Received: 8 July 2019 | Revised: 8 February 2020 | Accepted: 12 February 2020

DOI: 10.1002/pbc.28237

Pediatric Blood & Cancer | SIOP International Society of Paediatric Oncology | aspho The American Society of Pediatric Hematology/Oncology | WILEY

HEMATOLOGY: RESEARCH ARTICLE

Congenital neutropenia with variable clinical presentation in novel mutation of the SRP54 gene

Lior Goldberg[1,2] | Amos J. Simon[2,3] | Gideon Rechavi[3,4] | Atar Lev[2] | Ortal Barel[3,4] | Vered Kunik[5] | Amos Toren[1,6] | Ginette Schiby[3] | Hannah Tamary[1,7] | Orna Steinberg-Shemer[1,7]* | Raz Somech[1,2,3]*

[1]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

[2]Pediatric Department A, Pediatric Immunology Service, "Edmond and Lily Safra" Children's Hospital, Jeffrey Modell Foundation (JMF) Center, Tel HaShomer, Israel

[3]The Wohl Institute for Translational Medicine, Sheba Medical Center, Tel HaShomer, Israel

[4]Sheba Cancer Research Center, Sheba Medical Center, Tel HaShomer, Israel

[5]Bioinformatics Consulting, Tel Aviv, Israel

[6]Pediatric Hemato/Oncology Division and Bone Marrow Transplantation Unit, Tel HaShomer, Israel

[7]Departments of Hematology-Oncology, Schneider Children's Medical Center of Israel, Petach Tivka, Israel

Correspondence
Raz Somech, Pediatric Immunology, "Edmond and Lily Safra" Children's Hospital, Sheba Medical Center, Tel HaShomer, Sackler School of Medicine Tel Aviv University, Tel Aviv, Israel.
Email: raz.somech@sheba.health.gov.il

All coauthors have reviewed the manuscript and have contributed in a substantive and intellectual manner to the work described.

*Orna Steinberg-Shemer and Raz Somech had equal contribution.

Abstract

Background: The SRP54 (signal recognition protein 54) is a conserved component of the ribonucleoprotein complex that mediates cotranslational targeting and translocation of proteins to the endoplasmic reticulum. In 2017, mutations in the gene have been described as a cause of congenital neutropenia with or without pancreatic insufficiency, and since then, only limited cases were added to the literature.

Methods: Two patients with neutropenia underwent hematological, immunological, and genetic work-up, including lymphocyte phenotyping, immunoglobulins, and complement levels, antineutrophil and antinuclear antibodies, bone marrow FISH panel for myelodysplastic syndrome, whole-exome sequencing, and in silico proteomic analysis.

Results: Clinical findings in the two families revealed a wide spectrum of immunological and clinical manifestations, ranging from mild asymptomatic neutropenia during febrile illnesses to severe neutropenia and life-threatening infection requiring leg amputation. Immunological and hematological work-up showed isolated neutropenia with normal lymphocyte subpopulations, immunoglobulin and complement levels, and negative autoimmune tests. Bone marrow aspirations showed variability ranging from normal myelopoiesis to myeloid maturation arrest at the promyelocytic stage, with normal FISH panel for myelodysplastic syndrome. Genetic analysis identified a novel, *de novo*, in-frame deletion in the *SRP54* gene, c.342-344delAAC, p.T115del. In silico proteomic analysis suggested impaired SRP54 protein function due to reduced GTP activity and stability.

Conclusions: We describe congenital neutropenia with variable clinical presentation in novel mutation of the SRP54 gene.

KEYWORDS
congenital neutropenia, signal recognition particle, SRP54, variable expressivity

1 | INTRODUCTION

Neutropenia beyond one year of age is defined as an absolute neutrophil count (ANC) < 1500/μL and can be either acquired or congenital.[1] Acquired etiologies are more common and include infection related, drug induced and immune mediated. In contrast, congenital etiologies due to inherited genetic defects are extremely rare with an estimated incidence rate of 10 to 15 cases per million births. The congenital neutropenias are a heterogeneous group of syndromic or nonsyndromic blood disorders with broad genetic and phenotypic variability. Some of the disorders include extrahematological manifestations (e.g., Shwachman-Diamond syndrome [SDS]), whereas

Abbreviations: ANC, absolute neutrophil count; GTP, guanosine triphosphate; MDS, myelodysplastic syndrome; PMBCs, peripheral blood mononuclear cells; SDS, Shwachman-Diamond syndrome; SRP, signal recognition protein; WES, whole-exome sequencing.

others manifest as pure isolated neutropenia.[1-3] In 2017, Carapito et al[4] reported three patients with mutations in the signal recognition particle 54 (*SRP54*) gene as a cause of inherited neutropenia, and since then only a few cases were added to the literature.[5,6] The SRP54 protein is a conserved component of the ribonucleoprotein complex that mediates cotranslational targeting and translocation of proteins to the endoplasmic reticulum.[7,8] Bellanné-Chantelot et al[5] showed that *SRP54* mutations lead to ER stress and autophagy in myeloid precursor cells and neutrophil granulocytes. Our understanding of *SRP54* mutations is far from complete; reported cases were genetically and phenotypically heterogeneous, though are typically characterized by isolated severe neutropenia with a promyelocytic arrest. The minority of cases manifested with Shwachmam-Diamond like-syndrome including exocrine pancreatic insufficiency and clinically significant neurologic abnormalities.

