T-cell clonality and myelodysplasia without chromosomal fragility in a patient with features of Seckel syndrome

Seckel syndrome is a rare autosomal recessive disorder with characteristic craniofacial dysmorphism, skeletal defects, mental and prenatal growth retardation. About 50 cases have been reported in the literature. Hematologic abnormalities with associated chromosomal fragility have been noted in about 15% of the reported cases. We report a patient with Seckel syndrome with myelodysplastic features and clonal T-cells in the bone marrow but no evidence of chromosomal fragility. After 5 years of follow-up, this patient remains asymptomatic without any treatment and with stable peripheral blood counts.

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Introduction. Seckel syndrome is a rare autosomal recessive disorder first described by Seckel in 1960 , and is associated with characteristic craniofacial dysmorphism (prominent beaked nose and receding chin—bird headed dwarfism), mental deficiency, microcephaly, prenatal growth retardation and skeletal defects. About 50 cases have been described in the literature. Heterogeneity is consistently noted in the description of these cases, suggesting a spectrum of *Seckel* conditions that share common key features yet demonstrate a wide range of phenotypic variability. Many reports have cited anomalies in cardiovascular, hematologic , endocrine, gastrointestinal, and central nervous systems along with the characteristic skeletal deformities. Gene(s) responsible for this syndrome have not been identified. About 15% of the reported cases developed hematologic abnormalities, namely pancytopenia with hypoplastic marrow, myelodysplasia (MDS) or acute myelogenous leukemia , indicating that this syndrome may be part of a group of hereditary syndromes with primordial dwarfism and/or skeletal defects associated with hematologic defects. The best known syndrome of this class is Fanconi's anemia (FA), which is characterized by skeletal defects and aplastic anemia, often progressing to myelodysplasia and acute leukemia, and by chromosomal fragility , demonstrated *in vitro* as chromosomal breakage induced by mitomycin C (MMC) or diepoxybutane (DEB). There are at least eight FA complementation groups, of which genes for three (A, C, G) have been cloned. Mutations in these genes are considered to be responsible for the clinical syndrome in FA . Chromosomal fragility resulting in accumulation of genetic aberrations in FA has been proposed as a possible oncogenic mechanism. Several investigators have reported increased chromosomal fragility (by MMC breakage analysis) in the bone marrow cells of patients with Seckel syndrome and hematologic disorders and have suggested an analogy to the chromosomal fragility syndromes such as Fanconi's anemia. These reports raised the possibility that a mechanism similar to that of FA may be responsible for the pathogenesis of myelodysplasia or acute leukemia in these reported cases of Seckel syndrome. We report a patient with features of Seckel syndrome who developed myelodysplasia. This patient did not have chromosomal fragility by the DEB test but did have clonal T-cells in the bone marrow. *Case Report.* M.S. is a 25 year-old Hispanic male of Puerto Rican descent who was diagnosed with Seckel syndrome at the age of 6 weeks. There was no consanguinity, history of prenatal drug exposure, or history of any perinatal complications. Detailed birth history is not available, but he was very small at birth. In April 1993 he was noted to have mild macrocytic anemia with a normal white cell and platelet count (Table 1). In 1996 he was referred to us for a hematologic evaluation of leukopenia, thrombocytopenia and macrocytic anemia. Physical examination on presentation was notable for a thin young man, short stature for his age, and mental retardation. He had a beaked nose, micrognathia, and a receding forehead. Skeletal survey revealed multiple abnormalities including scoliosis and severe spinal stenosis of the lumbar spine, hypoplastic mandible, convex Tali and distal radii, epiphyseal dysplasia affecting the distal ends of long bones, hypoplasia of the lunate bones of both hands and abnormal knee and elbow joints. MRI of the brain showed atrophic cerebellum with a mild atrophy of the pons and mid brain. Ventricles, corpus callosum and brain parenchyma were noted to be normal. These findings are consistent with other reports of patients with Seckel Syndrome. Evaluation showed serum folate 7.3 ng/ml, ferritin 36 ng/ml, vitamin B12 485 pg/ml, Fe 162 mg/dl, TIBC 273 mg/dl, and TSH 1.0 IU/ml. Bone marrow aspiration and biopsy showed 25% cellularity, an increase in erythroid series with a mild degree of dyserythropoiesis, rare myeloid cells and atypical megakaryocytes (Figure 1). Cytogenetic analysis of the bone marrow revealed trisomy 8, deletion of part of the long arm of chromosome 1 and a ring chromosome (Figure 2). A small number of normal appearing lymphocytes were also identified in the bone marrow biopsy. Cell marker analysis by flow cytometry of this bone marrow aspirate showed these cells to be CD3+, CD5+ and CD45+ suggesting a T-cell origin (Figure 3). Using a semi-quantitative gene rearrangement assay based on our modification of a published procedure (13), these T cells were found to be clonal (Figure 4). DEB (diepoxybutane) test performed by Dr. Arleen Auerbach, Rockefeller University, showed no evidence of chromosomal fragility (Table 2) in 1996 and in 1998. Patient remained asymptomatic and was thus followed without treatment. Peripheral blood counts remained fairly unchanged over a follow-up period of 5 years (Table 1). A repeat bone marrow aspirate and biopsy showed persistent hypocellular marrow with myelodysplastic features without evidence of leukemic transformation. Interestingly, there was a significant increase in the clon-

