

## Mosaic Tetraploidy and Transient *GFI1* Mutation in a Patient With Severe Chronic Neutropenia

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This report presents the case of a 15-year-old male with severe chronic neutropenia, leukopenia, and persistent tetraploid mosaicism in the bone marrow and peripheral blood. His father had mild neutropenia and bone marrow tetraploidy. Flow cytometric analysis of DNA content peripheral blood showed tetraploidy in 20% of granulocytes and 15% of monocytes. Sequence analysis of the *ELA2*

gene was normal, but the *GFI1* gene exhibited transient appearance of single base changes the coding region and promoter. We speculate that an underlying genetic defect, inherited in an autosomal dominant pattern, leads to both disordered mitosis and neutropenia in this kindred. *Pediatr Blood Cancer* 2008;50:630–632. © 2006 Wiley-Liss, Inc.

**Key words:** agranulocytosis; congenital neutropenia; cytogenetics; DNA index; *GFI1*; leukopenia

### INTRODUCTION

Severe congenital neutropenia (SCN; OMIM #202700), also known as Kostmann disease, is a rare disorder characterized by peripheral blood agranulocytosis and maturation arrest of neutrophils within the bone marrow at the promyelocytic stage [1,2]. Most patients respond to treatment with granulocyte colony-stimulating factor (G-CSF; filgrastim), but retain an increased risk for death from sepsis and for development of myelodysplastic syndrome and acute myeloid leukemia (MDS/AML) [3].

Several reports have described karyotype abnormalities associated with this syndrome, most typically clonal loss of chromosome 7 or abnormalities of chromosome 21; these changes have been associated with transformation to MDS/AML [1,2,4]. We present a case with SCN and persistent tetraploid mosaicism in the bone marrow and peripheral blood leukocytes, and transient appearance of mutations in the *GFI1* gene.

### CASE HISTORY

The patient is a 15-year-old male, diagnosed with SCN at age 2 years after repeated upper respiratory infections. Infectious complications included frequent cellulitis, recurrent sinusitis, recurrent otitis media, mastoiditis, and one episode of typhlitis at age 14 requiring hospitalization and prolonged IV antibiotics. His total white blood cell counts, performed two to six times per year since infancy, ranged between 0.8 and  $1.1 \times 10^9/L$ , with absolute neutrophil counts of  $0.1–0.2 \times 10^9/L$  and absolute lymphocyte counts of  $0.5–1.2 \times 10^9/L$ . Other hematological parameters have been normal. Yearly bone marrow examinations showed marked myeloid hypoplasia with maturation arrest at the promyelocyte stage and large, dysmorphic promyelocytes and myeloblasts. Treatment with G-CSF, up to doses of 100  $\mu\text{g}/\text{kg}/\text{day}$ , failed to induce any increase in the neutrophil count. His medical history includes an auditory processing disorder with normal school performance. He has shown normal growth and development, with weight at the 95th percentile and height 25th percentile at age 15, and head circumference 98th percentile at age 13.

The family history includes mild neutropenia in his father, who also had a monoclonal gammopathy of unknown significance at age 44. Bone marrow examination showed mild myeloid hypoplasia with left-shifted myeloid maturation. His paternal grandfather died

of leukemia at age 40. His mother had a grade I follicular lymphoma at age 42. Two siblings have normal granulocyte counts.

### LABORATORY FINDINGS

Bone marrow, peripheral blood, and skin biopsy specimens were analyzed by routine cytogenetic methods [5]. Bone marrow cytogenetics, performed on 24–48 hr bone marrow cultures without mitogens, showed 34–68% tetraploid cells. Prior to 1997, the clinical cytogenetics laboratory reported several bone marrow specimens as 46, XY, but it is likely that tetraploid cells were misinterpreted as culture artifacts at that time. Peripheral blood cytogenetic analysis, performed on a specimen cultured with phytohemagglutinin, revealed tetraploidy in 14% of cells. No tetraploidy was detected in a culture of the patient's skin fibroblasts. Peripheral blood chromosomal breakage analysis using diepox-ybutane was normal. Among normal control samples, no tetraploid cells were detected in five pathologically normal bone marrow specimens; in five peripheral blood specimens referred for the