Here we describe two unrelated families, evaluated in two independent centers, presenting with congenital neutropenia, ultimately found to carry the same novel mutation in the *SRP54* gene. We describe their clinical characteristics, immunological, hematological, genetic, and proteomic investigation.

2 | METHODS

2.1 | Clinical date

We evaluated patients at the "Edmond and Lily Safra" Children's Hospital, Sheba Medical Center, in Tel Hashomer (patient 1) and at the Schneider Children's Medical Center (patient 2). The patients were interviewed and examined by the authors. Medical records were obtained from the electronic registry of the hospital. Informed consent was obtained, and all procedures were performed in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki declaration and its later amendments or comparable ethical standards.

2.2 | Immunological and hematological investigations

2.2.1 | Lymphocyte subset determination

Cell-surface markers of peripheral blood mononuclear cells (PBMCs) were determined by immunofluorescent staining and flow cytometry (Navios, Beckman Coulter, Brea, CA, USA) using anti-CD3, anti-CD20, anti-CD16, and anti-CD56 antibodies from BD Biosciences and anti-CD4 and anti-CD8 antibodies from Beckman Coulter.

2.2.2 | Measurement of immunoglobulins and complement levels

Serum levels of C3, C4, and immunoglobulins were measured by means of particle-enhanced immunonephelometry using the BN II System (Siemens Healthcare Diagnostics, Marburg, Germany) as per the manufacturer's instructions.

2.2.3 | Fluorescence in situ hybridization (FISH) analyses

FISH analyses of bone marrow cells were performed with the Cytocell Multiprobe MDS panel (Cytocell, http://www.cytocell.co.uk/) including probes for 5p/5q, i(17q), t(8,21), 11(q23), 7q/7q, and 20q.

2.2.4 | Whole-exome and Sanger sequencing

Whole-exome sequencing (WES) was performed using an Agilent v5 SureSelect capture kit and Illumina 2500 sequencing technology. For each sample, paired-end reads (2 × 100 bp) were obtained, processed, and mapped to the genome. We used the BWA mem algorithm (version 0.7.12)[9] for alignment of the sequence reads to the human reference genome (hg19). The HaplotypeCaller algorithm of GATK version 3.4 was applied for variant calling, as recommended in the best practice pipeline.[10] KGG-seq v.08 was used for annotation of identified variants[11] and in-house scripts were applied for filtering based on family pedigree and local data set of variants detected in previous sequencing projects. *SRP54* mutations were validated by dideoxy Sanger sequencing in patient and family members. PCR amplification products were directly sequenced using BigDye 3.1 Terminator chemistry (Applied Biosystems) and separated on an ABI 3500 genetic analyzer (Applied Biosystems). Data were evaluated using Sequencer v5.0 software (Gene Codes Corporation).

2.3 | Protein modeling and analysis

2.3.1 | Modeling of *SRP54* T115del mutation

The 3D structural model of wild-type and mutated GTPase heterodimer of human SRP54 and SR proteins was constructed using the SWISS-MODEL protein structure homology-modeling server,[12] using the PDB structure 5l3q as the template for structure prediction. Although the wild-type structures of SRP54 and SR proteins exist (PDB 5l3q), we used the same 3D modeling algorithm to construct both structures to avoid differences that may arise due to the modeling process.

2.3.2 | Structural analysis

Discovery Studio Visualizer (version 16.1.0.15350); BIOvIA, D. S. (2015).]Discovery studio modeling environment. San Diego, Dassault Systemes, Release, 4] was used to calculate the hydrogen bonds between SRP54 and GTP.

2.3.3 | Stability analysis

We used the FoldX algorithm[13,14] to assess the effect of the T115del mutation on the stability of the SRP54-SR complex. FoldX is based on empirical energy terms that were compared and correlated with experimental data of a large number of mutations in monomeric proteins and protein-protein complexes.[13] FoldX (version 4) was applied to the modeled SRP54-SR complex in the following manner: (i) the 3D structures of wild-type and mutated SRP54 in complex with SR were optimized using the FoldX RepairPDB function, (ii) the stability of the wild-type and mutated SRP54-SR structures were calculated using

