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FANCD2 Pseudogene Interference in Fanconi Anemia: Lessons from a Seven-Patient Turkish Cohort

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ABSTRACT

Purpose: To investigate the molecular basis of FA in patients with unresolved genetic diagnoses and to highlight diagnostic pitfalls related to pseudogene interference and autozygosity.

Methods: Seven patients presenting with bone marrow failure and clinical features compatible with FA were evaluated. All underwent whole-exome sequencing (WES), FA-targeted gene panel analysis, copy-number variation (CNV) assessment, and runs of homozygosity (ROH) mapping. In cases with inconclusive NGS results, targeted Sanger sequencing was performed for candidate regions.

Results: Four patients harbored clearly pathogenic or likely pathogenic biallelic variants in *FANCA*, including truncating, splice-site, and exon-deletion variants, establishing a definitive molecular diagnosis. In contrast, three unrelated patients (P5–P7) from consanguineous families remained without a confirmed genetic diagnosis for several years despite positive chromosomal breakage tests. All three shared the same intronic *FANCD2* splice-site variant, c.1948-16T>G, located within a genomic region of high homology to flanking *FANCD2* pseudogenes. Initial external WES analyses either failed to detect this variant or reported it with low allelic balance, precluding confident interpretation. Re-analysis incorporating ROH mapping identified a shared autozygous region on chromosome 3 encompassing *FANCD2*. Subsequent gene-specific Sanger sequencing confirmed homozygosity for the c.1948-16T>G variant in all three patients, establishing *FANCD2* as the causative gene.

Conclusion: This study demonstrates that pseudogene interference can obscure pathogenic variants in standard exome sequencing pipelines and lead to prolonged diagnostic odysseys in FA. Integrating chromosomal breakage testing with pseudogene-aware NGS analysis, ROH and structural-variant assessment, and targeted Sanger confirmation is essential for accurate FA diagnosis, particularly in consanguineous populations and for genes embedded in complex genomic regions.

Keywords: Fanconi anemia, FANCD2, pseudogenes, High-Throughput Nucleotide Sequencing, runs of homozygosity, Copy Number Variation.

ÖZET

Amaç: Genetik tanısı aydınlatılmamış Fanconi anemisi (FA) olgularında hastalığın moleküler temelini araştırmak ve psödogen kaynaklı tanısal zorluklara dikkat çekmek.

Yöntemler: Kemik iliği yetmezliği ve FA ile uyumlu klinik bulgularla başvuran yedi hasta değerlendirildi. Tüm hastalara tüm ekzom dizileme (WES), FA-hedefli gen paneli analizi, kopya sayısı varyasyonu (CNV) analizi ve homozigozite bölgelerinin (ROH) haritalanması uygulandı. NGS sonuçlarının yetersiz veya belirsiz olduğu olgularda, aday bölgeler için hedefe yönelik Sanger dizileme yapıldı.

Bulgular: Dört hastada FANCA geninde bialelik patojenik veya olası patojenik varyantlar saptanarak kesin moleküler tanı konuldu. Buna karşılık, akraba evliliği öyküsü bulunan ve kromozomal kırılganlık testleri pozitif olan üç bağımsız hasta (P5–P7), yıllar boyunca genetik tanı almadı. Bu üç hastanın her birinde, FANCD2 psödogenleriyle yüksek homoloji gösteren bir genomik bölgede yer alan intronik FANCD2 varyantı c.1948-16T>G ortak olarak mevcuttu. Dış merkezlerde yapılan WES analizlerinde bu varyant ya saptanamadı ya da düşük allelik denge ile raporlandı ve güvenilir yorum yapılamadı. ROH haritalamasını içeren yeniden analiz, kromozom 3 üzerinde FANCD2 genini kapsayan ortak bir otozigotik bölgeyi ortaya koydu. Ardından yapılan gen-spesifik Sanger dizileme ile üç hastanın tamamında c.1948-16T>G varyantının homozigot olduğu doğrulandı ve FANCD2 geninin bu hastalardaki sorumlu gen olduğu belirlendi.

