Congenital Neutropenia With Specific Granulocyte Deficiency Caused By Novel Double Heterozygous SMARCD2 Mutations: is there a benefit of thrombopoietin receptor agonist therapy?

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Abstract

SMARCD2 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily D, member 2) is critical for myelopoiesis. Recently, bi-allelic SMARCD2 mutations have been reported in five children causing autosomal recessive congenital neutropenia with specific granulocytes deficiency (CN-SGD); a syndrome resulting in G-CSF resistant neutropenia, recurrent infections and dysplastic myelopoiesis. We report a new case with CN-SGD caused by two novel heterozygous pathogenic variants in the SMARCD2 gene (c.1081del (p.Gln361Argfs*15), and c.217C>T (p.Arg73*)). Treatment with weekly dosing of thrombopoietin receptor agonist, Romiplostim, along with daily G-CSF transformed her clinical course implying potential synergism. This report advances understanding about CN-SGD caused by SMARCD2 mutations.

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Abstract

SMARCD2 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily D, member 2) is critical for myelopoiesis. Recently, bi-allelic *SMARCD2* mutations have been reported in five children causing autosomal recessive *c* ongenital*n* eutropenia with *s* pecific *g* ranulocytes*d* eficiency (CN-SGD); a syndrome resulting in G-CSF resistant neutropenia, recurrent infections and dysplastic myelopoiesis. We report a new case with CN-SGD caused by two novel heterozygous pathogenic variants in the *SMARCD2* gene*c.1081del (p.Gln361Argfs*15), and c.217C>T (p.Arg73*))* . Treatment with weekly dosing of thrombopoietin receptor agonist, Romiplostim, along with daily G-CSF transformed her clinical course implying potential synergism. This report advances understanding about CN-SGD caused by *SMARCD2*mutations.

Abbreviations:

Abbreviation	Expansion	Abbreviation	Expansion
ANC	Absolute neutrophil count	**HSPC**	Hematopoietic stem progenito
SAA	Severe aplastic anemia	**MDS**	Myelodysplastic syndrome
CN-SGD	Congenital neutropenia with specific granulocyte deficiency	**SCN**	Severe congenital neutropenia
G-CSF	Granulocyte Colony Stimulating Factor	SMARCD2	SWI/SNF-related, matrix-asso
HSCT	Hematopoietic stem cell transplant		

Introduction

The *SMARCD2* (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily D, member 2) gene plays an important in role chromatin remodeling and myeloid differentiation in humans, zebrafish and mice.[1] Recently, homozygous*SMARCD2* mutations were reported to cause autosomal recessive*c* ongenital *n* eutropenia with *s* pecific*g* ranulocyte *d* eficiency (CN-SGD)[1-3], a syndrome characterized by dysplastic myelopoiesis and sub-optimal phagocytotic activity leading to recurrent skin and deep-seated pyogenic infections.[4] These children present with life-threatening infections and granulocyte colony stimulating factor (G-CSF) resistant neutropenia requiring treatment with hematopoietic stem cell transplantation (HSCT). Other features include facial and skeletal dysmorphism such as misaligned teeth and brittle nails in addition to developmental delay and learning difficulties.[1,2] To date, five patients with CN-SGD have been reported and understanding about the genotype-phenotype relationship is still evolving.[2,5,6] In this report, we elaborate the clinical course and management of a pediatric patient with CN-SGD caused by novel double heterozygous mutations within*SMARCD2.* This is the sixth case with CN-SGD caused by*SMARCD2* mutations.

Case presentation

A 12-year-old African American girl was diagnosed with severe congenital neutropenia (SCN) at the age of 2-years. She presented with recurrent infections since early infancy, absolute neutrophil count (ANC) $<$ 0.5 $10^3/mm^3$ and promyelocyte arrest in bone marrow. She had chronic mucopurulent rhinosinusitis, recurrent/chronic otitis media and an extensive history of multiple bouts of superficial skin abscesses and deep cellulitis requiring multiple hospitalizations. She had bilateral cervical and submandibular lymphadenopathy, gingival hypertrophy, and chronic stomatitis, "cauliflower ear" deformity. Her growth and development was normal for her chronological age. Genetic testing was negative, including normal karyotype, myelodysplastic syndrome (MDS) panel, elastase-2 gene, and *HAX-1* gene mutations. Her immunoglobulin levels and lymphocyte subset analyses were normal. She was commenced on G-CSF which required dose increment to 20 microgram/kg/day due to apparent G-CSF resistance. Multiple blood smears showed Pelger-Huet anomaly, monocytosis and intermittent circulating blasts. She had several surveillance bone marrow evaluations, cytogenetics and Fluorescence in situ hybridization (FISH) studies to rule out MDS/leukemia. Bone

marrow evaluations since age 6 years showed dysmegakaryopoiesis along with promyelocyte arrest and mild increase in reticulin fibrosis.

