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CRISTINA-LOREDANA PANTEA



**GENETICA ERORILOR ÎNNĂSCUTE DE IMUNITATE
ÎNTR-O POPULAȚIE ROMÂNEASCĂ**

REZUMAT

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LISTA PUBLICATIILOR

1. **Pantea CL**, Bataneant M, Zimbru CG, Dragulescu B, Munteanu CV, Chirita-Emandi A. „Genetic landscape of Romanian children with inborn errors of immunity via gene panels, exome, and genome sequencing”. Sci Rep 15, 18830 (2025).
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PARTEA GENERALĂ

Erorile înnăscute de imunitate (IEI) reprezintă un grup heterogen de peste 550 de afecțiuni distincte, majoritatea caracterizate prin compromiterea severă a răspunsului imun față de agenții patogeni, dar care asociază și patologii autoimune, autoinflamatorii și malignități (1). Prevalența colectivă raportată a acestor afecțiuni variază între 1/1000 și 1/5000, în funcție de defectul molecular și de regiunea geografică (2–8). Estimările actuale indică faptul că aproximativ 6 milioane de persoane la nivel global sunt afectate de IEI, însă se estimează că 70–90% dintre aceste cazuri rămân nediagnosticate (9,10). Acest deficit de diagnostic este probabil cauzat de factori multipli, inclusiv variabilitatea semnificativă a prezentării clinice și accesul restricționat la testarea genetică, în special în populațiile adulte (11). În plus, unele IEI pot fi cauzate de variante în gene care nu sunt încă pe deplin caracterizate sau catalogate în bazele de date, sau gene care nu sunt încă asociate cu IEI, contribuind la provocările diagnostice (12,13).

Neutropenia congenitală cuprinde un grup de IEI rare și se caracterizează prin diversitate clinică, variind de la forme oligosimptomatice până la sepsis sau infecții care duc la deces (15–18). Uniunea Internațională a Societăților Imunologice (IUIS) a clasificat neutropenia congenitală în categoria mai largă a defectelor congenitale ale numărului sau funcției fagocitelor (9,14–17). Au fost descrise peste 30 de entități genetice distincte asociate cu neutropenia congenitală severă (SCN) (14,15,18,19). Variantele moștenite autosomal recesiv în *JAGN1* (Jagunal homolog 1) conduc la neutropenie congenitală severă, infecții bacteriene cu debut precoce, aftoză și abcese cutanate din cauza diferențierii și maturării aberante a neutrofilelor (20,21).

Imunodeficiența omună variabilă (CVID) este cea mai frecventă imunodeficiență umorală în rândul adulților, însă în CVID multe gene sunt neidentificate, explicând parțial randamentul diagnostic inferior observat în cohorta adultă (22,23). Majoritatea pacienților sunt diagnosticați la vârste cuprinse între 20-40 de ani, însă 20% dintre cazuri sunt identificate încă din copilărie sau adolescență (22,24). În lipsa tratamentului, IEI pot deveni cronice, grave sau chiar letale.

Principalele semne/simptome sugestive de IEI sunt: cel puțin patru otite/an, ≥două sinuzite/an, ≥ două luni de antibioterapie orală cu efect minim, ≥două pneumonii/an, falimentul creșterii, infecții cutanate profunde/abcese recurente de organ, afte bucale/infecții cutanate fungice persistente, necesitatea tratamentului antibiotic intravenos pentru vindecarea infecției, infecții severe (inclusiv septicemii) și istoric familial pozitiv. La acestea se adaugă următoarele: dezvoltarea de granuloame, autoimunitate, febră recurentă, eczemă, limfoproliferare sau scaune diareice (25–28).

Diagnosticul IEI implică o evaluare imunologică etapizată: hemoleucogramă, imunoglobuline serice, imunofenotipare limfocitară, răspuns vaccinal, teste de proliferare, excluderea etiologiilor secundare (HIV, HCV, CMV, EBV) și investigații complementare adaptate contextului clinic (autoimunitate, funcție tiroidiană, evaluare gastrointestinală) (29,30). Explorarea genetică pentru diagnosticul IEI are următoarele roluri: scurtează timpul până la obținerea unui diagnostic de certitudine, permite înțelegerea căii patogenice și utilizarea unor terapii țintite ale bolii, permite evaluarea altor membrii ai familiei persoanei afectate, iar astfel diagnosticul unor persoane încă oligosimptomatice și nu în ultimul rând, stabilește riscul de transmitere a bolii, permițând astfel consilierea și testarea prenatală (31–33). Variantele genetice pot fi moștenite autozomal dominant sau recesiv, sau X-linkat. Secvențierea de nouă generație (NGS) a devenit mai accesibilă la nivel mondial (34), și mai recent și în România. Deși caracterizarea detaliată genetică prezintă un interes major, datele populaționale privind etiologia genetică a IEI la persoanele din România lipsesc.

Scopul prezentei lucrări constă în evaluarea randamentului diagnostic și a eficienței strategiei de testare genetică secvențiale (multistep, de la paneluri de gene țintite către secvențierea întregului exom WES și genom WGS) în identificarea etiologiei imunodeficiențelor congenitale (IEI) în cohorta pediatrică din România. În plus, studiul urmărește aprofundarea corelațiilor genotip-fenotip, cu un interes particular pentru caracterizarea variabilității clinice a variantei fondatoare *JAGN1* c.63G>T. Totodată, lucrarea își propune să analizeze utilitatea implementării WGS ca metodă de primă intenție în diagnosticul imunodeficiențelor cu debut la vârsta adultă.

