WHOLE EXOME SEQUENCING OF PATIENTS WITH ADULT ONSET EVANS SYNDROME: A COHORT OF 120 PATIENTS

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Cities :1.2.3.4.5.7.12. Paris (F), 6. Toulouse (F), 8. Amiens (F), 9. Marseille (F), 10. Corbeil Essonne(F), 11. Nancy (F), 13. Saint Nazaire (F), 14. Pessac, (F), 15. Dijon (F)

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INNOVATIONS IN IMMUNE THROMBOCYTOPENIA
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Disclosures of Crickx Etienne (unrelated to this presentation)

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Novartis						EC	
UCB						EC	
Sanofi							EC
Amgen							EC

Introduction

- Loss of tolerance toward several self-antigens suggests an underlying genetic predisposition

- In children with Evans syndrome (ES), 30-40% have an underlying monogenic disease

Hadjadj et al., Blood 2019

- In adult, next generation sequencing is not routinelly performed in ES, as diagnosis yield is considered to be low

Fattizzo et al., Blood Adv 2021

Jiang et al., Blood Advance 2023

Objectives

- **Identify genetic variants** responsible for adult onset Evans syndrome
- Study their **association with clinical or biological features**
- Perform functional studies of **new genetic variants**
- **Guide therapeutic strategy** with genetic findings

Patients and methods

- **Inclusion criteria** : Adult onset (≥18 years) Evans syndrome
  - Defined as at least 2 autoimmune cytopenia among:
  - Immune thrombocytopenia (ITP), warm autoimmune hemolytic anemia (wAIHA), autoimmune neutropenia (AIN)
  - Diagnosed according to international guidelines[1,2]

- **Exclusion criteria** :
  - Systemic lupus (according to Systemic Lupus International Collaborating Clinics criteria [3])
  - Indolent B cell malignancies at Evans syndrome onset
  - Other causes of cytopenia (drugs)

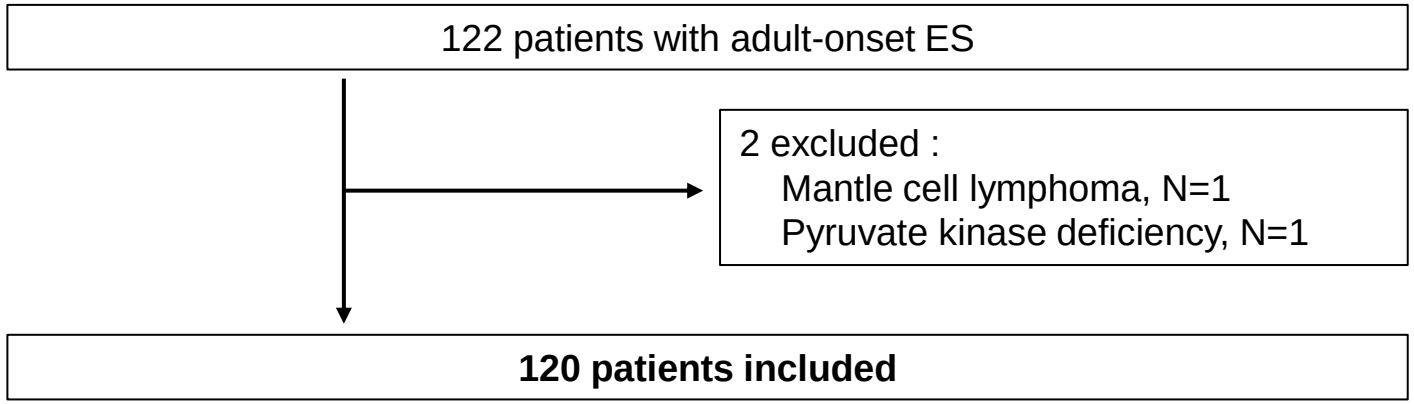
[1] *Provan D et al., Blood Adv 2019*
[2] *Jäger U et al., Blood Rev 2020*
[3] *Petri M et al., Arthritis Rheum 2012*

Patients and methods

- Patients identified in France in the network of the national reference center for adult autoimmune cytopenia (CERECAI) from November 2021 to June 2023
- Clinical and biological data were retrospectively collected using a standardized form
- Whole-exome sequencing after written and informed consent was obtained (except for patients with genetic diagnosis already available)
- Gene variants already published and/or with *in vitro* confirmed consequences were considered as genetic diagnoses. Clinical interpretation of variants was performed according to the ACMG guidelines[1]