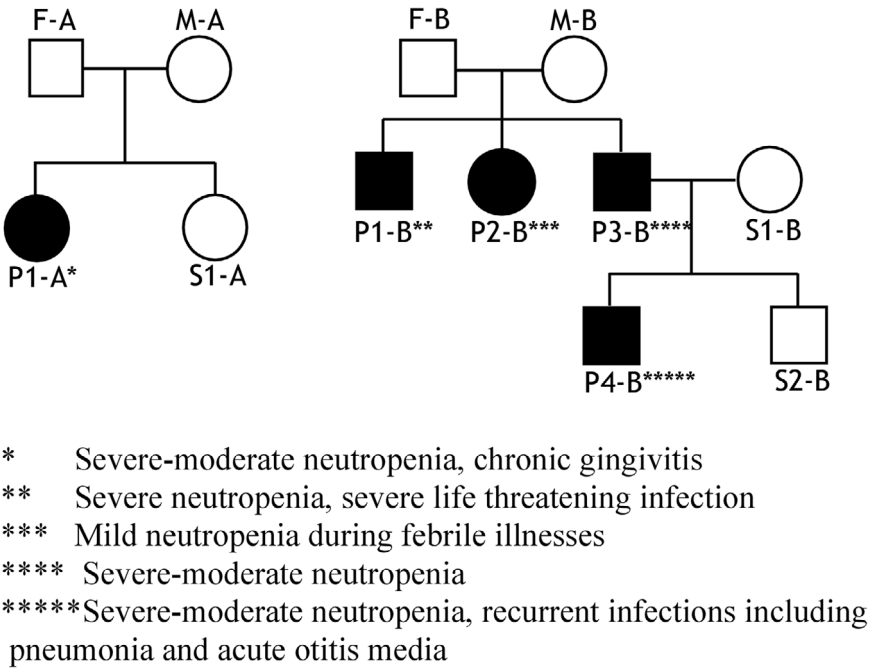

FIGURE 1 Family Pedigrees. Pedigrees of the two studied affected families (A and B), harboring the same *de novo* mutation. Filled black circles or squares depict the patients. Empty circles or squares depict WT genotypes with no clinical symptoms. F = father, M = mother, P = patient, S = sibling. Asterisks indicate clinical phenotypes of the five patients

the FoldX stability function, (iii) ΔΔG values were obtained using the following equation:

$$\Delta\Delta G = \Delta G_{\text{mutant}} - \Delta G_{\text{wild-type}}$$

The structure is considered to be destabilized if $\Delta\Delta G > 1$ kcal/mol, neutral if $0 \leq \Delta\Delta G \leq 1$ kcal/mol, and stabilized if $\Delta\Delta G < 0$ kcal/mol.

3 | RESULTS

3.1 | Clinical presentation

We describe here patients 1 and 2 from two unrelated families (P1-A and P4-B; Figure 1) which were evaluated for neutropenia. Patient 1, an eight-year-old female, was referred for evaluation of persistent neutropenia at one year of age and has been followed up since then. She was born at 42 weeks of gestation after an uneventful pregnancy. Birth weight was 3.1 kg (appropriate for gestational age); she had no dysmorphic features or visible bony abnormalities. The patient was the second child of healthy nonconsanguineous Israeli parents of mixed Ashkenazi and Iraqi Jewish descent, and there was no family history of hematological disease or primary immunodeficiency (Figure 1). At the age of eight months, she presented with severe isolated neutropenia (180/μL) that was initially attributed to Parainfluenza virus infection, yet upon follow-up tests, the severe-moderate isolated neutropenia persisted without cyclic pattern. She later presented with chronic gingivitis with recurrent episodes of flare-ups that responded well to antibiotic treatment. No life-threatening bacterial infections occurred up to date. Her growth parameters showed height and weight in the 20th to 25th percentiles, and no gastrointestinal, neurological, or abnormal neurodevelopmental manifestations were observed. Stool studies for fecal elastase were normal (632 μg/g, normal levels > 200 μg/g), and pancreatic ultrasound showed normal anatomy without lipomatosis. Echocardiography test showed normal heart structure and function. She currently receives granulocyte colony-stimulating factor (G-CSF) treatment at the dose of 5 μg/kg/day, with improvement in the neutrophil counts (> 1000/μL) and her chronic gingivitis.

Patient 2, a two-year-old male, was referred at the age of six months due to neutropenia and has been followed up since then. During the pregnancy, the mother had CMV seroconversion. Amniocentesis was not performed, and CMV infection was ruled out after birth. Brain sonography, after birth, demonstrated few cysts around the right thalamus and in the left caudothalamic groove. At the age of two months, one cyst was noted in the caudothalamic groove (6 mm). No further brain imaging has been done due to normal neurological development. He had no dysmorphic features or visible bony abnormalities. The absolute neutrophil count was 6200/μL after birth, and 930/μL at the age of 2 weeks. At four months, during febrile illness, his absolute neutrophil count was 600/μL. Since then, he suffered from recurrent infections including pneumonia and acute otitis media and was diagnosed with hyperactive airway disease. At the ages 7 and 10 months, he had two episodes of febrile seizures, and his neutrophil counts were 100-900/μL with normal hemoglobin and platelet counts. Upon follow-up tests, the severe-moderate isolated neutropenia persisted without a cyclic pattern. His growth parameters showed height and weight in the 10th to 20th percentiles, and no gastrointestinal, neurological, or abnormal developmental manifestations were observed. Stool studies for fecal elastase showed moderate to mild exocrine pancreatic insufficiency (186 μg/g, normal levels > 200 μg/g) and pancreatic ultrasound showed normal anatomy without lipomatosis. Echocardiography test