PARTEA SPECIALĂ

Material și metode

Am realizat un studiu retrospectiv și prospectiv asupra unei populații de copii (0-18 ani) și adulți (cu vârste între 18-60 ani) care prezentau fenotip sugestiv pentru IEI. Evaluările clinice ale pacienților pediatrici au fost efectuate în perioada septembrie 2018- septembrie 2024, într-un spital terțiar din Timișoara, România. Evaluările clinice ale pacienților adulți au fost efectuate din septembrie 2020 până în septembrie 2021, în spitale terțiare din Timișoara și București, România.

Studiul a fost aprobat de Comitetul de Etică (nr. 11, 08/05/2020). Finanțarea a fost asigurată printr-un grant de cercetare postdoctorală de la Universitatea de Medicină și Farmacie „Victor Babeș” (nr. 5 POSTDOC/2020, 02.2020–02.2022) pentru WGS și în completare de Asociația Română a Pacienților cu Imunodeficiențe Primare (ARPID).

Tehnicile genetice utilizate au fost următoarele: secvențiere Sanger, secvențiere de nouă generație (NGS) prin panel de gene (până la 474 gene asociate cu IEI), secvențierea întregului exom (WES) și secvențierea întregului genom (WGS) (35–37). Testarea genetică a fost efectuată după consiliere genetică adecvată și obținerea consimțământului informat al pacientului sau al părintelui (în cazul pacienților pediatrici).

Toți bolnavii cu suspiciune de IEI au fost evaluați astfel: istoric detaliat, antecedente de infecții (frecvență, severitate, etiologie), manifestări autoimune, limfoproliferare, malignitate și istoric familial (35,36). Indicii diagnostice de includere în studiu pentru pacienții adulți au inclus: infecții recurente sau neobișnuite (precum abcese profunde, sinuzită cronică sau bronșiectazii), manifestări autoimune, boală granulomatoasă, limfadenopatie sau limfoproliferare inexplicabilă, istoric familial de imunodeficiență sau mortalitate precoce, răspunsuri slabe la vaccinare sau hipogamaglobulinemie și malignitate la vârstă tânără (14,37).

Metodologia a presupus izolarea automatizată a ADN-ului genomic și controlul calitativ al acestuia prin metode spectrofotometrice și fluorometrice. Caracterizarea genetică a lotului a fost realizată prin tehnologii de secvențiere de nouă generație (NGS), utilizând o abordare flexibilă: paneluri de gene țintite, secvențierea întregului exom (WES) și a întregului genom (WGS). Secvențierea a fost optimizată pentru o acoperire înaltă, asigurând fidelitatea datelor, în timp ce prelucrarea bioinformatică a urmat un pipeline standardizat de aliniere și adnotare. (38–40).

Variantele au fost interpretate utilizând baze de date populaționale (gnomAD v4, ClinVar), rapoarte curate ClinGen și Human Phenotype Ontology (HPO) pentru corelația fenotip-genotip (41–45). Variantele cu MAF <1% sau raportate anterior ca patogene în HGMD® au fost prioritizate. Clasificarea a urmat ghidurile ACMG/AMP în patogenă (P), probabil patogenă (LP), VUS, probabil benignă (LB) sau benignă (B) (30,46). Efectele asupra splicing-ului au fost evaluate utilizând SpliceAI și Human Splicing Finder; datele WGS au fost analizate cu software-ul MOON. Variantele candidate au fost confirmate prin secvențiere Sanger. Descoperirile secundare au fost raportate conform recomandărilor ACMG (The American College of Medical Genetics and Genomics) (47,48). Analiza statistică a utilizat SPSS v12.0. Analiza corelației genotip-fenotip a fost efectuată utilizând NetworkX v3.4.2 și Matplotlib v3.10.0. O cercetare sistematică în literatura de specialitate a fost efectuată în PubMed, Scopus și Google Scholar utilizând cuvintele cheie „JAGN1”, „c.63G>T”, „p.Glu21Asp” și „neutropenie congenitală severă” pentru a caracteriza spectrul fenotipic al deficitului de JAGN1.

Rezultate

I. Cohorta pediatrică

Un total de 92 de pacienți pediatrici consecutivi (raport masculin:feminin 1:1, vârstă 0-18 ani) cu fenotipuri clinice sugestive pentru IEI au fost evaluați genetic. Din punctul de vedere al vârstei la diagnostic, vârsta medie la testarea genetică a fost de 4,38 ani (IQR 2,0-12,8), cu 27,1% dintre cazuri prezentând simptome înaintea vârstei de 1 an. Întârzierea diagnostică medie a fost de 1,67 ani (IQR 0,3-4,8), iar 40,5% dintre pacienții cu variante patogene aveau un istoric familial de IEI.

Cele mai utilizate tehnici genetice au fost panelurile de gene asociate cu IEI (43/92; 46,7%), urmate de WES (29/92; 31,5%) și WGS (20/92; 21,7%), iar 12 pacienți au necesitat mai mult de un test genetic din cauza rezultatelor inițial negative. Gena *JAGN1* a fost cea mai frecvent identificată (5 pacienți), urmată de *AIRE* (3 pacienți). O analiză detaliată a acestor 5 pacienți cu varianta c.63G>T din gena *JAGN1* a evidențiat o variabilitate fenotipică semnificativă: neutropenie congenitală severă, infecții bacteriene cu debut precoce, cât și manifestări extra-hematologice (gingivite, distrofii dentare, deficit statural, trăsături faciale caracteristice).

