STRONG EVIDENCE FOR AUTOSOMAL DOMINANT INHERITANCE OF SEVERE CONGENITAL NEUTROPENIA ASSOCIATED WITH *ELA2* MUTATIONS

LAURENCE A. BOXER, MD, STEVEN STEIN, BA, DANIELLE BUCKLEY, PHD, AUDREY ANNA BOLYARD, BA, AND DAVID C. DALE, MD

Objective To investigate cases of severe congenital neutropenia (SCN) to ascertain SCN inheritance after determining that the same sperm donor was used by 4 different families to impregnate mothers.

Study design Because the donor sperm was not available, alternative methods were used to determine whether the sperm donor transmitted SCN. DNA isolated from leukocytes was used to sequence the *ELA2* gene in the affected children and their mothers. *ELA2* was amplified by polymerase chain reaction (PCR), and the product was sequenced. PCR was also performed with genomic DNA from the mothers and affected children using a set of 22 microsatellite PCR primers on chromosomes 14 and 19 to establish linkage to the paternal allele.

Results None of the mothers had a mutation in *ELA2*, but all 5 affected children had the same mutation affecting the fourth exon at site S97L. Linkage mapping analysis confirmed that all affected children had the same paternal allele on chromosome 19, which contains *ELA2*.

Conclusions Our findings indicate that the father provided consistent haplotypes leading to the expression of SCN in all affected children, supporting an autosomal dominant inheritance in which *ELA2* mutations occur. *(J Pediatr 2006;148:633-6)*

Severe congenital neutropenia (SCN) was originally described in 1956 as an autosomal recessive disorder characterized by severe neutropenia and recurrent bacterial infections and subsequently called Kostmann syndrome.[1] Kostmann syndrome is attributed to autosomal recessive inheritance, based on careful genealogic records of the original family and the incidence and pattern of this disease in a sparsely populated region of Northern Sweden where it was first detected. Most cases of SCN appear to be sporadic and are clinically similar.[2,3] The original cases that Kostmann described are also similar to cases occurring with an autosomal dominant pattern of inheritance,[4] which are now attributed to heterozygous mutations in the gene for neutrophil elastase (the *ELA2* gene).[5,6] Recently, our disease registry (the SCN International Registry) reported several cases of SCN associated with the identical mutation in *ELA2*. Further investigation revealed a common parent for these cases conceived through either in vitro fertilization or donor insemination (in 1 family).

METHODS

Case Reports

Over a 42-month period, the SCN International Registry and Tissue Repository received clinical information and request for sequencing for the *ELA2* gene from 401 patients, 5 of whom had the same heterozygous mutation in *ELA2*. At the University of Michigan, the physician responsible for managing patients with neutropenia identified that 3 of the 4 families used in vitro fertilization and 1 family used donor insemination to conceive. Further review of the clinical information revealed that all 5 children were conceived using the same donor sperm, identified by the same code from the sperm bank by all 4 families (Figure 1). Molecular studies were initiated to test whether the 5 affected children shared the same sperm donor by initially evaluating the site of *ELA2* mutation.

The 5 children were developmentally normal at birth. All were found to have recurrent fevers, severe neutropenia, and evidence suggesting bacterial infections, leading to blood testing and the subsequent diagnosis of SCN during infancy. Bone marrow

ANC	Absolute neutrophil count	PCR	Polymerase chain reaction
G-CSF	Granulocyte colony–stimulating factor	SCN	Severe congenital neutropenia

From the Divison of Pediatric Hematology/Oncology, Department of Pediatrics, C.S. Mott Children's Hospital, University of Michigan, Ann Arbor, Michigan and the Division of Hematology, Department of Medicine and Department of Genetics, University of Washington, Seattle, Washington.

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Reprint requests: Laurence A. Boxer, MD, L2112 Women's Hospital, Box 0238, University of Michigan, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0238. E-mail: laboxer@umich.edu.

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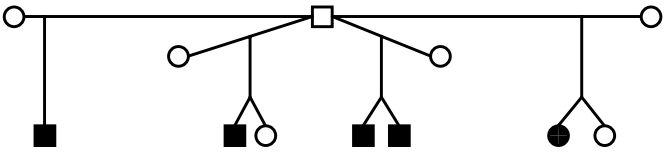

Figure 1. Pedigree of the affected children. The darkened symbols indicate the affected children.

examination in all of the children revealed similar arrest at the promyelocyte– myelocyte stage of development.

Based on the recurrent fevers and evidence of infection, all patients were treated with recombinant human granulocyte colony-stimulating factor (G-CSF) at a median dose of 6.7 ± 1.3 μg/kg/day with similar increases in neutrophil count. The mean for median blood absolute neutrophil count (ANC) at diagnosis was 84 ± 32 × 10^9/L (range, 0 to 166 × 10^9/L). After treatment with G-CSF, the mean for median ANC increased to 3290 ± 10^9/L (range, 420 to 8600 × 10^9/L). The clinical course for these 5 patients over the next 12 months improved, with no further bacterial infections in 3 children and infrequent episodes of otitis media in the other 2.