Sonuç: Bu çalışma, psödogen etkileşiminin standart ekzom dizileme yaklaşımlarında patojenik varyantları maskeleyebileceğini ve FA'da uzun süren tanısal gecikmelere yol açabileceğini göstermektedir. Özellikle akraba evliliğinin sık olduğu popülasyonlarda ve genomik olarak kompleks bölgelerde yer alan genler için, kromozomal kırık testlerinin psödogen farkındalığı olan NGS analizleri, ROH ve yapısal varyant değerlendirmesi ile birlikte kullanılması ve hedeflenmiş Sanger doğrulaması yapılması, doğru FA tanısı için kritik öneme sahiptir.

Anahtar Kelimeler: Fanconi anemisi, FANCD2, psödogenler, yüksek verimli (yüksek kapasiteli) nükleotid dizileme, homozigotluk bölgeleri, kopya sayısı varyasyonu

Fanconi anemia (FA) is a rare inherited bone-marrow failure syndrome characterized by congenital anomalies, progressive cytopenias, and a markedly increased risk of myelodysplastic syndrome, acute myeloid leukemia, and specific solid tumors (1, 2). FA results from pathogenic variants in a group of FA/BRCA pathway genes that cooperate in the repair of DNA interstrand cross-links and maintenance of genome stability (3, 4). Early and accurate diagnosis is essential because patients require tailored hematologic management and timely hematopoietic cell transplantation (HCT), and because molecular confirmation enables cascade testing and reproductive counseling (2).

For decades, the diagnostic gold standard has been chromosomal breakage analysis of peripheral lymphocytes after exposure to diepoxybutane or mitomycin C (5, 6). While highly informative, this assay can be inconclusive in patients with hematopoietic somatic mosaicism or in those tested after transfusions, and it does not identify the underlying gene defect (5-7). The increasing availability of next-generation sequencing (NGS) has therefore shifted FA diagnostics toward multigene panels and whole-exome sequencing (WES), which allow simultaneous interrogation of all known FA genes and can uncover unexpected genotypes (8).

However, the FA locus architecture is complex: several FA genes have closely related paralogs or pseudogenes, segmental duplications, or GC-rich regions that complicate read mapping and variant calling. As a result, NGS data may yield false-negative or ambiguous results, especially for intronic splice variants or alleles residing in regions with high sequence homology. *FANCD2* exemplifies these challenges. Pseudogene sequences and highly homologous regions within and around *FANCD2* can cause misalignment of short reads, artificially low allelic balances, or erroneous attribution of variants to nonfunctional loci (5, 8, 9).

In this study, we describe seven patients who presented with bone-marrow failure and clinical features compatible with FA and were ultimately shown to harbor pathogenic variants in FANCA or FANCD2 by comprehensive genomic evaluation. Particular attention is given to three unrelated patients sharing the same intronic FANCD2 splice-site variant, c.1948-16T>G, in whom initial external WES failed to detect the variant precluding a definitive molecular diagnosis for several years. By outlining the clinical and molecular features of this cohort and dissecting the diagnostic pitfalls introduced by FANCD2 pseudogene interference, our work underscores the importance of integrated functional testing and refined NGS pipelines for accurate FA diagnosis, particularly in consanguineous populations and in settings where standard WES pipelines may underestimate critical variants.

Methods

Patients and Ethics Statement

We evaluated seven patients who presented with pancytopenia and clinical features suggestive of Fanconi anemia. This study did not require formal ethics committee approval according to national and institutional regulations, as it involved only the retrospective analysis of routine clinical genetic testing results and anonymized clinical data. No additional procedures, interventions, or sample collection were performed for research purposes, and no identifiable personal information was used.

All patients (or their legal guardians) had previously provided written informed consent at the time of their clinical evaluation, permitting the use of their anonymized clinical and genetic data for scientific and educational purposes in accordance with institutional policy. All procedures were conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

No statistical analysis was performed, as the study is based on descriptive genetic findings without comparative or inferential statistical evaluations.

Whole Exome Sequencing

Genomic DNA was extracted from proband blood samples using the QiaAmp system (Qiagen, Hilden, Germany). Exome sequencing was performed with the Twist Comprehensive Exome kit (Twist Bioscience, San Francisco, CA, USA). Bioinformatic analysis of raw data was performed with Varsome Clinical Software (Saphetor SA, Lausanne, Switzerland) as described for family 1 in our previous study (10). Variants were filtered according to ClinVar (pathogenic/likely pathogenic/conflicting interpretations of pathogenicity) (11), pathogenic/likely

pathogenic variant classification by Varsome Clinical Software (12) and Human Phenotype Ontology terms (13) associated with the clinical findings of the patient. Also, an internal Fanconi anemia gene panel comprising 23 genes was applied, including FAAP100, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG (XRCC9), FANCI, FANCL, FANCM, PALB2, RAD51, RAD51C, REV7 (MAD2L2), RFWD3, SLX4, UBE2T, XRCC2, BRCA1, BRCA2, BRIP1, and ERCC4. Computational splice prediction for all intronic and exonic boundary variants was performed with SpliceAI Visual (14).

