



11-13  
SEPT.  
2024

LILLE  
GRAND PALAIS

# CONGRÈS FRANÇAIS d'HÉMOSTASE



## Identification de biomarqueurs plasmatiques impliqués dans la régulation des taux de Neutrophil Extracellular Traps

Gaëlle Munsch  
*Chercheur Post-Doctoral*



# Neutrophil Extracellular Traps (NETs)

NETs = filaments extracellulaires de chromatine des neutrophiles

→ Attraper & Eliminer les pathogènes

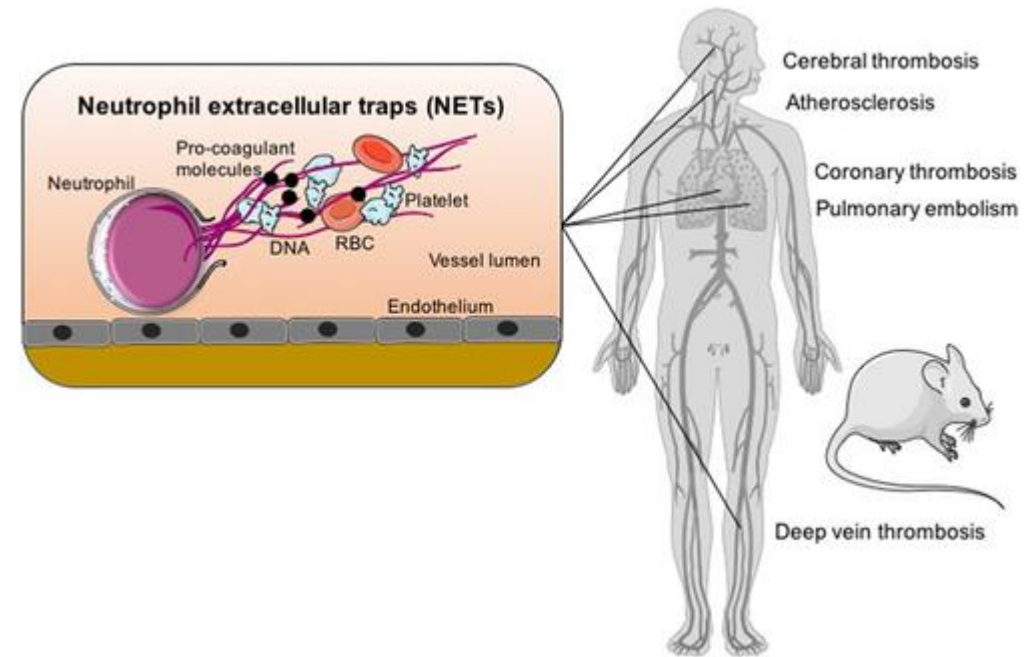
**NETs favorisent la coagulation**

→ **Inhiber les inhibiteurs naturels de la coagulation**

(TFPI, thrombomoduline)

→ **Activer les facteurs de la coagulation**

(FXII, vWF)



*Thalin et al., ATVB (2019)*

# Genetics of NETs

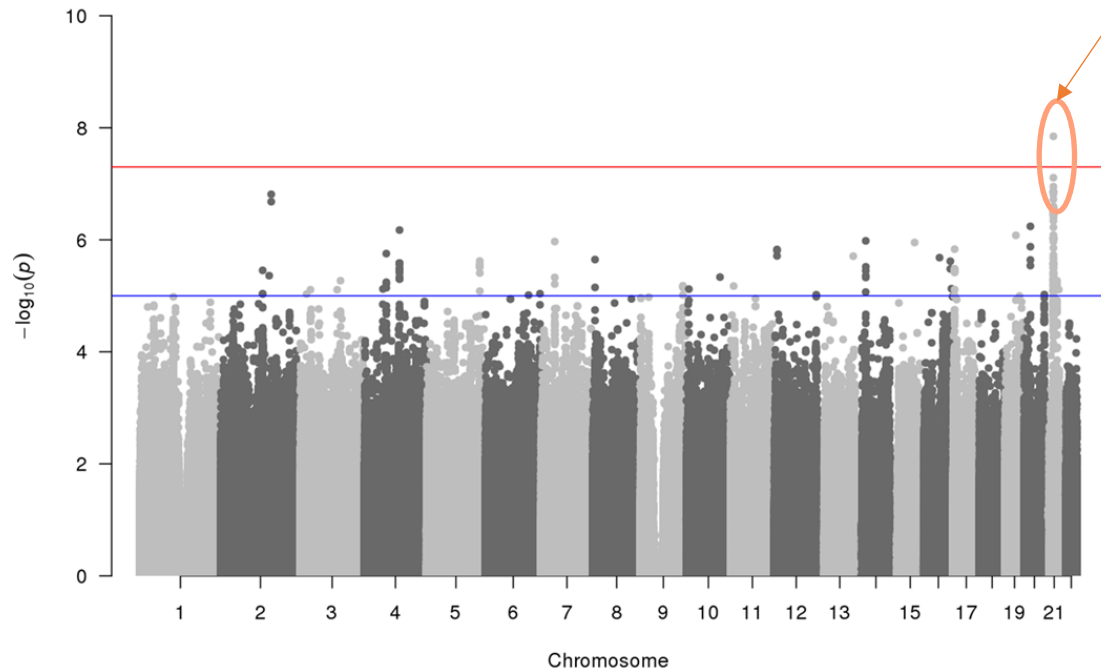
NAR  
 Genomics and Bioinformatics

[NAR Genom Bioinform.](#) 2023 Jun; 5(2): lqad062.  
 Published online 2023 Jun 28. doi: [10.1093/margab/lqad062](#)

PMCID: PMC10304785  
 PMID: [37388819](#)