At age 11-years, she had sixteen hospitalizations with pseudomonas sepsis, Escherichia coli sepsis, candida infection and rotavirus gastroenteritis. She had sacral abscess, multiple vaginal and perianal ulcerations, rectal abscess, upper and lower GI bleeding requiring multiple red cell transfusions. One of her hospitalizations was complicated by acute kidney injury and hypertension. Renal biopsy confirmed diagnosis of post-infectious glomerulonephritis potentially from a skin infection and was treated with steroids.

Since she had suboptimal and inconsistent response to G-CSF treatment throughout this period, the option of haploidentical HSCT from her relative was considered as she lacked full matched donor. To improve her ANC, thrombopoietin receptor agonist (TPO-RA) Romiplostim was started at 5 microgram/kg/week due to its promising action on HSCs in patients with severe aplastic anemia(SAA).[5,7] Romiplostim was chosen due to its parenteral mode of administration ensuring absorption. Upon addition of Romiplostim along with G-CSF (10 mcg/kg/day), her ANC recovered in 8- weeks (Figure 1) with improvement in her gingival hypertrophy and oral lesions (Figure 2). An attempt to reduce her G-CSF dose to 5 mcg/kg/day quickly plummeted her ANC requiring increment in dosing. She remained on this combination regimen with G-CSF at 10 mcg/kg/day and Romiplostim at 5 mcg/kg/week for 6-months. Her bone marrow showed mild to moderate reticulin fibrosis. She relocated and Romiplostim was discontinued. She underwent myeloablative haplo-identical HSCT from her half-sibling 3-months after discontinuation of Romiplostim. Pretransplant bone marrow testing showed reduction in her reticulin fibrosis. She is 1-year since her HSCT and had full engraftment.

Due to complexity of her clinical course, exome sequencing was performed using genomic DNA[8] which revealed two heterozygous pathogenic variants in geneNM_001098426.1 [c.1081del (p.Gln361Argfs*15)and c.217C>T (p.Arg73*)] explaining the etiology of her SCN.

Discussion

This report shares the clinical course of a patient with CN-SGD caused by compound heterozygous mutations within the *SMARCD2* gene. The clinical characteristics and bone marrow findings of our patient align with the previously described phenotype except she had normal growth and neuro-development. Unlike previously reported cases, our patient developed clinical findings like Bechet disease and post-infectious glomerulonephritis. Our patient showed evidence of early myelofibrosis at the age of 6-years, even before commencement of TPO-mimetic therapy. As expected, the myelofibrosis worsened after commencement of Romiplostim but reduced within 3-months after discontinuation of Romiplostim.[9,10]

Table 1 summarizes the previously mutations within *SMARCD2* in five cases with CN-SGD. The two heterozygous novel variants that were identified in our case are considered pathogenic as both sequence changes create a premature translational stop signal in the gene that result in an absent or disrupted protein product. Both variants in the gnomAD population database (https://gnomad.broadinstitute.org/) and had not been reported in the literature in individuals with -related conditions. The molecular mechanisms contributing to of *SMRACD2*related SCN and immune deficiency are well characterized.[2,3] *SMARCD2* -deficient bone marrow-derived CD34+ cells have impaired in vitro expansion and differentiation toward the neutrophilic lineage causing SCN.[3] Furthermore, the *SMARCD2* -deficient neutrophils are shown to have sub-optimal phagocytic activity due to severely impaired chemotactic response which in turn causes abnormal disaggregation, locomotion, and defective in-vitro-killing of Staphylococcus aureus, lack lactoferrin leading to susceptibility towards infection.[2,4] Interestingly,*SMARCD2* -deficient neutrophils show normal levels of myeloperoxidase and normal oxidative burst response. Furthermore, The phenotype of CN-SGD caused by *SMARCD2* [1] is similar to that caused by five-base pair deletion within the second exon of the *CEBPε* (CCAAT/enhancer binding protein epsilon) gene.[11] The *SMARCD2* gene product functions as a controller of early myeloid–erythroid progenitor cellular differentiation via interaction with transcription factor *CEBPε*. [1] As a result, mutations within these two genes lead to similar downstream effects and cause clinically similar phenotypes.[12] Unlike patients with *CEBPε*deficiency, most of the reported patients who had *SMARCD2*deficiency showed evidence

of myelodysplasia. Therefore, it is critical to identify the underlying molecular events to better characterize the outcome in children with CN-SGD. These differences can be attributed to the role of *SMARCD2* in controlling early stage differentiation of HSCs toward neutrophil granulocytes, while *CEBPε* controls terminal differentiation of neutrophils.[13]

