Novel *HAX1* gene mutations associated to neurodevelopment abnormalities in two Italian patients with severe congenital neutropenia

We read with interest the recent perspective article by Klein.[1] Genetic analysis in individuals with severe congenital neutropenia (SCN) indicates that 60% of cases were attributable to heterozygous mutation in *ELA2* gene encoding neutrophil elastase.[2] Homozygous mutation in *HAX1* gene has been identified in patients with autosomal recessive form of SCN (Kostmann syndrome).[3] Patients with *ELA2* or *HAX1* mutations reveal a similar morphological and similar clinical phenotype suggesting that there may be common downstream molecular events caused by both mutations.

Interestingly, 28 out of 39 (72%) patients with *HAX1* mutations reported were in SCN kindred of Middle eastern descent indicative of consanguinity or intermarrying within specific population groups.[3-5] Five Japanese SCN patients are described[6] and only a few cases of true European descent have been documented so far.[3,5,7]

In the present study we describe the first 2 Italian SCN patients carrying two novel *HAX1* mutations associated to neurodevelopment abnormalities.

Genomic DNA was extracted from peripheral blood of the patients and the components of their families. Sequencing analysis of *HAX1* was performed as previously reported,[3] after excluding mutations in *ELA2* gene.[8]

In the first kindred the patient, a 4-year old boy, started presenting infections at the age of nine days. After persistent neutropenia and recurrent infections at seven months of life a Kostmann syndrome was diagnosed on the basis of neutrophil count (0.1×10^9/L) and on bone marrow analysis showing severe ipoplasia of myeloid serie, hypereosinophilia and arrest at the promyelocyte stage. No cytogenetic abnormalities were found. He was started on G-CSF therapy with an average dose of 7.5 µg/kg once a day in order to maintain neutrophil count between 1.5 to 3.0×10^9/L. At 18 months of age, signs of a developmental delay were noted involving motor, language and social areas. Neurological diagnostics revealed pathological EEG but normal MRI; he has not presented seizures.

A compound heterozygous mutation within exon 3 of *HAX1* gene has been found. It consisted of a frame-shift

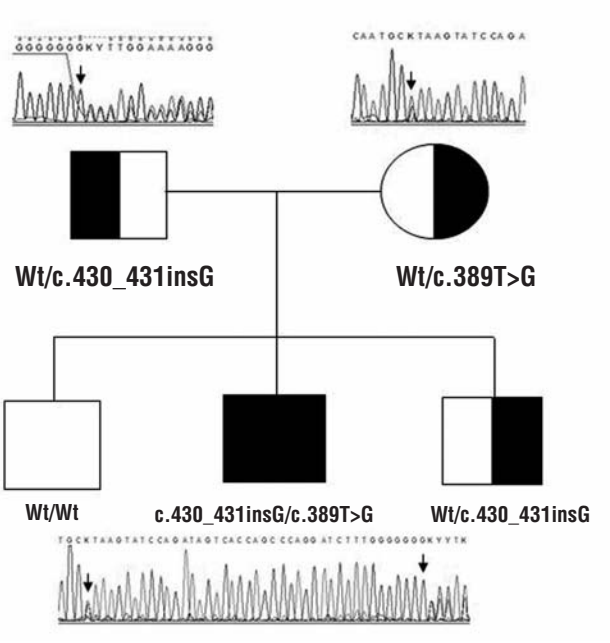

Figure 1. Sequence chromatograms of kindred 1. The filled symbol denotes the affected patient who is compound heterozygous for two mutations, each of them inherited from one of the parents. Both parents and one brother are heterozygous carriers (half-filled symbol). Mutations are indicated with black arrows. Square symbols denote male family members, the circle denotes a female.

Table 1. Known mutations in *HAX1*.

Location	Nucleotide change	Protein level	Phenotype§	N. patients	Origin	Ref.
Exon 2a	c.91delG	p.Glu31LysfsX54	1	2	British	(5)
Exon 2a/5*	c.91delG/ c.568 C>T	p.Glu31LysfsX54/ p.Gln190X	1	1	Swedish	(7)
Exon 2a	c.121-125insG	p.Ser43LeufsX	1	1	Ashkenazi Jewish	(5)
Exon 2a	c.130-131insG	p.Trp44X	1	19 2 1	Middle East	(3) (4) (5)
Exon 2a	c.174-175insG	p.Glu59X	1	1	Middle East	(4)
Exon 2a	c.180delA	p.Glu60AspfsX25	1	1	Middle East	(4)
Exon 2b	c.256C>T	p.Arg86X	2	1 3	Iranian Japanese	(3) (6)
Exon 2b/3*	c.256C>T/ c.376-434del	p.Arg86X / p.Arg126fsX128	2	2	Japanese	(6)
Exon 3	c.368-381del	p.Gln123LeufsX4	2	1	Middle East	(4)
Exon 3*	c.389T>G/ c.430-431insG	Leu130Arg/ p. p.Val144GlyfsX5	2	1	Italian	This report
Exon 3	c.409C>T	p.Gln137X	2	1	Italian	This report
Exon 3	c.430-431insG	p.Val144GlyfsX5	2	1	Middle East	(4)
Exon 5	c.568 C>T	p.Gln190X	2	3	Swedish	(3)