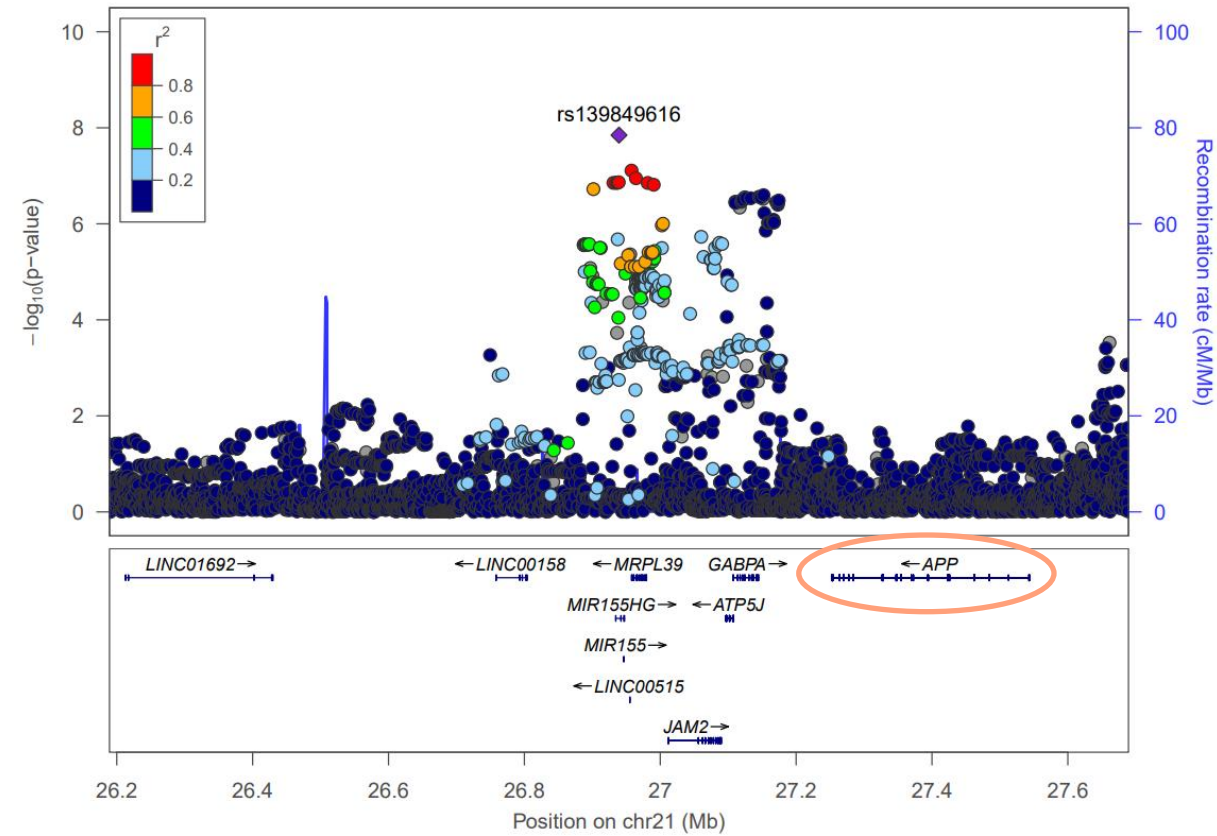
Genome-wide association study of a semicontinuous trait: illustration of the impact of the modeling strategy through the study of Neutrophil Extracellular Traps levels

Gaëlle Munsch,<sup>✉</sup> Carole Proust, Sylvie Labrousche-Colomer, Dylan Aïssi, Anne Boland, Pierre-Emmanuel Morange, Anne Roche, Luc de Chaisemartin, Annie Harroche, Robert Olaso, Jean-François Deleuze, Chloé James, Joseph Emmerich, David M Smadja, Héliène Jacqmin-Gadda, and David-Alexandre Tréguët



rs57502213 ( $p = 1.4 \times 10^{-8}$ ) chr21q21.3

miR-155 (*MIR155HG*)

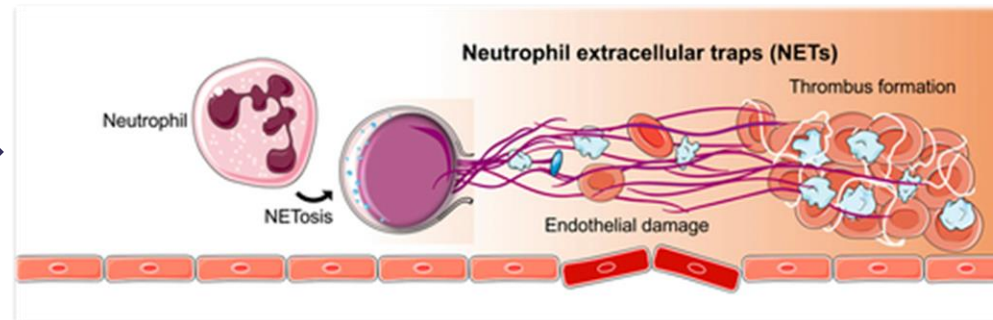


# Objectif

Identifier des **biomarqueurs moléculaires** pouvant être impliqués dans la **régulation des NETs** en utilisant des méthodes de **Randomisation Mendélienne**

