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RESEARCH LETTER

A *WAS* promoter variant underlying Wiskott-Aldrich syndrome in two kindreds

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The global expansion of genomic medicine has improved access to genetic testing for patients with suspected inborn errors of immunity (IEI). Despite these advances, diagnostic rates remain relatively low, with current estimates suggesting that <50% of patients with suspected IEI receive a confirmed genetic diagnosis. This limitation is partly due to the focus of most clinical laboratories on variants within protein-coding regions or those impairing splicing (1). Expanding variant analysis beyond these protein-coding regions and variants leading to aberrant splicing is promising to improve the diagnosis of IEI. Such variants can be pathogenic through different mechanisms, such as disruption of the 5′ untranslated region (UTR) and 3′ UTR, or by disrupting cis-regulatory elements (CREs), including promoters or enhancers (2, 3). While the availability of tools to screen for variants disrupting 5′ UTR and 3′ UTR remains limited, many scores or strategies were developed recently for variants located in CREs, including the use of tailored cutoffs for combined annotation dependent depletion (CADD) and regulatory Mendelian mutation (REMM) scores (2). A specific tool to screen for such variants, promoterAI, was also recently published.

In this context, we investigated four male patients from two unrelated kindreds with features of IEI (Fig. 1 A). P1 and P2, from kindred A, are of French descent and were born to non-consanguineous healthy parents. P1 is 4 years old and has a history of eczema, celiac disease, and recurrent hemorrhagic immune thrombocytopenic purpura (ITP) (Fig. 1, B and C). P2 is 7 years old and suffered at the age of 2 years from an acute episode of ITP (Fig. 1, B and C). He also has a history of atopic dermatitis. P3 and P4 from kindred B were born to non-consanguineous Eritrean parents and currently reside in Switzerland. Both patients presented with eczema. P3, now 24 years old, was referred in early childhood for lymphadenopathy. At the age of five, he was diagnosed with EBV-driven Hodgkin lymphoma (mixed-cellularity subtype, EBV positive, stage IIAE) involving the cervical, supraclavicular, and mediastinal regions. Almost 2 years after completing chemotherapy, he experienced a relapse of Hodgkin's disease with axillary, mediastinal, intra-abdominal, and pulmonary involvement (stage IVa). At 9 years of age, he developed autoimmune hemolytic anemia and ITP, initially treated with steroids. P3 has since experienced multiple ITP relapses and received treatment with steroids, intravenous immunoglobulins, and rituximab. He is currently stable under immunosuppressive therapy with mycophenolate-mofetil, prednisone, and sirolimus. The brother of P3, P4, is 32 years old and suffered from diffuse large cell lymphoma, classified as Murphy stage III (equivalent to Ann Arbor stage III), and was treated with chemotherapy. Until now, he has been in remission but has persistent inguinal lymphoproliferation, where biopsies were taken without any signs of malignancy. The sister and parents of P3 and P4 are healthy.

Given the suspicion of IEI, both kindreds were explored through high-throughput sequencing (HTS) approaches capturing exons, e.g., a panel for P1, and whole-exome sequencing in both P3 and P4. Screening of small nucleotide variants or copy

