# 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)

## INTRODUCTION

• A high prevalence of primary immunodeficiencies / dysregulations (PIDD) have been described in children with Evans syndrome (ES).

•In this study, we aimed to assess the prevalence of pathogenic, constitutional genetic variants in a cohort of patients with adult-onset ES, and their association to clinical and biological features.

## METHODS

• We consecutively included from November 2021 to December 2023 patients that had been diagnosed with adult onset (≥18 years) ES (defined as an association of two or more autoimmune cytopenia among immune thrombocytopenia (ITP), warm autoimmune hemolytic anemia (wAIHA) and/or autoimmune neutropenia (AIN)).

• We excluded patients with systemic lupus or indolent B cell malignancies at ES onset.

•Patients underwent whole-exome sequencing (WES) after written and informed consent was obtained.

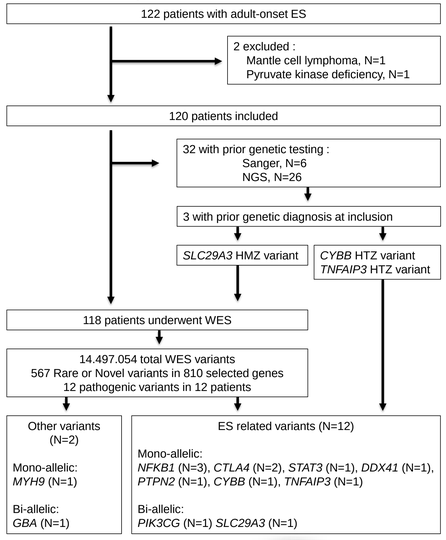
•A first analysis was performed using a panel of 810 genes involved in PIDD, constitutional thrombocytopenia, congenital hemolytic anemias and neutropenias.

•We identified rare/novel variants of interest as variants with frequency <1/10000 in gnomAD and with a CADD score ≥ 20. A second analysis was performed on all genes after filtering for variants with allele frequency >1/1000 in gnomAD and prioritized using CADD scores.

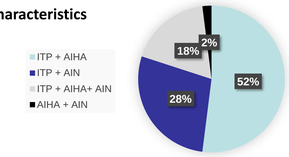
•We performed an unsupervised multiple correspondence analysis (MCA) to identify clusters of patients based on categorical variables of interest. Hierarchical clustering on principal component was conducted based on the Ward method.