Performanța diagnostică în rândul cazurilor pediatrice cu IEI a fost de 40,2%, variante cauzatoare de boală (patogene/probabil patogene) fiind identificate în 37/92 participanți, în 25 de gene diferite, utilizând o abordare de escaladare de la panel de gene la teste largi, WES și WGS. Variante cu semnificație incertă (VUS) au fost determinate în 14/92 (15,2%) dintre participanți. Pacienții cu variante patogene au prezentat debut mai precoce al simptomelor, întârziere diagnostică mai mare și istoric familial semnificativ comparativ cu pacienții cu rezultate negative. Defectele congenitale ale numărului/funcției fagocitelor au avut cel mai mare randament diagnostic, în timp ce tulburările autoinflamatorii au avut randamente mai scăzute.

Variante patogene au fost identificate în 25 de gene, *JAGN1* fiind cea mai frecventă (5 pacienți), urmată de *AIRE* (3 pacienți), *ATAD3A*, *ATM*, *CFTR*, *CYBB*, *FAS* și *TRNT1* (câte 2 pacienți). Au fost descoperite nouă variante anterior neraportate. Modurile de transmitere au inclus: heterozigot compus (15 pacienți), homozigot (10), heterozigot (9) și hemizigot (3). Cele mai frecvente prezentări clinice au fost: febra recurentă (25 pacienți), splenomegalia (19), infecțiile recurente (16) și neutropenia (15). Descoperiri secundare au fost identificate la cinci pacienți, incluzând variante asociate cu risc de cardiomiopatie dilatativă, ihtioză congenitală, risc de pancreatită cronică, hematurie familială benignă și predispoziție la cancer.

II. Corelația genotip-fenotip în cohorta *JAGN1*

Au fost analizați cincisprezece pacienți cu varianta *JAGN1* c.63G>T (p.Glu21Asp) (5 din România, 10 din literatura de specialitate). Vârsta medie la diagnostic a fost de $2,6 \pm 2,8$ ani. Toți pacienții erau în viață la ultima evaluare, cel mai în vârstă având 34 de ani. Patruzeci la sută au fost de etnie romă, iar 86% au avut istoric de consangvinitate.

Testarea genetica a identificat *JAGN1* c.63G>T în status homozigot la 93% dintre pacienți; un pacient a avut variante în status heterozigot compus. Întârziere diagnostică pentru pacienții din România a fost în medie de $10,5 \pm 0,8$ ani. Analiză detaliată a pacienților purtători ai variantei c.63G>T în gena *JAGN1* a relevat o variabilitate fenotipică semnificativă. Clinic, 80% au avut infecții severe, inclusiv abcese cu localizări profunde, tuberculoză și boală parodontală/gingivita (20%). Trăsături sindromice au fost prezente la 20%, inclusiv deficit statual, dismorfism sau întârziere în dezvoltare. Nu au fost observate malformații scheletice sau convulsii. Neutropenia severă a fost prezentă la 88,8% (cel mai scăzut $223 \pm 180,5$ neutrofile/ μ L). Toți pacienții au prezentat monocitoză, hipergamaglobulinemie policlonală (75%) și oprirea maturării măduvei osoase. Niciun pacient nu a dezvoltat sindrom mielodisplazic sau leucemie pe perioada de urmărire.

Comparativ cu spectrul fenotipic complet *JAGN1*, varianta c.63G>T a fost asociată cu manifestări non-hematologice mai ușoare, dar cu neutropenia și susceptibilitatea la infecții ca manifestări predominante. Toți pacienții au primit tratament cu factor de stimulare a coloniilor granulocitare (G-CSF), deși majoritatea au prezentat răspuns slab. Tipizarea HLA a fost efectuată pentru evaluarea în vederea transplantului cu celule stem hematopoietice (HSCT), iar 44,4% au fost supuși în cele din urmă HSCT.

III. Cohorta adultă cu IEI

Douăzeci și unu de pacienți adulți (54,5% femei, vârsta 18-60 ani) au fost evaluați prin WGS. Vârsta medie la debutul simptomelor a fost 16,5 ani, cu 52,3% dintre ei având debutul înaintea vârstei de 18 ani. Întârzierea medie a stabilirii diagnosticului de certitudine a fost de $16,9 \pm 11,3$ ani.

Cele mai frecvente semne și simptome identificate au fost: hipogamaglobulinemia (76,19%), infecțiile recurente (66,66%) și bronșiectaziile (28,57%). Patologiile autoimune au fost prezente la 42,85%, inclusiv trombocitopenie autoimună, tiroidită autoimună, neutropenie și anemie hemolitică. Simptomele neurologice și vasculare au apărut la 28,75% dintre cazuri.

Variante patogene au fost identificate la 9,5% (2/21) dintre pacienți: o variantă *BTK* hemizigotă (asociată cu Agamaglobulinemia X-linkată) și variante *LRBA* în status heterozigot compus (deficitul de LRBA, o formă de CVID). Variante cu semnificație incertă (VUS) relevante clinic au fost identificate la 14,28% (*GATA2*, *COPA*, *TNFRSF13B*, *SCNN1A*). În

cohorta noastră am identificat două variante încă nepublicate și le-am raportat ulterior în ClinVar: *BTK*, NM000061.3:c.1921C>G (p.Arg641Gly) și *SCNN1A*, NM001159576.2:c.744_745delinsAA (p.Arg249Ser). Descoperiri secundare fără asocieri cu IEI au fost identificate la 14,28% dintre pacienți, inclusiv variante *FLG* (ihtioză vulgară) și *HBB* (beta-talasemie).