Our patient's clinical course was altered after addition of Romiplostim, TPO-RA. The rationale behind using Romiplostim in this desperate clinical setting deserves discussion. Thrombopoietin and TPO-RA induce hematopoietic stem and progenitor cells (HSPCs) through binding with the receptor *c-mpl* [14] and regulate hematopoiesis through its pleotropic actions.[15] These findings led to clinical trials evaluating TPO-RA as a therapeutic option for SAA with encouraging outcomes.[5,7,16-18] The dosing regimen in SAA was higher than immune thrombocytopenic purpura and ranged from 5 to20 mcg/kg/dose.[19] We hypothesized that Romiplostim will help induce HSPCs and provide synergistic effect to G-CSF, a lineage-specific chemokine.[20] An attempt to reduce G-CSF dose immediately dropped our patient's ANC which indirectly provides evidence that Romiplostim helped induce proliferation of HSPCs, yet higher dose of G-CSF was needed for subsequent maturation of granulopoiesis. While we do not have mechanistic data to support our hypothesis, it is possible that Romiplostim may offer a synergistic therapeutic benefit to children with SCN/CN-SGD who are resistant to G-CSF. Clearly caution needs to be applied prior to using combination therapy as it may increase risk of progression towards MDS/leukemia. In conclusion, we share the clinical course of a patient with CN-SGD caused by novel heterozygous mutations within *SMRCD2* and short-term benefit of combination therapy with G-CSF and Romiplostim.

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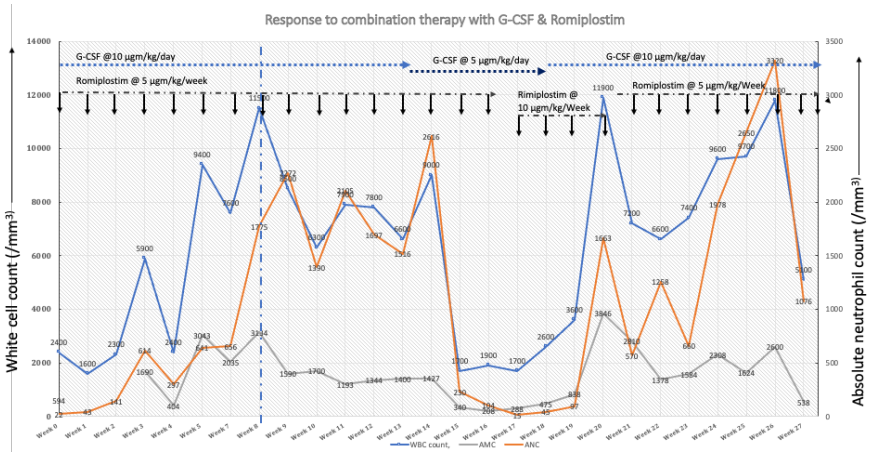
Table 1: Review of literature about severe congenital neutropenia caused by *SMARCD2* mutations. The two siblings, the first mutation produces three aberrant transcripts. All mutations resulted in frameshifts and premature sequence termination.

Author	Case number	Molecular mutations within *SMARCD2* gene	Molecular mutations within
		Nucleotide change	**Protein change**
Witzel, and Engelhardt et al[2]	Case 1	c.1181+1G>A	Ile362Cysfs*3 Ser394Argfs*
Witzel et al[1]	Case 2	c.401+2T>C	p.Arg73Valfs*8
Witzel et al[21]	Case 3 & 4	c.414_438dup*	p.Gln147Glufs*5
Yucels et al[3]	Case 5	c. 93del	p.Ala32Argfs*80
Our case	Case 6	c.217C>T c.1081del	p.Arg73* p.Gln361Argfs*15

Abbreviations: SNS: single nucleotide substitution,

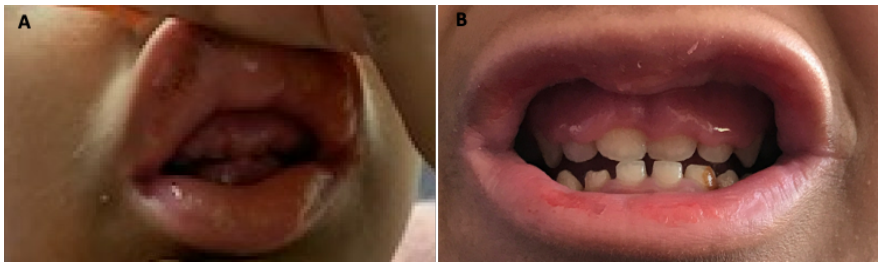
Figure 1: White cell, neurtophil and monocyte response addition of Romiplostim during first 6-months of