## RESULTS

### A. Flow Chart of study

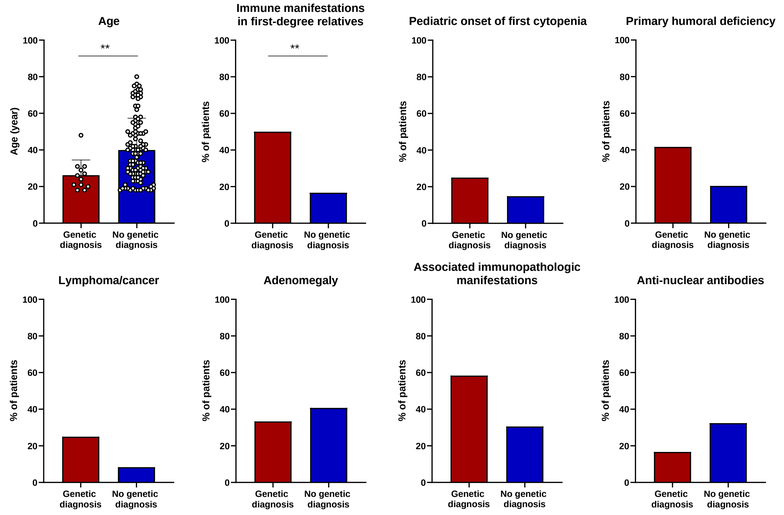



### B. Patients characteristics

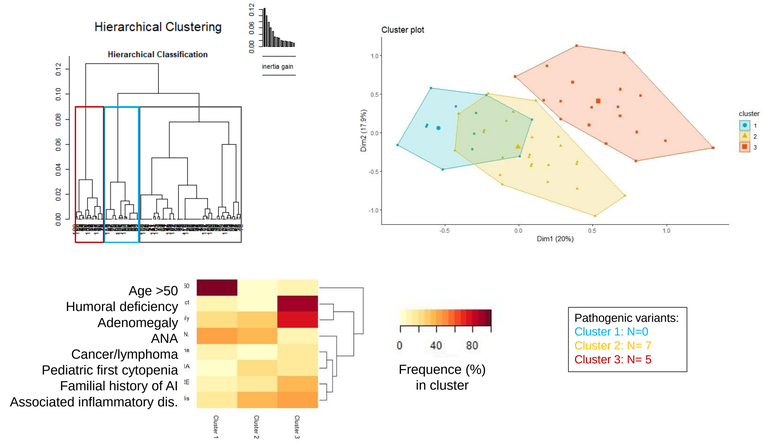



| | All patient (N=120) |
|---|---|
| Female | 44 (37) |
| Age at Evans syndrome diagnosis (median, range) | 33 [18 - 80] |
| Age at first cytopenia (median, range) | 30 (1 - 80) |
| Age < 18 at first cytopenia | 19 (16) |
| Immunopathologic manifestations in first-degree relatives | 24 (20) |
| Severe 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) |
| Lymphoproliferation | 64 (53) |
| Adenomegaly | 48 (40) |
| Splenomegaly | 37 (31) |
| Hepatomegaly | 12 (10) |
| Organ lymphoproliferation | 7 (6) |
| Malignancies | 13 (11) |
| Associated immunopathologic manifestations | 40 (33) |
| Antinuclear antibody (titer ≥ 1/160) | 37 (31) |

### C. Age and proportion of patients with or without genetic diagnosis according to the presence of various clinical features




### D. Hierarchical clustering of patients (multiple correspondence analysis, MCA)




### E. Variants characteristics

| Patient # | Gene | Gender | Evans syndrome onset (age) | cDNA mutation | Protein level mutation | CADD | Consequence | Zygosity | Allele frequency (GnomAD) | Other clinical features |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *CYBB* | M | 18 | c.925G>A | p.E309K | 29 | Loss of function | HTZ | 8.27e-7 | CGD |
| 2 | *TNFAIP3* | F | 27 | Exons 2-9 deletion | NA | . | Loss of function | HTZ | 0 | Rash, lupus like disease, granulomatosis |
| 3 | *SLC29A3* | M | 26 | c.1309G>A | G437R | 27 | Loss of function | HMZ | 3.47e-5 | H syndrome |
| 4 | *STAT3* | F | 21 | c.1082A>G | Q361R | 25 | Gain of function | HTZ | 0 | lymphoproliferation, infections, hypogammaglobulinemia |
| 5 | *CTLA4* | F | 21 | c.151 C>T | p.R51* | 34 | Stop gained | HTZ | 0 | psoriasis, cervical cancer, hypogammaglobulinemia |
| 6 | *CTLA4* | M | 31 | c.380A>G | Y127C | 26 | Loss of function | HTZ | 0 | |
| 7 | *NFKB1* | M | 48 | c.1550delT | p.L517fs | . | Stop gained | HTZ | 0 | hypogammaglobulinemia |
| 8 | *NFKB1* | F | 24 | c.928-2A>G | F310_Q333del | 34 | Loss of function | HTZ | 0 | hypogammaglobulinemia |
| 9 | *NFKB1* | F | 29 | c.899_900dupT | p.DF300fs | 1 | Stop gained | HTZ | 0 | hypogammaglobulinemia |
| 10 | *PTPN2* | F | 20 | c.376T>A | Y126N | 28 | Loss of function | HTZ | 0 | |
| 11 | *DDX41* | M | 31 | c.490C>T | R164W | 23 | Unknown | HTZ | 1.47e-4 | Poppema lymphoma Diffuse large B cell lymphoma |
| 12 | *PIK3CG* | M | 18 | c.2207T>C | V736A | 24 | Unknown | HMZ | 2.48e-6 | Crohn disease, alopecia areata |
| 13 | *MYH9* | M | 30 | c.4270G>A | D1424N | 29 | Missense | HTZ | 0 | Deafness |
| 14 | *GBA* | F | 19 | c.1226A>G c.[1448T>C;1483G>C;1497G>C] | p.N409S p.[L483P;A495P;V499=] | . | Loss of function | Compound HTZ | 8,05e-06 | splenomegaly |

## DISCUSSION

- **This large series of patients with ES and genetic workup revealed a high proportion (10%) of pathogenic variants for an adult-onset auto-immune disease.**
- **Patients with genetic diagnosis were younger and had more immune manifestations in first degree relatives.**
- **Clinical benefit of genetic diagnosis included use of JAK inhibitors in one patient with *STAT3 gain of function* and identification of hypogammaglobulinemia in relatives of families with *NFKB1* variants.**
- **Many variants of unknown significance warrant further *in vitro* studies.**
- **Overall these results suggest that genetic testing should be considered in every adult patient diagnosed with ES.**

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