Table 1. Hematologic findings in patient

	April 1993	Feb 1996	Aug 1998	May 2000
Hemoglobin (g/dl)	13.2	13.6	12.1	14.1
Hematocrit (%)	37.6	39	34.3	39.9
Mean corpuscular volume (fl)	105	107	100	109.5
Platelet count (10⁹/L)	153	122	135	-
White-cell count (10⁹/L)	4.3	2.9	3.1	4.8
Differential count (%)				
Neutrophils	38	44	46	57
Bands	0	0	0	0
Lymphocytes	48	39	38	32
Monocytes	10	15	16	9
Eosinophils	1	1	0	1
Basophils	3	1	0	1

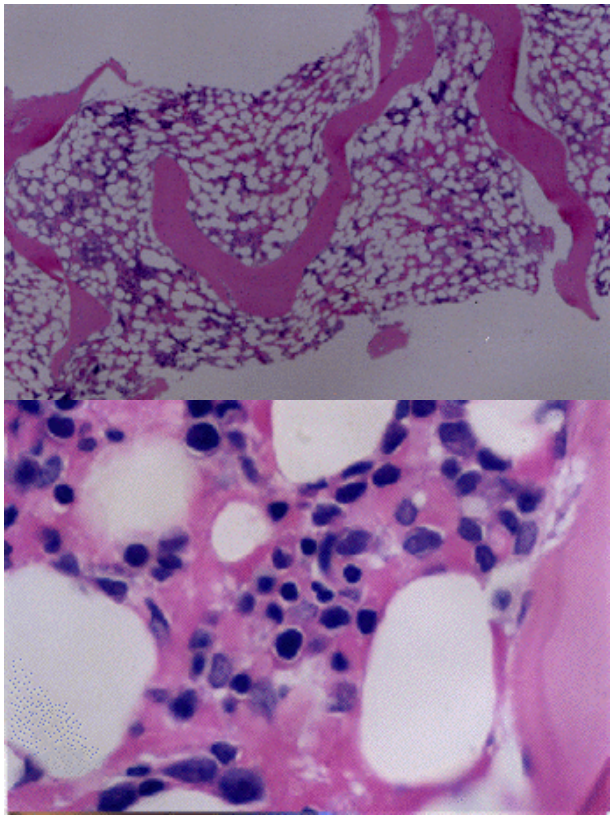

Figure 1. Photomicrographs of patient's bone marrow biopsy stained with Hematoxylin & Eosin (Aug. 1998).
A: Decrease in hematopoietic cells (hypocellular marrow) x 4.
B: Dyserythropoiesis x 100.