TABLE 1 Immunological investigation

Lymphocyte subpopulations (cells/μL)	Patient 1	Patient 2	References values
Lymphocytes	5197	N.D.	2300-5400
T ($CD3^+$)	2910	N.D.	1400-3700
T helper ($CD4^+$)	1923	N.D.	700-2200
T cytotoxic ($CD8^+$)	987	N.D.	490-1300
B ($CD20^+$)	1559	N.D.	390-1400
NK ($CD3^-CD16^+/CD56^+$)	520	N.D.	130-720
Double-negative ($CD4^-CD8^-CD3^+\alpha\beta^+$)	67	N.D.	16-140
Serum immunoglobulins (mg/dL)		N.D.	
IgG	743.00	448.00	360-1500
IgA	30.50	29.20	21-180
IgM	70.80	115.00	24-170
Serum complement (mg/dL)			
C3	227.00	N.D.	90-180
C4	37.60	N.D.	10-40
Antineutrophil antibody	Negative	Negative	
Antinuclear antibody	Negative	Negative	

N.D., not done.

showed normal heart structure and function. At the age of 11 months, G-CSF treatment was initiated. His current treatment is 7.5 μg/kg/day leading to normalization of the absolute neutrophil counts and resolution of his recurrent infections. Family history revealed four out of eight members with congenital neutropenia and variable phenotype (Figure 1). Patient's 2 father (P3-B; Figure 1) was diagnosed with congenital neutropenia and epilepsy, with neutrophil counts of 100-600/μL. He is being treated with G-CSF at a dose of 2 μg/kg/day with a good clinical and laboratory response. His brother (P1-B; Figure 1) had severe congenital neutropenia with neutrophil count of 200/μL. He suffered from a severe life-threatening infection that led to leg amputation. Bone marrow aspiration revealed a hypoplastic marrow with severe hypoplasia of the myeloid lineage and maturation arrest. His sister (P2-B; Figure 1) has a tendency to develop mild neutropenia during febrile illnesses, with no severe infections. Patient's 2 father (P3-B; Figure 1) is of a Jewish Libyan origin and the mother healthy (S1-B; Figure 1) is of Jewish Ashkenazy/Iranian origin. Patient 2 has one healthy older sibling (S2-B; Figure 1).

3.2 | Immunological and hematological investigation

Both patients' blood counts revealed isolated neutropenia, normal serum concentrations of immunoglobulins, and negative tests for antineutrophil antibodies as well as for antinuclear antibodies (Table 1). Patient 1 also had immunological assessment of peripheral blood lymphocyte subpopulations, including double-negative T-lymphocytes and serum complement components C3 and C4, which were all normal (Table 1). In patient 1, bone marrow evaluation showed mild hypocellularity with promyelocytic maturation arrest, whereas other cell lineages were normal (Figure 2). Patient's 2 bone marrow evaluation revealed normal myelopoiesis. For both patients, no cytogenetic abnormalities were detected by the MDS FISH panel. Patient 1 had normal karyotype study; patient 2 had unsuccessful karyotype study due to nonevaluable metaphases.

3.3 | Genetic investigation

WES was performed to both families (Figure 1) and revealed a novel, de novo, in-frame deletion in the SRP54 gene, c.342-344delAAC; p.T115del, which was confirmed by direct Sanger sequencing (Figure S1A). WES did not reveal any other mutations known to be involved in congenital neutropenia. The mutation putatively affects a highly conserved amino acid located in the GTPase domain of the protein (Figure S1B). The variant is exceedingly rare and could not be found in gnomAD nor in our in-house cohort of 2000 Israeli exomes.

The presence of the same mutation in two unrelated white families, in the relatively small population of Israel, prompted exploration of identity by descent. However, no evidence of a familial relationship between the two families was found. In addition, using the 1000 Genomes Project SNPs, we investigated common SNPs surrounding the mutation. The SNP analysis for both families revealed a lack of a common haplotype, suggesting that families are unrelated to each other.