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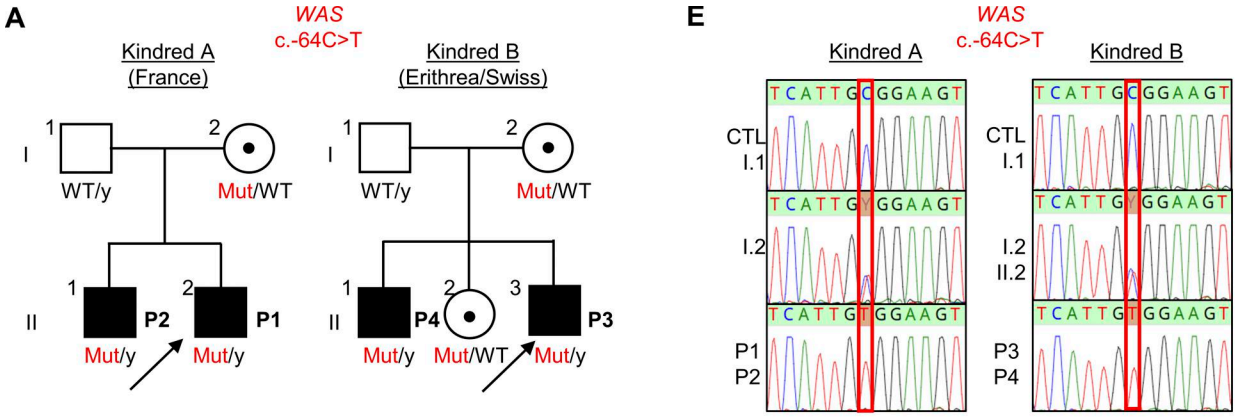
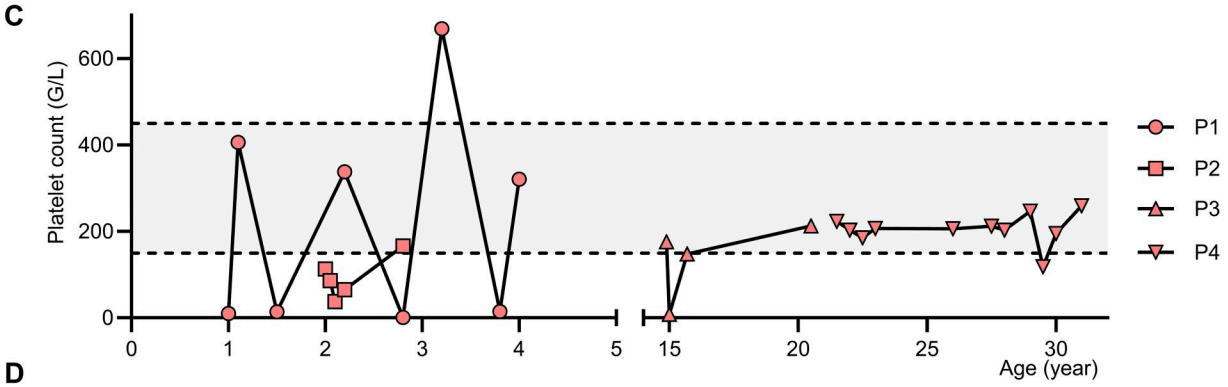


B

	Age of disease onset	Clinical phenotype	Nadir platelet counts
P1	4 months old	Eczema Celiac disease Immune thrombocytopenic purpura	1 G/L
P2	2 years old	Immune thrombocytopenic purpura Atopic dermatitis Cardiopathy	38 G/L
P3	1 year old	Lymphadenopathy EBV-driven Hodgkin lymphoma Recurrent hemolytic anemia and thrombocytopenia	8 G/L
P4	32 years old	Diffuse large cell lymphoma	118 G/L