Discuții

Prezentul studiu oferă prima caracterizare sistematică din punct de vedere genetic al erorilor înnașcute ale imunității (IEI) într-o populație din România, cuprinzând o cohortă de 92 de copii evaluați într-un centru terțiar de referință pentru medicină genomică din Timișoara într-o perioadă de șase ani și o cohortă de 21 de adulți în Spitale terțiare din Timișoara și București, România. Acest studiu arată că NGS, utilizând paneluri de gene, WES și WGS poate identifica etiologia genetică pentru un procent important de copii cu IEI.

Studiul a evidențiat o mare varietate de gene identificate ca fiind cauzatoare pentru IEI. Având în vedere fenotipul foarte variabil, împreună cu absența istoricului familial în majoritatea cazurilor, prioritizarea investigațiilor genetice oferă cea mai bună opțiune pentru diagnosticul și tratamentul în timp util.

Studiul privind WGS ca primă abordare diagnostică pentru IEI la adulți arată că o proporție mare de adulți cu suspiciune de IEI rămân fără o explicație moleculară definitivă, în ciuda utilizării testării genetice cuprinzătoare, precum WGS cu citiri scurte. Rezultatele arată o performanță diagnostică de 9,5% la 21 de adulți cu IEI din cohortă. Aceasta delimitează genotipuri și fenotipuri distincte la adulți, completând datele pediatrice. Rata de diagnostic genetic mai scăzută la adulți ar putea fi explicată prin faptul că IEI sunt determinate de variante în gene încă neidentificate sau etiologia este de multe ori multifactorială și nu monogenică. Prețul de secvențiere este mult mai mare pentru WGS față de panelul de gene, fiind mai puțin accesibil în practica clinică și mai mult în cercetare, iar interpretarea este de asemenea semnificativ mai dificilă. De asemenea în cohorta de adulți, lipsa disponibilității părinților pentru analiza de segregare sau pentru testarea „trio” (caz index și părinții) a limitat posibilitatea stabilirii dacă variantele cu semnificație incertă sunt moștenite sau sunt apărute de novo în vederea confirmării diagnosticului.

Testarea genetică a transformat fundamental diagnosticul și clasificarea IEI, deoarece clasificarea IUIS este acum centrată pe gene, iar genetic definește adesea entitatea bolii în sine. În IEI sporadice (sau non-familiale), diagnosticul genetic este dificil. Eficacitatea diagnostică a NGS prezintă o variabilitate considerabilă între studii, cu randamente raportate variind de la 10% la 79%, și un randament diagnostic mediu de 29% pentru toate modalitățile NGS (13). WGS permite identificarea variantelor structurale

complexe, inclusiv deleții în regiunile reglatoare și inversii, care nu sunt identificate prin WES. Variante intronice (non-codante) patogene au fost documentate în multiple gene asociate IEI (38,39).

Mai multe studii mai mici și prezentări de cazuri au sugerat că neutropenia congenitală severă (SCN) asociată cu *JAGN1* (autosomal recesivă) reprezintă până la 10% din toți pacienții cu SCN, cu grade variabile de severitate a bolii (20,40–47). Anumite variante ale *JAGN1* sunt raportate în alte studii ca apărând mai frecvent în populații specifice (48–50). SCN cauzată de varianta *JAGN1* c.63G>T (p.Glu21Asp) reprezintă o patologie clinic heterogenă, caracterizată prin neutropenie severă cu debut precoce, infecții recurente sau severe, manifestări extra-hematologice și răspuns suboptimal sau absent la terapia cu G-CSF. Seria de cazuri prezentate contribuie la înțelegerea rezultatelor clinice diverse asociate cu variantele *JAGN1* și subliniază importanța tehnicilor avansate de secvențiere genetică în diagnosticarea precisă a acestor cazuri. Dovezile derivate din cohorta noastră și din literatura existentă, sugerează că această variantă ar putea fi variantă fondatoare—în cadrul grupului de etnie rroma, justificând studii genetice suplimentare, inclusiv analiza haplotipului.

Concluzii

Cercetarea de față reprezintă prima investigație sistematică a substratului molecular al erorilor înnăscute de imunitate (IEI) în România, demonstrând utilitatea clinică a implementării tehnologiilor de secvențiere de nouă generație (NGS). Rezultatele obținute evidențiază un randament diagnostic de 40,2% în cohorta pediatrică, confirmând faptul că o strategie de testare genetică etapizată - de la paneluri țintite la WES și WGS - este eficientă pentru scurtarea intervalului până la diagnosticul de certitudine, în special în cazurile cu debut precoce și istoric familial sugestiv.

Un aport original semnificativ al tezei constă în caracterizarea variantei *JAGN1* c.63G>T (p.Glu21Asp), identificată ca fiind o varianta (mutație) recurentă și probabil fondatoare în populația românească, având o prevalență crescută în rândul comunităților de etnie romă. Studiul a relevat o variabilitate fenotipică importantă asociată acestei variante, marcată de neutropenie congenitală severă și susceptibilitate înaltă la infecții bacteriene, dar și prin prezența unor manifestări extra-hematologice precum distrofia dentară și dismorfismul facial, date ce extind spectrul clinic cunoscut al acestei patologii rare.