3.4 | Protein modeling and analysis

Following our genetic results, we analyzed the spatial location of the identified mutation and the hydrogen bonds formed with the corresponding GTP molecule. The number of hydrogen bonds between wild-type SRP54 and GTP is presented in Figure 3B. Figure 3A shows the spatial location of SRP54 (cyan, flat ribbon) residues K114, T115, T116, and T117 (by element, ball and stick) as well as the location of its SR (green, flat ribbon) and GTP molecules (purple, stick). Figure 3B shows the close network of hydrogen bonds formed between SRP54 residues K114, T115, and T116. The GTP molecule forms three hydrogen bonds with residue K114 (yellow), three hydrogen bonds with T115 (dashed green), and two hydrogen bonds with T116 (orange). Figure 3D shows the change in hydrogen bond formation upon T115del mutation. Similar to Figure 3A, Figure 3C shows the spatial location of SRP54 (cyan, flat ribbon) residues K114, T116, and T117 (by element, ball and stick) as well as the location of its SR (green, flat ribbon) and GTP molecules (purple, stick). Figure 3D shows the hydrogen bonds formed between the GTP molecule and residues K114, T116, and T117. It can be seen that only one of the three hydrogen bonds between K114 and the GTP molecule is left and one of the two hydrogen bonds formed between the GTP molecule and T116 remains upon the deletion of T115. In total, six hydrogen bonds are lost between SRP54

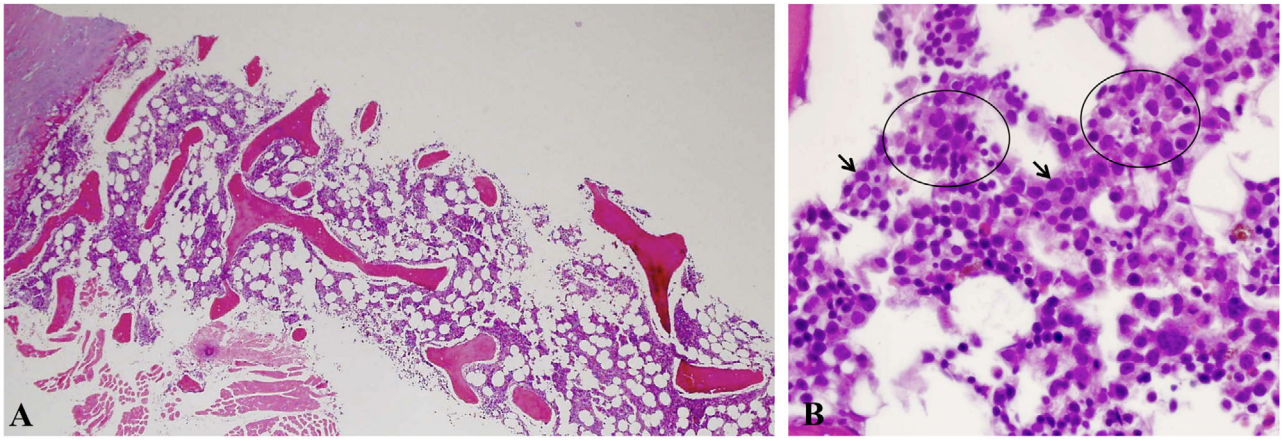

FIGURE 2 Bone marrow biopsy of patient 1. (A) Histology of the bone marrow biopsy with cellularity about 60% (hypocellular for the patient`s age). H&E, ×100. (B) Magnification of a more cellular area shows maturation arrest in promyelocytic stage (marked by ovals and arrows). H&E, ×600

residues K114, T115, T116, and the GTP molecule upon p.T115del mutation.

Overall, our structural analysis indicated that one GTP molecule forms a total of 19 hydrogen bonds with the wild-type SRP54 protein, whereas there are a total of 18 hydrogen bonds with the mutated protein. However, the residues that form the hydrogen bonds in the mutated protein are different from those of GTP molecule bonds in the wild-type structure (data not shown).

Moreover, our stability analysis indicated a ΔΔG value of 2.85. Namely, the mutation affects the stability of the complex by destabilizing it.

4 | DISCUSSION

In this study, we identified a novel, de novo, mutation in the *SRP54* gene resulting from in-frame deletion of the amino acid threonine in the highly conserved G1 GTPase domain of the protein, as the cause of congenital neutropenia in five patients from two different kindreds. Patients were of different ages and ethnicities, with a wide spectrum of immunological and clinical manifestations, ranging from mild asymptomatic neutropenia during febrile illnesses to severe neutropenia and life-threatening infection. Currently, 27 mutated cases of the SRP54 gene were reported in the literature.[4-6] Most of them resulted from *de novo* mutations as in our report. Indeed, small insertions and deletions are the second most abundant form of human genetic variation after single-nucleotide variants and comprise 18% of all recorded mutations causing human inherited disease.[15]

The SRP54 gene encodes a highly conserved protein, a key part of the ribonucleoprotein complex that mediates cotranslational targeting and translocation of proteins. SRP54 is a multidomain protein consisting of three parts: N-terminus (N domain), GTPase domain (G domain), which contains five specific G elements (G1-G5), and a C-terminal domain (M domain). The M domain attaches SRP54 to the ribosome-nascent chain complex, whereas the G and N domains target the complex to the endoplasmic reticulum by GTP-dependent interaction between SRP54 and the membrane-bound SRP54 receptor. Thus, this dynamic mechanism couples synthesis of proteins to their translocation across membranes or insertion into membranes.[16,17]