## Biomarqueurs inflammatoires

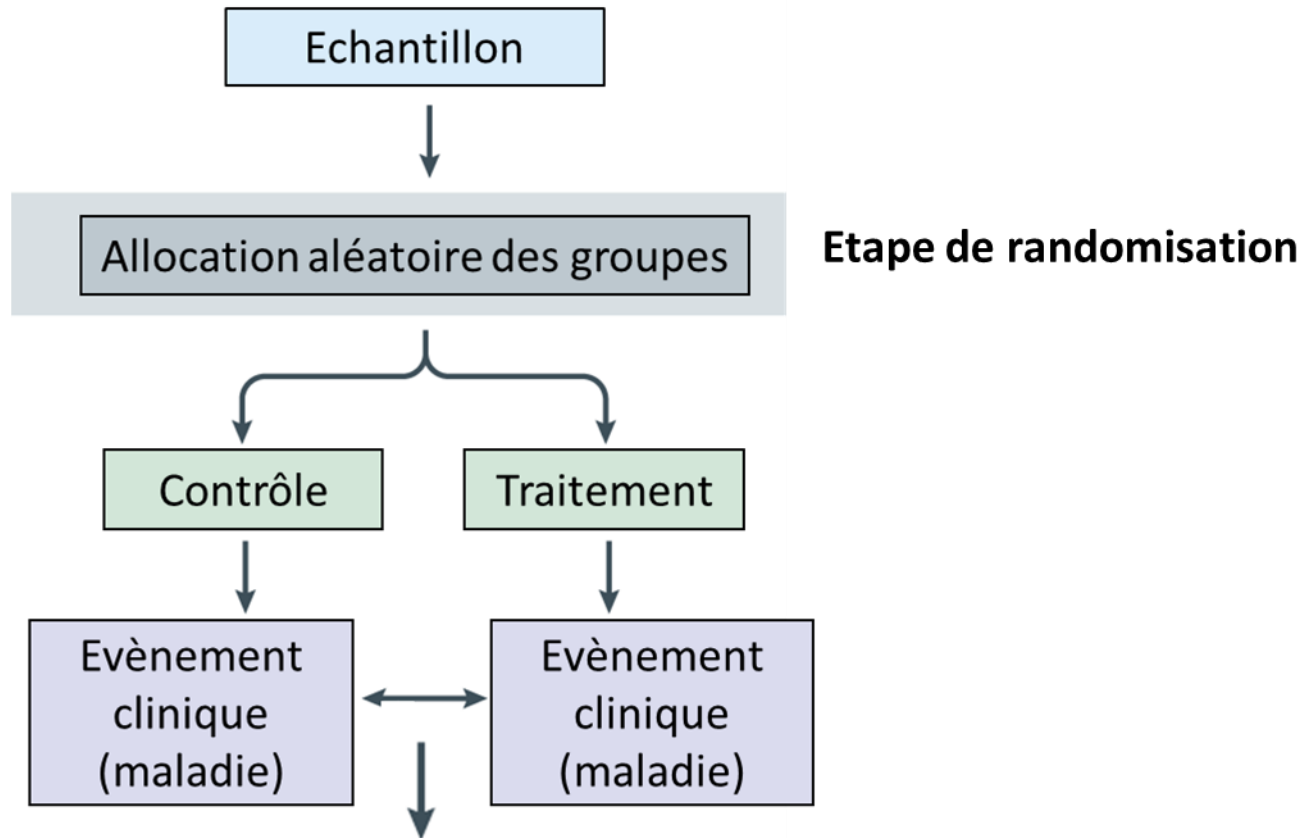
Interleukines,  
CRP,  
TNF- $\alpha$ ,  
...



Ng et al., ATVB (2020)

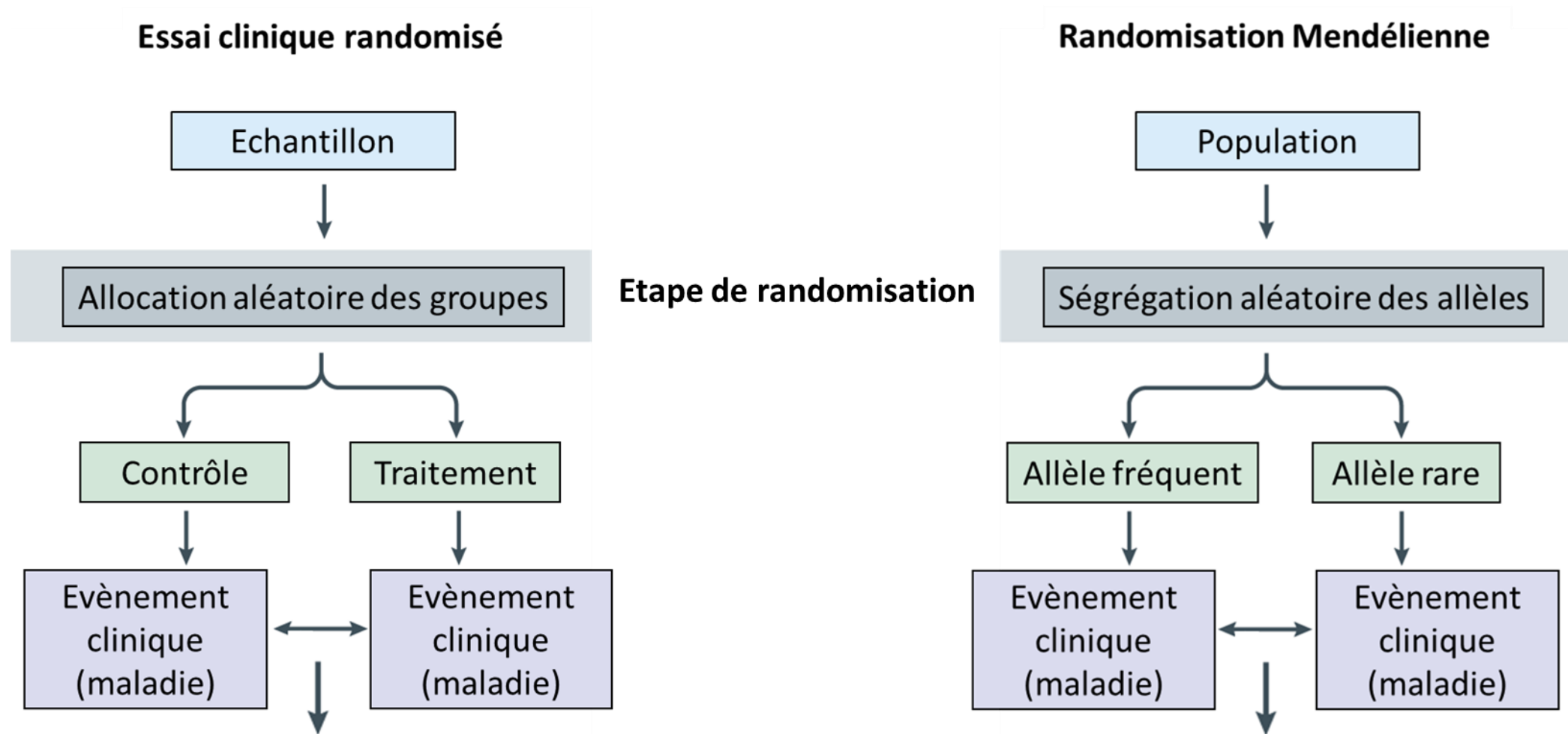
# La Randomisation Mendélienne (RM)