Laboratory Studies

Sequencing the gene for neutrophil elastase. Genomic DNA was isolated from peripheral blood cells from the neutropenic patients and their mothers. Written informed consent was obtained in every case as well as approval from the Institutional Review Board at the Universities of Michigan and Washington. DNA was not available from the patients' sperm donor. Direct sequencing analyses were performed on all 5 exons of the gene for neutrophil elastase and the minimal promoter using specific sense and antisense primers. In brief, 50-μL reactions containing 2.0 μL of each primer (12.5 pmol/μL), 28.5 μL of ddH_2O, 5 μL of buffer (10 ×), 1 μL of dNTPs (10 mmol), 0.5 μL of HotStar Taq polymerase (Qiagen, Santa Clarita, Calif), 10 μL of Q-solution (Qiagen), and 1 μL of genomic DNA (50 ng) were amplified, and the polymerase chain reaction (PCR) products were visualized on a 2% agarose gel. PCR products were purified using a Multiscreen 96 PCR plate (Millipore, Billerica, Mass). Sequencing was performed with the Big Dye Terminator version 3 (Applied Biosystems, Foster City, Calif) using standard techniques. All identified mutations were confirmed by repeat sequencing from a freshly prepared PCR product.

Microsatellite markers. Microsatellites are 2 to 5 nucleotide repeats (eg, GTGTGTGTGTGT) frequently occurring in the human genome. The number of repeats for a particular microsatellite varies within populations and within the alleles of an individual, and these repeats are heritable. Fluorescent PCR primers designed around the sites of known microsatellites will produce PCR products of varying sizes, depending on the number of repeats that an individual has for each allele. Linkage mapping is carried out by comparing the alleles in a family or a population over a series of closely spaced microsatellite markers.

PCR was performed with genomic DNA from the mothers and affected children using a set of 22 fluorescent-labeled PCR primers on chromosomes 14 and 19, spaced 5 to 10 centimorgans apart, equal to a statistical probability of 5% to 10% likelihood of crossing over between adjacent markers. Chromosome 14 served as a paternity confirmation control for the presence of the paternal allele in the affected children. Chromosome 19, which contains the elastase gene, was analyzed to confirm paternal association with the affected allele. PCR products were analyzed using ABI 3700 sequencers and ABI Gene Mapper software (Applied Biosystems). The following reagents were used: primer pair mix (5 μM of each primer), 1.0 of genomic DNA (3 mg/mL), 4.5 mL of True Allele PCR premix (Applied Biosystems), and 1.9 mL of sterile deionized water per reaction. Then 1.0 mL of each PCR product was added to a mixture of 9.0 μL of Hi-Di formamide and 0.0 2 μL of Gene Scan-500 L1 Z dye standard (both from Applied Biosystems) for fragment analysis on the ABI 3700.

RESULTS

Sequencing of the *ELA2* gene showed the identical mutation in exon 4 in all 5 patients; no mutation was detected in *ELA2* or in any of the mothers of these patients. The sequence studies identified 5 young children with the same mutation in exon 4 who exhibited a TCG→TTG change in their nucleotide sequence at nucleotide 4534 leading to amino acid change S97L.

The results of the microsatellite marker studies using a set of 22 fluorescent-labeled PCR primers on chromosomes 14 and 19 are shown in Figure 2. Linkage mapping analysis of the markers confirmed that all affected children had the same paternal alleles. Using markers D19S886 and D19S209, it was found that all of the affected children shared a single paternal allele across a 20-centimorgan region adjacent to the *ELA2* gene, a region equal to approximately 5 million DNA bases. As a control, there was no evidence of linkage on chromosome 14 using marker D14563, D145276, or D14574. Because the markers on chromosomes 14 and 19 were closely spaced, this reduced the likelihood of not detecting cross-over events between adjacent markers. The dominant paternal haplotypes over the 20-centimorgan region analyzed on chromosome 19 was B/B/C/C. In the dataset, the closest cross-over event on chromosome 19 can be seen in child 4 between marker D19S209 and marker D19S894, a distance of 2.5 to 3.5 million bases away from the site of the *ELA2* gene, resulting in haplotype B/B/(A or D)/D. A separate cross-over occurs in child 3 between markers D19S894 and D19S216, resulting in haplotypes B/B/C/D.

DISCUSSION

Severe congenital neutropenia is a rare hematologic disorder associated with severe infections and the risk of evolution to acute myelogenous leukemia.[7] The prognosis for children with this disorder has changed dramatically with the availability of recombinant human G-CSF, which has proven