From a protein functional point of view, Carapito et al[4] suggested that the p.T115A missense mutation directly affected GTP binding due to a loss of hydrogen bonds between T115 and the GTP molecule. Additionally, bone marrow mononuclear cells from the reported patient had a nearly complete abolished GTPase activity. Given that the novel p.T115del mutation affects the same highly conserved position within the GTPase domain of the protein, which has a crucial rule in GTP and receptor binding, and causes the loss of six hydrogen bonds between SRP54 and the GTP molecule, it is most likely that GTP binding is impaired. Moreover, our stability analysis results indicate a less stable complex, which further support the possibility of impaired interaction with the GTP molecule. Previous studies have shown that GTP binding to both SRP54 and SR leads to the formation of a stable "docked complex," which is necessary[18] but not sufficient[19] to promote release of the signal peptide from SRP. Considering the probable reduced affinity to the GTP molecule, our stability analysis may further support the influence of reduced GTP binding on complex stability and protein activity.

Our findings further highlight the phenomenon of variable expressivity in primary immunodeficiencies in general and in congenital neutropenia specifically. Previous studies, in genes associated with SCID, including *RAG1/2*, *ADA*, and *DCLRE1C*[20-25] and genes associated with CN, including *ELANE*[26,27] and *SBDS*,[28] revealed a variable clinical phenotypes in individuals with the same genetic mutation, even in the same family. Moreover, subanalysis of the cohort published by Bellanné-Chantelot et al[5] reveals intrafamilial and interfamilial immunological and clinical variability within patients harboring the same mutation. Currently, the reason for variable expressivity in

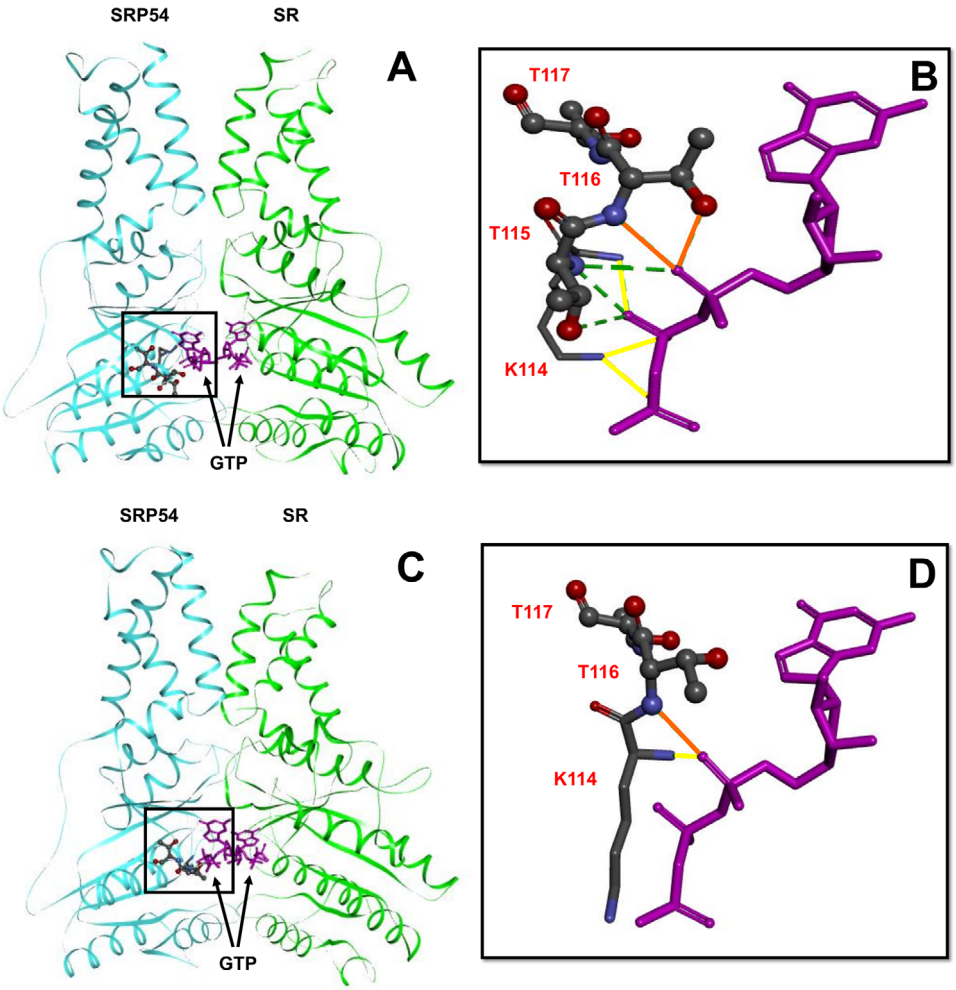

FIGURE 3 Molecular model of the N and G domains of WT and mutated SRP54, its SR, and corresponding GTP molecules. (A) SRP54, SR, and GTP are in cyan, green, and purple, respectively. Residues K114, T115, T116, and T117 are shown ball and stick by charge. (B) Close-up of the WT residues K114, T115, T116, and T117. Hydrogen bonds between the corresponding GTP and K114, T115, and T116 are presented in yellow, dashed green, and orange, respectively. According to the model, eight hydrogen bonds are formed between these residues and the GTP molecule. (C) SRP54, SR, and GTP are in cyan, green, and purple, respectively. Residues K114, T115, T116, and T117 are shown ball and stick by charge. (D) Close-up of mutated residues K114, T116, and T117. Hydrogen bonds between the corresponding GTP and SRP54 residues K114 and T116 are presented in yellow and orange, respectively. According to the model, all hydrogen bonds between deleted T115 and the corresponding GTP molecule are lost. Moreover, two of three hydrogen bonds formed with K114, and one of the two hydrogen bonds between the GTP molecule and T116 are lost