## Essai clinique randomisé



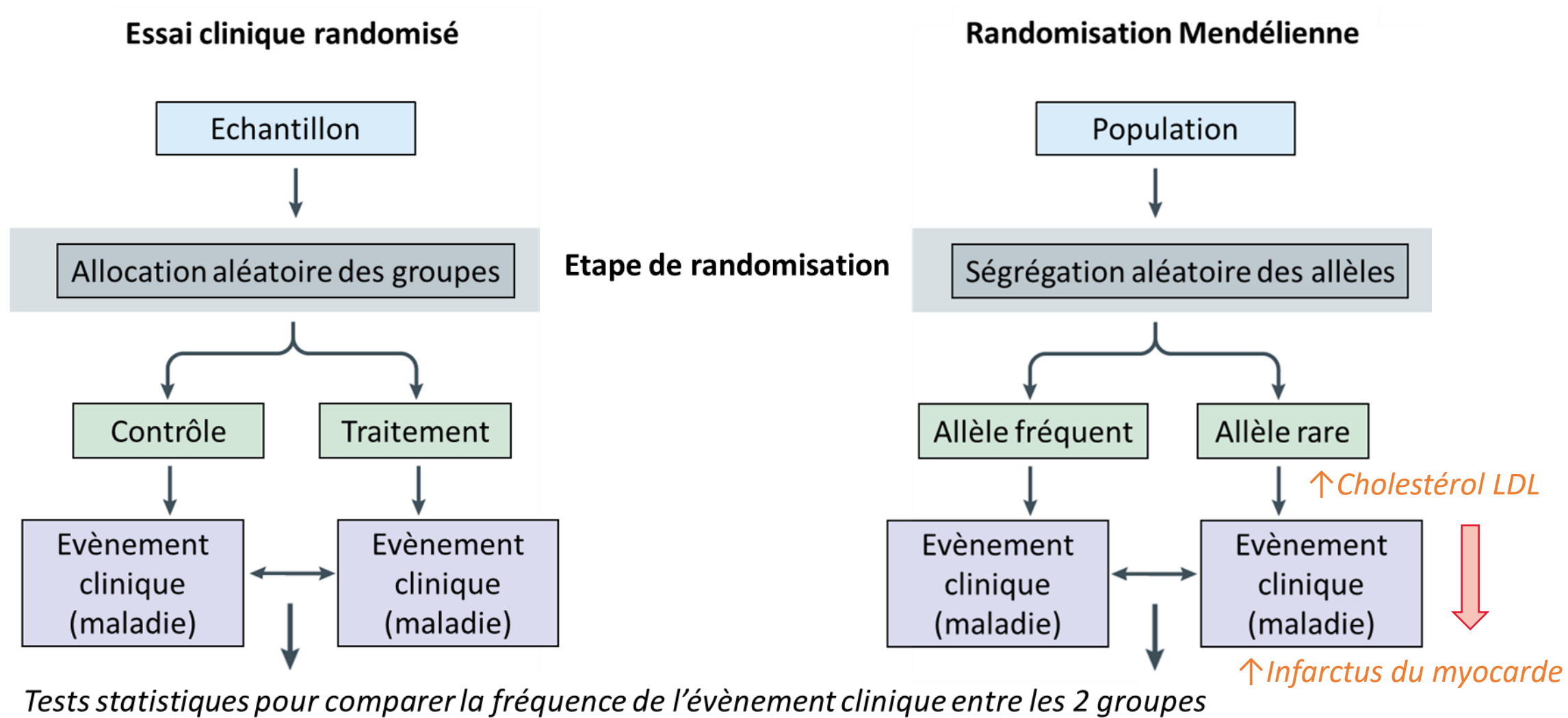
*Tests statistiques pour comparer la fréquence de l'évènement clinique entre les 2 groupes*

# La Randomisation Mendélienne (RM)

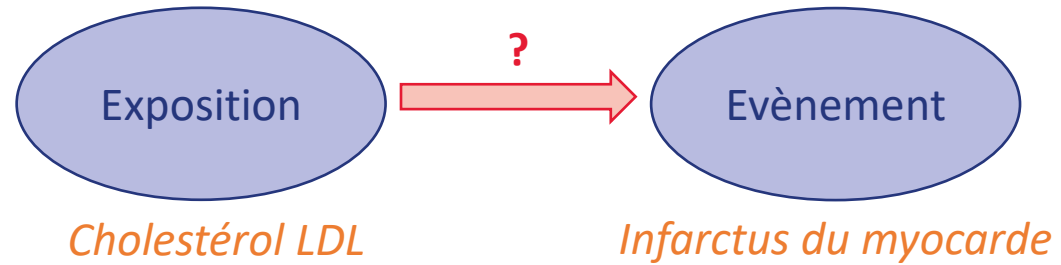


*Tests statistiques pour comparer la fréquence de l'évènement clinique entre les 2 groupes*