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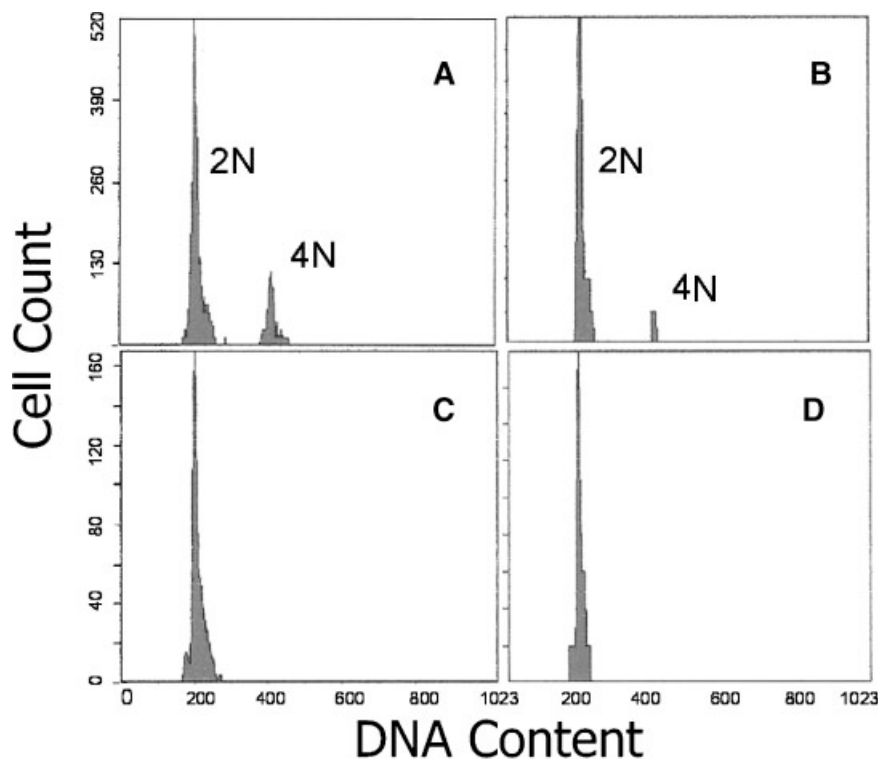
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**Fig. 1.** Flow cytometric analysis of cellular DNA content. Studies were performed on whole blood as previously described [6], on a FC 500 flow cytometer (Beckman Coulter, Hialeah, FL). Single cells were discriminated from cell clumps by a doublet discrimination model (pulse area vs. pulse width). Panel (A): CD13+ granulocytes, Panel (B): CD14+ monocytes, Panel (C): CD3+ T cells, Panel (D): CD20+ B cells. 2N and 4N refer to diploid and tetraploid DNA indices, respectively.

indication of repeat spontaneous abortion, 2% tetraploidy was found in one sample and no tetraploid cells in the others.

Flow cytometric analysis of the DNA content was performed on unstimulated peripheral blood using a double staining technique for surface CD45, CD13, CD14, CD20, CD3, and nuclear DNA, as previously described [6]. Analysis of CD45+ nucleated cells by lineage specific markers (Fig. 1) showed that 20% of CD13+ granulocytes (panel A), and 15% CD14+ monocytes were tetraploid (panel B), while CD3+ T cells and CD20+ B cells were all diploid (panels C and D).

Sequence analysis for mutations in the *ELA2* gene [7], revealed no mutations in the patient's genomic DNA. However, two mutations appeared transiently in the *GFII* gene, a rare site of dominant negative mutations causing SCN [8]. As shown in Table I (sequencing chromatograms are available as Figure 2 in the Supplemental Materials link), two mutations were repeatedly detected in bone marrow and peripheral blood samples from 2003, but not in samples from 2001, 2004 or 2006. The 2006 materials also included DNA from a buccal swab. In the 2003 sequences, the G → A base transition in exon 1 of the coding region results in a missense substitution from a serine amino acid residue to asparagine in position 36 (S36N). The A → C transversion in the promoter region of the *GFII* gene (lower panels) may alter the binding of the myeloid zinc finger gene transcription factor MZF1, which is expressed in hematopoietic progenitor cells committed to myeloid lineage differentiation [9]. Sequence analysis of the G-CSF receptor gene—performed on samples from 2001, 2003, and 2006—showed no abnormalities.