patients with SRP54 gene mutations is unknown. A possible explanation could be the influence of genetic modifiers in neutrophils. For example, expression quantitative trait loci (eQTL) are genomic regions that regulate gene expression at the genetic and epigenetic levels.[29] Neutrophil eQTL are markedly enriched for trait-associated variants especially in patients with autoimmune, immune dysregulation, and infectious diseases.[30] Furthermore, age and sex affected the human transcriptional responses to microbial challenges.[31] Moreover, several studies report that the transcription factors, LEF-1,[32] GFI-1,[33] C/EBPβ,[34] the granulocyte colony-stimulating factor receptor[35] and the activation of genes related to the Wnt3a/β-catenin pathway[36] are all linked to granulocytes homeostasis and severe congenital neutropenia phenotype and therefore may act as modifying genes.

5 | CONCLUSIONS

We describe a novel mutation in the *SRP54* gene resulting from an in-frame, de novo, deletion of the amino acid threonine in position 115 of the protein as a cause of congenital neutropenia. Our analysis suggests that the mutation impair protein activity through reduced GTP binding and complex stability. Moreover, we show that the same genetic mutation can lead to congenital neutropenia with variable phenotypic expressivity.

HUMAN AND ANIMAL RIGHTS STATEMENT

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or

national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interests.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Lior Goldberg https://orcid.org/0000-0002-1643-7616
Orna Steinberg-Shemer https://orcid.org/0000-0002-1680-2388