# La Randomisation Mendélienne (RM)



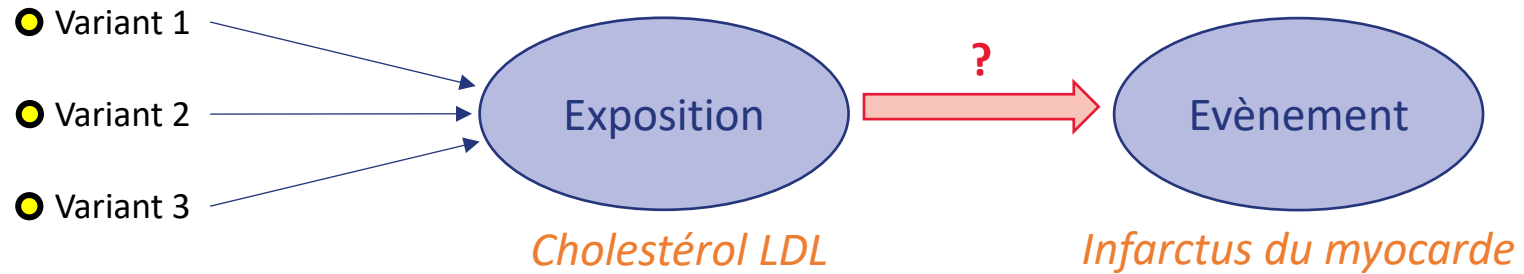
# La Randomisation Mendélienne (RM)



**Objectif:** Inférer l'effet causal de l'exposition sur l'évènement

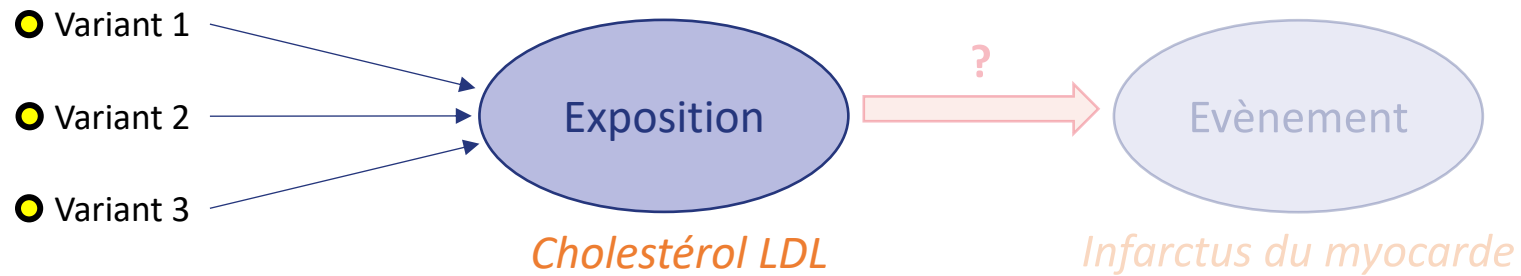


# La Randomisation Mendélienne (RM)



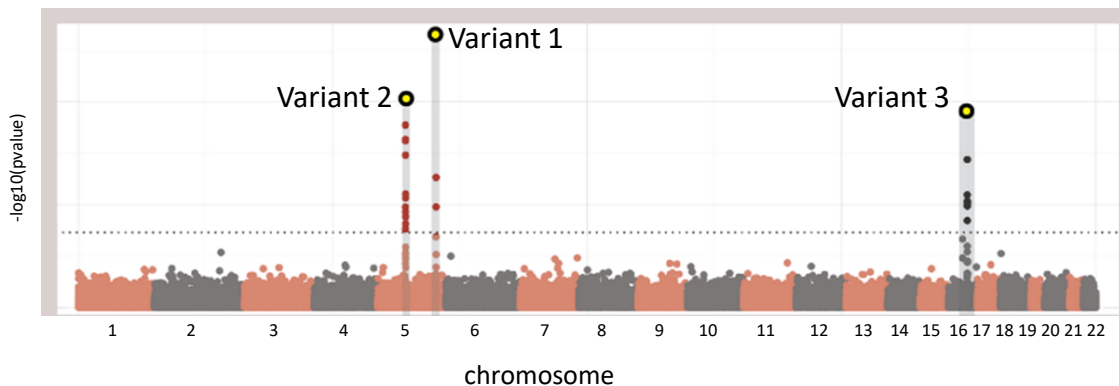
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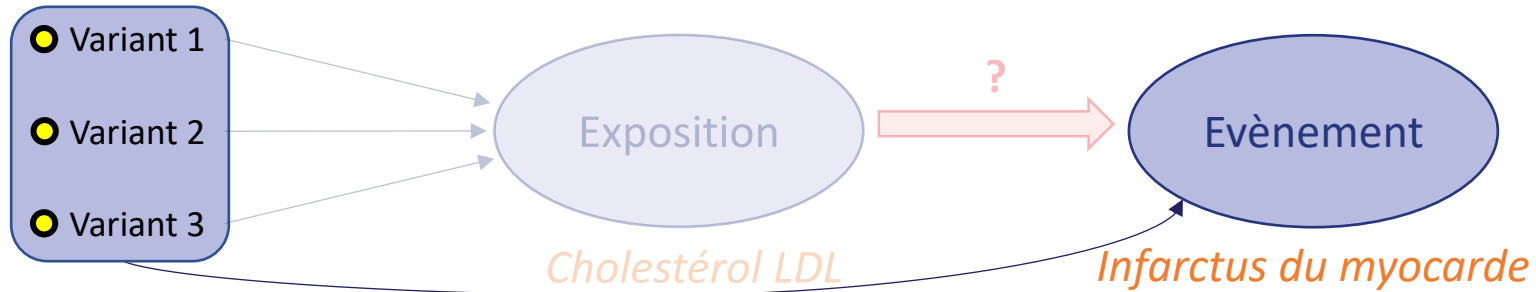