În ceea ce privește cohorta adultă, studiul a indicat un randament diagnostic mai scăzut, de 9,5%, subliniind complexitatea etiologică a formelor cu debut tardiv, cum este cazul imunodeficienței comune variabile (CVID). Totuși, utilizarea secvențierii întregului genom (WGS) s-a dovedit a fi un instrument valoros de screening, permițând identificarea unor variante rare în gene precum *BTK* și *LRBA*.

În întreaga cohortă pediatrică și de adulți, au fost identificate 11 variante genetice noi și raportate în baze de date internaționale (ClinVar), contribuind astfel la îmbogățirea resurselor genomice globale legate de IEI.

În concluzie, implementarea acestor protocoale avansate de diagnostic în centrele terțiare din România fundamentează tranziția către medicina de precizie în domeniul imunologiei clinice. Identificarea precoce a defectelor moleculare nu doar că optimizează managementul terapeutic — oferind indicații clare pentru terapii țintite sau transplant de celule stem hematopoietice — dar permite și o consiliere genetică riguroasă a familiilor afectate, reducând impactul medical și psihosocial al acestor afecțiuni cronice severe.

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**“VICTOR BABEȘ” UNIVERSITY OF MEDICINE AND PHARMACY
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CRISTINA-LOREDANA PANTEA



**GENETIC CHARACTERIZATION OF INBORN ERRORS OF IMMUNITY
IN A ROMANIAN POPULATION**

ABSTRACT

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LIST OF PUBLISHED SCIENTIFIC PAPERS

1. **Pantea CL**, Bataneant M, Zimbru CG, Dragulescu B, Munteanu CV, Chirita-Emandi A. „Genetic landscape of Romanian children with inborn errors of immunity via gene panels, exome, and genome sequencing”. Sci Rep 15, 18830 (2025). <https://doi.org/10.1038/s41598-025-03492-9>, PMID: 40442269, (IF 3.9)
2. **Pantea CL**, Bataneant M, Zimbru CG, Serban M, Puiu M, Chirita-Emandi A. „Phenotypic Variability Associated with Jagunal Homolog 1 (JAGN1) Deficiency Caused by the c.63G>T Variant”. Int J Mol Sci. 2026 Feb 11;27(4):1735. doi: 10.3390/ijms27041735. PMID: 41751872; (IF 4.9, Q1)
3. **Pantea CL**, Bataneant M, Jurcut C, Cochino A, Ioan A, Munteanu CV, Zimbru CG, Urtila P, Chirita-Emandi A. Whole Genome Sequencing as first diagnostic approach for Inborn Errors of Immunity in adults: Diagnostic Yield and Clinical Correlations, Int. J. Mol. Sci. 2026, 27(8),3415; <https://doi.org/10.3390/ijms27083415>, 10 April 2026; (IF 4.9, Q1)

GENERAL PART

Inborn errors of immunity (IEI), previously termed “primary immunodeficiencies”, encompasses heterogeneous disorders with variable immune dysfunction and diverse clinical manifestations (1). IEI are characterized by the presence of susceptibility to infections, autoimmunity, autoinflammatory diseases, allergy, bone marrow failure, and/or malignancy (2). Reported collective prevalence of these conditions is likely to range between 1/1000–1/5000 depending on the specific primary genetic defect and geographical region (3–9). Current estimations indicate that approximately 6 million individuals globally are affected by IEI, yet it is estimated that 70–90% of these cases go undiagnosed (10,11). This diagnostic gap is probably caused by multiple factors, including significant variability in clinical presentation, and restricted access to genetic testing, particularly in adult populations (12). Additionally, some IEI may be caused by variants in genes that are not yet fully characterized or catalogued in current databases, or genes not yet associated with IEI, contributing to diagnostic challenges (13,14).

Congenital neutropenia encompasses a group of rare IEI and is characterized by clinical diversity, ranging from oligosymptomatic to sepsis or fatal infections (15–18). The International Union of Immunological Societies (IUIS) has categorized congenital neutropenia under the broader category of congenital defects of phagocyte number or function (10,15,16,19,20). More than 30 distinct genetic entities associated with severe congenital neutropenia (SCN) have been described (15–17,21).

The clinical presentation of IEI can vary widely depending on the specific genetic defect involved. IEI is suspected in the presence of: $\geq$ four new ear infections within one year; $\geq$ two serious sinus infections within one year, $\geq$ two months of oral antibiotic treatment with little effect, $\geq$ two episodes of pneumonia within one year; failure to thrive; recurrent, deep skin or organ abscesses; granuloma; persistent oral thrush or fungal infection on skin, need for intravenous antibiotics to clear infections; $\geq$ two severe infections within one year, including septicemia; hepatosplenomegaly; lymphadenopathy; chronic diarrhea; severe eczema; severe recurrent allergies; hematologic and oncologic conditions; autoimmunity; autoinflammation; recurrent angioedema and/or a family history of primary immunodeficiency (2,22–24). SCN is defined by a peripheral absolute neutrophil count of less than 1000 cells/ μ L blood in the first year of life and less than 1500 cells/ μ L blood after this age (15–17,25,26), measured on at least three occasions with at least one of the following: deep-seated infection due to bacteria and/or fungi, recurrent pneumonia, buccal and/or genital aphthous lesions or ulcerations, an affected family member, and exclusion of secondary causes of neutropenia (15,16,18). Hypogammaglobulinemia, lymphopenia, and

monocytosis or monocytopenia can be associated with neutropenia (15–17,21). Long-term hematologic complications of congenital neutropenia include an increased risk for myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) (15–17,21,27,28). Severe combined immunodeficiency (SCID), the most severe IEI, is fatal within the first two years of life unless treated with potentially curative bone marrow transplantation, optimally performed before three months of age (29). Therefore, early diagnosis is life-saving.