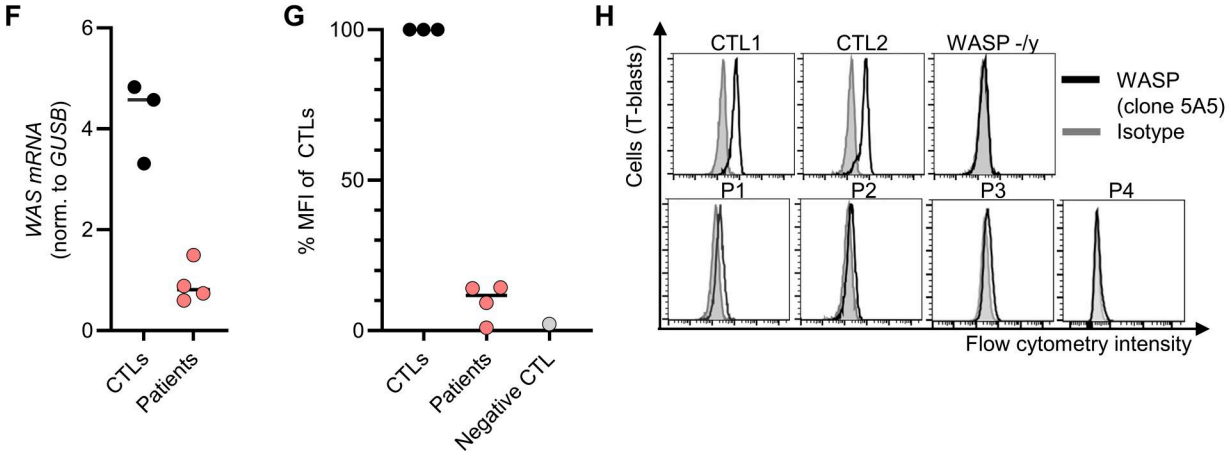


Figure 1. **A single nucleotide promoter variant underlying Wiskott-Aldrich disease. (A)** Pedigree tree. Male and female individuals are represented by squares and circles, respectively. Each generation is designated by a Roman numeral, and each individual by an Arabic numeral. Individuals with immune

dysregulation are shown as closed black symbols, and the index case is indicated by an arrow. Black dots represent heterozygosity. Mut, mutated; WT, wild-type; y, Y chromosome. **(B)** Summary of the phenotype of the four patients. **(C)** Counts of platelets in whole blood of the patients. Normal range is displayed in grey. **(D)** Scheme of *WAS* promoter adapted from Petrella et al. (4). Arrows indicate transcription factor–binding sites identified by a combination of computational predictions and experimental validation. The c.-64C>T variant is displayed in red, and the 5′UTR and transcription start site of *WAS* in green and blue, respectively. **(E)** Electropherograms for the sequencing of representative *WAS* nucleotide sequences present in the hemizygous state in the four patients, in the heterozygous state in their mothers and sister, and in the homozygous wild-type state in control (CTL) and in their fathers. **(F)** *WAS* quantitative PCR (normalized to *GUSB*) in T-activated cells from three controls and in the four patients. **(G)** Quantification of WASP protein expression derived from panel D. **(H)** Intracellular protein expression of WASp (clone 5A5) or isotype, in T-activated cells derived from two controls (CTL1 and CTL2), P1, P2, P3, and P4, and from a patient previously reported as deficient in WASP (1).

number variants within the coding sequences and essential splice site variants of captured genes failed to identify any pathogenic variants. We thus reanalyzed for variants covered in noncoding regions that are partially covered even by exon-captured based HTS (1). We did not detect any noncoding variants predicted to impair splicing by tools such as spliceAI. We further screened on noncoding variants with high CADD score, adapting the algorithm by Villani et al. (2). We ended up identifying in both kindreds a single nucleotide variant located upstream of the *WAS* gene (hg38 X-48683790-C-T; NM_0000377.3; c.-64C>T). This variant is very rare because it is absent from gnomAD v4.1. The CADD v1.7 score was high (=20.5). In addition, this variant was predicted as deleterious by REMM v0.4 (=0.999) and promoterAI v1.0 (=−0.746). The variant was indeed located on a conserved nucleotide as highlighted by elevated phastCons (=1) and phyloP (=3.90) scores. This variant is predicted to be located in a CRE by ENCODE, and the c.-64C position lies within the *WAS* promoter region shown to be essential for hematopoietic-specific expression, as it contains critical transcription factor–binding sites (e.g., FLI1, GATA family motifs, and GC boxes that bind Sp1/Sp3) (Fig. 1 D) (4). By Sanger sequencing, this variant was confirmed to be present at a hemizygous state in P1, P2, P3, and P4, at a heterozygous state in the mothers of both kindreds and in the sister of P3 and P4, and was absent in the fathers of both kindreds (Fig. 1 E). At immunological level, P1, P2, P3, and P4 displayed T CD8 lymphopenia, with increased CD4/CD8 ratio (data not shown). In T-activated blasts of the patients, WASP expression was 22% and 10% of normal at the mRNA and protein levels, respectively (Fig. 1, G and H). Overall, according to criteria from the American College of Medical Genetics, these data are further supportive of categorizing the c.-64C>T variant as pathogenic and causal for the clinical phenotype of the four patients.

