaciones de la diabetes



Z. Rodriguez



M. Grau



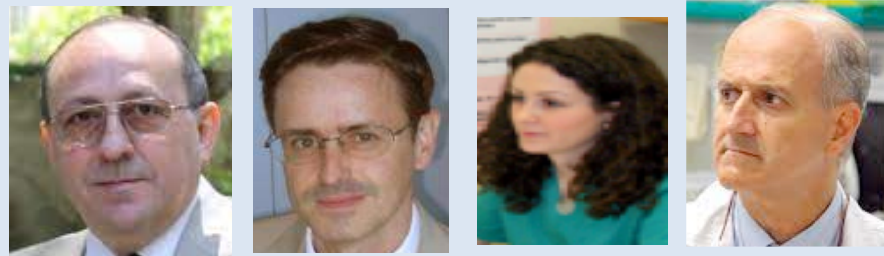
R. Pastor

The Metal-GWAS Initiative

STUDIES FROM SPAIN



Jose A. Casasnovas; Martin Laclaustra Belen Moreno, Fernando Civeira, Instituto Investigación Sanitaria de Aragon (IIAS)



Aragon Workers Health Study



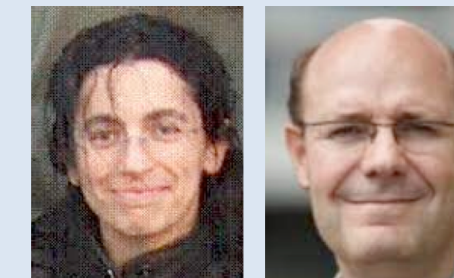
Juan C. Martin-Escudero (Hospital Rio Hortega); Josep Redón (and Daniel Monleon (INCLIVA))



Hortega Study



Beatriz Perez-Gomez (ISCI); V. Moreno (Instituto Catalán de Oncología)



Co-MCC-Spain

> 15000
participants with metal biomarkers and genome wide SNPs

STUDIES FROM THE US



Shelley A. Cole (Texas Biomedical Research Institute, San Antonio)



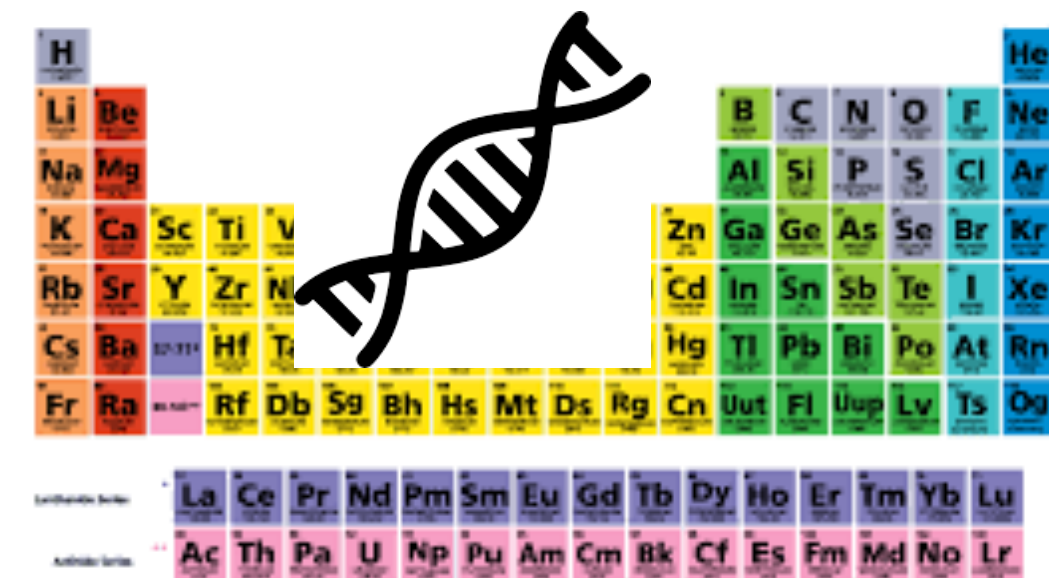
Strong Heart Study and Strong Heart Family Study



Ana Navas-Acien (Columbia University, New York)



Multi-Ethnic Study of Atherosclerosis





Estilo de Vida Saludable, Metabolómica e Incidencia de Diabetes

Table 4 Difference in type 2 diabetes incidence per 10,000 person-year per 1-point HLS increase^a