Genetic testing is valuable for treatment decision, risk stratification, and disease prevention and management of IEI are dependent on confirmation of the underlying molecular defect (7,30–32). According to the most recent update by the International Union of Immunological Societies (IUIS) expert committee the last few decades have seen the identification of more than 550 genetics causes (variants) of IEI (1,33). IEI are caused by variants in single genes that alter both the adaptive and the innate immune response. Variants can be dominantly or recessively inherited, autosomal, or X-linked, and with complete or incomplete penetrance of the clinical phenotype. Next generation sequencing (NGS) has become more accessible worldwide,(34) and more recently also in Romania. While detailed mapping of the genetic landscape is of great interest for describing founder variants as well as disease prevalence at a country level, population data regarding the genetic etiology of IEI in Romanian people are lacking.

The aim of the present work is to evaluate the diagnostic yield and efficiency of a sequential (multistep) genetic testing strategy, from targeted gene panels to whole exome sequencing (WES) and whole genome sequencing (WGS), in identifying the etiology of inborn errors of immunity (IEI) in a Romanian pediatric cohort. Additionally, the study seeks to deepen genotype-phenotype correlations, with particular focus on characterizing the clinical variability associated with the *JAGN1* c.63G>T founder variant. Furthermore, this work aims to analyze the utility of implementing WGS as a first-line diagnostic approach for inborn errors of immunity with adult onset.

SPECIAL PART

Materials and methods

The studies investigated a cohort of children and adults (18-64 years old) presenting with symptoms suggestive of an IEI. Pediatric patient's clinical evaluations were performed between September 2018 and September 2024, in a tertiary hospital in Timisoara, Romania. Clinical assessments of the adult patients were performed from September 2020 to September 2021 at tertiary hospitals in Timisoara and Bucharest, Romania. The study cohort

included a consecutive series of adult patients (aged 18-60 years) exhibiting an IEI phenotype who underwent genetic analysis using WGS as a first-tier genetic test.

The study was approved by the institutional Ethics Committee (no. 11, 08/05/2020). Funding was provided by a postdoctoral research grant from "Victor Babes" University of Medicine and Pharmacy (no. 5 POSTDOC/2020, 02.2020–02.2022) for WGS and additionally the Romanian Association of Patients with Primary Immunodeficiencies (ARPID).

Genetic testing for the diagnosis of IEI comprised: the IEI gene panel, Whole exome sequencing (WES), whole genome sequencing (WGS) and single gene testing for a specific IEI phenotype (Sanger sequencing), and was performed after appropriate counselling and obtaining the informed consent of the patient or parent (for pediatric patients).

Clinical assessments for patient with suspected IEI implied: detailed patient history focusing on infection patterns (frequency, severity, causative organisms), autoimmune manifestations, lymphoproliferation, malignancy, and family history (35,36). Key diagnostic clues in adults include: recurrent or unusual infections (such as deep-seated abscesses, chronic sinusitis, or bronchiectasis), autoimmune manifestations, granulomatous disease, unexplained lymphadenopathy or lymphoproliferation, family history of immunodeficiency or early mortality, poor vaccine responses or hypogammaglobulinemia, and malignancy at a young age (14,37).

The methodology involved automated genomic DNA isolation and quality control using spectrophotometric and fluorometric methods. Genetic characterization of the cohort was performed using next-generation sequencing (NGS) technologies, employing a flexible approach: targeted gene panels, whole exome sequencing (WES), and whole genome sequencing (WGS). Sequencing was optimized for high coverage to ensure data fidelity, while bioinformatic processing followed a standardized alignment and annotation pipeline (38–40).

Variants were interpreted using population databases (gnomAD v4, ClinVar), ClinGen curated reports, and Human Phenotype Ontology (HPO) for phenotype-genotype correlation (41–45). Variants with MAF <1% or previously reported as disease-causing in HGMD® were prioritized. Classification followed ACMG/AMP guidelines into pathogenic (P), likely pathogenic (LP), VUS, likely benign (LB), or benign (B) (30,46). Splicing effects were assessed using SpliceAI and Human Splicing Finder; WGS data were analyzed with MOON software. Candidate variants were confirmed by Sanger sequencing. Secondary findings were reported per ACMG recommendations with patient consent (47,48). Continuous variables were expressed as median (IQR); categorical variables as numbers and proportions. Mann-Whitney U-test was used for non-parametric comparisons; chi-squared or Fisher's exact test for categorical variables (significance: $p < 0.05$). Statistical analysis used SPSS v12.0. Gene-phenotype network analysis was performed using NetworkX v3.4.2 and Matplotlib v3.10.0. A systematic literature search was conducted in PubMed, Scopus, and

Google Scholar using keywords "JAGN1", "c.63G>T", "p.Glu21Asp", and "severe congenital neutropenia" to characterize the phenotypic spectrum of JAGN1 deficiency.

Results

I. Pediatric Cohort with IEI

A total of 92 consecutive pediatric patients (male:female ratio 1:1, age 0-18 years) with clinical phenotypes suggestive of IEI underwent genetic evaluation. Median age at genetic testing was 4.38 years (IQR 2.0-12.8), with 27.1% presenting symptoms before age 1 year. Median diagnostic delay was 1.67 years (IQR 0.3-4.8). One-fifth had a positive family history of IEI.