The patient was also evaluated for possible dyskeratosis congenita [10]. The *hTERC* gene was normal in sequence and the *DKC1* gene contained a novel sequence alteration in the 3' non-coding region. However, leukocyte telomere length [11] was normal.

## DISCUSSION

The patient described in this report presents a unique combination of phenotype and genotype, with an unusual form of G-CSF-unresponsive SCN with mosaic tetraploidy in bone marrow and peripheral blood, plus transient appearance of mutations in the *GFII* gene. Constitutional chromosomal abnormalities have not been reported in association with SCN, but patients progressing to MDS/AML often acquire clonal loss of chromosome 7 or other changes.

Autosomal dominant and sporadic in occurrence SCN has been associated with mutations in *ELA2*, found in 35–84% of cases. The disease has also been attributed to mutations in the *GFII* and *WAS* (Wiskott-Aldrich syndrome) [12]. *GCSFR* (G-CSF receptor) gene mutations in SCN generally represent acquired changes preceding evolution to MDS/AML [1]. The transient appearance of two mutations in the *GFII* gene in the present case may represent the emergence and loss of a hematopoietic clone with the mutations. The finding raises the possibility that *GFII* mutations reported in other neutropenic patients [8] may also be transient.

The finding of mosaicism in our patient and his father suggests that it represents a congenital propensity towards acquiring

TABLE I. Transient, Novel *de novo* Mutations in the *GFII* Gene

	Year of study			
	2001	2003	2004	2006
Coding region	CCTAG <u>CC</u> CGA	CCTA <u>ACC</u> CGA	CCTAG <u>CC</u> CGA	CCTAG <u>CC</u> CGA
Amino acid 36	Serine	Asparagine	Serine	Serine
Promoter region	GGAAAGTGC	GGAA <u>C</u> GTGC	GGAAAGTGC	GGAAAGTGC

DNA sequencing demonstrated a missense mutation in exon 1 of the *GFII* gene resulting in a substitution of serine amino acid residue in position 36 to asparagine (S36N), as well as a mutation in the promoter region, at nucleotide -45 of the *GFII* gene. Underlining indicates the codon for amino acid 36 and the rectangular box outlines the substituted nucleotide in each mutated sequence. As indicated in the top margin, these mutations were detectable only in samples from 2003, but not from 2001 or 2006.

tetraploidy. However, cytogenetic evaluation of the patient's skin fibroblasts detected no tetraploidy, indicating failure of the putative tetraploid embryonic cells to contribute to that lineage. As the patient showed no phenotypic feature of constitutional mosaic tetraploidy [13–17], the mosaicism is probably limited to few or no lineages beyond hematopoietic cells, as noted previously in a case with isolated bone marrow tetraploidy [16]. Two studies have reported maternal tetraploid mosaicism in families of children with pure or mosaic tetraploidy, suggesting the presence of a hereditary predisposition to mitotic non-disjunction [13,14].

Our patient's father also had a history of neutropenia, suggesting a common underlying genetic cause for both conditions in this kindred. The finding of tetraploidy in peripheral blood granulocytes and monocytes indicates that the affected hematopoietic progenitor cells are capable of differentiation, so maturational failure of the cells is unlikely to have produced the observed neutropenia. A proliferative defect limited to the subpopulation of tetraploid cells also fails to explain the hematological findings, as all myeloid progenitors are hypoplastic and unresponsive to G-CSF.

We speculate that an underlying genetic defect leads to both leukopenia and disordered mitosis (but not genomic instability leading to aneuploidy or chromosomal breakage) in this kindred. Although tetraploidy has been associated with AML, and this patient's refractoriness to G-CSF suggests an increased risk of leukemic conversion [3], so far no evolution to MDS/AML has been detected.

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