[1] *Richards S et al., Genet Med 2015*

Flow Chart of study



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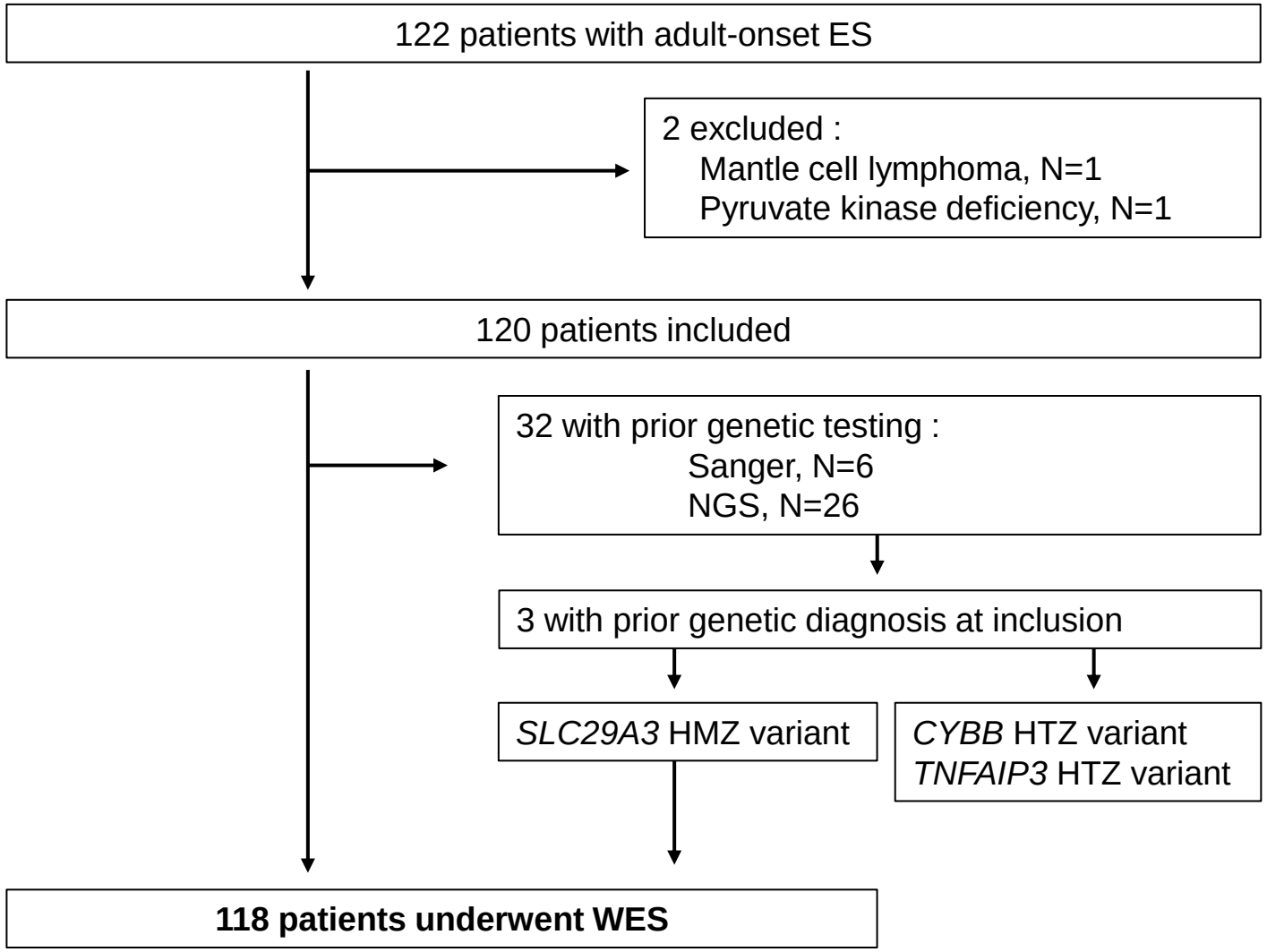
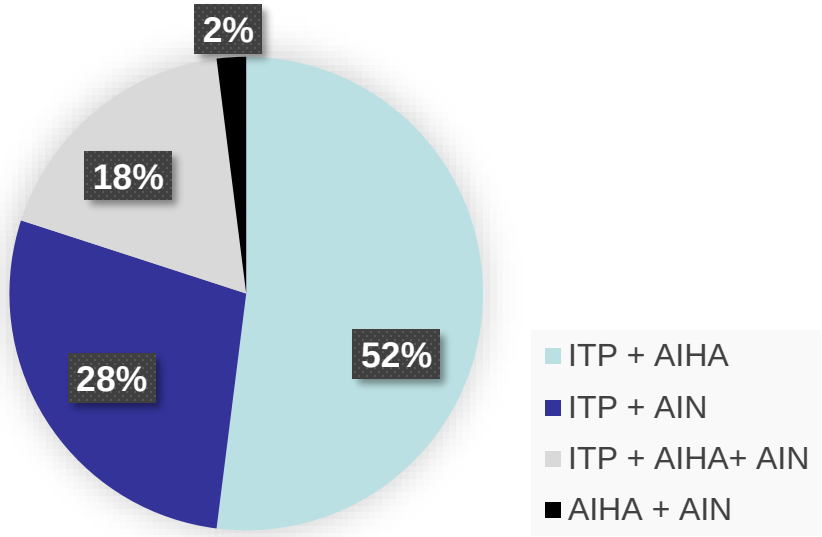