NGS panels were applied to nearly half of patients (43/92; 46.7%), followed by WES (29/92; 31.5%) and WGS (20/92; 21.7%), with 12 patients requiring more than one genetic test due to initially negative results with suggestive phenotypes. Median age at genetic testing was 4.38 years, and 40.5% of patients with disease-causing variants had a family history of IEI.

The diagnostic yield among pediatric IEI cases was 40.2%, with disease-causing variants (pathogenic/likely pathogenic) identified in 37/92 participants across 25 different genes, using a stepwise escalation approach from targeted gene panels to comprehensive testing (WES and WGS). Variants of uncertain significance (VUS) were identified in 14/92 (15.2%) participants. Patients with disease-causing variants showed earlier symptom onset, longer diagnostic delay, and more significant family history compared to those with negative results. Congenital defects of phagocyte number/function had the highest diagnostic yield, while autoinflammatory disorders had lower yields.

Disease-causing variants were identified across 25 genes, with *JAGN1* being most frequent (5 patients), followed by *AIRE* (3), *ATAD3A*, *ATM*, *CFTR*, *CYBB*, *FAS*, and *TRNT1* (2 each). Nine previously unreported variants were discovered. Inheritance patterns included compound heterozygous (15 patients), homozygous (10), heterozygous (9), and hemizygous (3). The most frequent clinical presentations were recurrent fever (25 patients), splenomegaly (19), recurrent infections (16), and neutropenia (15). Secondary findings were identified in five patients, including variants associated with dilated cardiomyopathy risk, congenital ichthyosis, chronic pancreatitis risk, benign familial hematuria, and cancer predisposition.

II. *JAGN1* Cohort Genotype-Phenotype Correlation

Fifteen patients with the *JAGN1* c.63G>T (p.Glu21Asp) variant were analyzed (5 Romanian and 10 from literature). Mean age at diagnosis was 2.6±2.8 years. All patients

were alive at last follow-up (mean 15 ± 8.1 years), with the oldest being 34 years. Forty percent were Roma ethnicity, and 86% had consanguineous backgrounds.

Molecular testing identified homozygous *JAGN1* c.63G>T in 93% of patients; one patient had compound heterozygous variants. Romanian patients experienced a mean diagnostic delay of 10.5 ± 0.8 years.

A detailed analysis of patients carrying the c.63G>T variant in the *JAGN1* gene revealed significant phenotypic variability. Clinically, 80% had severe infections including deep abscesses, tuberculosis, and periodontal disease, causing premature tooth loss (20%). Syndromic features were present in 20%, including short stature, mild dysmorphism, and developmental delay. Notably, no skeletal malformations or seizures were observed. Severe neutropenia was present in 88.8% (lowest ANC: 223 ± 180.5 cells/ μ L). All patients showed monocytosis, polyclonal hypergammaglobulinemia (75%), and bone marrow maturation arrest. No patient developed MDS or leukemia during follow-up.

Compared to the full *JAGN1* phenotypic spectrum, the c.63G>T variant was associated with a milder extra-hematological phenotype, with neutropenia and infectious susceptibility as predominant manifestations. All patients received G-CSF (median 13 μ g/kg/day), though most showed poor response. Mean age at treatment initiation was 8.2 ± 4.93 years. HLA typing was performed for transplant evaluation, and 44.4% ultimately underwent HSCT as definitive therapy.

III. Adult Cohort with IEI

Twenty-one adult patients (54.5% female, age 18-60 years) underwent WGS. Median symptom onset was 16.5 years, with 52.3% experiencing onset before age 18. Median diagnostic delay was 16.9 ± 11.3 years.

The most frequent findings were hypogammaglobulinemia (76.19%), recurrent infections (66.66%), and bronchiectasis (28.57%). Autoimmune features were present in 42.85%, including immune thrombocytopenia, autoimmune thyroiditis, neutropenia, and hemolytic anemia. Neurological and vascular symptoms occurred in 28.75%.

Disease-causing variants were identified in 9.5% (2/21) of patients: one hemizygous *BTK* variant (X-linked agammaglobulinemia) and compound heterozygous *LRBA* variants (*LRBA* deficiency). Clinically relevant VUS were identified in 14.28% (*GATA2*, *COPA*, *TNFRSF13B*, *SCNN1A*). In our cohort we identified two previously unreported variants and subsequently submitted them to ClinVar: the *BTK*, NM_000061.3:c.1921C>G (p.Arg641Gly) and the *SCNN1A*, NM_001159576.2:c.744_745delinsAA (p.Arg249Ser). Secondary findings unrelated to IEI were identified in 14.28%, including *FLG* (ichthyosis vulgaris) and *HBB* (beta-thalassemia) variants.

Discussion

The present study provides the first systematic characterization of the genetic landscape of inborn errors of immunity in Romanian cohort, based on a cohort of 92 children evaluated at a tertiary referral center for genomic medicine in Timisoara over a six-year period and a cohort of 21 adults at tertiary medical institutions in Timisoara and Bucharest, Romania. This study shows that NGS (using gene panels, WES and WGS) can identify the genetic etiology for an important percentage of children with IEI. Our comprehensive phenotypic characterization delineates the genetic architecture and associated clinical phenotypes unique to adult-presenting IEI. We report a diagnostic yield of 9.5% in 21 adults. This delineates distinct genetic architectures and clinical phenotypes in adults, complementing pediatric data.