1 Identifier les variants génétiques associés à l'exposition

*Etude d'association pan-génomique (GWAS)  
sur les taux de Cholestérol LDL*

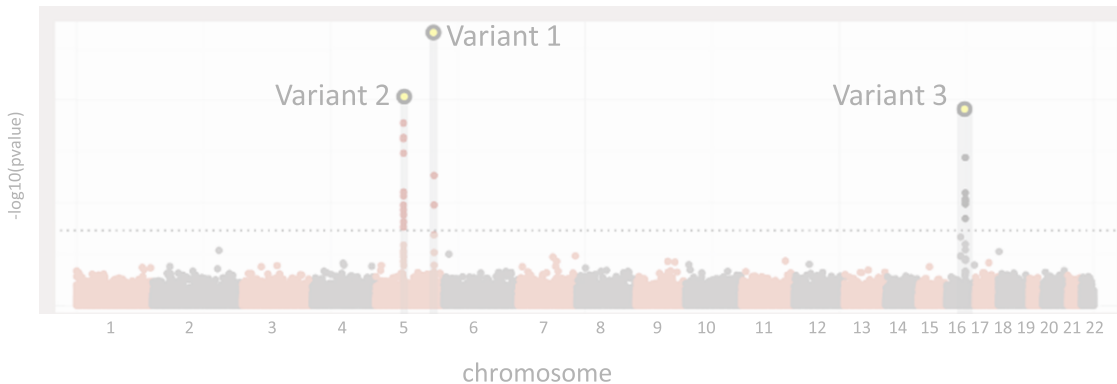


# La Randomisation Mendélienne (RM)



① Identifier les variants génétiques associés à l'exposition

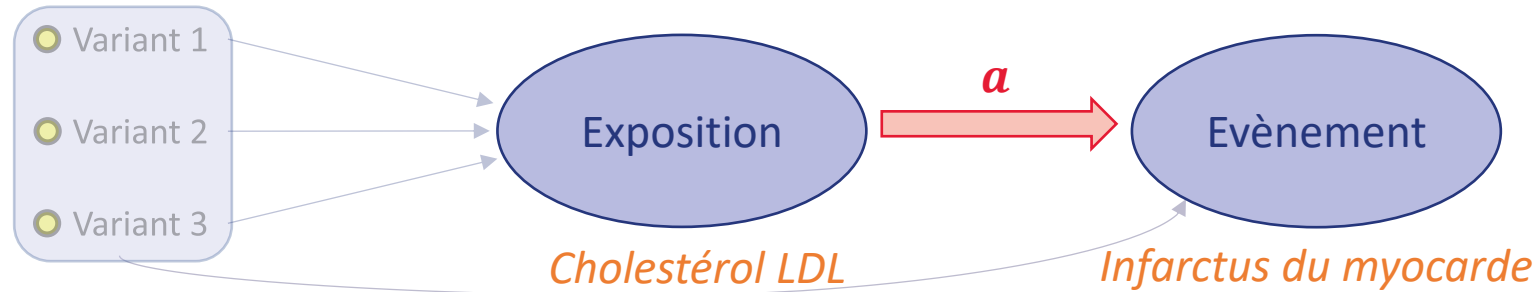
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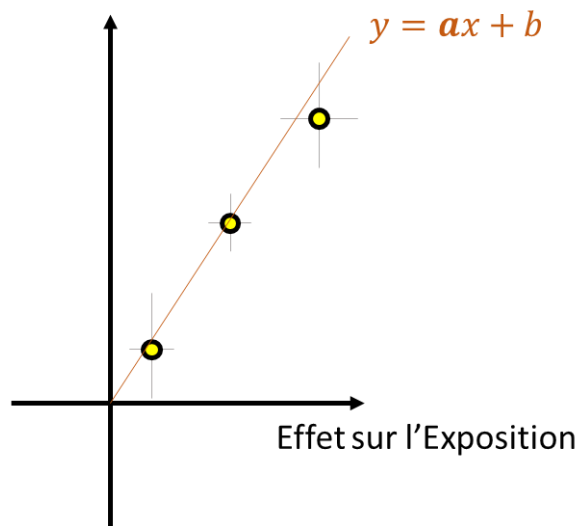
② Estimer l'association entre les variants identifiés et l'évènement

*Effet individuel des 3 variants  
sur le risque d'Infarctus du myocarde  
(régression logistique, Khi-2...)*

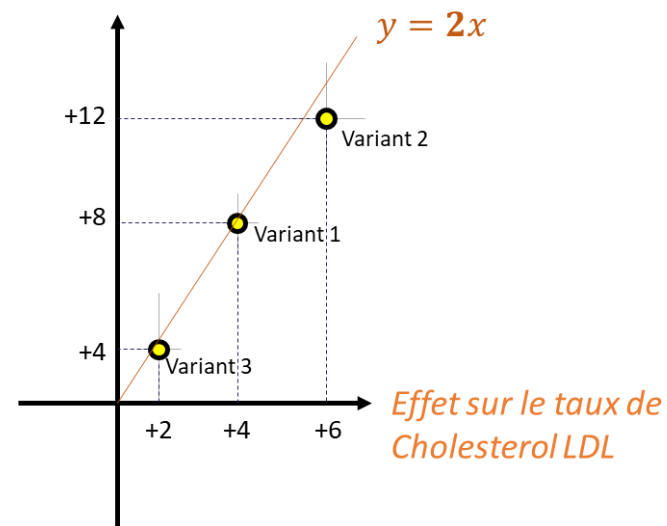
# La Randomisation Mendélienne (RM)



Effet sur l'Evènement



Effet sur le risque d'Infarctus du myocarde



# Protéines influençant les taux de NETs

« Exposition »

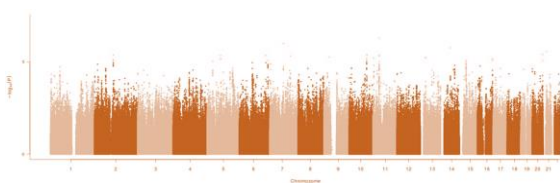
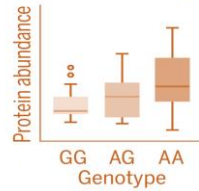
« Evènement »