In families with consanguinity, analysis of homozygous and compound heterozygous variants supporting a presumed autosomal recessive inheritance pattern was prioritized. Variants were classified according to the Association for Clinical Genomic Science (ACGS) (15) and Clinical Genome Resource (ClinGen) Guidelines (16).

Confirmation of Variants with Low Allelic Balance

Given the known challenges in FA genes—particularly FANCD2—due to pseudogene interference and mosaic allelic imbalance, variants with low sequencing allele fractions were confirmed through Sanger sequencing.

Sanger validation was performed for the FANCD2 c.1948-16T>G variant detected in Patients 5–7. PCR amplification and sequencing were carried out using standard clinical laboratory protocols to verify variant presence and zygosity. This approach was essential due to the variant's location in a region prone to pseudogene-related misalignment and its initial detection as a mosaic-level finding in WES data.

Run of Homozygosity and Copy Number Variant Analysis

Run of homozygosity (ROH) analysis was performed in patients from consanguineous families to identify extended homozygous regions suggestive of autosomal recessive inheritance and potential founder variants. ROH intervals were assessed based on contiguous stretches of homozygous markers across the genome, with particular attention to regions encompassing known Fanconi anemia genes.

Copy number variant (CNV) analysis was conducted using the ExomeDepth CNV Caller, which evaluates exon-level deletions and duplications by comparing observed read depth against an expected reference generated from other samples included in the same sequencing run. At least five samples were used as internal references for CNV calling whenever possible. Differences between expected and observed sequencing depth across targeted genomic regions were calculated to identify potential copy number changes.

Results

Clinical characteristics

Seven patients with bone marrow failure and clinical suspicion of Fanconi anemia (FA) were identified in our center. Age at presentation ranged from early infancy to adulthood, and all patients eventually developed pancytopenia except patient 5 (Table 1). Most had additional congenital or developmental anomalies, including growth restriction, microcephaly, radial ray or skeletal abnormalities, skin pigmentation changes, and endocrinologic or gynecologic features. Consanguinity was documented in five of the seven families, and three patients had a positive family history for FA-related phenotypes or myelodysplastic syndrome.

Chromosomal breakage testing with DEB/MMC was available for five patients. Three (P4, P6, and P7) showed the typical increased chromosomal breakage consistent with FA, whereas one infant (P5) had a normal result despite a classic FA phenotype.

FANCA-associated patients (P1–P4)

Four patients carried biallelic pathogenic or likely pathogenic variants in *FANCA*(Table 2). In P1, a combination of nonsense and frameshift variants was detected, both absent from population databases and classified as pathogenic. P2 harbored a homozygous canonical splice-site variant that has been previously reported in FA. P3 carried a homozygous exon 3 deletion detected by CNV analysis of WES data. P4 had a homozygous missense variant with very low population frequency and supportive in silico predictions; this change was therefore classified as likely pathogenic despite only borderline functional scores. Together, these findings mirror the known predominance of *FANCA* in FA cohorts and illustrate the spectrum of truncating, splice-disrupting, and structural variants in this gene.

Table 1. Clinical and Genetic Features of the Seven Patients with Fanconi Anemia

Patient	Age/Sex	Consanguinity	Onset	Clinical Findings	FA Gene	DEB/ MMC Test	Additional Findings
P1	21 y / M	No	19 y	Pancytopenia	FANCA	NA	Sibling with FA
P2	8 y / F	Yes	8 y	Pancytopenia; short stature; unilateral radius shortening; hypopigmented macules	FANCA	NA	–
P3	7 y / F	Yes	7 y	Pancytopenia; dysmorphic face; microcephaly; nasal bridge flattening; café-au-lait macules	FANCA	Positive	–
P4	29 y / F	Yes	29 y	Macrocytic anemia; primary ovarian insufficiency with low AMH, embryo arrest and oocyte maturation defect leading to infertility; hypothyroidism; periocular brown hyperpigmentation.	FANCA	Positive	Sister diagnosed with MDS at age 25
P5	3 mo / M	No	—	Prematurity; microcephaly; respiratory distress; IUGR; café-au-lait; VSD; PS; PFO	FANCD2	Normal	–
P6	5 y / M	Yes	5 y	Pancytopenia; dysmorphic face; microcephaly; café-au-lait macules	FANCD2	Positive	–
P7	7 y / F	Yes	2 y	Pancytopenia; short stature; microcephaly; pigmentary lesions; bilateral clinodactyly; hearing loss	FANCD2	Positive	Sibling with FA, Prior negative WES