Patients characteristics

	All patient (N=120)
Female	44 (37)
Age at Evans syndrome diagnosis (median, range)	33 [18 - 80]
Age at first cytopenia (median, range)	30 (1 - 80)



Patients characteristics

	All patient (N=120)
Age < 18 at first cytopenia	19 (16)
Immunopathologic manifestations in first-degree relatives	24 (20)
Severe and/or recurrent infections	31 (26)
Humoral immune deficiency	30 (25)
CVID-like	20 (17)
IgA deficiency	3 (3)
IgG subclass deficiency	2 (2)
Secondary humoral immune deficiency	5 (4)
Organomegaly	64 (53)
Adenomegaly	48 (40)
Splenomegaly	37 (31)
Hepatomegaly	12 (10)
Tissue lymphocyte infiltration	7 (6)
Malignancies	13 (11)
Associated immunopathologic manifestations	40 (33)
Antinuclear antibody (titer ≥ 1/160)	37 (31)

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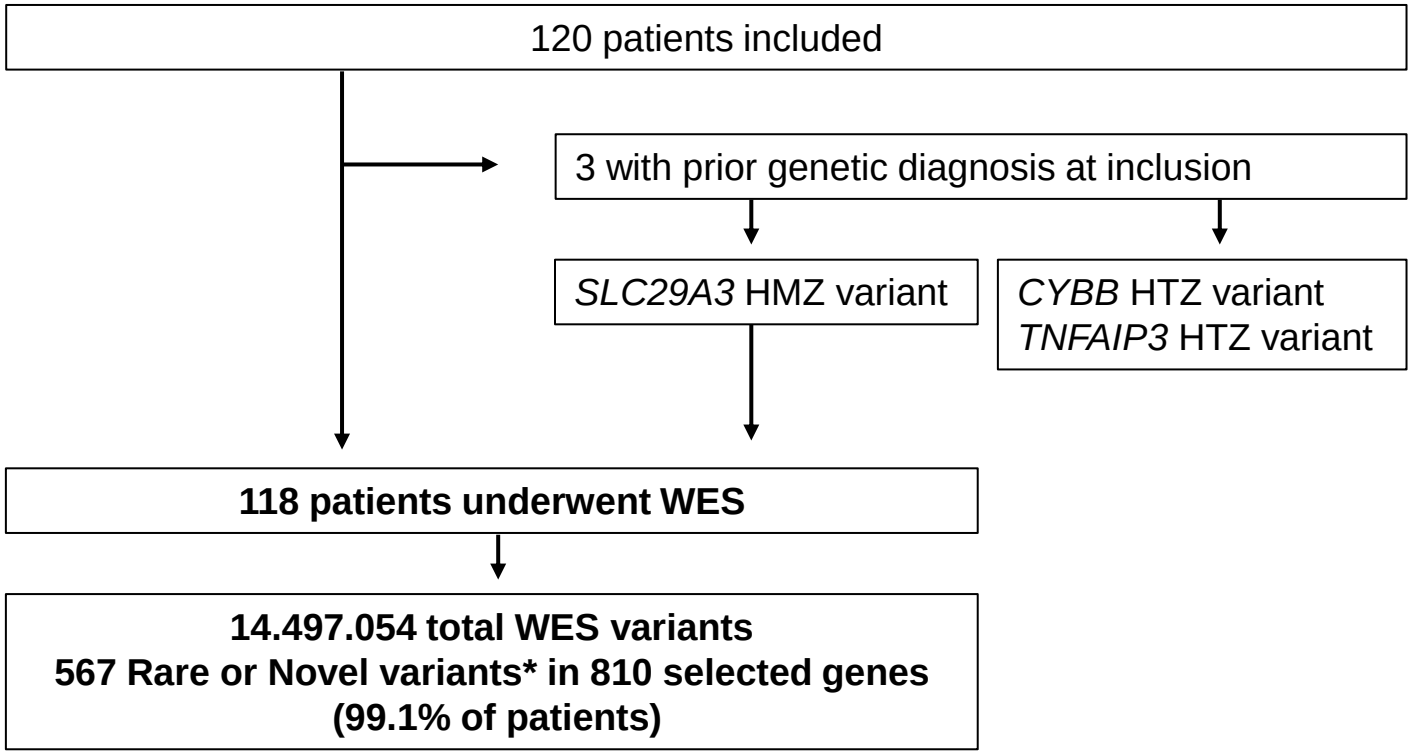
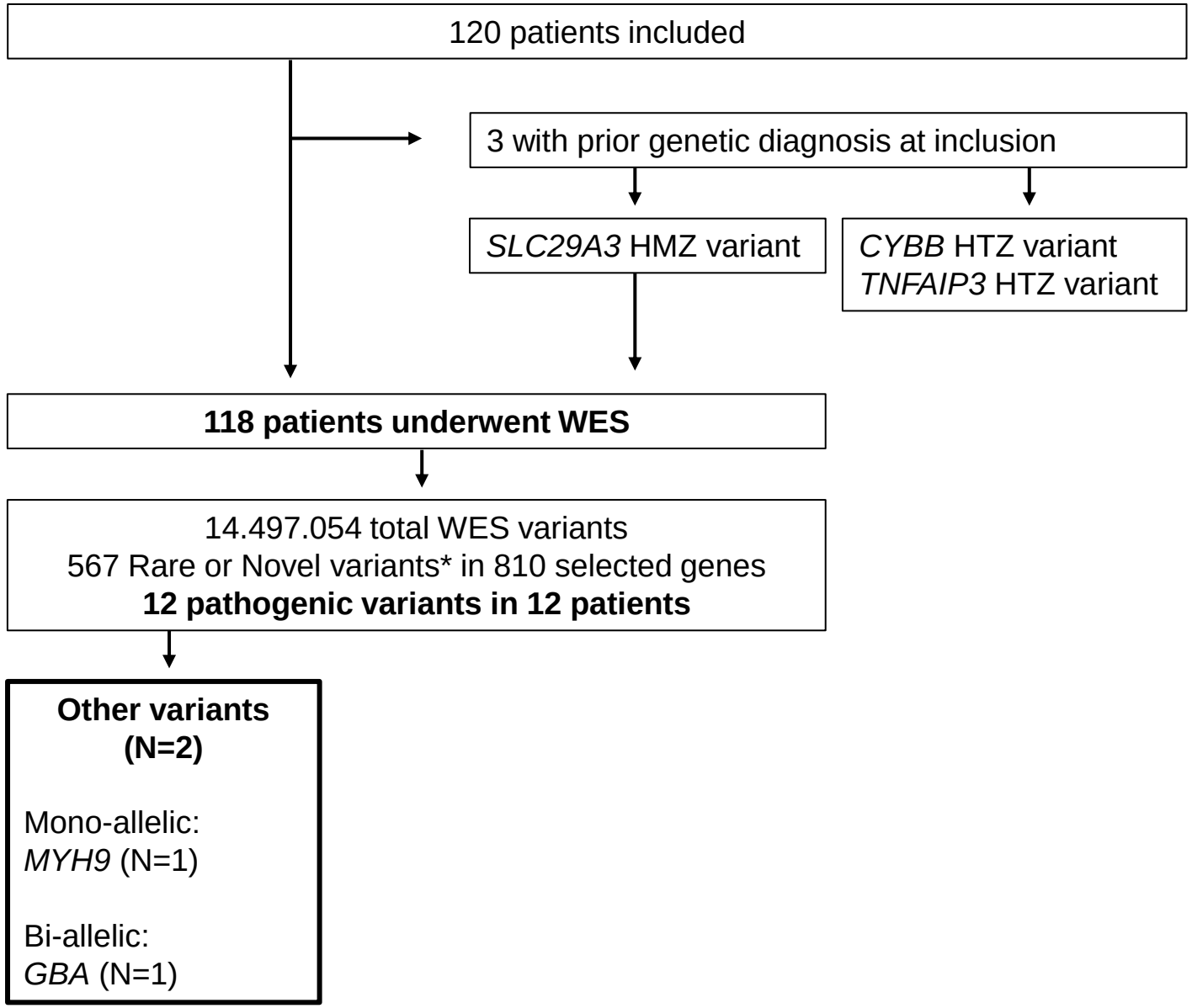
Genetic testing