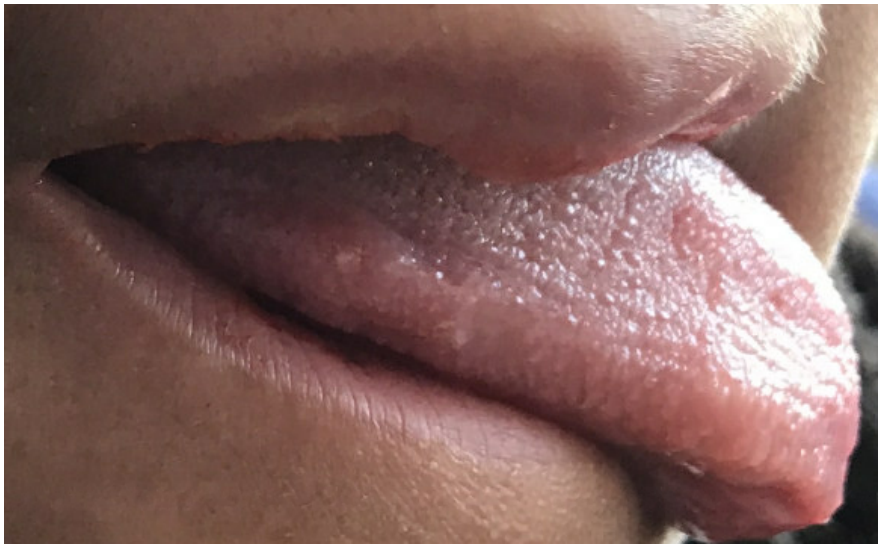
therapy. Reduction in dosage of G-CSF plummeted neutrophil response during week 17 through 19th.

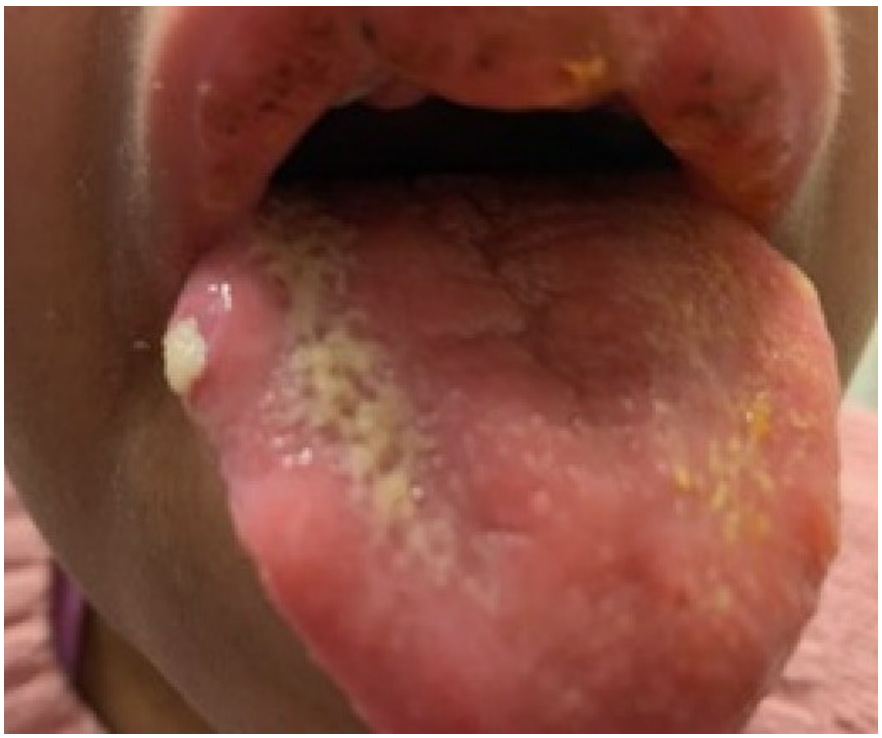


Abbreviations: G-CSF: Granulocyte colony stimulating factor; WBC: white blood cell count; ANC: absolute neutrophil count; AMC: absolute monocyte count

Figure 2: Clinical photographs showing improvement in gingival hypertrophy after addition of Romiplostim: A. Gingival hypertrophy covering teeth before commencement of Romiplostim; B. Improvement in gingival hypertrophy after 12 weeks of therapy with Romiplostim and G-CSF; C: Stomatitis before therapy; D. Resolution of stomatitis 8 weeks after Romiplostim therapy







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