Protéines  
plasmatisques

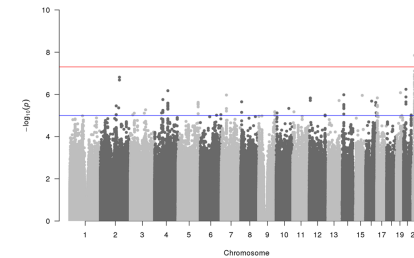


Taux de  
NETs

Genome-proteome-wide  
association study



Etude FARIVE (N = 657)



Munsch, NAR Genom. Bioinform. (2023)

Projet deCODE (N = 35 559)

~ 3 739 protéines mesurées par Somascan

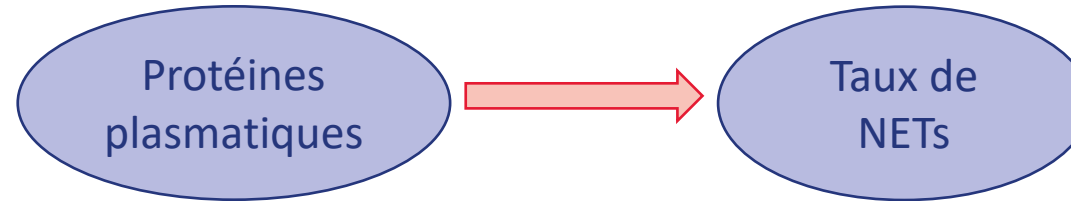
Ferkingstad, Nat Genet (2021)

Etude UK Biobank (N = 34 557)

~ 2 308 protéines mesurées par Olink

Sun, Nature (2023)

# Protéines influençant les taux de NETs

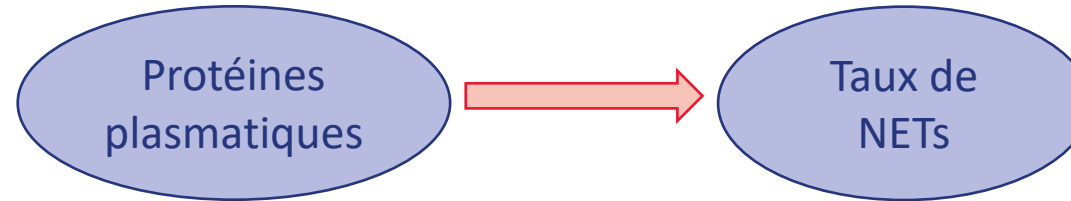


→ Pas de protéines passant le seuil de significativité ( $P < 2 \times 10^{-5}$ )

**Mais de bons candidats :**

Beta	Pvalue	Gène	Protéine
-1,22	6,31E-05	PPBP	Platelet basic protein
-1,62	7,41E-05	TINAGL1	Tubulointerstitial nephritis antigen-like
1,10	1,02E-04	ENOB	Beta-enolase
-1,86	1,83E-04	ATP6V1G2	V-type proton ATPase subunit G 2
-3,08	2,88E-04	APOA4	Apolipoprotein A-IV
-0,37	3,02E-04	PON2	Serum paraoxonase/arylesterase 2
-1,11	3,04E-04	SPARC	SPARC
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-1,82	4,70E-04	LAT	Linker for activation of T-cells family member 1
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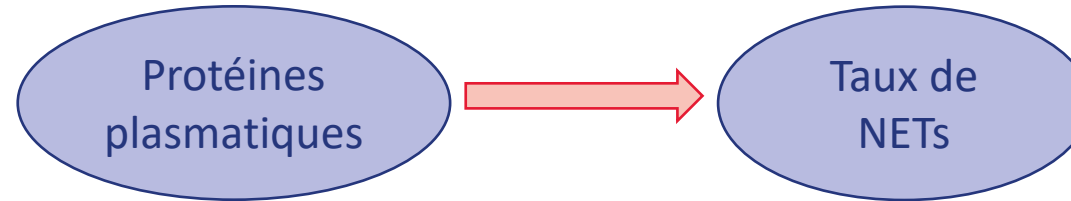
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*“Platelet interaction with activated neutrophils is a potent inducer of NETs”*

*Kim, Semin Immunol (2016)*

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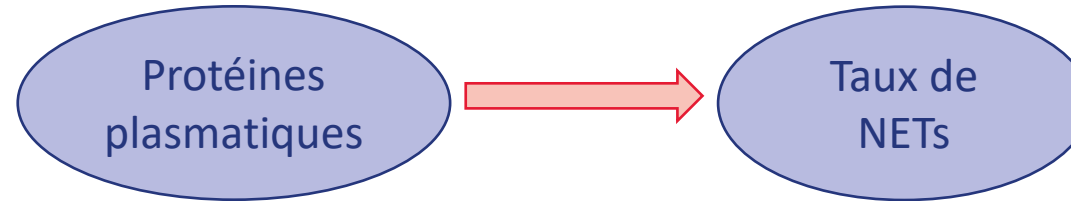
*“Platelet factor 4 limits NETs”*

*Ngo, JCI Insight (2023)*

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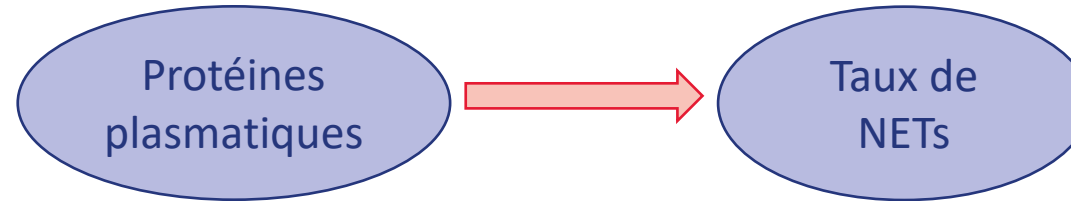
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*“Neutrophil can be attracted to amyloid-beta plaques [...] and release NETs”*

Canalli Kretzschmar, *Front Mol Biosci* (2021)

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*KNG1* & *SERPINE2* sont des facteurs de risque génétique de la **thrombose veineuse**