Adjustment models	Difference in type 2 diabetes rates per 10,000 person-year (95% CI)	Percent explained, % (95% CI) ^c
Age, sex, education, prevalent hypertension, total plasma cholesterol, use of lipid lowering medication (Reference Model)	-8.23 (-16.3, -0.13)	Reference
Reference model + lipoprotein subclasses	-4.45 (-12.65, 3.75)	45.9 (3.6, 238.7)
Reference model + amino acids	-4.49 (-13.06, 4.08)	45.4 (7.2, 274.0)
Reference model + fatty acids	-6.38 (-15.00, 2.24)	22.5 (-11.2, 164.1)
Reference model + fluid balance	-6.12 (-14.58, 2.34)	25.6 (4.4, 223.7)
Reference model + energy	-5.20 (-13.93, 3.54)	36.8 (0.3, 285.2)
Reference model + products of bacterial co-metabolism	-5.10 (-13.82, 3.62)	38.0 (2.8, 266.2)
Reference model + O-phosphoethanolamine	-6.24 (-14.79, 2.31)	24.2 (2.9, 211.3)
Reference model + phenylpropionate, and medium HDL and small LDL particle concentrations ^b	-3.91 (-12.25, 4.44)	52.5 (15.9, 384.3)

Abbreviations: CI, confidence interval

^a Aalen additive models with progressive degrees of adjustment; $n = 830$, 51 incident type 2 diabetes cases and 779 non-cases

^b Relevant metabolites defined as showing a PIP > 20% in BKMR-P regression

^c Bias-corrected and accelerated 95% confidence intervals from bootstrap based on 1000 resamplings as conducted by the *boot* R package

El “score” de vida saludable mostró una asociación inversa fuerte con la incidencia diabetes tipo 2 incidente, que se explicó en gran medida por los metabolitos plasmáticos medidos años antes del diagnóstico clínico

Source: Delgado-Velandia, 2021. International Journal of Behavioral Nutrition and Physical Activity. In press.



M. Delgado



I. Galán



M. Sotos

CONCLUSION

La investigación mecanística en epidemiología de las enfermedades crónicas puede ayudarnos a mejorar la comprensión de cómo nuestros antecedentes familiares, comportamientos, medioambiente y genes funcionan juntos y además ayudar a encontrar nuevas herramientas para la prevención y el control.

Socioeconomic impact:

Understanding metal-related cardiometabolic risk



Benefits for the society:



Health providers



Companies



citizens

CP12/03080; PI15/00071; Proyecto
PID2019-108973RB-C21; La Caixa InPHINIT



II Jornadas del CNE

National Center of Epidemiology (Madrid, Spain): A. Domingo-Relloso, M. Galvez-Fernandez, Z. Rodriguez-Hernandez, R. Pastor, B. Pérez, P. Fernandez, I. Galán, R. Ramis....

Universidad Autónoma de Madrid: M. Sotos, F. Rodriguez-Artalejo, E. García-Esquinas

INCLIVA Biomedical Research (Valencia, Spain): J. Redón, M. Grau-Perez, M. Herreros, S. Zuñiga, P. Rentero

Hospital Rio Hortega/Valladolid University: J.C. Martin-Escudero

Instituto Investigación Sanitaria de Aragón: J.A. Casanovas, F. Civeira

Columbia University: A. Navas-Acien

Johns Hopkins Bloomberg School: E. Guallar

Texas Biomedical Research Foundation: S. Cole, K. Haack, S. Smith, D. Newman, J. Kent

The Metal-GWAS Initiative

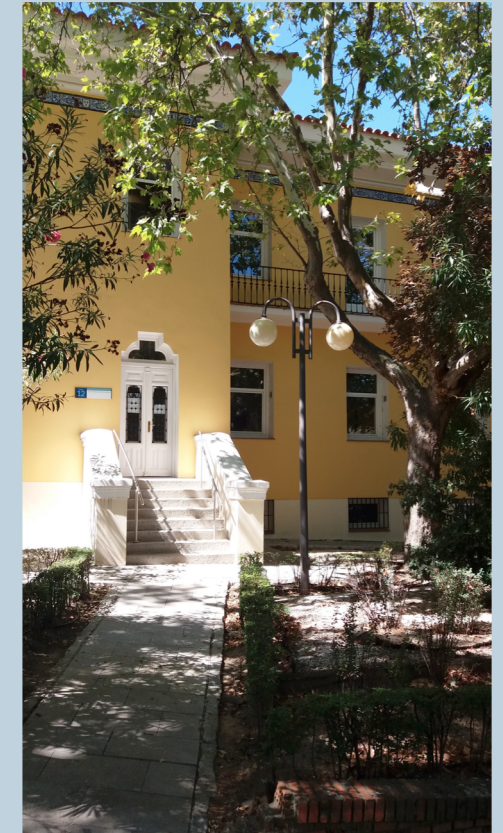
Hortega Study (J.C. Martin-Escudero, J. Redon, D. Monleon)

AWHS (J.A. Casanovas, F. Civeira, B. Moreno, M. Laclaustra).

MCC-Spain (B. Perez, V. Moreno)

MESA (A. Navas-Acien, J. Rotter)

SHS and SHFS (S. Cole, K. Haack, S. Smith)



i GRACIAS

POR SU ATENCIÓN !