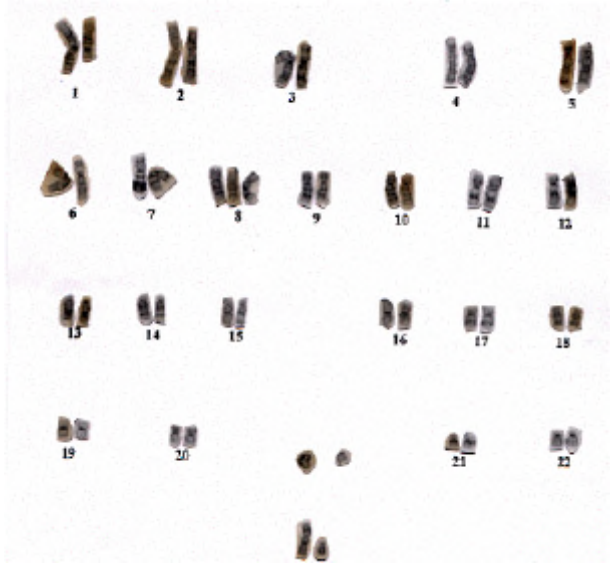

Figure 2. Photomicrograph of metaphase chromosomal spreads of bone marrow taken from patient (Aug 1998). Normal male karyotype with five clonal cell populations: (1) 47 chromosomes with trisomy 8 [47, XY, +8]; (2) 47 chromosomes with duplication of long (q) arm of chromosome 1[47, XY, dup (1), +8]; (3) 48 chromosomes with 1q deletion and ring chromosome [48, XY, del (1), +r, +8]; (4) 49 chromosomes with additional copy of ring chromosome [49, idem, +r]. (5) Karyotypically normal [46, XY]. This figure shows clone 3. Arrowheads point at 1q deletion, trisomy 8 and ring chromosomes.

Table 2. Diepoxybutane sensitivity analysis in the bone marrow of patients

	1996		1998	
	Spontaneous	DEB*-induced (0.1g/ml)	Spontaneous	DEB*-induced (0.1g/ml)
Patient	---	0.12	---	0.12
Control Rang	0.00-0.05	0.00-0.10	0.00-0.05	0.00-0.10
FA Range	0.02-0.80	1.06-23.9	0.02-0.80	1.06-23.9

* Diepoxybutane.
‹ 50 DEB treated cells were counted.

Table 3. Summary of Seckel patients with hematologic abnormalities reported in the literature

Case	Diagnosis	Abnorm. cyto-genetics	Chrom. fragility*	Bone marrow biopsy	Ref.
1.	AML	+	NA	NA	(2)
2.	Pancytopenia	-	+	NA	(4)
3.	Pancytopenia		+	Normal	(10)
4.	Myelodysplasia	+	+	NA	(21)
5.	Aplastic Anemia	-	+	NA	(3)
6.	Pancytopenia	-	-	Hypoplastic/ absent megakaryocyte	(5)
7.	Pancytopenia	-	-	Hypoplastic	(5)
8.	Pancytopenia	-	+	NA	(6)
9.	Myelodysplasia	+	-	Hypoplastic	This Report

*Determined by MMC and/or DEB test.
Abbreviations: NA, not available

al T-cell infiltration of the marrow.

Discussion. The patient reported here has many features of Seckel syndrome, although our patient is somewhat taller than the most reported cases and is only mildly mentally retarded. Thus, he would be classified as a patient with primordial dwarfism with features of Seckel syndrome as suggested by Buebel *et al.* Various hematologic abnormalities have been described in patients with Seckel syndrome (Table 3). When reviewed, most patients reported previously had chromosomal fragility, suggesting an association between chromosomal instability and hematologic abnormalities, as is suggested for Fanconi's anemia. Lilleyman has reported on two patients with Seckel like features and pancytopenia that lacked chromosomal fragility. This report lacks a complete description of clinical features in these patients and thus an accurate diagnosis of Seckel syndrome cannot be definitely ascertained. The author also recognized features in these patients that were more suggestive of FA than Seckel syndrome. Clinical findings described in our patient are those of Seckel syndrome and despite these features and hematologic defect, he did

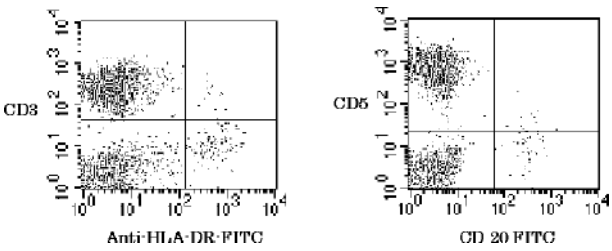

Figure 3. Flow cytometric analysis of bone marrow aspirate. Flow cytometric analysis of bone marrow aspirate was done to phenotype the abnormally present lymphocytes. These cells were strongly CD3+ (A) and CD5+ (B) but did not express CD20 antigen.