*frequency <1/10.000 (gnomAD)
and CADD score ≥ 20

Genetic testing



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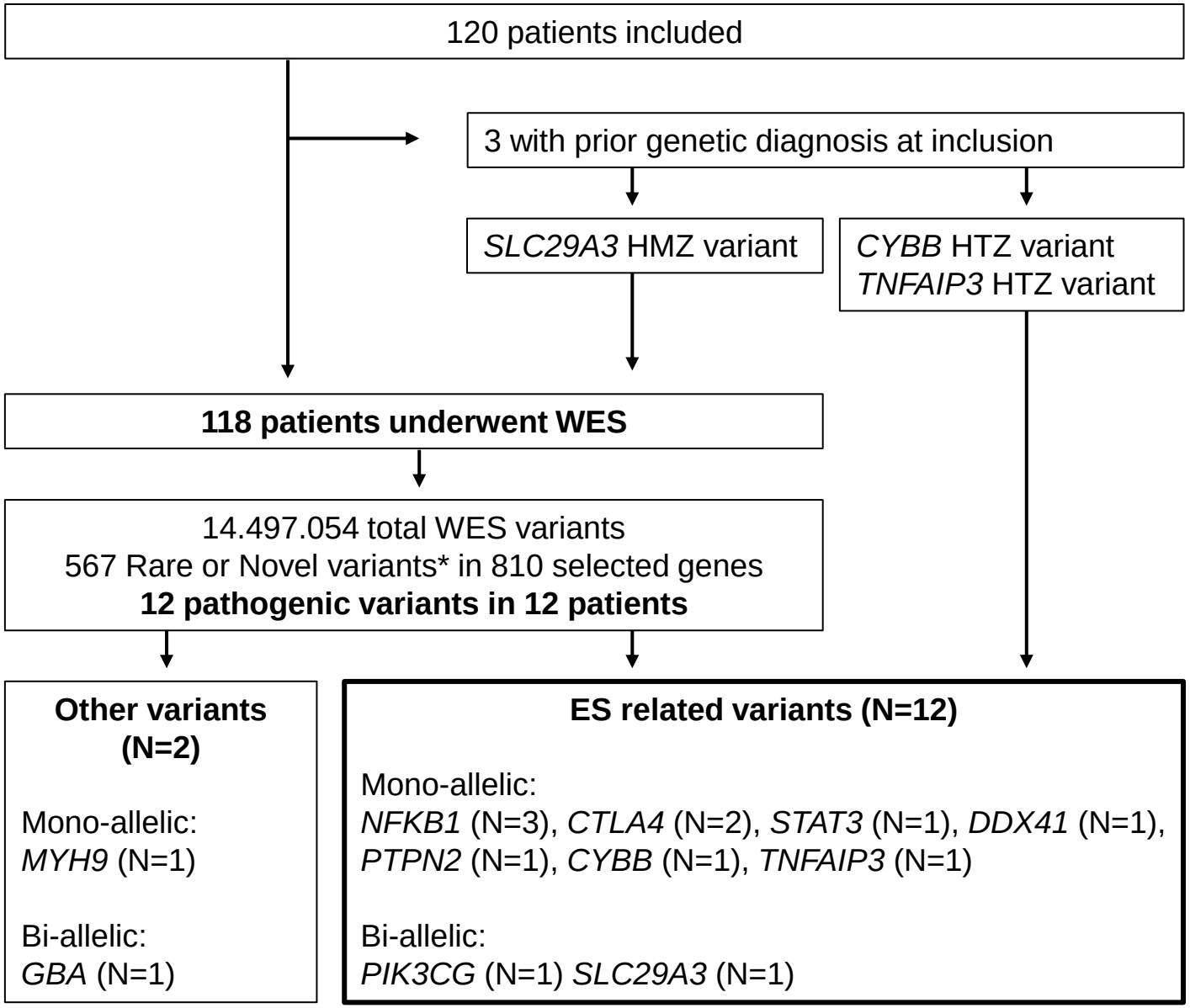
Genetic testing