Table 2. Genomic Variants Identified in the Fanconi Anemia Cohort

Patient	Gene	Variant	Zygosity	Variant Type	Population Frequency (gnomADv4.1.0)	ClinVar ID	Classification	In silico Tools	Allelic Balance	Reference
P1	FANCA	c.3586G>T (p.Glu1196*)	Heterozygous	Nonsense	Absent	592052	Pathogenic	–	–	Cagnan et al., 2018 (27)
P1	FANCA	c.1812dup p.(Glu605Argfs*8)	Heterozygous	Frameshift	Absent	–	Pathogenic	–	–	Not reported
P2	FANCA	c. [2778+1G>A]; [2778+1G>A];	Homozygous	Splice-site	0.00001	635518	Pathogenic	SpliceAI: 0.77	–	Park et al.,2013; De Rocco et al., 2014; Kimble et al., 2018 (5, 28, 29)
P3	FANCA	Exon 3 deletion (del16q24.3)	Homozygous	Exon deletion	Absent	–	Pathogenic	–	–	Madjunkova et al., 2014 (30)
P4	FANCA	c.1015G>A (p.Ala339Thr)	Homozygous	Missense	0.00000248	–	Likely pathogenic	REVEL: 0.262; BayesDel: 0.117	–	Not reported
P5	FANCD2	c.[1948-16T>G]; [1948-16T>G]	Homozygous	Splice-site	Absent	929659	Pathogenic	SpliceAI: 0.55	**%84**	Kalb et al., 2007(9)
P6	FANCD2	c.[1948-16T>G]; [1948-16T>G]	Homozygous	Splice-site	Absent	929659	Pathogenic	SpliceAI: 0.55	**%59**	Kalb et al., 2007 (9)
P7	FANCD2	c.[1948-16T>G]; [1948-16T>G]	Homozygous	Splice-site	Absent	929659	Pathogenic	SpliceAI: 0.55	**%70**	Kalb et al., 2007(9)

Recurrent FANCD2 splice-site variant and pseudogene-related diagnostic complexity (P5–P7)

The remaining three patients (P5–P7) were found to share the same intronic *FANCD2* variant, c.1948-16T>G, which affects the acceptor splice site of intron 22 (Table 2). Initial WES analysis performed at external laboratories for patient 7 and her affected brother failed to detect this variant. Despite strong FA clinical suspicion and, in P6 and P7, clearly positive DEB/MMC tests, no molecular diagnosis had been reached for several years.

Reanalysis of the WES data using our internal FA gene panel and CNV/ROH tools showed that all three patients carried the c.1948-16T>G variant in an apparently homozygous state. Read-depth–based CNV and ROH plots demonstrated extended ROH tracts on chromosome 3 in P5, P6 and P7, ranging from 2.3 to 12.5 Mb in length. Specifically, ROH intervals were identified at chr3:8,746,798–11,018,936 (2.3 Mb) in P5, chr3:6,673,281–19,148,749 (12.5 Mb) in P6, and chr3:4,814,915–13,619,604 (8.80 Mb) in P7. . These segments overlapped in a shared 2.3-Mb interval (chr3:8,746,798–11,018,936; hg38) encompassing FANCD2 (Figure 1). The presence of this common ROH, together with the high but variable allelic balances (approximately 59–84%) observed in the exome data, strongly supported true homozygosity of the variant rather than somatic mosaicism.

To exclude pseudogene artefacts and confirm the genomic location of the variant, targeted Sanger sequencing of *FANCD2* was performed in all three patients. The chromatograms showed a clean homozygous c.1948-16T>G change in each case, with no evidence of the reference allele (Figure 2), confirming that the variant resides in the functional *FANCD2* gene and establishing its biallelic status.