REFERENCES

1. Segel GB, Halterman JS. Neutropenia in pediatric practice. *Pediatr Rev*. 2008;29(1):12-23. quiz 24.
2. Donadieu J, Beaupain B, Fenneteau O, Bellanne-Chantelot C. Congenital neutropenia in the era of genomics: classification, diagnosis, and natural history. *Br J Haematol*. 2017;179(4):557-574.
3. Donadieu J, Beaupain B, Mahlaoui N, Bellanne-Chantelot C. Epidemiology of congenital neutropenia. *Hematol Oncol Clin North Am*. 2013;27(1):1-17.
4. Carapito R, Konantz M, Paillard C, et al. Mutations in signal recognition particle SRP54 cause syndromic neutropenia with Shwachman-Diamond-like features. *J Clin Invest*. 2017;127(11):4090-4103.
5. Bellanné-Chantelot C, Schmaltz-Panneau B, Marty C, et al. Mutations in the SRP54 gene cause severe congenital neutropenia as well as Shwachman-Diamond-like syndrome. *Blood*. 2018;132(12):1318-1331.
6. Carden MA, Connelly JA, Weinzierl EP, Kobrynski LJ, Chandrakasan S. Severe congenital neutropenia associated with SRP54 mutation in 22q11.2 deletion syndrome: hematopoietic stem cell transplantation results in correction of neutropenia with adequate immune reconstitution. *J Clin Immunol*. 2018;38(5):546-549.
7. Focia PJ, Shepotinovskaya IV, Seidler JA, Freymann DM. Heterodimeric GTPase core of the SRP targeting complex. *Science*. 2004;303(5656):373-377.
8. Elvekrog MM, Walter P. Dynamics of co-translational protein targeting. *Curr Opin Chem Biol*. 2015;29:79-86.
9. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25(14):1754-1760.
10. McKenna A, Hanna M, Banks E, et al. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20(9):1297-1303.
11. Li MX, Gui HS, Kwan JS, Bao SY, Sham PC. A comprehensive framework for prioritizing variants in exome sequencing studies of Mendelian diseases. *Nucleic Acids Res*. 2012;40(7):e53.
12. Waterhouse A, Bertoni M, Bienert S, et al. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res*. 2018;46(W1):W296-W303.
13. Guerois R, Nielsen JE, Serrano L. Predicting changes in the stability of proteins and protein complexes: a study of more than 1000 mutations. *J Mol Biol*. 2002;320(2):369-387.
14. Schymkowitz J, Borg J, Stricher F, Nys R, Rousseau F, Serrano L. The FoldX web server: an online force field. *Nucleic Acids Res*. 2005;33(Web Server issue):W382-388.
15. Zhang X, Lin H, Zhao H, et al. Impact of human pathogenic micro-insertions and micro-deletions on post-transcriptional regulation. *Hum Mol Genet*. 2014;23(11):3024-3034.
16. Halic M, Beckmann R. The signal recognition particle and its interactions during protein targeting. *Curr Opin Struct Biol*. 2005;15(1):116-125.
17. Wild K, Halic M, Sinning I, Beckmann R. SRP meets the ribosome. *Nat Struct Mol Biol*. 2004;11(11):1049-1053.
18. Gowda K, Chittenden K, Zwieb C. Binding site of the M-domain of human protein SRP54 determined by systematic site-directed mutagenesis of signal recognition particle RNA. *Nucleic Acids Res*. 1997;25(2):388-394.
19. Pool MR, Stumm J, Fulga TA, Sinning I, Dobberstein B. Distinct modes of signal recognition particle interaction with the ribosome. *Science*. 2002;297(5585):1345-1348.
20. Delmonte OM, Schuetz C, Notarangelo LD. RAG deficiency: two genes, many diseases. *J Clin Immunol*. 2018;38(6):646-655.
21. Notarangelo LD, Kim MS, Walter JE, Lee YN. Human RAG mutations: biochemistry and clinical implications. *Nat Rev Immunol*. 2016;16(4):234-246.
22. Picard C, Bobby Gaspar H, Al-Herz W, et al. International union of immunological societies: 2017 primary immunodeficiency diseases committee report on inborn errors of immunity. *J Clin Immunol*. 2018;38(1):96-128.
23. Shovlin CL, Hughes JM, Simmonds HA, et al. Adult presentation of adenosine deaminase deficiency. *Lancet*. 1993;341(8858):1471.
24. Volk T, Pannicke U, Reisli I, et al. DCLRE1C (ARTEMIS) mutations causing phenotypes ranging from atypical severe combined immunodeficiency to mere antibody deficiency. *Hum Mol Genet*. 2015;24(25):7361-7372.
25. Buchbinder D, Baker R, Lee YN, et al. Identification of patients with RAG mutations previously diagnosed with common variable immunodeficiency disorders. *J Clin Immunol*. 2015;35(2):119-124.
26. Germeshausen M, Deerberg S, Peter Y, Reimer C, Kratz CP, Ballmaier M. The spectrum of ELANE mutations and their implications in severe congenital and cyclic neutropenia. *Hum Mutat*. 2013;34(6):905-914.
27. Newburger PE, Pindyck TN, Zhu Z, et al. Cyclic neutropenia and severe congenital neutropenia in patients with a shared ELANE mutation and paternal haplotype: evidence for phenotype determination by modifying genes. *Pediatr Blood Cancer*. 2010;55(2):314-317.
28. Kuijpers TW, Alders M, Tool AT, Mellink C, Roos D, Hennekam RC. Hematologic abnormalities in Shwachman Diamond syndrome: lack of genotype-phenotype relationship. *Blood*. 2005;106(1):356-361.
29. Chen L, Ge B, Casale FP, et al. Genetic drivers of epigenetic and transcriptional variation in human immune cells. *Cell*. 2016;167(5):1398-1414 e1324.
30. Naranbhai V, Fairfax BP, Makino S, et al. Genomic modulators of gene expression in human neutrophils. *Nat Commun*. 2015;6:7545.
31. Piasecka B, Duffy D, Urrutia A, et al. Distinctive roles of age, sex, and genetics in shaping transcriptional variation of human immune responses to microbial challenges. *PNAS*. 2018;115(3):E488-E497.
32. Skokowa J, Fobiwe JP, Dan L, Thakur BK, Welte K. Neutrophil elastase is severely down-regulated in severe congenital neutropenia independent of ELA2 or HAX1 mutations but dependent on LEF-1. *Blood*. 2009;114(14):3044-3051.
33. Person RE, Li FQ, Duan Z, et al. Mutations in proto-oncogene GFI1 cause human neutropenia and target ELA2. *Nat Genet*. 2003;34(3):308-312.

34. Hirai H, Yokota A, Tamura A, Sato A, Maekawa T. Non-steady-state hematopoiesis regulated by the C/EBPbeta transcription factor. *Cancer Sci*. 2015;106(7):797-802.
35. Qiu Y, Zhang Y, Hu N, Dong F. A truncated granulocyte colony-stimulating factor receptor (G-CSFR) inhibits apoptosis induced by neutrophil elastase G185R mutant: implication for understanding csf3r gene mutations in severe congenital neutropenia. *J Biol Chem*. 2017;292(8):3496-3505.
36. Hiramoto T, Ebihara Y, Mizoguchi Y, et al. Wnt3a stimulates maturation of impaired neutrophils developed from severe congenital neutropenia patient-derived pluripotent stem cells. *PNAS*. 2013;110(8):3023-3028.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Goldberg L, Simon AJ, Rechavi G, et al. Congenital neutropenia with variable clinical presentation in novel mutation of the SRP54 Gene. *Pediatr Blood Cancer*. 2020;67:e28237. https://doi.org/10.1002/pbc.28237