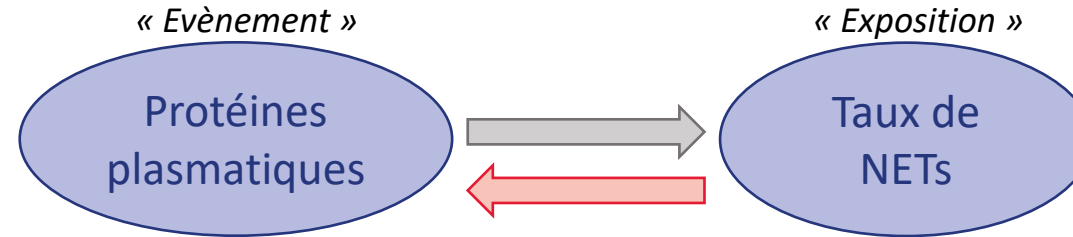
*Morange, Blood (2011)*

*Thibord, Circulation (2022)*

*KNG1* influence les taux de **FXI**

*Sabater-Lleal, ATVB (2012)*

# Protéines influencées par les NETs



Beta	Pvalue	Gène	Protéine
0,01	9,53E-05	CD74	HLA class II histocompatibility antigen gamma chain
0,01	4,10E-04	SULT2A1	Bile salt sulfotransferase
-0,01	4,81E-04	HAVCR1	Hepatitis A virus cellular receptor 1
-0,01	5,78E-04	IMPACT	Protein IMPACT
-0,01	7,68E-04	HDDC2	5'-deoxynucleotidase HDDC2
0,01	9,88E-04	AKR7L	Aflatoxin B1 aldehyde reductase member 4

➔ Aussi connue sous le nom de TIM-1

[J Clin Invest](#). 2022 May 16; 132(10): e154225.  
Published online 2022 May 16. doi: [10.1172/JCI154225](https://doi.org/10.1172/JCI154225)

PMCID: PMC9106355  
PMID: [35358095](https://pubmed.ncbi.nlm.nih.gov/35358095/)

## Neutrophil extracellular traps regulate ischemic stroke brain injury

Frederik Denorme,<sup>1</sup> Irina Portier,<sup>1</sup> John L. Rustad,<sup>1</sup> Mark J. Cody,<sup>1</sup> Claudia V. de Araujo,<sup>1</sup> Chieko Hoki,<sup>1</sup> Matthew D. Alexander,<sup>2,3</sup> Ramesh Grandhi,<sup>3,4</sup> Mitchell R. Dyer,<sup>5</sup> Matthew D. Neal,<sup>5</sup> Jennifer J. Majersik,<sup>2</sup> Christian C. Yost,<sup>1,6</sup> and Robert A. Campbell<sup>1,7</sup>

[Review](#) > [Mol Neurobiol](#). 2022 Jan;59(1):643-656. doi: [10.1007/s12035-021-02635-z](https://doi.org/10.1007/s12035-021-02635-z).  
Epub 2021 Nov 8.

## Neutrophil Extracellular Traps Exacerbate Ischemic Brain Damage

Congqin Li<sup>1</sup>, Ying Xing<sup>1</sup>, Yuqian Zhang<sup>1</sup>, Yan Hua<sup>1</sup>, Jian Hu<sup>1</sup>, Yulong Bai<sup>2</sup>

Affiliations + expand  
PMID: 34748205 DOI: [10.1007/s12035-021-02635-z](https://doi.org/10.1007/s12035-021-02635-z)

[Review](#) > [Pharmacol Ther](#). 2023 Jan;241:108328. doi: [10.1016/j.pharmthera.2022.108328](https://doi.org/10.1016/j.pharmthera.2022.108328).  
Epub 2022 Dec 6.

## Neutrophil extracellular traps: A novel target for the treatment of stroke

Ziyuan Zhao<sup>1</sup>, Zirong Pan<sup>1</sup>, Sen Zhang<sup>1</sup>, Guodong Ma<sup>1</sup>, Wen Zhang<sup>1</sup>, Junke Song<sup>1</sup>, Yuehua Wang<sup>1</sup>, Linglei Kong<sup>2</sup>, Guanhua Du<sup>3</sup>

Affiliations + expand  
PMID: 36481433 DOI: [10.1016/j.pharmthera.2022.108328](https://doi.org/10.1016/j.pharmthera.2022.108328)

[Observational Study](#) > [Arterioscler Thromb Vasc Biol](#). 2020 Jul;40(7):1777-1786.  
doi: [10.1161/ATVBAHA.120.314269](https://doi.org/10.1161/ATVBAHA.120.314269). Epub 2020 May 28.

## Association of TIM-1 (T-Cell Immunoglobulin and Mucin Domain 1) With Incidence of Stroke

Lu Song<sup># 1</sup>, Jiangming Sun<sup># 2</sup>, Martin Söderholm<sup>2 3</sup>, Olle Melander<sup>2</sup>, Marju Orho-Melander<sup>2</sup>, Jan Nilsson<sup>2</sup>, Yan Borné<sup>2</sup>, Gunnar Engström<sup>2</sup>

Affiliations + expand

PMID: 32460577 DOI: [10.1161/ATVBAHA.120.314269](https://doi.org/10.1161/ATVBAHA.120.314269)

# Conclusion & Perspectives

Utilisation de la **Randomisation Mendélienne** pour **identifier des protéines associées aux taux de NETs**

Limite principale : taille de l'échantillon (N = 657) & mesure des NETs dans FARIVE (MPO-DNA)

→ Effet des **plaquettes** sur les taux de NETs :  $\uparrow$  *PPBP* & *PF4*  $\longrightarrow$   $\downarrow$  NETs

→ Relation **Thrombose veineuse** – *KNG1/FXI* – *SERPINE2* : rôle des **NETs** ?

→ Relation **AVC** – **NETs** : explorer davantage le lien entre *TIM-1* et les taux de NETs



*Pr Joseph Emmerich*

*Pr Pierre-Emmanuel Morange*

*Pr Chloé James*

*Pr David M Smadja*

*Dr David-Alexandre Trégouët*