Figure 1: Runs of Homozygosity (ROH) Encompassing the FANCD2 Locus in Patients P5, P6, and P7

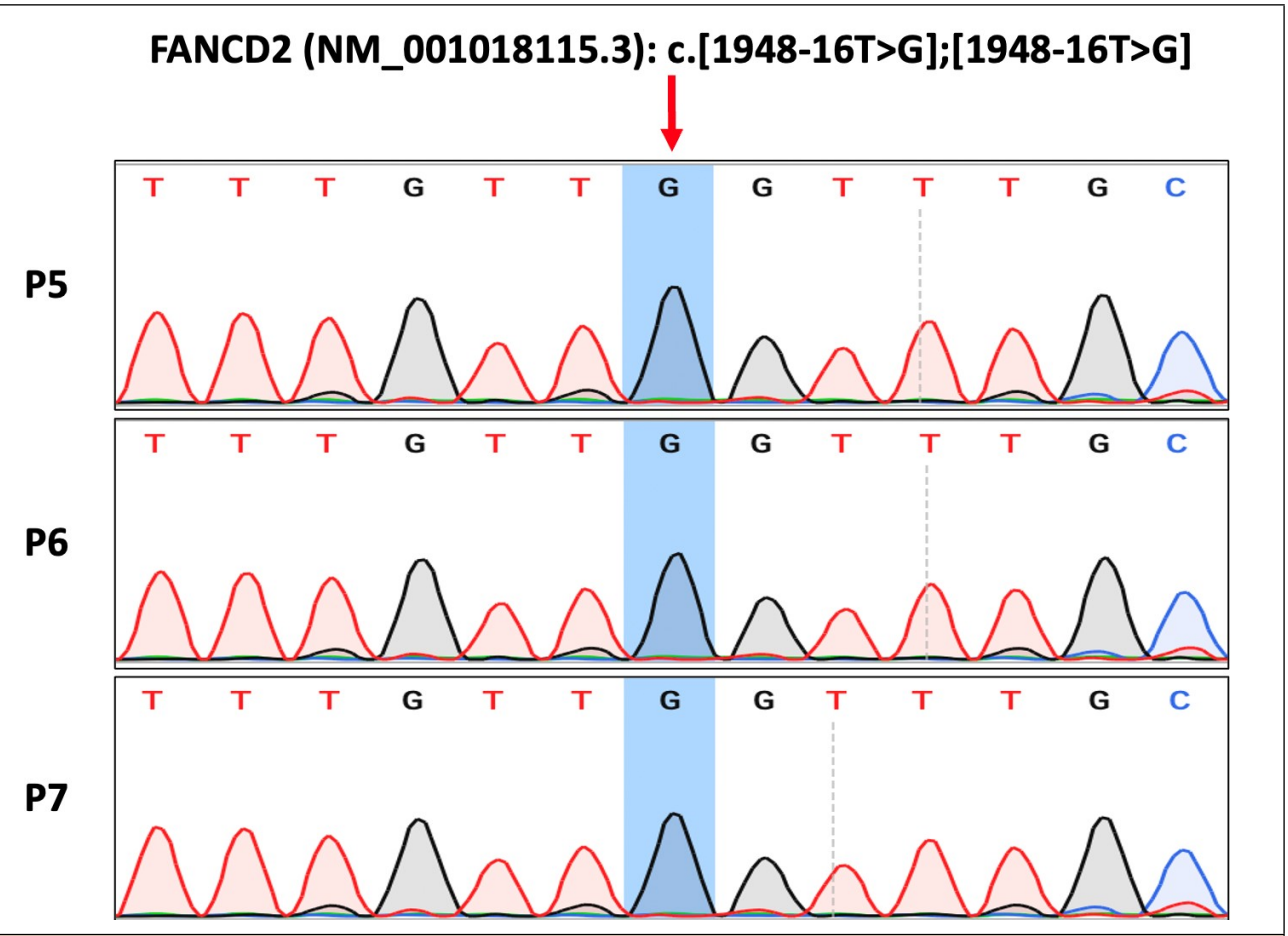

Figure 2: Sanger Sequencing Confirmation of the Homozygous FANCD2 Splice-Site Variant c.1948-16T>G in Patients P5–P7