*Compound heterozygous mutation. §1 = affecting transcript variant 1, pt with SCN. 2 = affecting transcript variant 2, pt with SCN and neurological symptoms.

mutation c.430_431insG leading to a premature stop codon Val144GlyfsX5 inherited from his father and a missense mutation c.389T>G generating a non-conservative amino acid substitution Leu130Arg inherited from his mother. This Leu130Arg substitution is a novel variation that was not detected in 90 healthy controls, so excluding the possibility of a polymorphic change. Both parents and one brother, heterozygous carriers of the c.430_431insG, had no detectable clinical phenotype (Figure 1).

In the second kindred, the patient, a 7-year old boy, was diagnosed at the age of 21 months after a persistent neutropenia and a long history of severe recurrent infections. Bone marrow aspiration showed myeloid dysplasia and arrest at myelocyte stage. Cytogenetic analysis was normal. He was started on G-CSF therapy with an average dose of 5 µg/kg once a day in order to maintain neutrophil count between 1.5 to 3.0×10^9/L. Patient development has been delayed since infancy, with mental and psychomotor retardation, serious walking impairment, and severe bilateral myopia. No episode of seizure was reported in this patient. The sequencing analysis of *HAX1* gene identified the homozygous mutation c.409C>T within exon 3, resulting in a premature stop codon p.Gln137X. This mutation is novel and heterozygous carrier status was confirmed in healthy parents and his sister. No consanguinity was reported among parents, but their origins are from the same geographical area.

So far only 10 *HAX1* mutations are described. The known *HAX1* mutations reported up to date, included the new mutations that we have described, are listed in Table 1.

As shown in the Table, analysis of the patients' genotypes and phenotypes revealed a striking correlation: mutations affecting transcript variant 1 only were associated with SCN, whereas mutations affecting both transcript variants 1 and 2 caused SCN and neurological symptoms, including epilepsy and neurodevelopmental delay.[7] This correlation is confirmed also in our patients. In fact, all the mutations are founded within exon 3, affecting transcript variants 1 and 2 of *HAX1* gene; both patients presented neurodevelopment abnormalities with mental and psychomotor retardation.

Our study describing the first 2 Italian patients with SCN due to *HAX1* mutations indicates that mutations of this gene are not limited to patients with specific ethnic origin. *HAX1* is a ubiquitously expressed gene but its mutations are relatively uncommon. Given this, the description of all new patients and the determination of whether the type of mutation impacts on phenotype and/or susceptibility to leukemic transformation, adds new and precious information needed to better characterize the clinical features of SCN-*HAX1* mutated patients and the role of HAX1 protein.

Marina Lanciotti,[1] Stefania Indaco,[1] Sonia Bonanomi,[2] Tiziana Coliva,[2] Elena Mastrodicasa,[3] Gianluca Caridi,[4] Michaela Calvillo,[1] and Carlo Dufour[1]

[1]Hematology Unit, Pediatric Hemato-Oncology Department, G. Gaslini Institute, Genova; [2]Clinic of Pediatrics, University of Milano Bicocca, San Gerardo Hospital, Monza; [3]Unit of Pediatric Oncology and Haematology, S. Maria della Misericordia Hospital, Perugia; [4]Laboratory on Pathophysiology of Uremia, G.Gaslini Children's Hospital, Genova, Italy. On behalf of the Italian Registry of Neutropenia and of the Marrow Failure Syndrome group of the AIEOP (Associazione Italiana Emato-Oncologia Pediatrica)

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Correspondence: Marina Lanciotti, Hematology Laboratory, Department of Pediatric Hemato-Oncology, G. Gaslini Institute, L.go G. Gaslini 5, Genova Italy. Phone: international +39.10.5636693. Fax: international +39.10.5636693. E-mail: marinalanciotti@ospedale-gaslini.ge.it

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Molecular or cytogenetic monitoring and preemptive therapy for central nervous system relapse of acute promyelocytic leukemia

We read with great interest the article by Montesinos *et al.*[1] concerning central nervous system (CNS) relapse of acute promyelocytic leukemia (APL). They reported a low incidence of CNS involvement at first relapse in APL patients following therapy without CNS prophylaxis.[1] The optimal management of APL relapse in CNS has taken on increasing significance.[2] Here we report our experience concerning CNS relapse of APL and introduce a new approach with molecular or cytogenetic monitoring in cerebrospinal fluid (CSF) as contrasted with the observations by Montesinos *et al.*[1]

Since 2005, we experience of 6 patients with first relapse of APL at The University of Tokyo Hospital. These patients received different first-line therapies with or without prophylactic intrathecal chemotherapy (IT) and high-dose cytarabine (HDAraC), which are effective for CNS leukemia. All these first-line therapies included all-trans retinoic acid (ATRA) and anthracycline. Patients' characteristics are shown in Table 1. The patients with relapsed APL received arsenic trioxide and prophylactic IT except case 1, who received ATRA,