doi: 10.4081/reumatismo.2026.2408

SUPPLEMENTARY MATERIAL

Atypical adult polyarteritis nodosa unmasking deficiency of adenosine deaminase 2: a case report about the importance of genetic testing in adults

Sofia Antolini,[1] Denise Cassandrini,[2] Sara Bindoli,[3,4] Vincenzo Bronte,[2] Angelo Fassio[1]

[1]Rheumatology Unit, Verona Integrated University Hospital, Verona; [2]Immunology Unit, University Hospital, Azienda Ospedaliera Universitaria Integrata, Verona; [3]Department of Medicine - DIMED, University of Padua; [4]Rheumatology Unit, Azienda Ospedale Università di Padua, Italy

Correspondence: Sara Bindoli, Rheumatology Unit, Department of Medicine - DIMED, University of Padua, Via Giustiniani, 2 35128 Padua, Italy.
Tel.: +390498212083. E-mail: sara.bindoli@unipd.it

Key words: ADA2 enzyme, adult-onset DADA2, PAN, medium- and small-sized vasculitis, TNF alfa-inhibitors, case report.

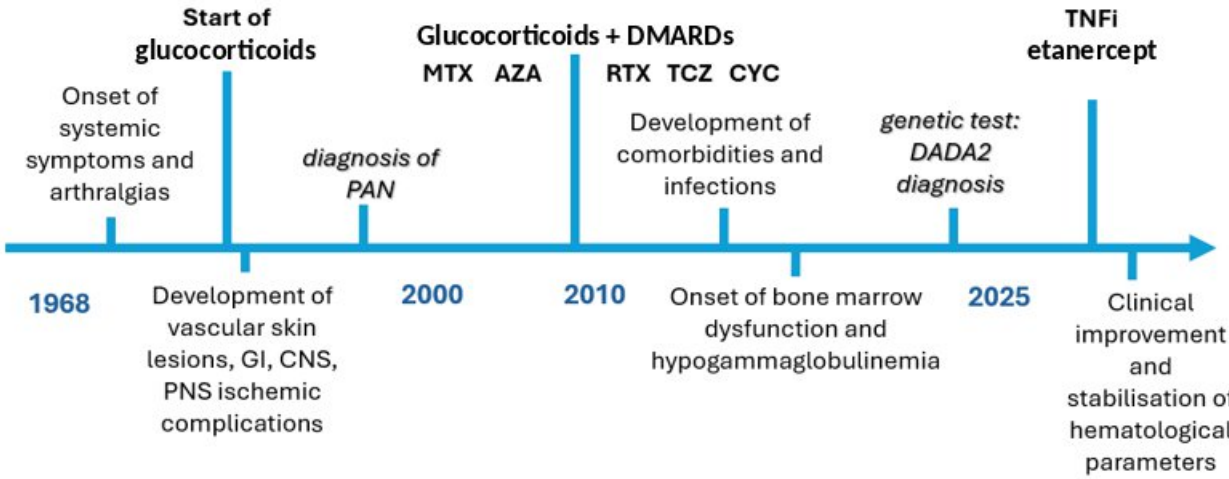
Note: The publisher is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries should be directed to the corresponding author for the article

Supplementary File 1. Detailed description of genetic analysis.

The missense variant c.1358A>G (p.Tyr453Cys) in the *ADA2* gene (reference transcript: NM_001282225.2) was identified by whole-exome sequencing. Raw sequencing data were converted to FASTQ format using bcl2fastq software (Illumina) and aligned to the human reference genome (GRCh37/hg19) using the BWA-MEM algorithm. Data processing followed standard guidelines: 1-Marking of duplicate reads. Local realignment around small insertions/deletions (indels). 2-Recalibration of base quality scores (BQSR). 3-Nuclear variant calling performed with GATK algorithms (Sentieon implementation). Average sequencing depth and coverage of target regions were calculated by excluding reads with zero mapping quality (MQ0). Rigorous quality control (QC) procedures ruled out contamination or sample mix-ups, validated by exceeding the sensitivity and specificity thresholds of internal reference samples. Copy number variations (CNVs), defined as single- and multiexonic deletions or duplications, were identified using a proprietary bioinformatics pipeline. The algorithm segmented the genome by calculating the deviation between the observed and expected read depth (normalized to the profile of other samples from the same run and corrected for GC content bias). The annotation and functional interpretation of genomic and mitochondrial (mtDNA) variants were performed using VcfAnno, VEP, and Alamut Visual software, querying multiple reference databases: Population frequencies and pathogenicity: gnomAD, ClinVar, HGMD Professional, 1000G. Mitochondrial databases: Mitomap, HmtVar. CNV databases: Database of Genomic Variants (DGV) and DECIPHER. The homozygous pathogenic variant c.1358A>G (p.Tyr453Cys) was successfully confirmed by Sanger sequencing. Familial segregation analysis could not be performed due to the unavailability of blood relative samples.

The ACMG criteria applied are as follows: PS1, PM3, PM2, PM1, PP3, PP2, PP1, and PP5, which classify the variant as pathogenic. The combination of these criteria (1 Strong, 3 Moderate, 4 Supporting) creates an extremely robust pathogenicity profile, which far exceeds the formal threshold for Pathogenic classification. In the ACMG algorithmic system, even the combination of two Strong criteria, or one Strong criterion combined with three Moderate criteria, is sufficient to determine pathogenicity. In our specific case, the variant accumulates an extremely robust score distributed across different levels of evidentiary strength. The criteria applied can be described in detail:

- Strong Evidence PS1: Same amino acid change as a variant already known and previously established as pathogenic, regardless of the nucleotide change.
- Moderate Evidence (Moderate) PM1: The variant is located within a mutational hotspot or a critical and well-documented functional domain of the protein, where a benign variation is not observed. PM2: The variant is absent (or extremely rare) in healthy general population databases, such as gnomAD. PM3: For autosomal recessive diseases, the variant has been identified in trans (on the other chromosome) to an established pathogenic variant.
- Supporting Evidence (PP1): The variant co-segregates with the disease within the family (affected family members analyzed have the same mutation).PP2: This is a missense variant in a gene that has a low frequency of benign missense variations and where such mutations are a known disease mechanism.PP3: Multiple predictive bioinformatics tools (in silico) agree that the variant causes structural or functional damage to the protein.PP5: A reliable source or another laboratory has previously reported this variant as pathogenic, although the original evidence was not directly accessible in detail.



Supplementary Figure 1. Brief timeline summarizing the main clinical events of our patient. GI, gastrointestinal; CNS, central nervous system; PNS, peripheral nervous system; PAN, polyarteritis nodosa; DMARDs, disease-modifying antirheumatic drugs; MTX, methotrexate; AZA, azathioprine; RTX, rituximab; TCZ, tocilizumab; CYC, cyclophosphamide; DADA2, deficiency of adenosine deaminase 2; TNFi, tumor necrosis factor-α inhibitor.

Supplementary Table 1. Summary of treatment, prednisone dosage, and key laboratory parameters at baseline and during follow-up.

	25 mg PDNeq + other immunosuppressive agents	5 mg PDNeq + etanercept (9 months of therapy)
CRP (mg/L)	27	3
Hb (g/dL)	8.5	9.4
Lymphocytes ($\times 10^9$/L)	0.43	0.61
IgG (g/L)	4.11	4.46
IgA (g/L)	0.25	0.15
IgM (g/L)	0.12	0.08

PDNeq, dose of prednisone equivalent; CRP, C-reactive protein; Hb, hemoglobin; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M.