Discussion

This study illustrates how FANCD2 pseudogenes can seriously complicate molecular diagnosis of Fanconi anemia and underscores the need for an integrated, stepwise diagnostic strategy. In our cohort of seven patients with bone marrow failure and clinical features compatible with FA, biallelic pathogenic variants in *FANCA* or *FANCD2* were identified in all cases. For four patients, exome-based analysis was straightforward. By contrast, in P5–P7 the presence of highly homologous *FANCD2* pseudogenes obscured the causal intronic splice-site variant c.1948-16T>G, leading to ambiguous or apparently negative NGS findings for up to approximately five years in P7 despite a typical FA phenotype and positive chromosomal breakage tests. In this context, the FANCA cases serve as an internal reference illustrating the broader diagnostic spectrum of Fanconi anemia and highlighting the contrast between relatively straightforward molecular diagnoses and the gene-specific complexity encountered in FANCD2.

The *FANCD2* locus is flanked by two pseudogenes, *FANCD2-P1* and *FANCD2-P2*, which retain exon order and >95% sequence identity across large parts of the gene, including introns 21–26. Kalb et al. showed that these duplicated regions co-amplify with conventional PCR and require long gene-specific "superamplicons" to achieve clean *FANCD2* sequencing (9). Kalb et al. further demonstrated that short-read exome pipelines can misalign *FANCD2-P2* reads to *FANCD2*, generating spurious variants in the pseudogene while masking the true gene defect (9).

Our experience with P5–P7 mirrors these reports: routine WES at external laboratories failed to flag a clearly pathogenic *FANCD2* variant, and in P6 the patient and her affected sibling remained without a molecular diagnosis for years despite strong clinical suspicion.

Re-analysis using an internal FA gene panel, with attention to copy-number and autozygosity, was key. All three patients came from consanguineous families and showed an extended run of homozygosity on chromosome 3 encompassing *FANCD2*, immediately suggesting a recessive founder allele. ROH mapping, combined with manual review of read alignments, prompted targeted Sanger sequencing that confirmed the same homozygous c.1948-16T>G variant in P5–P7. The Sanger traces and ROH plots clearly demonstrated zygosity at the true gene locus rather than the pseudogene, resolving the previously ambiguous NGS data. These confirmations, together with the patients' compatible phenotypes, firmly established *FANCD2* as the disease gene in these cases.

Functionally, the chromosomal breakage test provided the decisive anchor throughout the diagnostic odyssey. DEB/MMC-induced chromosome fragility remains the gold standard for FA diagnosis and is insensitive to sequence homology or platform-specific artefacts (6, 17). In our series, P3, P4, P6, and P7 showed clearly positive DEB/MMC results, confirming FA even when exome data were equivocal and in line with guideline recommendations that place breakage testing as the first-line diagnostic modality, with molecular testing as a complementary second tier (6, 7, 18). Our data reinforce that discordance between a positive breakage test and "negative" sequencing should automatically trigger deeper interrogation of genes with complex architecture such as *FANCD2*.

P5 exemplifies the limitations of this approach. This child was diagnosed at an early age on the basis of WES performed for congenital anomalies, before hematologic abnormalities had emerged, and the DEB test was unexpectedly normal—a finding that has also been reported in other FANCD2-related cases (19). Possible explanations include testing at a very young age with insufficiently stressed hematopoietic progenitors, transient suppression of breakage responses, somatic mosaicism, or gene-specific differences in pathway dysfunction (19, 20). Together, these observations indicate that while DEB/MMC assays are highly sensitive, they are not infallible; in individuals with compelling clinical or genetic evidence of FA, the diagnosis cannot be confidently excluded on the basis of a single normal DEB/MMC result.

At the same time, our cohort illustrates both the strengths and blind spots of current NGS-based FA diagnostics. Several groups have reported that comprehensive strategies combining WES or targeted NGS with CNV calling and functional assays can identify disease-causing variants in >90% of FA patients (21, 22). In our hands, exome sequencing rapidly solved four of seven cases but failed in the three patients whose pathogenic variants lay within a pseudogene-rich region. Such "NGS-negative" or "single-allele" FA cases are increasingly recognized and often harbour cryptic variants in GC-rich exons, duplicated regions, or deep intronic splice sites (23). Our results support recommendations that, whenever sequencing identifies no variant or only one mutant allele in a clinically definite FA case, laboratories should explicitly consider pseudogene interference and structural variants, and escalate to orthogonal methods such as long-range PCR/Sanger sequencing, MLPA or read-depth CNV analysis, and ROH mapping in consanguineous families (2, 24). Consistent with our observations, recent data from high-risk pediatric screening cohorts demonstrate that systematic early genetic evaluation—integrating chromosomal breakage testing with molecular analysis—substantially increases diagnostic yield and reduces diagnostic delay in Fanconi anemia (25). These findings support the notion that optimized and context-aware genetic workflows are critical not only for early detection but also for avoiding false-negative results inherent to standard sequencing approaches.