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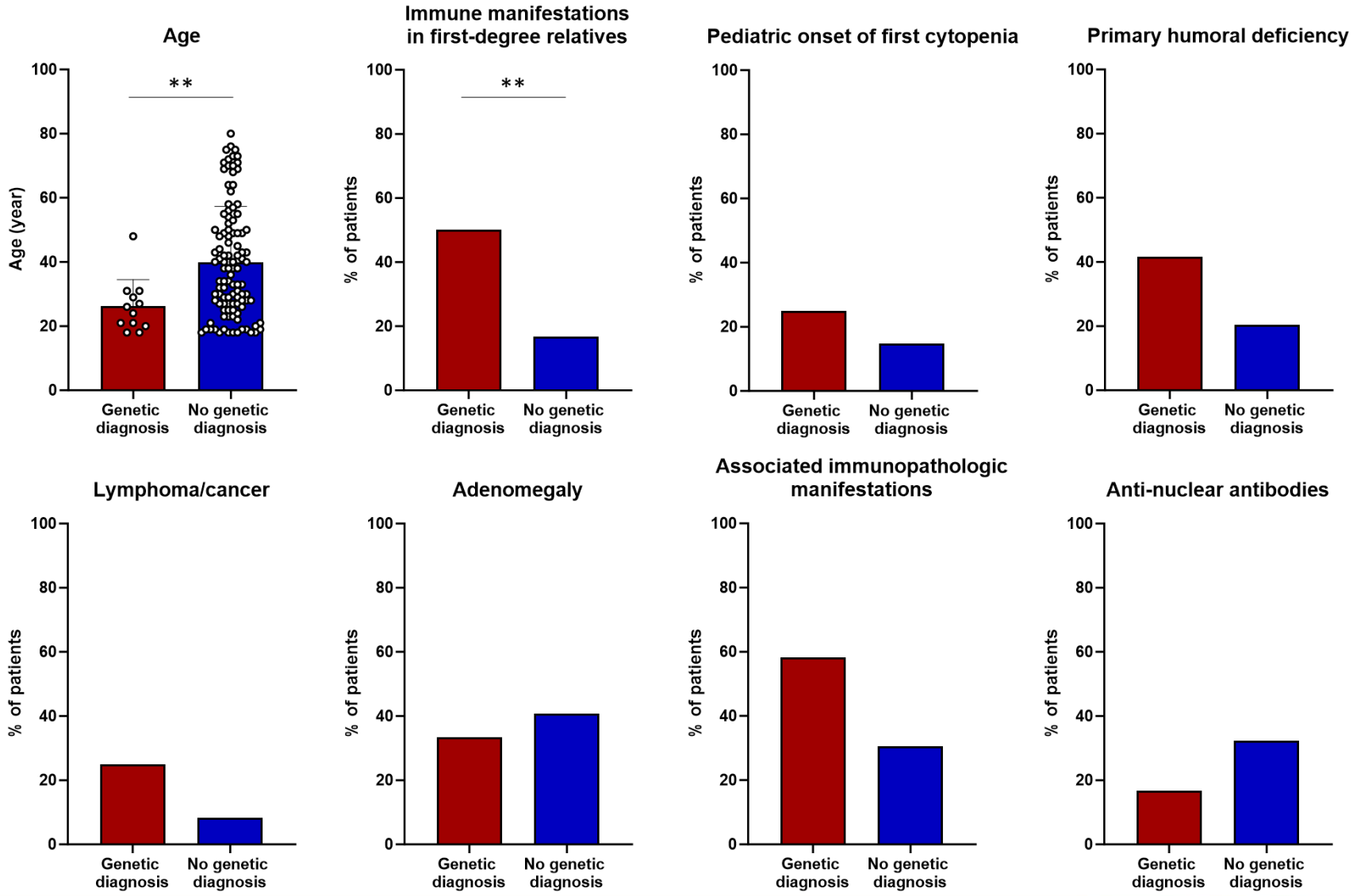
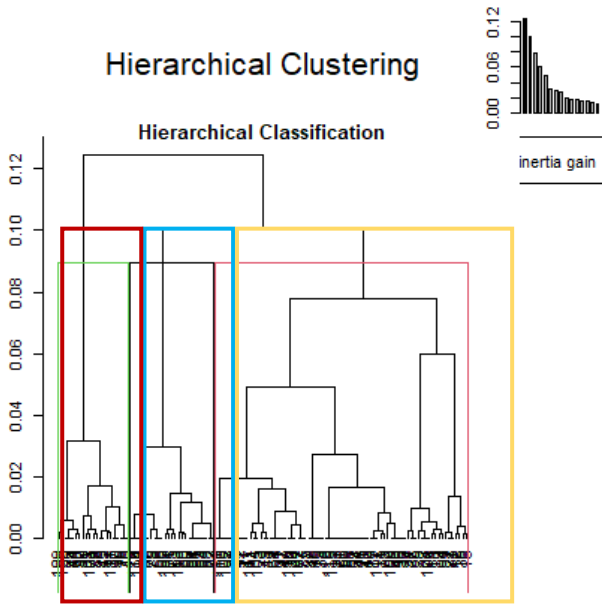