We thus report a pathogenic variant located within the promoter of *WAS*. We found this rare variant in two unrelated kindreds and evidenced decreased but not absent protein level amongst the T cells of the patients. This likely explained the clinical phenotype, characterized by intermittent periods of normal platelet counts and relatively mild but fluctuating disease manifestations such as eczema, autoimmunity, and lymphoproliferation. Although this presentation may appear mild and would historically have been considered "intermittent X-linked thrombocytopenia," it would now be better classified as a class I WAS variant (5), and the occurrence of malignancies in two patients highlights the potential severity of such phenotypes. Clinically, this emphasizes that WASP deficiency should also be considered in patients who do not present with the classic triad (eczema, thrombopenia, and immunodeficiency) and who may even have episodes of normal thrombocyte counts. Recognition of such atypical presentations is important for timely genetic counseling and screening of female carriers, as well as for the treatment of the patients, who may benefit from hematopoietic stem cell transplantation or gene therapy. Regarding *WAS* locus, variants located within the core promoter element c.-28 could also be candidate pathogenic variants. The mechanism of pathogenicity for variants in CRE is usually decreased transcription of otherwise normal gene products, resulting in a quantitative rather than qualitative defect (3). Furthermore, some variants in CRE may also lead to additional alternative splicing. Interestingly, variants in CRE that can also result in increased transcript products have been reported in other genetic diseases such as developmental disorders (3), but not, to the best of our knowledge, for IEI. A limitation of the study is that we did not address the possible cell-specific impact of the variant (3). Overall, the variant we report here further exemplifies that the implementation of bioinformatics tools for noncoding regions is warranted to systematically screen genetic data for variants in such regions in genes for IEI.

Data availability

All data are either included in the manuscript or available upon request.

Ethics statement

Ethics approval

Informed consent for participation in this study was obtained in accordance with local regulations, with approval from the institutional review board in France and in Switzerland.

Consent to participate

Written informed consent to participate was obtained from the patients or their parents.

Consent for publication

Consent to publish this report was obtained from the patients or their parents. All the authors approved the final version of the manuscript.

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References

1. Alioua, N., N. Lambert, M. Puel, S. Hanein, P. Bastard, M. Fusaro, M. Jaffray, B. Medel, L. Khellaf, Y. Seeleuthner, et al. 2025. Intronic branchpoint-to-acceptor variants underlying inborn errors of immunity. *J. Hum. Immun.* 1:e20250041. https://doi.org/10.70962/jhi.20250041
2. Villani, R.M., M.E. McKenzie, A.L. Davidson, and A.B. Spurdle. 2024. Regional-specific calibration enables application of computational evidence for clinical classification of 5' cis-regulatory variants in Mendelian disease. *Am. J. Hum. Genet.* 111:1301–1315. https://doi.org/10.1016/j.ajhg.2024.05.002
3. Cheng, Y.H.H., S.C. Bohaczuk, and A.B. Stergachis. 2024. Functional categorization of gene regulatory variants that cause Mendelian conditions. *Hum. Genet.* 143:559–605. https://doi.org/10.1007/s00439-023-02639-w
4. Petrella, A., I. Doti, V. Agosti, P.C. Giarrusso, D. Vitale, H.M. Bond, C. Cuomo, P. Tassone, B. Franco, A. Ballabio, et al. 1998. A 5′ regulatory sequence containing two ets motifs controls the expression of the Wiskott-Aldrich syndrome protein (WASP) gene in human hematopoietic cells. *Blood*. 91:4554–4560. https://doi.org/10.1182/blood.V91.12.4554
5. Vallée, T.C., J.S. Glasmacher, H. Buchner, P.D. Arkwright, U. Behrends, A. Bondarenko, M.J. Browning, D. Buchbinder, A. Cattoni, L. Chernyshova, et al. 2024. Wiskott-aldrich syndrome: A study of 577 patients defines the genotype as a biomarker for disease severity and survival. *Blood*. 143: 2504–2516. https://doi.org/10.1182/blood.2023021411