Looking ahead, several technical developments could help pre-empt the diagnostic difficulties encountered in P5–P7. Long-amplicon NGS approaches have already been applied successfully to *FANCA* and *FANCG*, reducing turnaround time and improving detection of point mutations in high-burden genes (7, 23, 26). A similar

design focusing on *FANCD2* segments that exclude pseudogene homology might minimize mis-mapping. In parallel, long-read sequencing platforms are increasingly used for Mendelian disorders with complex structural variation and could theoretically distinguish *FANCD2* from *FANCD2-P1/P2* by spanning the divergent regions in single reads. Although not yet standard for FA diagnostics, these technologies are promising for cases that remain unsolved despite optimized short-read pipelines.

Our findings also have implications at the population level. The same intronic *FANCD2* variant was present in three unrelated Turkish patients who shared a common ROH block, and has previously been reported in FA-D2 patients from this region, suggesting a possible founder allele (9). In settings with high consanguinity, pseudogene-prone genes such as *FANCD2* merit particular scrutiny, as long ROH segments can both facilitate mapping and amplify the impact of founder mutations. Early recognition of such alleles is important for carrier testing, prenatal diagnosis, and genetic counseling.

Finally, our study should be interpreted in light of certain limitations. Functional RNA studies were not performed, so the exact splicing consequence of c.1948-16T>G in our patients was inferred from in silico predictions and previous reports rather than directly demonstrated (9). In addition, our experience derives from a single-center cohort and may not capture the full spectrum of *FANCD2* variants or the diverse pseudogene architectures observed across global populations. Nonetheless, by combining detailed clinical characterization, DEB/MMC testing, ROH analysis, and gene-specific Sanger sequencing, we were able to unmask the underlying defect in all three pseudogene-challenged cases.

In conclusion, this cohort highlights *FANCD2* pseudogene interference as a concrete, clinically relevant cause of false-negative or ambiguous NGS results in FA. The diagnostic pitfalls seen in P5–P7 emphasize that robust FA work-up must couple chromosomal breakage assays with pseudogene-aware genomic analysis, especially in consanguineous populations and for genes embedded in complex genomic architecture. Adoption of such integrated algorithms will reduce diagnostic delay, enable timely institution of FA-specific management, and prevent patients from being left in a diagnostic "blind spot" despite access to advanced sequencing technologies.

Declarations

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Conflict of interest statement

The authors have no conflicts of interest to declare.

Ethics approval

This study did not require formal ethics committee approval according to national and institutional regulations, as it involved only the retrospective analysis of routine clinical genetic testing results and anonymized clinical data. No additional procedures, interventions, or sample.

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We would like to thank the patient and his family for their contribution.

Availability of data and material

The datasets generated and/or analyzed during the current study are not publicly available due to patient privacy and ethical restrictions but are available from the corresponding author on reasonable request.

Authors' contributions

EU and AY conceived and designed the study. EU, VT, AU, and AP collected the clinical data. ST and ABA contributed data and analysis tools. EU, HY, HM, FNE, and ST performed the molecular and bioinformatic analyses. EU and AY drafted the manuscript. All authors critically reviewed the manuscript, approved the final version, and agree to be accountable for all aspects of the work.

Declaration Regarding the Use of AI and AI-Assisted Technologies

During the preparation of this work, the author(s) utilized ChatGPT (OpenAI) to assist with language editing, structural refinement, and improving the clarity of the

manuscript. The scientific content, data interpretation, and all conclusions were generated entirely by the author(s), who verified the accuracy and validity of all AI-assisted outputs. After carefully reviewing and editing the content as necessary, full responsibility for the publication's final version is taken by the author(s). This incorporation of AI tool usage primarily impacted the language editing and organization of the article; no study data, analyses, or scientific results were generated or altered by the tool.

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