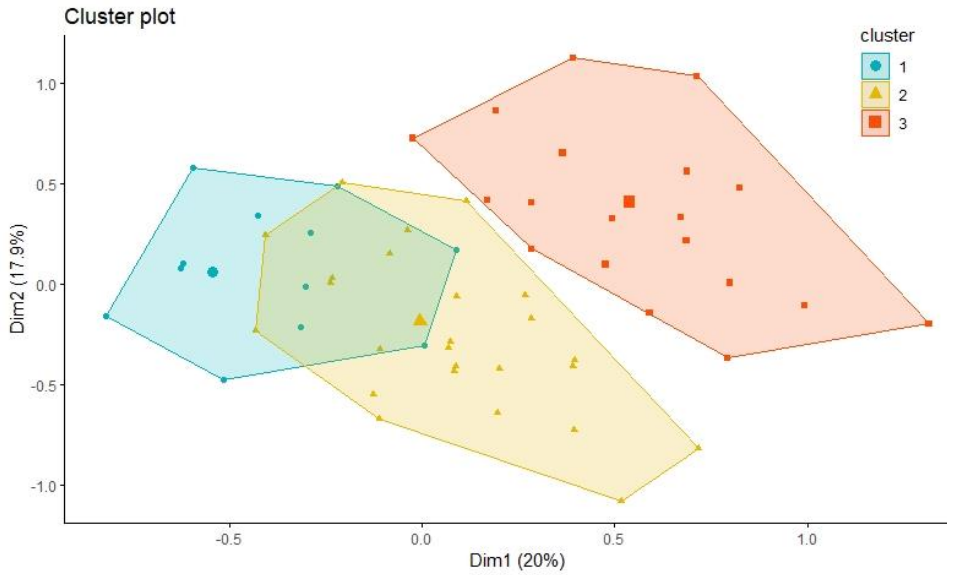
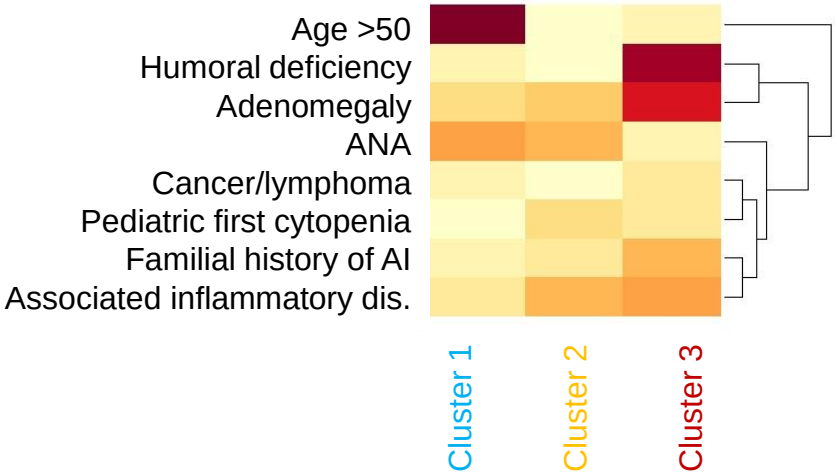
Clinical and biological features of patients with or without genetic diagnosis