Several smaller studies and case reports have suggested that autosomal recessive *JAGN1* deficiency accounts for up to 10% of all patients with SCN, with varying degrees of disease severity (49–57). Certain variants of *JAGN1* are reported in the scientific literature to occur more frequently within specific populations (49–53,55–57). Due to historical isolation, endogamy/consanguinity and genetic drift, some variants, possibly including *JAGN1* NM_032492.4:c.63G>T, have become more common in certain Roma subgroups compared to the general population (58–60).

In contrast to pediatric presentations, adult-onset IEI may arise from hypomorphic variants within IEI-associated genes that allow for partial immune functionality, leading to delayed clinical manifestations.

WES and WGS have emerged as useful diagnostic methodologies. The diagnostic efficacy of NGS exhibits considerable variability across studies, with reported yields ranging from 10% to 79%, and an average diagnostic yield of 29% for all NGS modalities (14). Comparative studies indicate that the diagnostic yield of genome sequencing alone in adult patients with IEI is generally in the range of 14–60%, depending on cohort selection, analytic strategy, and the inclusion of structural and noncoding variants (61,62). For example, WES in 1000 families with complex immune phenotypes yielded a molecular diagnosis in 32.7% of probands, with actionable findings in 69.5% (63). WGS facilitates a thorough search for pathogenic variants, including structural and deep intronic changes often missed by narrower sequencing methods.

Genetic testing has fundamentally transformed the diagnosis and classification of IEI, as the IUIS classification is now gene-centered and genetics often defines the disease entity itself. In sporadic (or non-familial) IEI, genetic diagnosis is difficult. WGS enables

identification of complex structural variants, including deletions in regulatory regions and inversions, which are missed by both WES and chromosomal microarray. Pathogenic intronic (non-coding) variants have been documented in multiple IEI-associated genes. WGS lacks the enrichment step that introduces bias in WES data, providing more consistent coverage of exonic sequences and improved CNV detection.

The SCN caused by the *JAGN1* c.63G>T (p.Glu21Asp) variant represents a multisystemic, clinically heterogeneous disorder characterized by the early-onset, severe neutropenia, recurrent or severe infections, extra-hematopoietic manifestations, and suboptimal or absent responsiveness to G-CSF therapy. The case series contribute to understanding the diverse clinical outcomes associated with *JAGN1* variants and stresses the importance of advanced genetic sequencing techniques in diagnosing these cases accurately. Evidence derived from our cohort, in conjunction with existing literature, suggests that this variant may be enriched—or potentially act as a founder variant—within the Roma ethnic group, warranting further genetic studies, including haplotype analysis. The cases contribute to understanding the diverse clinical outcomes associated with *JAGN1* variants and stresses the importance of advanced genetic sequencing techniques in diagnosing these cases accurately.

The study on WGS as a first-line diagnostic approach for IEI in adults demonstrates that a large proportion of adults with suspected IEI remain without a definitive molecular explanation, despite the use of comprehensive genetic testing such as short-read WGS. The results show a diagnostic yield of 9.5% among the 21 adults with IEI in the cohort. This delineates distinct genotypes and phenotypes in adults, complementing the pediatric data. The lower genetic diagnostic rate in adults may be explained by the fact that IEI are caused by variants in genes not yet identified, or the etiology is often multifactorial rather than monogenic. The sequencing cost is significantly higher for WGS compared to gene panels, making it less accessible in clinical practice and more suited to research settings, while interpretation is also considerably more challenging. Additionally, in the adult cohort, the lack of parental availability for segregation analysis or trio testing (proband and parents) limited the ability to determine whether variants of uncertain significance are inherited or de novo, thereby delaying diagnostic confirmation.

Conclusions

The present research represents the first systematic investigation of the molecular substrate of inborn errors of immunity (IEI) in Romania, demonstrating the clinical utility of implementing next-generation sequencing (NGS) technologies. The results highlight an effective diagnostic yield of 40.2% in the pediatric cohort, confirming that a stepwise genetic

testing strategy—from targeted panels to WES and WGS—is effective in shortening the time to definitive diagnosis, particularly in cases with early onset and suggestive family history.

A significant original contribution of this thesis consists in the characterization of the *JAGN1* c.63G>T (p.Glu21Asp) variant, identified as a recurrent and likely founder mutation in the Romanian population, with increased prevalence among Roma ethnic communities. The study revealed significant phenotypic variability associated with this variant, characterized by severe congenital neutropenia and high susceptibility to bacterial infections, but also by the presence of extra-hematological manifestations such as dental dystrophy and facial dysmorphism—findings that expand the known clinical spectrum of this rare disorder.

Regarding the adult cohort, the study indicated a lower diagnostic yield of 9.5%, underscoring the etiological complexity of late-onset forms, such as common variable immunodeficiency (CVID). Nevertheless, whole genome sequencing (WGS) proved to be a valuable screening tool, enabling the identification of rare variants in genes such as *BTK* and *LRBA*.

In the entire pediatric and adult cohorts, 11 novel genetic variants were identified and reported to international databases (ClinVar), thereby contributing to the enrichment of global genomic resources related to IEI.

In conclusion, the implementation of these advanced diagnostic protocols in tertiary centers in Romania establishes the foundation for the transition toward precision medicine in clinical immunology. Early identification of molecular defects not only optimizes therapeutic management—providing clear indications for targeted therapies or hematopoietic stem cell transplantation—but also enables rigorous genetic counseling for affected families, reducing the medical and psychosocial burden of these severe chronic conditions.

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