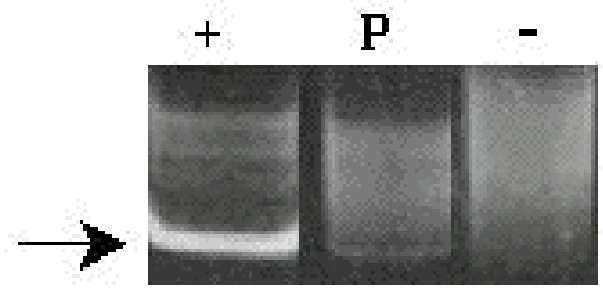

Figure 4. Gamma T-cell receptor (TCR) gene rearrangement assay to determine T-cell clonality. Presence of a discrete band is consistent with a monoclonal cell population. (+) positive control from a cancer line, distinct band signifies a clonal population of cells; (P) patient's bone marrow shows a faint but discrete band indicating a small population of clonal T cells (arrow) at the level of + control; (-) tonsillar tissue as negative control showing polyclonal pattern. Similar results were found on analysis of this patient's peripheral blood (data not shown).

not have any demonstrable chromosomal fragility. Our report complements the recent observation made by Abou-Zahr *et al*., who reported 2 patients with Seckel syndrome without hematologic abnormalities or MMC sensitivity. Taken together, these reports raise the possibility that at least a subset of patients with Seckel syndrome do not have any chromosomal fragility and further that hematologic defects in some patients with Seckel syndrome may be unrelated to chromosomal instability. Since there are known syndromes of dwarfism associated with the development of acute myelogenous leukemia or other hematologic abnormality, such as the Noonan syndrome Dubowitz syndrome and Shwachman-Diamond syndrome , and since myelodysplasia is rare in young patients, it remains possible that in our patient, the Seckel syndrome was the predisposing factor in the development of myelodysplasia. Of note here is the fact that our patient has abnormal cytogenetic findings that reveal five different clonal cell populations with a normal male karyotype (figure 2). Since we have not evaluated other tissues for similar cytogenetic abnormalities it is difficult to comment whether these abnormal clones of cells represent autosomal mosaicism as part of Seckel syndrome spectrum that took place earlier in the embryogenesis or are they changes reflecting the acquired myelodysplastic syndrome. Though former seems unlikely for two reasons. Firstly, autosomal mosaicism is extremely rare and secondly because, such extensive cytogenetic abnormalities with ring chromosome, are usually incompatible with fetal survival. An important yet unclear aspect of this patient's disease is the presence of T-cell clone in his bone marrow, identified by the T-cell receptor (TCR) gene arrangement assay (Figure 4). Presence of T-cell clone in association with MDS has been reported in the literature although their exact role in the pathogenesis of MDS remains elusive. Clinical data from MDS patients treated with antithymocyte globulin (ATG) suggest a T-cell mediated inhibition of hematopoiesis . These results are analogous to those seen in patients with aplastic anemia who are treated with ATG or cyclosporin. Interestingly, most of the MDS patients that responded to ATG or cyclosporin had hypoplastic bone marrow and belonged to the refractory anemia (RA) FAB subtype.

The development of MDS in this patient may be a result of inherent predisposition from Seckel syndrome rather than a primary T-cell disorder. In this patient the extent of T-cell infiltration of bone marrow was significantly increased on follow-up biopsy, clinical significance of which remains unclear. Improved understanding of the relationship between Seckel syndrome and hematologic disease will require the identification of the gene(s) responsible for the syndrome.

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