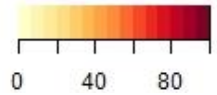
Hierarchical clustering of patients (multiple correspondence analysis)







Pathogenic variants:
Cluster 1: N=0
Cluster 2: N= 7
Cluster 3: N= 5



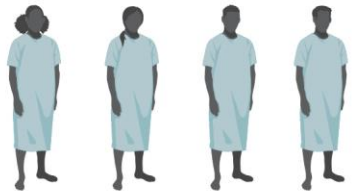
Frequence (%) in cluster

Discussion

- Large cohort of adult patients with ES and genetic testing, with **genetic diagnosis in 10%**
- Led to **targeted therapy** in some patients[1], **screening of relatives** (*NFKB1* families), and **new variants description** (e.g. *PTPN2*[2])
- Limitations include « solo » exome (can miss genetic diagnoses), and assessment of rare variants with high impact only
- Patients with genetic diagnosis were younger and had more immune manifestations in first-degree relatives
- Overall these results suggest that genetic testing should be considered in every adult patient diagnosed with ES

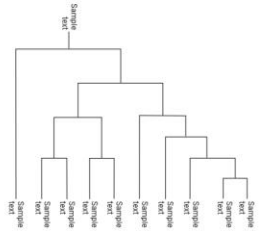
[1] *Fischer et al., JACI 2023*
[2] *Jeanpierre et al., JEM 2024*

120 patients with adult-onset Evans syndrome



Clinical and biological features

Whole exome sequencing



Hierarchical clustering analysis

14.10^6 variants

567 rare/novel variants in genes involved in primary immune deficiency/dysregulation or platelet/red blood cell/neutrophil biology

14 pathogenic variants in 14 patients

Evans syndrome related variants (N=12)

Other variants (N=2)
MYH9
GBA

Cluster 1	Cluster 2	Cluster 3
Aged patients *Antinuclear antibodies*	*Associated inflammatory diseases*	*Humoral deficiency* *Adenomegaly*
N = 0	N = 7 *CTLA4 (N=1)* *CYBB (N=1)* *DDX41 (N=1)* *PIK3CG (N=1)* *PTPN2 (N=1)* *SLC29A3 (N=1)* *TNFAIP3 (N=1)*	N = 5 *NFKB1 (N=3)* *CTLA4 (N=1)* *STAT3 (N=1)*

Created using biorender

FRL team « *Immunogenetics of Pediatric Autoimmune Diseases* » :

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- Aude Magerus
- Bénédicte Neven
- Marie-Claude Stolzenberg
- Victor Michel
- Malak Fakih
- Duong Ho-Nhat

CEDI Necker

- Capucine Picard
- Jérémie Rosain

Patients and their families

CERECAI network – French Evans study group

- Matthieu Mahévas, Bertrand Godeau, Marc Michel, Laetitia Languille (Créteil)
- Lionel Galicier, Claire Fieschi, Jehane Fadlallah, Marion Malphettes, David Boutboul, Laurence Gérard, Eric Oksenhendler (St Louis, Paris)
- Olivier Hermine, Morgane Cheminant, Felipe Suarez (Necker, Paris)
- Jérémie Dion, Thibault Comont, Guillaume Moulis (Toulouse)
- Clement Gourguechon (Amiens)
- Mikael Ebbo, Benoit Fauger (Marseille)
- Anne Marie Ronchetti (Corbeil)
- Thomas Moulinet (Nancy)
- Delphine Gobert, Jerome Hadjadj (Saint Antoine, Paris)
- Julie Graveleau (Nantes)
- Jean-Francois Viallard (Bordeaux)
- Sylvain Audia (Dijon)
- Marie Goussef (Vannes)
- Louis Terriou (Lille)
- Antoine Dossier (Bichat, Paris)
- Adrien Bigot (Tours)